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Pharmacognostic, Physicochemical and Phytochemical Analysis of Two Cultivars from *Hibiscus sabdariffa* L. in Cuba

Francis Brown[1], José González[2*], Max Monan[3]

[1]Dirección de Ciencia, Técnica e Innovación, Universidad de Ciencias Pedagógicas "Enrique Jose Varona", La Habana, Cuba
[2]Facultad de Educación de Ciencias Técnicas, UCP "Enrique Jose Varona", La Habana, Cuba
[3]ARVARNAM, Martinica, France
Email: *jgyaque@ucpejv.edu.cu, *jgyaque60@gmail.com

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Abstract

Roselle (*Hibiscus sabdariffa* L.) is an edible plant used in various applications including foods. Among them, the most popular are the fleshy red calyces used for making wine, juice, jam, syrup, pudding, cakes, ice cream or tea. Roselle flower and calyces are also known for its antiseptic, diuretic, antioxidant and antimutagenic properties. In order to offer aspects related to the quality and effectiveness of the plant, its pharmacognostic, physicochemical, and phytochemical parameters of the chalices were realized. Moisture content was (1.11% ± 0.07%), total acidity was (21.24% ± 0.31%), alcohol extractive value was at 95% (4.5 ± 0.03). The total phenolic content was 22.18 mg/g, while the total anthocyanidin content was 6.972 mg/g of dried matter. Phytochemical screening revealed the possible presence of saponins, phenolic compounds, glycosides, aminoacids, reductants sugars, anthraquinones, and alkaloids and the absence of terpenoids.

Subject Areas

Bioengineering, Plant Science

Keywords

Hibiscus sabdariffa, Pharmacognostic, Physicochemical Parameters, Phytochemical Screening, Cultivars

1. Introduction

Hibiscus sabdariffa Linn. is a shrub belonging to the family Malvaceae. It is thought as native to Asia (India to Malaysia) or Tropical Africa. The plant is

widely grown in tropics like Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and Philippines as a home garden crop. In Sudan, it is a major crop of export especially in western part where it occupies second place area wise after pear millet followed by Sesamum [1] [2].

The phenolic content in the plant consists mainly of anthocyanins like delphinidin-3-glucoside, sambubioside, and cyanidin-3-sambubioside; other flavonoids like gossypetin, hibiscetin, and their respective glycosides; protocatechuic acid, eugenol, and sterols like β-sitosterol and ergosterol. Roselle calyx extract is a good source of antioxidants from its anthocyanins [3] [4]. Anthocyanin is one type of flavonoid component that can be in Roselle calyces. Tsai *et al.* (2002) suggest that anthocyanin is the major source of antioxidant capacity in roselle petal extract [5] [6].

In Cuba, since 2002, the National Institute of Agricultural Sciences has been studying the plant to extend its crops. Among their pharmacological properties, it could be finding the antihypertensive and diuretic effect, anti-scorbutic and emollient. The chromosomal number is 2n = 72 [7] [8]. The common names in our country are: Agrio de Guinea, aleluya, aleluya roja de Guinea, flor de Jamaica, Jamaica, quimbombó chino, rosella, roselle, serení [7].

Cuban Educational System has proposed alternatives to increase the study and practical activities by the students of Agricultural and Industrial Chemistry's careers, being these activities a part of the integral formation and environmental education for a sustainable development of the students in those careers [9] [10] [11].

The aim of this study was to evaluate the pharmacognostic, physicochemical and phytochemical parameters of two cultivars of *Hibiscus sabdariffa* L. that grow in Cuba.

2. Materials and Methods

2.1. Plant Material and Reagents

The vegetable material was collected in the Experimental Station of Medicinal Plants "Dr. Juan Tomás Roig", located in San Antonio de los Baños, Artemisa Province, Cuba. The seeds were collected from the Horticole Research Center "Liliana Dimitrova" between April and June 2018 and 2019, planting the seeds (2 - 3) in two parcels of 5 m^2 (plantation surface: 0.90 × 0.60 cm). **Figure 1** shows the macromorphological characteristics of the calyces from the two cultivars used: Gerzy (a) and Liliana (b).

The flowers were collected after their mature and dried under the sun. The extracts were prepared with the ground material without screen. The ethanolic extracts were concentrated and evaporated under vacuum to 200 mL at 120 rpm, a temperature of 70˚C and 500 mbar. All reagents used were of analytical grade (Merck). All solvents were degassed prior to use in an ultrasonic bath without filtration.

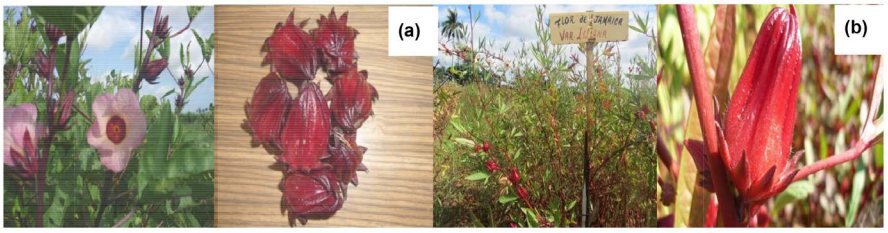

Figure 1. Morphological details of flowers and calyces of *H. sabdariffa* L. (from left to right (a) Gerzy; (b) Liliana).

2.2. Pharmacognostic Analysis

2.2.1. Macroscopic Studies

Ten plants were chosen randomly and were examined for morphological characters including high, number of branches and fresh weight of calyces. After post-harvest process, the calyces were weighted on analytical balance. The calyces were dried under the sun. The characters after the date of planting were monitoring weekly and at the moment of harvesting to get information about the behavior under our conditions. Both crops were panted in a Ferralitic hydrated soil (Ferrasol).

2.2.2. Physicochemical Parameters of the Calyces

Moisture content, total acidity and extractable matter were determined according to the standard procedures mentioned in the general rule of WHO and Miranda y Cuéllar [12] [13].

2.2.3. Phytochemical Screening

The chemical constituents were screened according to Chhabra [14] and Miranda y Cuéllar [13] to ascertain the presence of chemical components in diethyl ether, ethanol and water, respectively.

2.2.4. Determination of Total Phenolic and Total Anthocyanidins Contents

Total phenols were calculated by the Folin-Ciocalteu method, using as reference the gallic acid (Sigma-Aldrich) at concentrations of 10, 20, 30, 40 and 50 mg/mL as referred by Chlopicka *et al.*, 2012 [15]. On the other hands, the content of totalanthocyanidins was calculated by the spectrophotometric method according to Salinas *et al.* (2003) [16].

2.2.5. TLC

The freeze-dried powders were extracted by methanol/water/acetic acid (85:15:0.5, v/v, MeOH/H_2O/AcOH) as reported previously [17]. TLCP (thin-layer chromatography plate) was done on silica gel with fluorescent indicator 254 nm on aluminum cards (layer thickness 0.2 mm) (10 × 20 cm). A solvent system composed of concentrated hydrochloric acid (25%), formic acid (24%), and water (51%) (FHW) and using n-butanol: acetic acid: water (BAW 65:25:10) as developing agent (v/v/v) were used for the simultaneous separation of anthocyanidins. After running, the dried chromatograms were sprayed with

aluminum chloride (3% in methanol), concentrated sulfuric acid plus heat and $FeCl_3$ as chromogenic agents [18].

2.2.6. Statistical Analysis

Results are presented as mean ± SD. Statistical analyses were performed by Student's t-test. The values *of* $p < 0.05$ were considered significant. Duncan test was used utilizing the Statgraphics®Plus, version 5.0 program. The mean effective concentration (IC_{50}) was determined with the help of the Graphprism 5.0 statistical program.

3. Results and Discussion

Botanical Characteristics

1) Macroscopical parameters

Table 1 summarized the results of the evaluated parameters in both red roselle cultivars. Gerzy cultivar showed a lesser high than Liliana cultivar, as well as the branches number, except the weight of the calyces, where Gerzy cultivars reach the best results, indicating the differences between both cultivars.

To determine the best date of planting, the study was done in the same months (April and June) but in two different years (2018-2019). The flowering of Gerzy cultivar began in the second half of August while the flowering of Liliana started after October 15th. This result is according to McCaleb *et al.*, 2000, when they said that the plantation season of *H. sabdariffa* is stretching bind to the daylight and requires a monthly rainfall ranging from 130 to 260 mm in the first 3 to 4 months of growth. Rain and high humidity during harvest and drying can downgrade the quality of calyces and reduce the yield [19].

2) Physicochemical characteristics

Moisture content (11.1% ± 0.07%), was inside the limited index (8% - 14%). Extractable matter in ethanol (4.5% ± 0.03%) suggesting that the chemical constituents are less soluble in ethanol giving and idea that the drug has more non-polar components. Total acidity was (21.24% ± 0.31%). The acidity of the extraction is directly proportional to the content of the acids present in the solution. In the case of Jamaica they are citric, ascorbic, malic, stearic, and protocatechuic acid [20].

3) Phytochemical screening

Table 2 summarized the preliminary phytochemical screening suggesting the presence of saponins, phenolic compounds, flavonoids, aminoacids or amines,

Table 1. Behavior of parameters evaluated in the same date of 2018 and 2019.

Parameters	April/2018		June/2018		April/2019		June/2019	
	Gerzy	Liliana	Gerzy	Liliana	Gerzy	Liliana	Gerzy	Liliana
Plant higher (cm)	2.23	2.24	1.92	2.09	1.67	2.27	1.65	2.0
Number of branches	10	6	8	16	4	7	5	8
Weight of calyces (kg)	3.366	1.432	3.414	2.516	4.531	2.821	2.864	4.239

Table 2. Phytochemical screening of *H. sabdariffa* L. in Cuba.

Test for constituents groups	
Saponins (foam test)	++
Phenolics compounds ($FeCl_3$)	+++
Flavonoids (Shinoda)	++
Aminoacids (Ninhydrin)	+++
Reductants sugars (Fehling)	+++
Anthraquinones (Börntrager)	+
Terpenoids (Sudan III)	–
Alkaloids (Dragendorff)	+

reductants sugars, anthraquinones, and alkaloids. None triterpenes or steroids were found in the assays.

4) Total phenol and anthocyanins contents

Total phenolic content was 22.18 mg/g, but this result is lowest comparing with the same results using ethanol (87.7 mg/g) and water (72.22 mg/g) according to Al-Hashimi, 2012 [21]. Anthocyanidins content was 6972 mg/g, our result is better than the same evaluation done by Galicia-Flores*et al.*, 2008 when they got values using methanol (3649.8 - 6066.7 mg/kg^{-1}) and water (1725.8 - 2969.9 $mg{\cdot}kg^{-1}$), respectively.

According to Marco *et al.* (2005), the degradation of anthocyanins using the PARAFAC model (Parallel Factor Analysis) is directly proportional to the influence of pH in the equilibrium displacement among the chromophores species of anthocyanins (flavylium cation, quinoidal base, carbinol pseudobase, and chalcones), because of the pH in the medium the amount of those forms vary and the maximum absorption of wavelength [22]. The predominant form is flavilium cation at low pH (3.0 - 1.3) as is represented in **Figure 2**.

UV/Vis spectrophotometry is still widely used to study anthocyanidins, which change their form and colour depending on pH, concentration, metal ions and copigmentation. This multistate behaviour has been used to derive molecular machines based on flavonoids, particulary flavylium containing ones like anthocyanins [23].

5) TLC

To confirm the presence of anthocyanins, both total extracts were tested heating 2 mL of ethanolic extract with 1 mL of $HCL_{(conc)}$ during 10 min. After cooling, 1 mL of water and 2 mL of amilic alcohol were added, and then the mixture was shakes and separated in two phases. Was seen an intense red color in the amilic phase, that is indicative of a positive assay [13].

Total extracts were plotted on TLCP. After the chromatograms were dried, there were sprayed with aluminum chloride (3% in methanol), anthocyanins with their free adjacent hydroxyls on the B-ring of the aglycone (delphinidin, cyanidin, and petunidin derivatives) turn blue as shown in **Figure 3**. It is evident

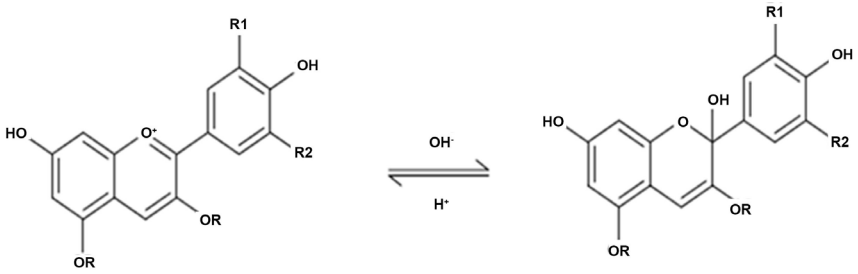

Figure 2. Structural change pH dependent in anthocyanins (R = H or glycosylation).

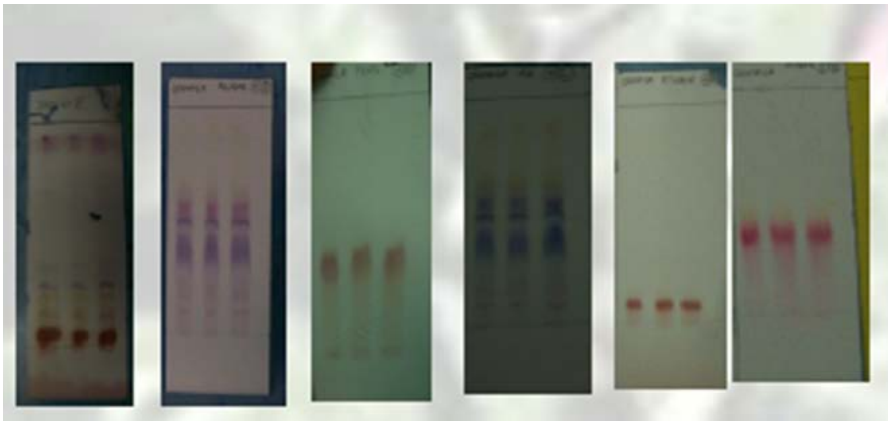

Figure 3. TLC of both cultivars (spots from left to right: under ordinary light; $AlCl_3$; $FeCl_3$).

that complete separation of anthocyanidins with closely related R_f values is difficult, and two-dimensional TLC may thus be preferable [18].

Anthocyanins are visible at the concentration levels encountered on chromatograms.

Examination under UV light is worthwhile, however, because the 3,5-diglycosides of pelargonidin, peonidin, and malvidin are distinguished by their fluorescence from the corresponding 3-glycosides.

After the dried chromatograms have been sprayed with aluminum chloride (3% in methanol), anthocyanins with their free adjacent hydroxyls on the B-ring of the aglycone (delphinidin, cyanidin, and petunidin derivatives) turn blue. The chemical components were separated in some way taking into account their polarities, suggesting the presence of phenolic compounds with $FeCl_3$ and H_2SO_4 plus heat.

4. Conclusion

The present study indicated that both cultivars used (Gerzy and Liliana) had very good indicators in plant higher, number of branches and weight of calyces. The results of this research demonstrated that the best date of plantation is between June 15 and November 15. The best yields crop was get with the Gerzy cultivar when their seeds were planted in the month of June. The high total

phenolic content suggests that the antioxidant capacity of both cultivars is prominent and the main chemical compound that has a special role in it is the anthocyanins present in the extract. The reported phytochemical and pharmacognostic studies support its traditional uses and may prove to be useful for clinical evaluation and development of commercial drugs. Introduction and commercial cultivation of its varieties in Cuba are also recommended.

Conflicts of Interest

The authors declare no conflict of interest.

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