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VARIABILITY OF THE PHYTOCHEMICAL PROFILE OF *Hibiscus Rosa-Sinensis* FLOWERS: INFLUENCE OF MANAGEMENT, MINERAL NUTRITION, AND ENVIRONMENTAL CONDITIONS

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ABSTRACT

Introduction: *Hibiscus rosa-sinensis* is a plant highly popular for medicinal purposes; it is increasingly used in research and studies, demonstrating its potential for phytotherapeutic applications. **Objective:** To analyze the phytochemical profile of *Hibiscus rosa-sinensis* flowers and evaluate the influence of management practices, mineral nutrition, and environmental factors on the variation of their secondary metabolites. **Methodology:** Three *Hibiscus rosa-sinensis* seedlings were cultivated in an experimental garden. Methanolic and ethanolic extracts were prepared from the flowers, followed by phytochemical screening using the Shinoda, ferric chloride, Salkowski, Keller-Kiliani, and foam formation tests. Subsequently, the results were compared with scientific literature to assess potential factors related to variations in the species' phytochemical profile. **Results and Discussion:** oth extracts showed the presence of flavonoids, tannins, terpenoids, and glycosides; however, the presence of saponins was not detected in either extract. These results showed both similarities and differences when compared with findings from other studies. **Conclusion:** The results, when compared to other studies, support the hypothesis that the findings vary depending on care and management practices.

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INTRODUCTION

Hibiscus rosa-sinensis (image 1), belonging to the Malvaceae family, is a shrub with serrated, lanceolate leaves, and perennial flowers featuring actinomorphic and pedicellate characteristics, with a corolla of five petals, generally measuring 3 to 12 cm in length and 2 to 5 cm in width. In scientific studies, this plant has been found to contain phytochemical compounds with cardioprotective, analgesic, and anti-inflammatory effects, as well as antibacterial activity, including glycosides, terpenoids, tannins, saponins, and flavonoids. These compounds have demonstrated inhibitory activity against the bacterium *Listeria monocytogenes* and efficacy against *Escherichia coli*. However, laboratory analyses of this plant tend to produce variable results regarding its phytochemical composition (Ruban; Gajalakshmi, 2012; Raza[1]., et al., 2025). Traditionally, all parts of *Hibiscus rosa-sinensis* can be used, with their phytochemical activities being notably described in the scientific literature. Consequently, this has further broadened the horizon for the use of traditional techniques and knowledge based on scientific analyses, expanding knowledge through the integration of traditional wisdom with current scientific understanding (Kainat et al., 2021). Accordingly, the present study aims to investigate the phytochemistry of *Hibiscus rosa-sinensis* flowers and analyze the possible influence of their cultivation practices on these compounds.

METHODOLOGY

Accordingly, the present study aims to investigate the phytochemistry of *Hibiscus rosa-sinensis* flowers and analyze the possible influence of their cultivation practices on these compounds.

SOIL ANALYSIS: Before planting the hibiscus, two soil samples were collected from the garden of the UNEF University Center for chemical and physical analyses by the Empresa Brasileira de Pesquisa Agropecuária (Embrapa). The purpose of this soil study was to assess the feasibility of growing *Hibiscus rosa-sinensis* in the area in question, which could, for example, be enriched if necessary. For the sampling, two holes approximately 10 cm deep were excavated. The collected soil was stored and identified in two plastic bags, with sample 1 weighing 2.3 kg and sample 2 weighing 2.4 kg. The soil samples in question were sent to Embrapa's chemical and physical analysis laboratories, specifically to the Embrapa Mandioca e Fruticultura, located in Cruz das Almas, Bahia, Brazil. The results of the physical (Figure 1) and chemical analyses revealed that the soil texture classification and acidity were suitable for hibiscus cultivation, consisting of a loamy sand with a pH of 6.2, that is, a slightly acidic sandy soil. However, the chemical analysis (Table 1)



Image 1. *Hibiscus rosa-sinensis*

also demonstrated a certain imbalance in the macronutrients, which was corrected through hibiscus management.

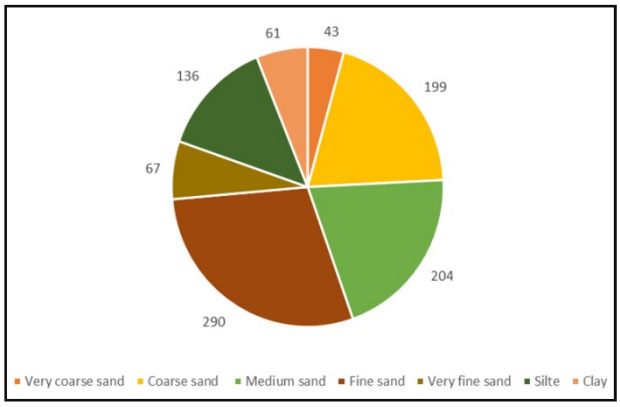


Table 1. Analytical results – macronutrient fertility

Macronutrient	Reference value	Result
Phosphorus (P)	>20 mg/dm³	10 mg/dm³
Potassium (K)	0,07 - 0,15 Cmolc/dm³	0,15 Cmolc/dm³
Calcium (Ca)	1,6 - 3,0 Cmolc/dm³	1,63 Cmolc/dm³
Magnesium (Mg)	0,4 - 1,0 Cmolc/dm³	0,68 Cmolc/dm³
Aluminum (Al)	0,5 - 1,0 Cmolc/dm³	0,0 Cmolc/dm³

PLANTING AND MANAGEMENT: After planting the hibiscuses (image 2) in February, it was necessary to enrich the soil with mixed mineral fertilizer NPK 04-14-08, suitable for flowering. The NPK was added at a proportion of approximately 10 g for each seedling, with this enrichment being carried out three times over the course of three months.



Image 2. Hibiscus planting

Due to the textural classification and pH proving to be viable for the development of hibiscus, no interventions were necessary in these aspects; it was only necessary to meet its water requirement through irrigation, on average, every two days, taking care to prevent the soil from becoming excessively drained. During the months of care, as expected, pests ended up emerging among the plants. Specifically, leaf-cutting ants, mealybugs, and aphids, with the presence of the species being noticed at the beginning of their infestation, and to eradicate these aggressors, different methods were used. For the ants, it was necessary to identify the anthills and add granular ant killer to them. This type of ant killer was chosen to avoid contact between the plants and the toxic agents that could influence the results of their phytochemistry, which would probably occur with the use of an ant killer that required spraying the plant and the environment where it is cultivated. The cochineal insects and aphids (Image 3) were eradicated through a homemade solution used in family farming to control these pests, this solution was made by mixing 30 ml of vinegar, 30 ml of detergent, 20 ml of 70% alcohol and 20 g of salt, the solution was gradually added to the affected areas, allowing the immediate death of the pests upon contact, these areas were thoroughly rinsed to remove the dead insects and the solution used in the area (Image 4).



Image 3. Scale insects and aphids



Image 4: Plant after the removal of the pests

The care provided ensured the plant's good development and excellent flowering, as its particular needs were met, in addition to preventing infestations by the pests previously mentioned, thus avoiding probable stress to the plant.

This development allowed for the preparation of an exsiccate, which was deposited in the herbarium of the State University of Feira de Santana in the state of Bahia (UEFS), under voucher number HUEFS No. 296537 (image 5).



Image 5. Exsiccate of *Hibiscus rosa-sinensis*

2.3 MATERIAL COLLECTION

In April 2025, the collection of flowers began (Image 6). These were weighed and dried in an oven at 60 °C for 24 h. The dried flowers were identified (Image 7) and stored in a freezer at -18 °C. On average, six flowers were dried per week; therefore, it is estimated that a total of 228 flowers of the red variety were collected, resulting in 44.7 g of material (Image 8).



Image 6: Flower collection

PHYTOCHEMICAL ASSAYS OF THE ETHANOLIC AND METHANOLIC EXTRACTS

The formulation of both extracts was based on the study by Ruban and Gajalakshmi (2012). For the analysis of the phytochemical profile, the studies by Odeja et al. (2016) and Sapunyo et al. (2023) were used. Shinoda, ferric chloride, Salkowski, Keller-Kiliani, and foam formation tests were performed to assess the presence or absence of flavonoids, tannins, terpenoids, glycosides, and saponins, respectively.



Image 7. Identification



Image 8. Final weighing

For each sample, 5 g of the powdered flower were used. This material was added to two separate beakers, to which 100 mL of the respective solvents were added. The beakers were sealed with plastic wrap to prevent solvent evaporation and then kept under magnetic stirring for 72 hours. After this period, the samples were filtered to obtain the extracts (Image 9), which exhibited distinct colors from one another. The ethanolic extract intensified the flower's natural red coloration, presenting a burgundy hue. In contrast, the methanolic extract exhibited a color closer to amber, which allowed for better visualization of the results.



Image 9. Filtration of the Ethanolic and Methanolic extracts

Table 2. Phytochemical profile of the ethanolic and methanolic extracts of *H. rosa-sinensis*

Test	Reagents	Phytochemicals	E. ethanolic	E. methanolic
Shinoda	Mg HCl	Flavonoids	+	+
Ferric chloride	$FeCl_3$ a 5%	Tannins	+	+
Salkowski	$CHCl_3$ H_2SO_4	Terpenoids	+	+
Keller kiliani	$CH3COOH$ $FeCl_3$ H_2SO_4	Glycosides	+	+
Foam	Agitation	Saponins	-	-

The Shinoda test was used to evaluate the presence of flavonoids in hibiscus. This method consisted of adding 2 mL of the extract to a test tube, followed by the insertion of a fragment of magnesium and, subsequently, 3 drops of HCl. The results were assessed by observing a change in color to intense red or orange, which was compared with the standard sample, prepared with 2 mL of extract and 2 mL of distilled water. The ferric chloride test was performed with the objective of evaluating the presence of tannins in the flower. To carry out the assay, 2 mL of the extract were added to a test tube, followed by 3 drops of a 5% $FeCl_3$ solution. A color change to dark blue or dark green was observed.

The evaluation of the presence of terpenoids was performed using the Salkowski test. To perform the assay, 2 mL of the extract and 2 mL of chloroform were added to a test tube. Then, 1 mL of concentrated sulfuric acid was carefully added along the wall of the tube. The formation of a brown halo was observed in the test.

The investigation of the presence of glycosides was carried out using the Keller-Kiliani test. To perform the assay, 2 mL of the extract was added to a test tube, followed by 1 mL of glacial acetic acid and one drop of $FeCl_3$. Subsequently, 1 mL of concentrated H_2SO_4 was carefully added. The formation of a brown ring at the interface between the liquids was observed. The presence of saponins was evaluated using the foam formation test. To perform the assay, 2 mL of the extract and 2 mL of distilled water were added to a test tube. The solution was then shaken. No foam formation was observed.

RESULTS AND DISCUSSION

In both extracts, the phytochemical investigation of the flowers of this hibiscus species evidenced the presence of flavonoids (Image 10 and Image 11, item 2), tannins (Image 10 and Image 11, item 1), terpenoids (Image 10 and Image 11, item 3), and glycosides (Image 10 and Image 11, item 4); however, it did not corroborate the presence of saponins (Image 10 and Image 11, item 5) (Table 2).

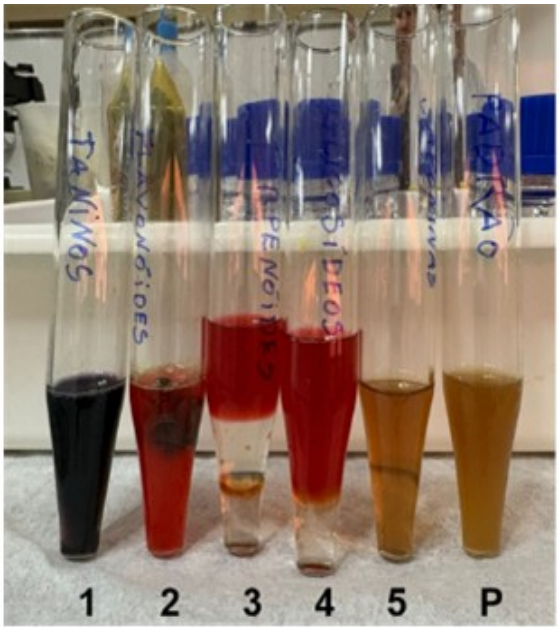


Image 10: Analysis of the methanolic E.

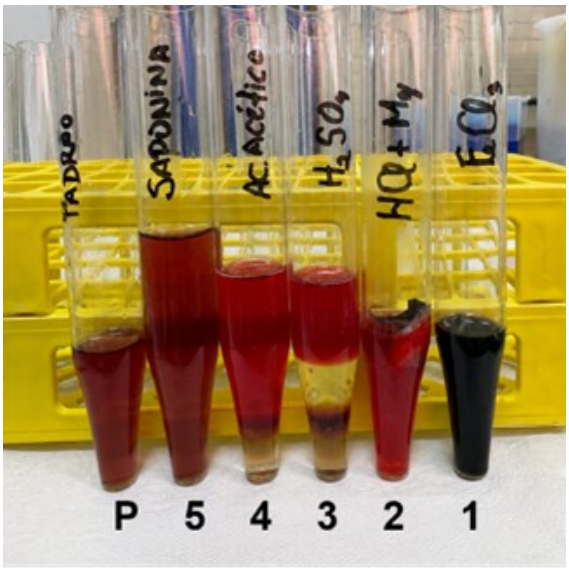


Image 11: Analysis of the ethanolic E.

These results present agreements and divergences in relation to studies conducted subsequently. Research such as that by Bhaskar and Nithya (2012) obtained positive results for tannins, flavonoids, terpenoids, and saponins in the analysis of the ethanolic extract, but presented negative results for other compounds, including glycosides. The study by Raza² et al. (2025) confirmed the presence of flavonoids and tannins in the methanolic extract. The study by Mejía et al. (2023) further emphasized the issue of flavonoids, reporting the presence of 20 types of these compounds, in addition to a glycosylated flavonoid in the hydroalcoholic extract of the flowers of this plant. In contrast, analyzing a different structure of the plant, the study by Barus et al. (2025), using the ethanolic extract of the leaves, reported only traces of flavonoids, but confirmed the presence of tannins and saponins. This "inconsistency" in the results of studies on the phytochemistry of *Hibiscus rosa-sinensis*, at first glance, could indicate a disparity in the scientific community regarding this plant and even raise doubts about the reliability of such distinct results across studies. However, in reality, these results demonstrate what was stated by Khan et al. (2017), according to whom this hibiscus species exhibits differences in its phytochemical profile due to the way it is managed and the variety of the plant. This finding was also corroborated by Mejía et al. (2023), whose research analyzed the phytochemistry of *Hibiscus rosa-sinensis* flowers of different colors and found a greater presence of anthocyanin-type flavonoids in red flowers compared with yellow, lilac, pink, and white flowers. In addition, the availability of macronutrients can also influence the presence of phytochemical compounds in plants in general. For example, nitrogen (N) availability can affect plant metabolism in different ways. N can induce the synthesis of saponins (Wei et al., 2019) and, when associated with phosphorus (P), can favor the production of terpenoids. On the other hand, under conditions of greater N availability, the plant may prioritize the synthesis of proteins that promote its growth (Bustamante et al., 2020); however, in adequate amounts, it can favor the formation of flavonoids and glycosides (Tavarini., et al, 2015). Other compounds influenced by N availability are tannins, whose response may vary according to their class. Proanthocyanidins, a class of condensed phenolic oligomers, exhibit a more pronounced response to N availability. In contrast, ellagitannins, a class of hydrolyzable phenolic oligomers, are not greatly influenced by the availability of this nutrient, even when their concentration is altered (Narvekar; Tharayil, 2021).

Moreover, it is also necessary to emphasize that a macronutrient will not necessarily be specifically associated with the formation of a particular phytochemical compound. For example, it is pertinent to mention potassium (K), which can also favor the synthesis of flavonoids (Jin et al., 2023), as can N (Tavarini et al., 2015). Another important point is that the presence of a compound may also be directly related to a plant's response to some stimulus. An appropriate example would be the increased biosynthesis and accumulation of saponins in response to some type of stress, due to their being a phytochemical compound involved in defense against different types of pathogens, such as fungi, bacteria, insects, etc. (Shakeel., et al, 2025). With these points presented, it is possible to hypothesize that studies in which the material used originated from an uncontrolled environment, where the plant may have been exposed to inadequate amounts of macronutrients and water, or may have been affected by pests without any intervention, could present results that differ from those of a study in which the material originated from management practices suited to the plant's needs, where it received appropriate macronutrients and water supply, in addition to pest prevention and the suppression of any pests that might have emerged..

CONCLUSION

The various forms of influence on the phytochemistry of *Hibiscus rosa-sinensis* flowers have a clear correlation with the way in which this plant developed, and may yield different results depending on whether this plant underwent controlled management, where its particular needs were met throughout its development; or whether it grew in an uncontrolled environment, where it may have been exposed to the most diverse variables that influenced it to adapt to its own reality. Thus, it is understood that research on *Hibiscus rosa-sinensis* in which its phytochemical compounds are evaluated may yield more uniform results if the plant used comes from a controlled environment, where it will receive appropriate care. The result of this study that most strongly supports this hypothesis was the absence of saponins in the phytochemical test used, where the lack of this compound demonstrates the influence of management and pest control, preventing stress in the plant.

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