Standard Operating Procedures (SOP) for the Spectrophotometric Determination of Phenolic Compounds Contained in Plant Samples

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Additional information is available at the end of the chapter

1. Introduction

The quality control of raw materials and products from plants is one of the topics most discussed by universities and health surveillance agencies. One of the primary tools used to ensure the reliability of production processes is the use of Standard Operating Procedures (SOPs). SOPs sequentially describe the steps of a particular methodology so that it can be reproduced by different analysts, which minimises variations in their implementation and improves the standardisation of the final product.

Several techniques, such as high performance liquid chromatography, gas chromatography and mass spectrometry, can be used in SOPs to control the quality of plant phenolic compounds [1,2]. However, these compounds have a characteristic spectrum produced by the double bonds in the aromatic rings and substituent positions that facilitates their identification and the development of spectrometric analytical techniques is easily accomplished. In this sense, spectrophotometric methods are more practical, reproducible and inexpensive than other techniques and are therefore favoured for the development of analytical methodologies for such determinations.

In addition to producing compounds such as carbohydrates, lipids, proteins and nucleic acids directly involved in their essential growth functions, plants have an arsenal of enzymes capable

of producing, processing and accumulating several other substances not necessarily related to the maintenance of their life [3]. All of these reactions can be defined as secondary metabolism, the products of which provide advantages for both survival and species perpetuation in the plant's ecosystem [4]. However, this protection has a cost for the plant because metabolic resources that could increase its biomass are used to produce these compounds. In addition to protection, secondary metabolites perform important ecological functions such as inhibiting the germination and growth of other plants, attracting both pollinators and seed-dispersing animals and providing chemical defences against microorganisms [5].

Phenolic compounds, which have one or more hydroxyl groups linked to an aromatic ring, stand out from other classes of plant secondary metabolites because they are widely distributed and have various ecological functions that are scientifically proven to have numerous pharmacological activities and are well represented by tannins, flavonoids and coumarins.

1.1. Tannins

Tannins are water soluble phenolic compounds with a molecular weight between 500 and 3000 Daltons and may be chemically classified into two groups: hydrolysable tannins and condensed tannins [6,7]. Hydrolysable tannins are connected by ester-carboxyl linkages, which undergo hydrolysis under acidic and basic conditions [8]. Figure 1 presents an example of hydrolysable tannin (gallotannin), connected through a polyol (usually β-D-glucose) with the hydroxyl group esterified by gallic acid. Polyphenols connected with ellagic acid are called ellagitannins [9].
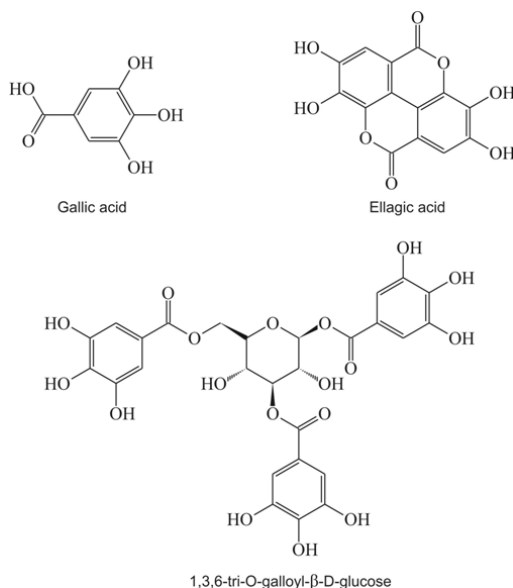

Figure 1. Structure of gallic acid, ellagic acid and 1,3,6-tri-O-galloyl-β-D-glucose, a hydrolysable tannin.

Condensed tannins, also known as proanthocyanidins (Figure 2), can contain dozens of units of flavan-3-ols (catechin) or flavan-3,4-diols (leucoanthocyanidins). These units have a complex structure and are resistant to hydrolysis; however, they can be soluble in aqueous organic solvents because of their structure [7].
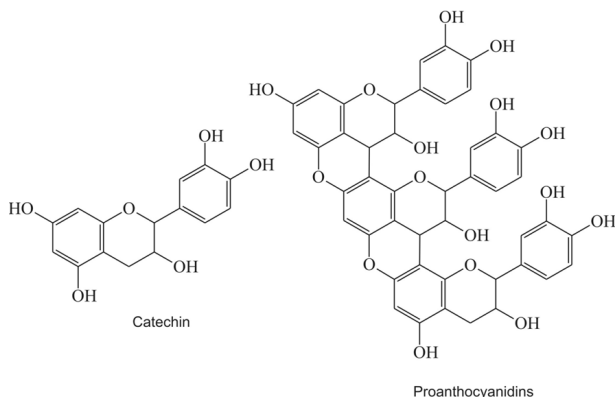

Figure 2. Basic structures of catechin and proanthocyanidins (condensed tannin).

Hydrolysable and condensed tannins may occur in the same plant simultaneously. However, the hydrolysable tannins are characteristic of Magnoliopsida herbaceous and woody plants and are restricted to certain taxonomic families. Ellagitannins have been used as taxonomic markers, particularly for Hemamelidaceae, Dilenidaceaa and Rosaceae. Condensed tannins have been identified in all plant groups, including Gymnosperms and Pteridophytes [10].

These secondary metabolites were initially identified by their astringent taste and capacity to bind proteins, which allows for the precipitation and formation of complexes with collagen skin fibres to increase their resistance to water and heat. Chemically speaking, hydrophobic interactions and hydrogen bonds between the phenolic groups in tannins and some macromolecules explain these features. However, the stability of the formed complexes only results after the formation of covalent bonds via the oxidation of tannins by quinones [11].

Since antiquity, plants containing tannins have been used medicinally as anti-inflammatory, antimicrobial, antitumor and antiviral agents and to treat both wound sand burns [7]. Tannins are also used to manufacture beverages and process animal skin into leather. Some researchers have shown that tannins protect plants against attack by herbivores and pathogens [12].

Although the use of tannin in the tanning industry has become restricted, interest in studying the ingestion of foods containing tannins to prevent diseases such as atherosclerosis or certain types of cancer has increased because of various epidemiological studies. Some studies report that the complexation of tannins with proteins gives them an important role in controlling bacteria, fungi and insects [13-15]. Other studies examined the inhibitory action of the enzyme reverse transcriptase [16] and the anticarcinogenic activity associated with

green tea and diets rich in fruits containing tannins [17]. It is generally believed that the pharmacological activity of tannins occurs via their complexation with metal ions, antioxidant activity or the ability to complex with macromolecules.

Maytenus ilicifolia (Schrad.) Planch. (Celastraceae), popularly known as "espinheira-santa", is a species native to Brazil traditionally used to treat digestive disorders and the literature cites tannins as compounds that act to protect the stomach by helping to treat ulcers and gastritis [18-20]. In addition to these activities, *M. ilicifolia* has other pharmacological applications, such as anticancer, antimicrobial and antioxidant activity and treatment of the central nervous system [21]. For these reasons, *M. ilicifolia* was chosen as a reference species in this study.

1.2. Flavonoids

Flavonoids comprise a group of natural substances with great structural diversity and there are currently more than nine thousand known flavonoids that do not occur in humans but can be found in various plant parts such as the leaves, fruits, bark, roots, stems and flowers [22,23]. Flavonoids (Figure 3) are composed of a simple skeleton containing two phenol rings connected by a propionic chain; where ring A is the acetate derivative and both ring B and the three-carbon bridge are derived via a shikimate pathway, which may be associated with carbohydrates (heterosides), un associated (aglycones) or polymerised further (anthocyanins) [24].
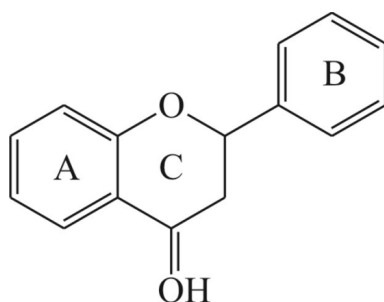

Figure 3. Basic structure of flavonoids.

This class of metabolites has several biological functions, such as defence against both herbivores and pathogens, the perpetuation of the species by attracting seed dispersing animals, protection from ultraviolet rays and allelopathy [24,25]. Flavonoids also possess important pharmacological properties, such as antioxidant, antiinflammatory, anti-thrombogenic, antimicrobial, anticancer, antidiabetic and hypocholesterolemic activities [23,26].

Studies show that flavonoids are chemical markers responsible for various pharmacological activities performed by the genus *Bauhinia* [27-29]. *Bauhinia forficata* Link (Fabaceae), popularly known as "pata-de-vaca", is a tree native to Brazil that prevails in the phytogeographical area of Mata Atlântica [30] and can also be found in Cerrado. Pata-de-vaca is used in folk

medicine as a hypoglycemic, diuretic and antihypertensive agent [31,32]. Several plant-based products are sold in open markets, pharmacies and natural product stores [33]. Though not officially recommended, herbal products from the leaves of pata-de-vaca (*Bauhinia* L.) are popularly used for therapeutic purposes. This plant was used in our study as a reference species for flavonoids.

1.3. Coumarins

Coumarins are lactones of *O*-hydroxy-cinnamic acid derived from trans-cinnamic acid via oxidation-reduction and isomerisation to produce 1,2-benzopyrone. Coumarins are divided into simple coumarins, furanocoumarins, pyranocoumarins, dimeric coumarins and chromones (Figure 4) [34]. The difference between these classes is the position of the radical in the coumarin lactone ring, which varies between C-6, C-7 and C-8. Chromones represent a group of natural isomers to coumarin that may be linearly and angularly prenylated at C-6 and C-8, respectively, in furanochromones and pyranochromones, respectively [34].
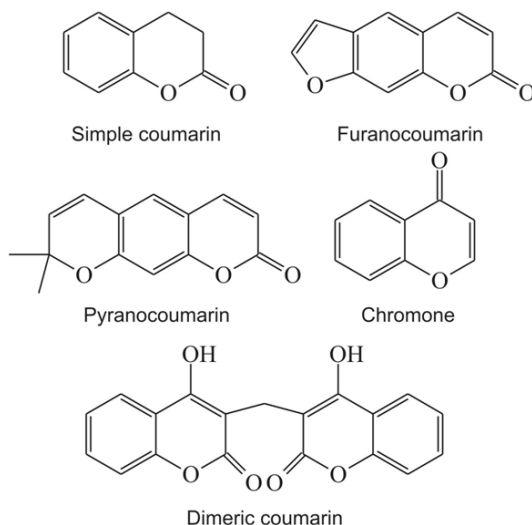

Figure 4. Basic structure of coumarins and chromones.

Coumarins are used as antioxidants, anti-HIV drugs, antispasmodics, spasmolytics, hypolipidemics, hypotensives and vasodilating agents [34]; however, they are also used in food flavouring, perfumes, tobacco and cosmetic products [35]. It is estimated that the daily human exposure to coumarins from cosmetics and perfumes is 0.04 mg/kg/day [36].

Coumarins are a class of secondary metabolites widely distributed throughout the Plantae kingdom and found in Fungi and Bacteria as well [37]. In plants, coumarins are found frequently in the families of Apiaceae, Rutaceae and Asteraceae, and less frequently in the families of Fabaceae, Oleaceae, Moraceae and Thymeleaceae [38].

Mikania glomerata Spreng. (Asteraceae), popularly known in Brazil as "guaco", has existed in the Brazilian Pharmacopoeia since 1929 and is used as an expectorant to treat respiratory problems. Most herbal products are marketed as some form of syrup; however, they are produced from fluid extracts and tinctures [39,40]. Chemical studies of this species show coumarins to be a major component that can be used as a chemical marker for the quality control of products based on guaco [41-43].

Coumarins have a characteristic UV spectrum due to the nature and position of their substituents, which facilitates both their identification and the development of analytical spectrophotometric techniques [11]. For these reasons, *M. glomerata* was chosen as the reference species to quantify the coumarin level.

2. Problem statement

Despite extensive literature presenting various analytical methods, the development of an SOP is often difficult for three reasons: 1) the work does not detail the difficulties and adjustments required to implement the methodology, 2) the steps are not clearly presented for reproduction and 3) the limits of interpretation are not discussed. One criterion recommended by the National Sanitary Surveillance Agency (Agência Nacional de Vigilância Sanitária - ANVISA) in Brazil for the standardisation of herbal drugs is the active compound content or chemical class, which is the total concentration of tannins for products based on *M. ilicifolia* (espinheira-santa) [44], flavonoids for *B. forficata* (pata-de-vaca) [45,46] and coumarins for *M. glomerata* (guaco) [44].

Thus, this paper presents research protocols adopted by our research group to study the levels of tannins, flavonoids and coumarins from plant extracts and the experimental application of these SOPs to analyse products sold in markets (pharmacies and natural product stores) as phytomedicines[1] or plant drugs[2] with high commercial value.

3. Standard operating procedures (POP)

The following SOPs describe the chemical classes to be analysed and the chemical basis of the methods. They provide a detailed list of all the reagents required for the preparation and describe the experimental procedure to be followed. Finally, there is a list of references used in the development of the SOP.

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1 "All medicine is obtained using solely active raw vegetables. It is characterised by knowledge of the effectiveness and risks of their use, as well as the reproducibility and consistency of its quality. Its efficacy and safety are validated through ethnopharmacological surveys of use, documentation, technical and scientific publications or clinical trial phase 3"[44].

2 "Medicinal plant or their parts, after collection processes, stabilisation and drying and can be full, erasures, crushed or powdered" [44].

3.1. Standard operating procedure for the quantification of tannins

Description: Tannins are phenolic compounds with the ability to bind macromolecules, especially proteins. This class of compounds has traditionally been divided into two groups: hydrolysable and condensed tannins. Hydrolysable tannins are characterised by a central polyol, usually β-D-glucose, containing hydroxy groups esterified with gallic acid and ellagic acid. Condensed tannins are oligomers or polymers formed by the condensation of two or more molecules of flavan-3-ol or flavan-3,4-diol [3] [7].

Principle of the method: The phenolic compounds in the sample are oxidised using the Folin-Ciocalteu reagent. This reagent is a mixture of phosphotungstic and phosphomolybdic acids that are reduced by the oxidation of phenolic compounds in a mixture of tungsten and molybdenum oxides. The blue colour produced by the oxides has a maximum absorption at 760 nm and is proportional to the total phenolic concentration [47]. The tannin content is calculated as the difference between the total and waste phenol content.

Reagents:

(1) Tannic acid (0.1 mg/mL, w/v): Dissolve 10 mg of tannic acid in 100 ml of distilled water.

(2) Folin-Ciocalteu reagent (10%, v/v): Dilute 5 ml of Folin-Ciocalteu reagent with 45 mL of distilled water.

(3) Sodium carbonate Na_2CO_3 (7.5%, w/v): Dissolve 7.5 g of Na_2CO_3 in 100 ml of distilled water. If necessary, solubilising the solution on a heating plate and magnetic stirrer.

(4) Methanol (80%, v/v): Dilute 800 ml of methanol with 200 ml of distilled water.

The reagent volume is sufficient to examine a maximum of 100 analyses.

Preparation of samples: The powdered sample (500 mg) should be extracted with 50 mL of 80% methanol for 30 minutes on a hot plate. The extract should be filtered through filter paper into a 50 mL volumetric flask and the volume should be completed using the same solvent. The final extract concentration will be 10 mg/mL. If the products are liquid, they must be evaporated. The dried extract should be dissolved in 80% methanol for a final concentration of 1 mg/mL.

Calibration curve: The calibration curve must be prepared using 100-500 μL aliquots of the tannic acid solution, 500 μL of the Folin-Ciocalteu solution and 1 mL of the sodium carbonate solution. The final volume should be adjusted to 10 mL with distilled water. The final tannic acid concentration will be 1-5 μg/mL.

Measurement procedure: To quantify the total phenol concentration (that is, all of the phenols present in the sample), 500 μL of the extract must be transferred to a test tube. Next, 500 μL of the Folin-Ciocalteu solution, 1 mL of the sodium carbonate solution and 8 ml of distilled water are added. The samples remain at room temperature for 30 minutes. The spectrophotometer should be adjusted to a wavelength of 760 nm and the equipment must be rinsed with distilled water. To quantify the phenol waste, (i.e., the phenols present in the sample except for the proteins precipitated with the tannin) 500 mg of casein is weighed and

transferred into a 25 ml Erlenmeyer flask before adding 5 ml of the extract and 5 ml of water distilled. After two hours (time required for the complexing of the tannins to the total protein), the extracts are filtered into a 10 mL volumetric flasks and its volume is adjusted with distilled water. The phenols are considered to be equal to the residue from the total phenol. These assays are performed at least in triplicate and the total phenolic content is expressed as milligrams of tannic acid equivalents per gram of sample or extract (mg TAE/g).

References:

Amorim E. L. C, Nascimento J. E., Monteiro J. M., Peixoto Sobrinho T. J. S, Araújo T. A. S., Albuquerque U. P. A simple and accurate procedure for the determination of tannin and flavonoid levels and some applications in ethnobotany and ethnopharmacology. Functional Ecosystems and Communities 2008; 2(1) 88-94 [47].

Santos S. C., Mello J. C. P. Taninos. In: Simões C. M. O., Schenkel E. P., Gosmanm, G., Mello J. C. P., Mentz L. A., Petrovick P. R. (ed.) Farmacognosia: da planta ao medicamento. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2004. p.615-656 [3].

3.2. Standard operating procedure for the quantification of flavonoids

Description: Flavonoids are the most important and diverse phenolic compounds. Most flavonoids have 15 carbon atoms and consist of two phenol rings connected by a chain of three carbons to form a tricyclic compound [24].

Principle of the method: The aluminium ion (Al^{3+}) is reacted with the flavonoids in the sample to form the stable flavonoid-Al^{3+}complex, which has a yellow colour and intensity proportional to the flavonoid concentration. This reaction causes a bathochromic shift and intensification in the absorption, which can be measured without influence from other phenolic compounds present in the sample [48].

Reagents:

(1) Rutin (0.1 mg/mL, w/v): Dissolve 10 mg of rutin in 100 ml of methanol.

(2) Acetic acid solution (60%, v/v): Dilute 30 ml of acetic acid with 20 ml of methanol.

(3) Pyridine Solution (20%, v/v): Dilute 40 ml of pyridine with 160 ml of methanol.

(4) Aluminium chloride solution $AlCl_3$ (5%, w/v): Dissolve 5 g $AlCl_3$ in 100 mL of methanol. If necessary, complete dissolution via magnetic stirring.

(5) Methanol (80%, v/v). Dilute 80 ml of methanol with 20 ml of distilled water.

The reagent volume is sufficient to examine a maximum of 100 analyses.

Preparation of samples: The powdered sample (500 mg) should be extracted with 50 mL of 80% methanol on a hot plate for 30 minutes. The extract should be filtered through filter paper into a 50 mL volumetric flask and the volume should be adjusted with the same solvent. The final extract concentration will be 10 mg/mL. Liquid products must be evaporated. The dried extract should be dissolved in 80% methanol to obtain a final concentration of 1 mg/mL.

Calibration curve: The calibration curve must be prepared using 100-1000 μL aliquots of the rutin solution, 500 μL of the acetic acid solution, 2 mL of the pyridine solution and 1 ml of the reagent aluminium chloride solution. The final volume should be adjusted to 10 mL with 80% methanol. The final rutin concentration will be 1-10 μg/mL.

Measurement procedure: To quantify the flavonoids, 500 μL of the extract should be transferred to a test tube. Next, 500 μL of the acetic acid solution, 2 mL of the pyridine solution, 1 ml of the reagent aluminium chloride solution and 6 ml of 80% methanol will be added. The samples remain at room temperature for 30 minutes. The spectrophotometer should be adjusted to a wavelength of 420 nm and the equipment must be rinsed with distilled water. The test shall be performed at least in triplicate and the flavonoid content is expressed as milligrams of rutin equivalents per gram of sample or extract (mg RE/g).

References:

Peixoto Sobrinho T. J. S, Silva C. H. T. P., Nascimento J. E., Monteiro J. M., Albuquerque U. P., Amorim E. L. C. Validação de metodologia espectrofotométrica para quantificação dos flavonóides de *Bauhinia cheilantha* (Bongard) Steudel. Brazilian Journal of Pharmaceutical Sciences 2008; 44 (4) 683-689 [48].

Zuanazzi, J. A. S.; Montanha, J. A. Flavonóides. In: Simões C. M. O., Schenkel E. P., Gosmanm, G, Mello J. C. P., Mentz L. A., Petrovick P. R. (ed.) Farmacognosia: da planta ao medicamento. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2004, p.577-614 [24].

3.3. Standard operating procedure for the quantification of coumarins

Description: Coumarins are lactones of *O*-hydroxy-cinnamic acid and are metabolites of phenylalanine. 1,2-benzopyrone is the simplest coumarin representative, others being furocoumarins, pyranocoumarins, dimeric coumarins and chromones [34].

Principle of the method: The Borntrager reaction is based on the solubility of free coumarin derivatives in polar organic solvents and the solubility of their soluble alkali phenolates. Coumarin absorbs at 280 nm; however, ionisation of phenolic hydroxyls in the molecule by alkaline hydroxide causes a bathochromic deviation to 320 nm, which is proportional to the coumarin concentration [40].

Reagents:

(1) 1,2-benzopyrone (1 mg/mL, w/v): Dissolve 10 mg of coumarin in 10 ml of distilled water.

(2) Lead acetate (5%, w/v): Dissolve 2,5 g of lead acetate in 50 ml of distilled water.

(3) Hydrochloric acid solution, HCl (0.1 M, v/v): Dilute 10 ml of concentrated hydrochloric acid with 1000 ml of distilled water.

(4) Methanol (80%, v/v): Dilute 80 ml of methanol with 20 ml of distilled water.

The reagent volume is sufficient to examine a maximum of 100 analyses.

Preparation of samples: The powdered sample (500 mg) should be extracted with 50 mL of 80% methanol for 30 minutes on a hot plate. The extract should be filtered through filter paper

into a 50 mL volumetric flask and the final volume should be adjusted with the same solvent. The final extract concentration will be 10 mg/mL. Liquid products must be evaporated. The dried extract should be dissolved in 80% methanol for a final concentration of 1 mg/mL.

Calibration curve: The calibration curve must be prepared using 50-500 μL aliquots of the coumarin solution, 2 ml of distilled water and 500 μL of the lead acetate solution. The sample should be shaken and the final volume should be adjusted to 10 mL with distilled water before transferring 2 mL of this solution to a new test tube and adding 8 mL of hydrochloric acid solution. The final concentration of rutin will be 1-10 μg/mL.

Measurement procedure: To quantify the coumarins, 500 μL of the extract should be transferred to a test tube. Next, 2 ml of distilled water and 500 μL of lead acetate solution will be added. The sample is shaken and then 7 ml of distilled water are added before transferring 2 mL of this solution to a new test tube and adding 8 mL of hydrochloric acid solution. The samples remain at room temperature for 30 minutes. The spectrophotometer should be adjusted to a 320 nm wavelength and the equipment must be rinsed with distilled water. The test should be performed in at least triplicate and the total coumarin content is expressed as milligrams of coumarin equivalents per gram of the sample extract (mg CE/g).

References:

Kuster R. A. M., Rocha L. A. M. A. Cumarinas, coronas e cantinas. In: Simões CMO, Schenkel EP, Gosmanm, G, Mello JCP, Mentz LA, Petrovick PR. (ed.) Farmacognosia: da planta ao medicamento. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2004, p. 537-556 [34].

Osório O. K., Martins J. L. S. Determinação de cumarina em extrato fluido e tintura de guaco por espectrofotometria derivada de primeira ordem. Brazilian Journal of Pharmaceutical Sciences 2004; 40 (4) 481-486 [40].

4. Results

Analysis of the active component levels in raw plant materials and phytomedicines is essential for the safety and efficacy of pharmaceutical products [49]. The quantification of active compounds in herbals is still only incidentally performed due to the presence of active phytocomplexes plants and their extracts [50], which complicates their analysis. Through this framework, the use of standardised extracts focusing on specific groups of active components ensures the chemical homogeneity of the product, which improves product quality [51]. The compounds selected for this quality adjustment process should be the same as the assets in the product [52].

In this way, five products containing *M. ilicifolia* (all plant drugs), four containing *B. forficata* (all plant drugs) and five containing *M. glomerata* (three plant drugs and two fluid extract) sold in pharmacies and health food stores in Recife/PE, Northeast Brazil were obtained.

Analysis of the active component concentrations were conducted as listed in the described standard operating protocols (SOPs) and the results are presented below.

4.1. Calibration curves

To quantify the active components, calibration curves with increasing concentrations proportional to their absorbance were constructed. A correlation equation was obtained from these curves (generally linear) of the type $y = ax + b$, where y corresponds to the absorbance of the sample and x the concentration. To convert the absorbance values   (nm) to sample concentration (µg/mL), it is necessary to place the sample absorbance into the equation as y. The correlation coefficient (R^2) shows the ability of the method to provide directly proportional results between the analyte concentration and the device response. The interval between the lowest and highest scalar values, which is also called the linearity range, should be determined with both precision and accuracy. The correlation coefficient must be equal to or greater than 0.98.

The calibration curve constructed for tannic acid and tannins and used to quantify *M. ilicifolia* demonstrated a correlation equation of y = 0.067x + 0.01 and a correlation coefficient of R^2 = 0.996 (Figure 5).
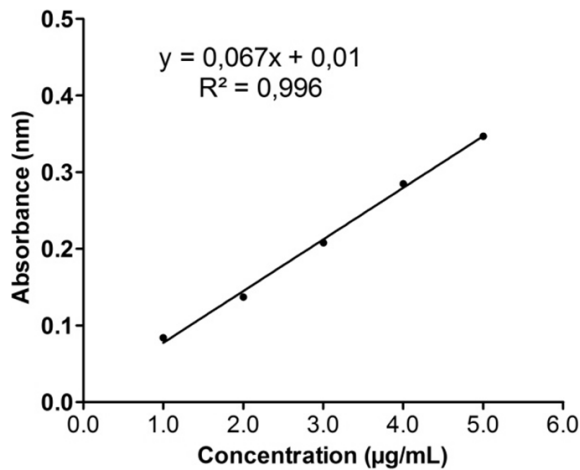

Figure 5. Calibration curve constructed using tannic acid concentrations of 1-5 µg/mL at 760 nm used to quantify the tannin content of *Maytenus ilicifolia* (Schrad.) Planch.

A calibration curve was constructed from rutin to quantify flavonoids in products from *B. forficata* and yielded the correlation equation y = 0.022 + 0.0039 x and correlation coefficient R^2 = 0.991 (Figure 6).
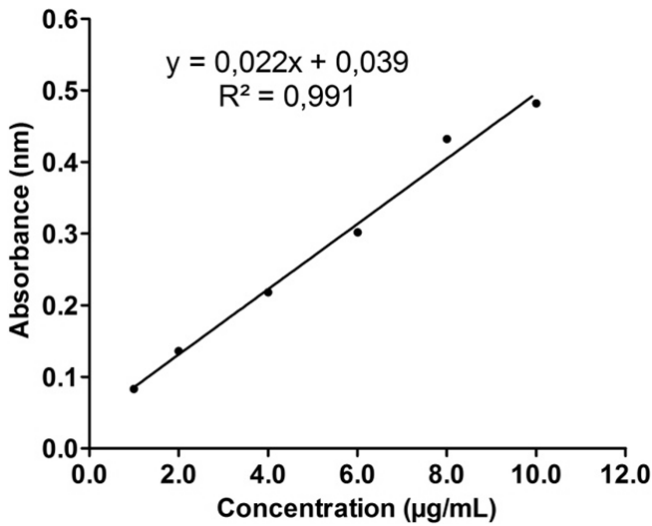
Figure 6. Calibration curve constructed from rutin for concentrations of 1-10 µg/mL at 420 nm used to quantify the flavonoids content in *Bauhinia forficata* Link.

The correlation equation and coefficient obtained from the calibration curve used to analyse coumarins in products containing *M. glomerata* were y = 0.049 x + 0.031 and R^2= 0.994 (Figure 7), respectively.
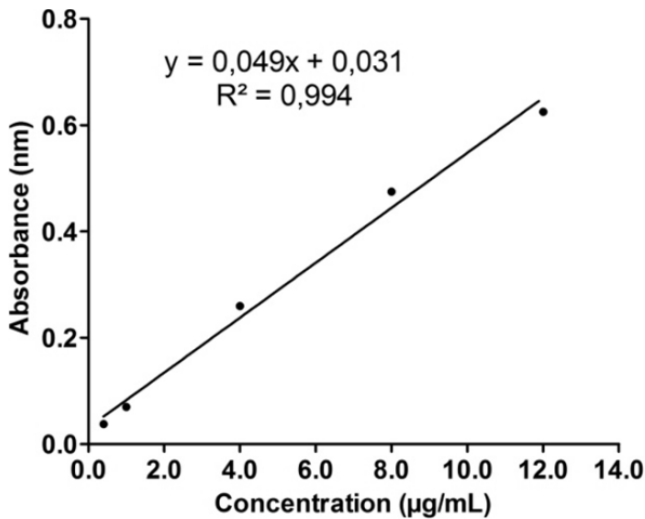

Figure 7. The calibration curve constructed using 1,2-benzopyrone in concentrations of 1-12 µg/mL using 320 nm excitation to quantify the coumarin content in *Mikania glomerata* Spreng.

4.2. Content of active principles

The results of quality control of tannins of *M. Ilicifolia*, flavonoids of *B. forficata* and coumarins of *M. glomerata* are shown in Table 1.

Specie / Marker	Sample	Concentration ± SD (mg/g)	CV (%)
Maytenus ilicifolia Tannins	MI1	12.57 ± 2.15a	17.12%
	MI2	4.04 ± 0.23b	5.75%
	MI3	5.61 ± 0.55bc	9.76%
	MI4	7.72 ± 0.84c	10.84%
	MI5	11.81 ± 1.00a	8.44%
Bauhinia forficata Flavonoids	BF1	4.89 ± 0.11a	2.33%
	BF2	7.27 ± 0.39a	5.41%
	BF3	50.38 ± 5.36b	10.64%
	BF4	65.98 ± 3.62c	5.49%
Mikania glomerata Coumarins	MG1	3.06 ± 0.20a	6.67%
	MG2	5.17 ± 0.59b	11.40%
	MG3	6.80 ± 0.24c	3.46%
	MG4	1.63 ± 0.20d	12.50%
	MG5	4.49 ± 0.20b	4.55%

Table 1. Results of quality control of tannins, flavonoids and coumarins contained in products based on *Maytenus ilicifolia (*Schrad.) Planch., *Bauhinia forficata* Link and *Mikania glomerata* Spreng. respectively, sold in pharmacies in Recife/PE, Northeast of Brazil.

Values are mean ± standard deviation. Values followed by the same letter in column are not statistically different (n = 6, $p<0.05$).

Analysis of variance (ANOVA) is one way to indicate significant differences ($p<0.01$) for the drugs of *M. ilicifolia*, which is made from five plants. Samples Mi1 (12.57 ± 2.15 mg TAE/g) and Mi5 (11.81 ± 1.0 mg TAE/g) both had higher concentrations of tannins and were not significantly different, whereas at least three of the other samples showed tannins (Figure 8). Comparing the average tannin concentration from different samples showed a low coefficient of variation (CV = 37.61%).

Analysis of four products containing *B. forficata* showed a significant difference ($p<0.01$). The sample Bf4 presented the highest flavonoid concentration (65.98 ± 3.62 mg RE/g), whereas samples Bf1 and Bf2 had the lowest concentrations (4.89 ± 0.11 and 7.27 ± 0.39 mg RE/g, respectively) with a content approximately 13 times lower. These results indicate that there is no standardisation regarding the flavonoid concentrations in products (Figure 9).
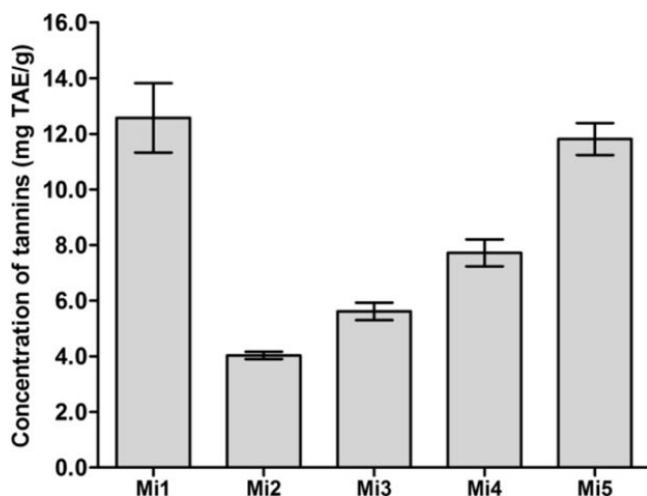
Figure 8. Concentration of tannin (mg TAE/g) contained in products from *Maytenus ilicifolia* (Schrad.) Planch. Sold in Recife/PE, Brazil.
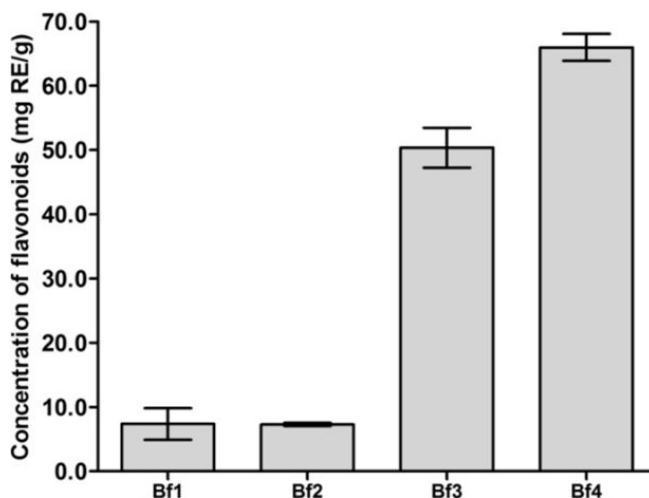

Figure 9. Concentration of flavonoids (mg RE/g) contained within *Bauhinia forficata* link. sold in Recife/PE, Brazil.

Of the five products from *M. glomerata*, three were plant drugs and two were fluid extracts. The plant drugs were extracted with ethanol as recommended by the Brazilian Pharmacopoeia 4th Edition to remove discrepancies from the results [53]. Both the extracts and liquids were evaporated to dryness. The one way ANOVA showed significant differences between the products ($p < 0.01$) and sample Mg3 had the highest level (6.80 ± 0.24 mg CE/g), whereas

sample Mg4 had the lowest level (1.63 ± 0.20 mg CE/g), with an approximately four times lower coumarin concentration.
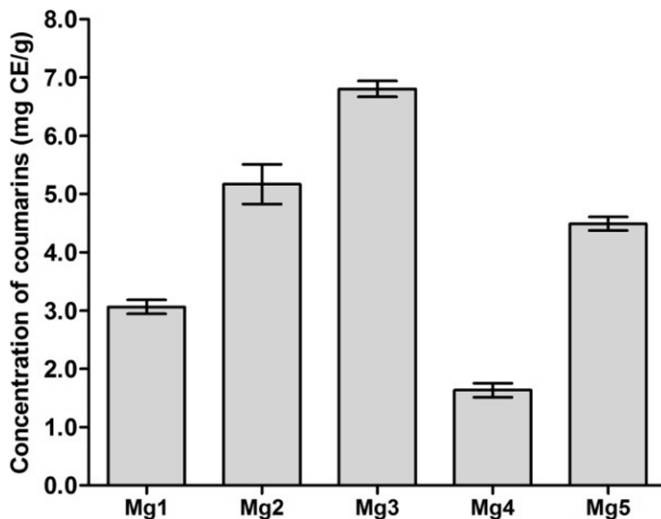

Figure 10. Concentration of coumarins (mg CE/g) contained in products from *Mikania glomerata* Spreng. sold in Recife/PE, Brazil.

The quantitative analysis of raw vegetables and phytomedicines is a fundamental quality control process that leads to security, stability, consistency and effectiveness in the produced phytomedicines [49]. It is important to emphasise the need for standardisation in analysing herbal medicines to determine the concentration of their active components in raw vegetable materials as well as for species identification.

5. Conclusion

This chapter provides easily reproducible standard operating procedures (SOPs) for the quality control of raw materials and herbal plants to ensure a minimal standard of quality in products sold. The implementation of these SOPs allows for the analysis of samples sold in establishments in Recife/PE and reveals an inconsistency in the concentration of tannins, flavonoids and coumarins within these products.

The low level of these metabolites may alter their effectiveness and more rigorous quality control and standardisation of these products is required to prevent compromising their therapeutic activity.

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