Scientific Research Publishing

Open Journal of Applied Sciences, 2022, 12, 714-722
https://www.scirp.org/journal/ojapps
ISSN Online: 2165-3925
ISSN Print: 2165-3917

An Alternative Tool for Determining Flavonoid Compounds in *Markhamia tomentosa* and *Bunchosia glandulifera* Using Digital Image Analysis

Luciana M. Santos[1], Antonio J. Demuner[1*], Daiane E. Blank[1], Cristiane I. Cerceau[1], Iara F. Demuner[2], Marcela R. Coura[2], Maria J. M. Firmino[3], Marcelo H. Santos[1], Neusa F. Moura[3]

[1]Chemistry Department, Federal University of Viçosa, Viçosa, Brazil
[2]Pulp and Paper Laboratory, Federal University of Viçosa, Viçosa, Brazil
[3]Research Group in Natural Product, Federal University of Rio Grande, Rio Grande, Brazil
Email: *ademuner@ufv.br

How to cite this paper: Santos, L.M., Demuner, A.J., Blank, D.E., Cerceau, C.I., Demuner, I.F., Coura, M.R., Firmino, M.J.M., Santos, M.H. and Moura, N.F. (2022) An Alternative Tool for Determining Flavonoid Compounds in *Markhamia tomentosa* and *Bunchosia glandulifera* Using Digital Image Analysis. *Open Journal of Applied Sciences*, **12**, 714-722.
https://doi.org/10.4236/ojapps.2022.125048

Received: April 6, 2022
Accepted: May 15, 2022
Published: May 18, 2022

Copyright © 2022 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Bunchosia glanduliera stands out because of the high content of flavonoid compounds in the pulp, contributing to the antioxidant potential of fruit extracts. Another plant species rich in flavonoid compounds is *Markhamia tomentosa*. However, to perform such an assay, a high-cost instrument is needed. To develop a simple and low-cost method for the determination of flavonoid compounds in *M. tomentosa* and *B. glandulifera* with PhotoMetrix® program application use pixels of digital imaging as an alternative method and linear correlation techniques for univariate analysis implementing systems of RGB colors (red, green, and blue). To determine the total flavonoids, the reaction with ferric chloride and quercetin was used as a control. For the acquisition of data or smartphones, low-cost materials were used, demonstrating the applicability of this analytical tool while comparing its cost to other analytical instrumentation. The total flavonoid content was also determined using a spectrophotometry technique in the visible ultraviolet spectrum (UV-Vis). The pulp of *B. glandulifera* showed the highest content of flavonoid compounds. The content of flavonoid compounds found in the fruit of *B. glandulifera* was 259.54 mg 100 g^{-1}. In relation to the results found in the analysis of total flavonoids of *M. tomentosa* can be observed in the flower *in natura* has a higher content of these compounds. The use of PhotoMetrix® for the determination of flavonoid compounds in *M. tomentosa* and *B. glandulifera* reduced expense and analysis time. The method is reproducible and efficient. The pro-

posed method can be adopted in different species.

Keywords

Markhamia tomentosa, *Bunchosia glandulifera*, PhotoMetrix®, Flavonoid, Smartphone

1. Introduction

The investigation of the use of a new analytical tool for determining flavonoid compounds in fruits, flowers, leaves, seeds, and peels is an analysis of great interest. Since this phytochemical group is widely distributed in the plant kingdom and has pharmacological importance attributed to its various biological properties [1] [2]. Among the plant species, *Bunchosia glandulifera* stands out because of the high content of flavonoid compounds in the pulp, contributing to the antioxidant potential of fruit extracts [3]. The three most abundant flavonoids are rutin, vitexin, and quercitrin, with rutin having the highest concentration [4]. *Bunchosia glandulifera* is native to the north-western portion of South America (Venezuela and Colombia), and is cultivated in Brazil (Amazon, Atlantic Forest, and Pantanal) [5]. In the municipality of Santo Antonio da Patrulha, the fruit is typically consumed *in natura* [6]. The pulp has an intense red color due to the presence of lycopene, while the seed exhibits a green color, which when roasted and ground, can be consumed similarly to guaraná powder because of its caffeine content [6]. Another plant species rich in flavonoid compounds is *Markhamia tomentosa* which grows as a perennial tree with yellow flowers. In folk medicine, its roots are used for the treatment of hookworm in some parts of Tanzania [7]. Species of the genus *Markhamia* possess anti-inflammatory, analgesic, and antiviral activity [8] [9]. The chemical composition of *M. tomentosa* from the stem bark has been reported as 2-acetylnaphto[2,3-b]furan-4,9-dione, 2-acetyl-6-methoxynaphto[2,3-b]furan-4,9-dione, oleanolic acid, pomolic acid, 3-acetylpolic acid, tormentic acid, β-sitosterol, and β-sitosterol-3-O-β-D-glucopyranoside. Antiprotozoal activities are attributed to the first two constituents, which also exhibited high toxicity in the presence of mammalian cell lines [10]. Reports in the literature indicate the detection of flavonoid compounds in *B. glandulifera* and *M. tomentosa* through several analytical methods [3] [6] [8]. However, these methodologies present low mobility, and require large amounts of chemical reagents and expensive equipment. Evidencing greater speed, mobility, and cost in the analyses, colorimetric analysis software has been developed, with many types of chemical analyses being equipped with digital imaging [11] [12]. The aim of the study is to show an analysis performed for the first time using the pixels of the digital image in the determination of flavonoid compounds in *M. tomentosa* and *B. glandulifera* using the smartphone application of the Program PhotoMetrix®.

2. Materials and Methods

2.1. General Experimental Procedures

All chemicals were used without further purification. The reagents are: ferric chloride hexahydrate (99%) was purchased from Merck®, quercetin (≥95%) was purchased from Sigma Aldrich®, and commercial ethanol was purchased from local businesses. Quercetin stock solution (100 $mg{\cdot}mL^{-1}$) in ultrapure water was prepared to determine the total flavonoids. A solution of 1% ferric chloride (w/v) in ultrapure water was prepared for the determination of total flavonoids and quercetin. The solutions were prepared with ultrapure water (resistivity > 18.0 MΩ·cm) obtained from a Millipore Milli-Q® system (USA). A single-beam Ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu, UV Mini 1240) equipped with a 1-cm quartz cuvette was used to perform UV-Vis analysis. A Samsung Galaxy J2 Prime cell phone with an 8.0 MP resolution camera containing the PhotoMetrix® applicative was used to perform digital image analysis.

2.2. Plant Material

The leaves, fresh flowers, dried flowers, and bark of the stem of *M. tomentosa* were provided by on-site cultivation at the Federal University of Viçosa-MG, being the same collected in January 2019. The pulp and seed *B. glandulifera*, were collected in February 2019, from crops grown in orchards in the municipality of Santo Antonio da Patrulha-RS. A voucher specimen was deposited in the Herbarium of UFRGS-Federal University of Rio Grande do Sul under number 167276 ICN designation *Bunchosia glandufliera* (Jacq.) Kunth (Malphiguiaceae).

2.3. Extraction and Determination of Flavonoid Compounds

The sample (20 g) was thawed and extracted with an alcohol solution (ethanol/water, 1:1, v/v) under stirring for 60 min. The fruit with the extraction solution was filtered using a Buchner funnel. To determine total flavonoids, the quercetin standard was used to construct the analytical curve with concentrations of quercetin ranging from 0 to 30 $mg{\cdot}L^{-1}$ (n = 7 points) prepared from the quercetin stock solution (100 $mg{\cdot}L^{-1}$) in water. For reading in the PhotoMetrix® application, 340 µL of the quercetin solutions were pipetted, in different concentrations, to which 10 µL of the 1% ferric chloride solution were added. The determination of the flavonoid content in the extracts was carried out by pipetting 340 µL of each extract and adding 10 µL of the 1% (w/v) ferric chloride solution. Then, the reading was performed in the PhotoMetrix® application [13] [14]. The total flavonoid content was also determined using the traditional technique of spectrophotometry in the visible ultraviolet (UV-Vis). To construct the analytical curve, the same quercetin solutions prepared to obtain the analytical curve using PhotoMetrix® were used. 2.0 mL of the quercetin solutions and the extracts, respectively, were used to obtain the analytical curve and to determine the total flavonoid content in the extracts. 70 µL of the 1% ferric chloride solution

(m/v) was added to the quercetin solutions. The reading was performed at λ = 294 nm.

2.4. Apparatus

Images were made with Android smartphones through the PhotoMetrix® app. This application presents the options of univariate analysis (univariate analysis), multivariate analysis (settings), and settings about this app. The "univariate analysis" feature with two options, Multiple Channels (Vector channels) and RGB vectors for data collection. Running Multiple Channels automatically opened the third screen with the "Calibration", "Sampling", "Saved Results" and "Help" options. Accessing the "Calibration" option, the fourth screen appeared, where the number of samples used for the construction of the analytical curve was added. Also, the location and name of the sample were informed on the screen. Using the "Capture Images" option, the concentration of the solutions prepared for the construction of the analytical curve was reported [14]. The measurements of the analytes of interest in the samples were performed using the sampling option. At the end of the analyses, the analytical curve was selected, allowing the conversion of the signal obtained in the analysis of the samples to the concentration of the analyte of interest.

3. Results and Discussion

The simple, portable, and low-cost method, using digital imaging, was used to determine total flavonoids in different samples. For the capture of digital images, the support booth was designed to guarantee the fixation of the cell phone, ensuring reliability, accuracy/precision, and reproducibility of the RGB data obtained. First, the best position for fixing the plate of Light Emitting Diode (LED) lamps located on the upper wall of the compartment was evaluated, as well as the intensity of the light emitted to improve image capture. It was common to find shadows, which influenced the method's repeatability. Thus, the best positioning of the dividing plate inside the chamber was carried out to reduce shadows or excessive reflections on the sample and other regions in order to avoid obtaining erroneous results. Thus, the adjustment of the smartphone camera with the container holding the sample was carried out. The RGB values found and the UV absorbances of the extracts can be seen in **Table 1**, the results of the analysis of flavonoid compounds in fresh flower, dry flower, leaf, *M. tomentosa* bark, and pulp, and the seed of *B. glandulifera* in **Table 2**.

The results in **Table 2** of the analysis of total flavonoids in samples of *M. tomentosa* and *B. glandulifera* show that both have flavonoid compounds. However, the pulp of *B. glandulifera* exhibited the highest content of flavonoid compounds (769.86 ± 0.02 mg 100 g^{-1}). Other studies have also found high content of flavonoid compounds in the pulp extracts of this fruit [3] [4] [6]. This high quantity of bioactive compounds in *B. glandulifera* has been related to its antioxidant capacity [4]. The content of flavonoid compounds found in the seed of

Table 1. RGB values and absorbances of *M. tomentosa* and *B. glandulifera* extracts.

Sample	Absorbance in UV-Vis	R	G	B
Fresh flower	0.357	189	180	132
Fresh flower	0.356	190	183	135
Fresh flower	0.357	189	180	132
Dry flower	0.314	184	178	147
Dry flower	0.315	182	175	143
Dry flower	0.315	182	175	143
Leaf	0.288	185	179	150
Leaf	0.285	187	182	154
Leaf	0.288	185	179	150
Bark	0.132	171	187	156
Bark	0.130	172	188	158
Bark	0.130	172	188	158
Pulp	0.406	159	150	152
Pulp	0.406	159	153	155
Pulp	0.406	158	149	152
Seed	0.214	174	177	174
Seed	0.214	172	177	174
Seed	0.214	172	177	174

Table 2. Phenolic compounds in *M. tomentosa* and *B. glandulifera*.

Sample	PhotoMetrix app (mg 100 g^{-1})*	UV-Vis (mg 100 g^{-1})*
Fresh flower of *M. tomentosa*	364.01 ± 0.60	364.10 ± 0.51
Dry flower of *M. tomentosa*	301.20 ± 0.54	317.66 ± 0.57
Leaf of *M. tomentosa*	287.40 ± 0.73	287.42 ± 0.56
Bark of *M. tomentosa*	107.0 ± 0.89	107.01 ± 0.01
Pulp of *B. glandulifera*	769.86 ± 0.02	770.41 ± 0.06
Seed of *B. glandulifera*	257.99 ± 0.51	259.54 ± 0.08

*(mg 100 g^{-1}) of fruit and flower *in nature*.

B. glandulifera was 257.99 ± 0.51 mg 100 g^{-1}. Relative to the results found in the analysis of the total flavonoids of *M. tomentosa*, the *in natura* flower has a higher content of flavonoid compounds (364.01 ± 0.60 mg 100 g^{-1}). The dried flower had a 301.20 ± 0.54 mg 100 g^{-1}, the leaf had a concentration of 287.40 ± 0.73 mg 100 g^{-1}, and the peel with a concentration of 107.0 ± 0.89 mg 100 g^{-1}. These results indicate that during the dry process may have degraded the bioactive compounds. Studies report that plant drying degrades flavonoid compounds [3]. With this easily accessible method, it was possible to determine flavonoid com-

pounds in two plants from different families, totaling six concentration results using Photometrix® and six results using the spectrophotometer for comparison, giving us a viable method for performing other tests with several samples. The similarity between the Photometrix® and UV-Vis determination methods was proven using analysis of variance (ANOVA) and Tukey's test with 95% confidence. Therefore, this method stands out as a viable alternative for determining total flavonoids in plant species, being particularly useful in laboratories with limited financial and analytical resources while using techniques that are in accordance with Green Chemistry. Additionally, the method for determining flavonoids used in the present study has a low cost, generates little waste, and can be easily used outside of the laboratory. The images can be captured from any cell phone, provided that a collection booth compatible with the cell phone is built. For this reason, the dimensions and model of the cell phone must be informed. The images in the present study were captured with a digital camera from Android smartphones, for example. A recent study performed monitoring of the degradation of dyes using digital images, compared with UV-Vis, showing efficient results and a viable alternative for analysis of environmental samples [12]. Admittedly, the use of gas or liquid chromatography is extremely sensitive techniques that allow the identification of chemical compounds present in the extracts. However, these methods are laborious, involve expensive equipment, consume many reagents, and cannot be used for in situ analysis due to their low portability. To overcome these disadvantages, simple, inexpensive methods that utilize minimal amounts of reagents for spectrophotometric techniques associated with image analysis have recently been used in the determination of different compounds in diverse matrices. They are usually simple devices such as cameras [15], scanners [16], and cell phones [17]. PhotoMetrix® is an application that can be purchased for free on the Play Store for Android and Windows smartphones. The application performs data processing using pixels from digital images for analytical applications in the determination of flavonoid compounds in plant species through simple linear correlation techniques for univariate or multivariate analysis [18]. Digital images can be decomposed in the colors using different models, with RGB being the most used: red (R), green (G), and blue (B) [18] [19]. To investigate the best channel for assessing linearity, a concentration range of 5 to 30 ppm was studied. The results showed that the channel which presented the most prominent results was G, with a correlation coefficient of 0.9934, followed by the individual channels R (r^2 = 0.9787) and B (r^2 = 0.9908). The results obtained in this work show the applicability of Photometrix® as a new analytical tool for the determination of flavonoid compounds in extracts of plant species, enabling new studies and analytical applications while reducing costs related to analytical instrumentation. This method is particularly useful for laboratories with limited analytical financial resources, contributes to green chemistry, and can be applied in practical chemistry classes in elementary and high schools. Thus, the performance of experiments in the classroom using this simple and accessible tool makes it possible to increase students' comprehension

and interest by offering a more dynamic and practical lesson [14].

4. Conclusion

In conclusion, the present study uses simple, rapid, and accessible methods to determine flavonoid compounds in *M. tomentosa* and *B. glandulifera*. The similarity between the digital method using the PhotoMetrix® application and that of spectrophotometry in UV-Vis was confirmed by statistical tests. The results of the determination of total flavonoids of plant species using the PhotoMetrix® application were satisfactory. The method is reproducible and can be an efficient and economical alternative for determining these parameters in diversified foods. The method developed during this work consumes a smaller quantity of reagents when compared to traditional methods, contributing to the preservation of the environment. Additionally, this method is easy to reproduce because it is economical and only requires a cell phone as a technological resource. The results of the chemical composition *of M. tomentosa* and *B. glandulifera* support the performance of biological activities.

Acknowledgements

We are grateful the Professional Master's Program in National Network Chemistry (PROFQUI), Brazil, for their support in project. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Funding

This work was supported by the Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)—research fellowships (AJD, MHS, and MJMF), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)—research fellowships (DEB).

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Torres, D.S., Pereira, E.C.V., Sampaio, P.A., Souza, N.A.C., Ferraz, C.A.A., Oliveira, A.P., Moura, C.A., Almeida, J.R.G.S., Rolim-Neto, P.J., Oliveira-Júnior, R.G. and Rolim, L.A. (2018) Influência do método extrativo no teor de flavonoides de *Cnidoscolus quercifolius* Pohl (Euphorbiaceae) e atividade antioxidante. *Química Nova*, **41**, 743-747. https://doi.org/10.21577/0100-4042.20170236

[2] Pereira, G.A., Araujo Peixoto, N.M., Arruda, H.S., Faria, D.P., Molina, G. and Pastore, G.M. (2019) Phytochemicals and Biological Activities of Mutamba (*Guazuma ulmifolia* Lam.): A Review. *Food Research International*, **126**, Article ID: 108713. https://doi.org/10.1016/j.foodres.2019.108713

[3] Blank, D.E., Bellaver, M., Fraga, S., Lopes, T.J. and Moura, N.F. (2018) Drying Kinetics and Bioactive Compounds of *Bunchosia glandulifera*. *Journal of Food Process Engineering*, **41**, e12676. https://doi.org/10.1111/jfpe.12676

[4] Silva, S.F., Blank, D.E., Peixoto, C.R., Moreira, J.J.S. and Moura, N.F. (2016) Bioactive Compounds and Antioxidant Activity of *Bunchosia glandulifera*. *International Journal of Food Properties*, **19**, 467-473. https://doi.org/10.1080/10942912.2015.1033547

[5] Quattrocchi, U. (2019) Peanut Butter Tree-*Bunchosia glandulifera*. https://www.growables.org/information/TropicalFruit/peanutbuttertree.htm

[6] Blank, D.E., Justen, D., Fraga, S., Peixoto, C.R. and Moura, N.F. (2018) Chemical Composition and Antioxidant Activity of *Bunchosia glandulifera* Fruit at Different Ripening Stages. *Food and Nutrition Sciences*, **9**, 1147-1159. https://doi.org/10.4236/fns.2018.910083

[7] Nchu, F., Githiori, J.B., McGaw, L.J. and Eloff, J.N. (2011) Anthelmintic and Cytotoxic Activities of Extracts of *Markhamia obtusifolia* Sprague (Bignoniaceae). *Veterinary Parasitology*, **183**, 184-188. https://doi.org/10.1016/j.vetpar.2011.06.017

[8] Kernan, M.R., Amarquaye, A., Chen, J.L., Chan, J., Sesin, D.F., Parkinson, N., Ye, Z., Barrett, M., Bales, C., Stoddart, C.A., Sloan, B., Blanc, P., Limbach, C., Mrisho, S. and Rozhon, E.J. (1998) Antiviral Phenylpropanoid Glycosides from the Medicinal Plant *Markhamia lutea*. *Journal of Natural Products*, **61**, 564-570. https://doi.org/10.1021/np9703914

[9] Temdie, G., Fotio, A.L., Dimo, T., Beppe, J. and Tsague, M. (2012) Analgesic and Anti-Inflammatory Effects of Extracts from the Leaves of *Markhamia tomentosa* (Benth.) K. Schum. (Bignoniaceae). *Pharmacologia*, **11**, 565-573. https://doi.org/10.5567/pharmacologia.2012.565.573

[10] Tantangmo, F., Lenta, B.N., Boyom, F.F., Ngouela, S., Kaiser, M., Tsamo, E., Weniger, B., Rosenthal, P.J. and Vonthron-Sénécheau, C. (2010) Antiprotozoal Activities of Some Constituents of *Markhamia tomentosa* (Bignoniaceae). *Annals of Tropical Medicine & Parasitology*, **104**, 391-398. https://doi.org/10.1179/136485910X12743554760180

[11] Shawky, E. and Kheir, R.M.A. (2018) Rapid Discrimination of Different Apiaceae Species Based on HPTLC Fingerprints and Targeted Flavonoids Determination Using Multivariate Image Analysis. *Phytochemical Analysis*, **29**, 452-462. https://doi.org/10.1002/pca.2749

[12] Böck, F.C., Helfer, G.A., Costa, A.B., Dessuy, M.B. and Ferrao, M.F. (2020) PhotoMetrix and Colorimetric Image Analysis Using Smartphones. *Journal of Chemometrics*, **34**, e3251. https://doi.org/10.1002/cem.3251

[13] Santos, M.H., Lemos, B.B., Duarte, S.M.S., Abreu, C.M.P. and Gouvêa, C.M.C.P. (2007) Influência do processamento e da torrefação sobre a atividade antioxidante do café. *Química Nova*, **30**, 604-610. https://doi.org/10.1590/S0100-40422007000300020

[14] Bazani, E.J.O., Barreto, M.S., Demuner, A.J., Santos, M.H., Cerceau, C.I., Blank, D.E., Firmino, M.J.M., Souza, G.S.F., Franco, M.O.K., Suarez, W.T. and Stringheta, P.C. (2021) Smartphone Application for Total Phenols Content and Antioxidant Determination in Tomato, Strawberry, and Coffee Employing Digital Imaging. *Food Analytical Methods*, **14**, 631-640. https://doi.org/10.1007/s12161-020-01907-z

[15] Paquet-Durand, O., Solle, D., Schirmer, M., Becker, T. and Hitzmann, B. (2012) Monitoring Baking Processes of Bread Rolls by Digital Image Analysis. *Journal of Food Engineering*, **111**, 425-431. https://doi.org/10.1016/j.jfoodeng.2012.01.024

[16] Sorouraddin, M.H., Saadati, M. and Mirabi, F. (2015) Simultaneous Determination of Some Common Food Dyes in Commercial Products by Digital Image Analysis. *Journal of Food and Drug Analysis*, **23**, 447-452. https://doi.org/10.1016/j.jfda.2014.10.007

[17] Intaravanne, Y., Sumriddetchkajorn, S. and Nukeaw, J. (2012) Cell Phone-Based Two-Dimensional Spectral Analysis for Banana Ripeness Estimation. *Sensors and Actuators B*: *Chemical*, **168**, 390-394. https://doi.org/10.1016/j.snb.2012.04.042

[18] Helfer, G.A., Magnus, V.S., Böck, F.C., Teichmann, A., Ferrão, M.F. and Costa, A.B. (2017) PhotoMetrix: An Application for Univariate Calibration and Principal Components Analysis Using Colorimetry on Mobile Devices. *Journal of the Brazilian Chemical Society*, **28**, 328-335. https://doi.org/10.5935/0103-5053.20160182

[19] Yu, H. and MacGregor, J.F. (2003) Multivariate Image Analysis and Regression for Prediction of Coating Content and Distribution in the Production of Snack Foods. *hemometrics and Intelligent Laboratory Systems*, **67**, 125-144. https://doi.org/10.1016/S0169-7439(03)00065-0