GREEN CHEMISTRY: EXTRACTING SOLUTION OF DYES AND BIOACTIVES FROM HIBISCUS FLOWERS (*Hibiscus rosa-sinensis L.*, *Hibiscus rosa-sinensis* 'VARIEGATA', *Hibiscus rosa-sinensis* 'CARNATION')

Aila T. Castelo Branco[1*], Fernanda L. A. Silva[2], Maria A. M. Castelo Branco[3], Maria J. B. Silva[4], José de A. R. do Rêgo[5], Davi do S. B. Brasil[6], Nathiel S. Moraes[7].

[1] *Chemical Engineering, UFPA/ITEC, Belém/Brazil.*
[2] *Chemical Engineering, UFPA/ITEC, Belém/Brazil.*
[3] *Biotechnology, UFPA/ICB, Belém/Brazil.*
[4] *PPGCMA, UFPA/ICEN, Belém/Brazil.*
[5] *UFPA/ICEN, Belém/Brazil.*
[6] *UFPA/ITEC, Belém/Brazil.*
[7] *PPGCMA, UFPA/ICEN, Belém/Brazil.*
* *ailacastelobranco@gmail.com*

ABSTRACT

Hibiscus is a plant from the Malvaceae family, and its dyes and bioactives are very relevant to the pharmaceutical and food industries. In short, their extraction is carried out using hydroalcoholic solutions acidified with HCl or supercritical acid. The aim of this study is to use an acidic, non-toxic, low-cost and azeotropic solution for extraction and to analyze its responses. To do this, a solution of ethanol:distilled water:acetic acid was produced in a ratio of 1:8:3 (v/v/v), using 2 g of flower petals from three hibiscus in oven-dried and fresh conditions, immersed in 10 mL of solution for 24 hours. After carrying out Ultraviolet-Visible (UV-vis) scanning, 1,1-diphenyl-2-picrylhydrazyl (DPPH) inhibition, total phenolic and anthocyanin quantification, the result was monomeric anthocyanins ranging from 85. 00-414.30 mg/L, total phenolics 41.40-291.59 mgAGE/g, DPPH inhibition varied greatly between 7.01-57.19% when comparing all the samples, indicating that each condition used extracted a greatger quantity of certain bioactive compounds of interest. It can be concluded that the responses were satisfactory for the extraction of the different flowers, under the conditions employed and within the volumes used.

Keywords: Hibiscus. Extraction. Dyes. Colorants. Bioactives.

1 INTRODUCTION

Hibiscus is a plant of the Malvaceae family, originating from East Africa, with annual cultivation, which blooms throughout the year, introduced to Brazil by Africans[7,9]. It is recognized for its beneficial food properties in Asian countries (China, Japan, Taiwan, and Korea) due to its rich composition in bioactive substances[8]. Various research studies on hibiscus have revealed a variety of therapeutic effects, such as liver protection, antibacterial action, antioxidant properties, cholesterol reduction, antimutagenic action, and antihypertensive effect. These benefits are attributed to antioxidant compounds such as vitamin C, phenolic acids, flavonoids, and anthocyanins present in hibiscus[6,10,15].

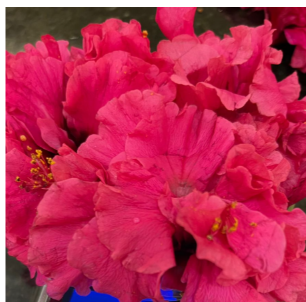

Figure 1 *Hibiscus rosa-sinensis L*. **Figure 2** *Hibiscus rosa-sinensis* 'variegata'. **Figure 3** *Hibiscus rosa-sinensis* 'carnation'.

However, effective extraction and stabilization are the obstacles to capturing bioactive substances in anthocyanin matrices, since these pigments are relatively unstable and highly degradable. Thus, their stability is more effective in acidic environments, and the main elements that influence the deterioration of anthocyanins are pH, temperature, enzymes such as polyphenol oxidase and peroxidase, oxygen levels and ascorbic acid[13], so most extractions are carried out using hydroalcoholic solutions acidified with hydrochloric acid (HCl) or supercritical acid. Therefore, the aim of this study is to employ a new acidic, non-toxic and azeotropic solution extraction methodology to obtain vibrant dyes and large quantities of compounds from three types of hibiscus under different conditions

2 MATERIAL & METHODS

The extracting solution was obtained using ethanol: distilled water: acetic acid in a ratio of 1:8:3 (v/v/v) called sol-Z. The three

hibiscus flowers were collected near the Federal University of Pará, each species was indicated by taxonomic comparison3, the samples were identified as follows: *Hibiscus rosa-sinensis L.* as F1, *Hibiscus rosa-sinensis* 'variegata' as F2 and *Hibiscus rosa-sinensis* 'carnation' as F3. The removal methods applied consisted of: subjecting 2 g of fresh flowers to 10 mL of extracting solution at room temperature for 24 hours; and subject 0.8 g of flowers after oven (30 minutes at 50°C and 110 minutes at 70°C) to 10 mL of extracting solution in a water bath at 40°C for 1 hour, and then condition them for 24 hours outside the bath at room temperature. After both processes, filter the resulting dyes on qualitative paper.

The analyzes carried out on the mixture samples were:

The first, a UV-vis scanning using a Shimadzu UV-1800 Ultraviolet-visible (UV-vis) spectrophotometer;

The second, on the antioxidant potential by percentage using the 1,1-diphenyl-2-picrylhydrazyl (DPPH)[2] inhibition method, taking readings of 0, 15, 30, 45, and 60 minutes in the range of 517 nm on the UV-Vis spectrophotometer, using 0.5 mL of sample in 1.95 mL of methanolic DPPH solution (AA), the blank (AB) consisting of 0.5 mL of sample in 1.95 mL of methanol P.A., and the control (AC) consisting of 0.5 mL of methanol in 1.96 mL of DPPH[2] solution;

The third, on the total phenolics[4], which consisted of forming the gallic acid curve at concentrations of 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL of the stock solution (20 mg/L of gallic acid diluted in the extracting solution) in 10 mL volumetric flasks, distributed in cuvettes with 0.25 mL in triplicate of each concentration, with 2.75 mL of 3% Folin-Ciocalteu reagent. Succeeding 10 minutes, 0.25 mL of sodium carbonate solution ($Na2SO3$) was added, and readings were taken at 765 nm on the UV-vis spectrophotometer after 60 minutes of this mixture being kept away from light. The same procedure was repeated on all the samples, replacing the gallic acid concentrations with 0.25 mL of the samples;

The fourth, on the concentration of monomeric anthocyanins[5], which consisted of diluting in sextuplicate 0.1 mL of the fresh samples and 0.3 mL of the samples after oven drying in 3 mL of two solutions. The first set of triplicates was diluted in a standard sodium chloride (KCl) solution at pH 1, and the second set of triplicates was diluted in a standard sodium acetate solution at pH 4.5 for each sample. Readings were then performed on all samples at wavelengths of 700 nm and 510 nm;

The last, on color density[5], percentage[5], and polymeric color[5], performing dilutions, in sextuplicate, of 0.1 mL of the fresh samples and 0.3 mL of the samples after over drying in 3 mL of standard KCl solution at pH 1. In three of these dilutions, 0.2 mL of distilled water was added, and in the other three, 0.2 mL of potassium metabisulfite solution, at a dilution of 1 g to 5 mL of water, was added. All were then read at 700 nm, 510 nm, and 420 nm bands.

3 RESULTS & DISCUSSION

The samples F1, F2, and F3 were differentiated for fresh samples, as F1N, F2N, and F3N, and for samples after the drying process, as F1S, F2S, and F3S, as can be seen in table 1 and figure 4.

Table 1 Results of anthocyanins quantification, total polymerics, polymeric color, color density, total phenolics, and DPPH inhibition from each sample.

Sample	Monomeric anthocyanins (mg/L)	Total polymerics (%)	Polymeric color	Color density	Total phenolics (mg GAE/g)	% of DPPH radical inhibition
F1N	410.79	4.54	1,74	38.28	56.57	50.09 - 34.29
F1S	89.01	9.48	1,77	18.66	291.59	27.14 - 25.85
F2N	129.25	4.40	0,63	14.31	31.98	23.73 - 19.03
F2S	59.45	1.46	0,96	8.16	272.59	6.37 - 7.01
F3N	414.30	3.52	1,35	38.34	41.40	55.14 - 57.19
F3S	85.00	7.17	1,97	17.76	267.07	11.54 - 10.58
TEIXEIRA 2008[7]	22.975					
SILVA 2014[11]	8.571				155,85	-

Source: Authors (2024).

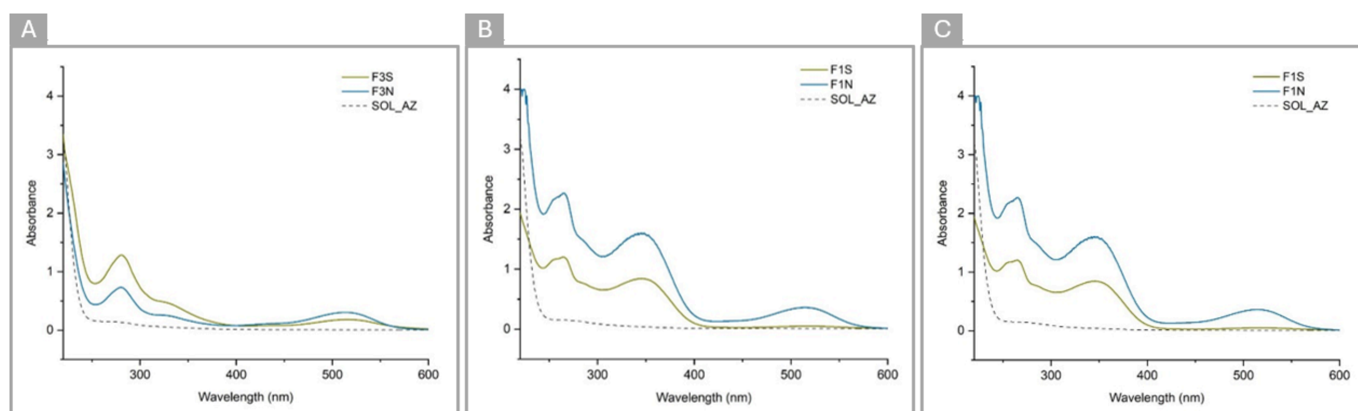

Figure 4 UV-vis scanning graphs of different flowers comparing the extractions of each flower in different conditions and the extracting solution, all comparing the absorbance capacity with wavelength, in nanometers, of each one. **A:** UV-vis scanning of *Hibiscus rosa-sinensis L.* **B:** UV-vis scanning of *Hibiscus rosa-sinensis* 'variegata'. **C:** UV-vis scanning of *Hibiscus rosa-sinensis* 'carnation'. **Source:** Authors (2024).

The results showed that, to the three fresh flowers, there was higher extraction of monomeric anthocyanins (using cyanidin-3-glucoside as a standard) and a reduction in total phenolic levels. This fact resulted in an increase in the inhibition of the DPPH radical, since anthocyanins react more favorably compared to phenols[2]. On the other hand, dried samples presented opposite results, low DPPH inhibition was observed, along with a low concentration of anthocyanins and a high concentration of total phenolics.

When analyzing the graphs in figure 4, it can be identified the presence of phenolic compounds and organic acids[1,12] in the 230-300 nm range, along with other dyes and flavonoids[1,12] in the 300-400 nm range, with better presentation in dried samples, and anthocyanin peaks in the range of 400-600 nm, with better presentation in fresh samples. The dilution of the samples (1:100 v/v of the sample in distilled water) to perform this analysis may have influenced the expressiveness of the anthocyanin results. The image analysis, also including scanning the solution used (sol-Z), didn't show significant peaks. Comparing the literature, the hydroalcoholic extraction[11] 7:3 obtained a reduction in the extraction of anthocyanins when compared to total phenolics, while the hydroalcoholic extraction[7] 7:3, acidified with HCl until pH 2, captured the extraction of anthocyanins better, relating the proportionality of solvents to the extracted bioactives.

As for dyes, the pink flower (F1) obtained the highest results, while the white and red striped flower (F2) demonstrated low levels of compounds, regardless of extraction. The red flower (F3) showed similar results to F1 in terms of anthocyanins and lower in terms of other dyes, such as flavonoids. As for color density, it was significantly higher in extractions from fresh flowers compared to dried flowers. As for polymeric color, there was intense bleaching in the samples with potassium metabisulfite, indicating low presence of polymeric anthocyanins and, consequently, low polymeric color. However, total polymer values were higher in dry samples, indicating possible degradation of monomeric anthocyanins during the drying process.

4 CONCLUSION

The extracting solution achieved the goal of extracting a variety of compounds from hibiscus flowers in a stable manner and with distinct characteristics. For fresh flowers, there was higher isolation of anthocyanins, as evidenced by the monomeric results, and higher DPPH inhibition. Furthermore, a comparatively low proportion of phenolic compounds was presented. In contrast, to dried flowers, there was a higher concentration of phenolic compounds, a reduction in all anthocyanin results and a lower DPPH inhibition. It is inferred that the solution captures several important bioactives at optimal working temperatures using low-cost substances, thus demonstrating its success and viability for replication and further study in future research.

REFERENCES

1. BERNARDI, F., NICOLINI, K. P., and NICOLINI, J. 2017. Phytochemical study of *Hydrangea sp.* using classic methods of analysis by visible ultraviolet spectroscopy (UV-Vis) and column and paper chromatography. 29 (1). Infarma. 68-80.
2. BRAND-WILLIANS, W., CUVELIER, M. E. and BERSET, C. 1995. Use of a free radical method to evaluate antioxidant activity. 28 (1). LWT-FOOD SCI TECHNOL. 25-30. doi:10.1016/s0023-6438(95)80008-5.
3. ESTEVES, G. L., DUARTE, M. C., and TAKEUCHI, C. 2014. Synopsis of *Hibiscus L.* (Malvoideae, Malvaceae) in São Paulo State, Brazil: native and ornamental species. 41 (4). Hoehnea. 529-539.
4. GEORGÉ, S., BRAT, P., ALTER, P., and AMIOT, M. J. 2005. Rapid Determination of Polyphenols and Vitamin C in Plant-Derived Products. 53 (5). J Agric Food Chem. 1370-1373. doi:10.1021/jf048396b.
5. GIUSTI, M. M., and WROLSTAD, R. E. 2001. Characterization and Measurement of Anthocyanins by UV-Visible spectroscopy. *In:* Current Protocols in Food Analytical Chemistry. John Wiley & Sons, New York. Unit F1.2.1-13.
6. LIN, T.-L. *et al*. 2007. *Hibiscus sabdariffa* extract reduces serum cholesterol in men and women. 27 (3). Nutrition Research. 140-145.
7. LIU, K. S., TSAO, S. M., and YIN, M. C. 2005. In vitro antibacterial activity of roselle calyx and protocatechuic acid. Phytotherapy Research. 19 (11). 942-945.
8. LORENZI, H. and MATOS, F. J. A. 2002. Plantas medicinais no Brasil: nativas e exóticas [Medicinal plants of Brazil: Native and exotic]. Instituto Plantarum de Estudos da Flora, Nova Odessa.
9. PANIZZA, S. 1998. Plantas que curam: cheiro de mato [Plants that heal: smell of the forest]. 21st ed. IBRASA, São Paulo.
10. PRENESTI, E. *et al*. 2007. Antioxidant power quantification of decoction and cold infusions of *Hibiscus sabdariffa* flowers. 100 (2). Food Chemistry. 433-438.
11. SILVA, A. B. *et al*. 2014. Antibacterial and phytochemical characterization of flowers of *Hibiscus rosa-sinensis L.* (treat-of-venus) and *Hibiscus syriacus L.* (hibiscus-the-syrian). 73 (3). Rev Inst Adolfo Lutz. doi: 10.18241/0073-98552014731614
12. SOUSA, I. J. O. *et al*. 2018. Creeaning phytochemical and evaluation n of the hemolytic and antimicrobial capacity of an extract of crude bark of *Ziziphus joazeiro* Mart. stem. (Rhamnaceae). 14 (4). Biofarm. 208-225.
13. SCHWARTZ, S. J., VON ELBEE, J. H., and GIUSTI, M. M. 2010. Corantes [Colorants]. *In:* Química de Alimentos de Fennema [Fennema's Food Chemistry]. DAMODARAN S., PARKIN K. L., and FENNEMA O. R. (eds.). 4th ed. Artmed, Porto Alegre. 445–498.
14. TEIXEIRA, L. N., STRINGHETA, P. C., and ALVES, F. O. 2008. Comparation of methods for anthocyanin quantification. 55 (4, July-August). Revista Ceres. 297-304.
15. WANG, W.-D. and XU, S.-Y. 2007. Degradation kinetics of anthocyanins in blackberry juice and concentrate. 82 (3). J Food Eng. 271-275.

ACKNOWLEDGEMENTS

I am greatly thankful to the laboratories LABA, LEPRON, and of the UV spectroscopy, all laboratories from the chemical engineering building (LEQ/UFPA), and their coordinators for all the support in conducting this work, especially to my supervisor Nathiel Sarges Moraes and laboratory technicians Rafaela Pinheiro and Samara Marques for their teachings, collaboration, patience, and dedication to the advancement of natural product science.