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Analysis of Vitamins, Lipid, Ashes and Ph of Leaves and Antibacterial Capacity of Ora-Pro-Nóbis Extract (*Pereskia aculeata* Miller)

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Abstract

The species *Pereskia aculeata* Miller, belonging to the Cactaceae Juss family, is popularly known in Brazil as ora-pro-nóbis. The leaves of this plant contain large amounts of proteins, vitamins, minerals, fibers and hemicellulose, important for human nutrition. The ora-pro-nóbis, also offers antioxidant and antibacterial action, for containing in its composition sources of bioactive substances found among secondary metabolites, especially carotenoids and phenolic compounds. The antioxidants present, act against cell oxidation and free radical damage, helping to prevent some degenerative diseases. In addition, the antibacterial action found in this plant can be used to treat different diseases caused by bacteria and to help in the conservation of food. However, there is still little information on the benefits of ora-pro-nóbis, consequently, it is important to carry out new studies on the biological activity of this plant. Therefore, this study aimed to identify and determine the vitamins B_6 and C, the ash content, lipid and potential of hydrogen (pH) of fresh leaves and the antibacterial capacity of ora-pro-nóbis extract.

Keywords: *Pereskia aculeate*; Vitamins; Lipid; Ashes; pH; Antibacterial activity

Abbreviations

ATCC: American Type Colection Culture; $C_8H_{11}NO_3$·HCl: Pyridoxine Hydrochloride; DMSO: Dimethyl Sulfoxide; g (gram); $HClO_4$: Perchloric Acid; m: meters; M: Molar; mg: milligram; mL: milliliter; mm: millimeter; nm: nanometer; pH: potential of hydrogen; rpm: revolutions per minute; UFP: Unconventional Food Plant; w/v: weight/volume; %: percent; °C: degree Celsius; °: degree

Introduction

Food is related simultaneously to people's health, being associated with nutritional risks or benefits according to individual dietary habits [1]. A diet rich in natural foods is generally more efficient at converting natural resources into calories and nutrients for human consumption [2] and can help with weight management and lower the risk of chronic diseases such as cancer, cardiovascular disease, high blood pressure and diabetes [3].

The human body needs different nutrients in order to be able to perform its functional role properly and to be able to provide enough energy to perform its daily activities [4]. These nutrients are found in fruits, plants and other natural foods which are capable of activating a positive and desirable physiological effect on the body, bringing health benefits the people who consume them [5].

The plants contain several functional properties and natural compounds, such as resins, monoterpenes, terpenoids and biopolymers, which have various biological effects as antibacterial, antifungal and antioxidant activity generating promotion of human health [6].

The use of Unconventional Food Plants (UFPs) is considered a strategy to maintain diversity in the diet and encourage forest maintenance, as it has a low impact on agricultural practices [7], in addition to improving the nutritional value of the diet in relation to vitamins, minerals and fiber supply [8].

An example of UFP that can improve the diet is the species *Pereskia aculeata* Miller, belonging to the family Cactaceae Juss, popularly known in Brazil as ora-pro-nóbis. It was also known as "meat for the poor" because of its high protein content compared to other plants commonly used as food. Its leaves also contain large amounts of vitamins (A, C and folic acid), minerals (manganese, zinc, calcium, iron and magnesium), fiber and hemicellulose, important for human nutrition [9].

Ora-pro-nóbis also contains antioxidant and antibacterial action, because of its bioactive substances, especially carotenoids, vitamins and phenolic compounds [10]. The antioxidants content in this plant protect the human body against cell oxidation and free radical damage, helping to prevent degenerative diseases [11]. The antibacterial action found in ora-pro-nóbis can be used to treat different diseases promoted by bacteria, including infectious diseases of the respiratory tract, gastrointestinal, urinary and biliary systems [12] and also for food preservation [6].

However, this plant is not very known, because it was considered for a long time as a "weed plant", and grows alone in places where it is not cultivated [7,13]. In this context, there is still little information on the benefits of ora-pro-nóbis, consequently. This requires further research on the biological activity of this plant. Therefore, this study

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aimed to identify and determine the vitamins B_6 and C, the ash content, lipid and the potential of hydrogen (pH) of fresh leaves and to analyze the antibacterial capacity of ora-pro-nóbis extract.

Materials and Methods

The ora-pro-nóbis leaves (Pereskia aculeata Miller) were collected in União da Vitória, in the Brazilian state of Paraná in the following geographic coordinates: Latitude: -26.2304; Longitude: -51.0866 26° 13′ 49″ South, 51° 5′ 12″ West; and Elevation: 751 m.

Vitamin analysis

In order to perform the analysis of vitamins B_6 and C of the ora-pro-nóbis leaves, it was used the technique described by the Institute Adolfo Lutz [14].

Determination of vitamin B_6

For the analysis of Vitamin B_6, 0,4 g of the fresh leaves were weighed on an analytical scale with an accuracy of 0,001 gram, grounded in a knife mill and quantitatively transferred to a porcelain Gral. 40 mL of acetic acid and 5 mL of mercury acetate at 6% were added. Then macerated with the pistil and filtered with filter paper number 15 to remove the remaining leaves that were not dissolved. Afterwards, it was transferred to a 250 mL Erlenmeyer flask and then titrated with 0,1 M perchloric acid ($HClO_4$), using the violet crystal indicator. This analysis was performed in triplicate and with the use of an analyte-free sample (white).

At the end, the content in percentage of vitamin B_6 present in the ora-pro-nóbis leaves was calculated, considering that each mL of $HClO_4$ spent on titration is equivalent to 20,56 mg of vitamin B_6 ($C_8H_{11}NO_3$·HCl).

Determination of vitamin C

For the analysis of vitamin C, 5 g of fresh ora-pro-nóbis leaves were grounded in a knife mill and placed in a porcelain Gral for complete maceration, with the aid of 3 portions of 30 mL aqueous solution of oxalic acid at 1 g% was transferred to a 250 mL volumetric flask. Gral was washed with 2 portions of 30 mL of 1 g% oxalic acid solution, passing them to the same volumetric flask. In addition, the last wash performed with 2 servings of 25 mL of distilled water, transferring them to the same volumetric flask. After washing, the volume of the flask was completed with distilled water and 50 mL of the sample was transferred to a 250 mL Erlenmeyer, adding 2 mL of 0,01 M iodine solution and stirring for 30 seconds. Thus, 3 mL of starch solution was added at 2 g%. A 0,01 M sodium thiosulfate solution was placed in a burette and titrated until the blue coloration disappeared. The analysis was performed in triplicate.

In order to obtain the vitamin C content present in the ora-pro-nóbis leaves, the following calculation was made:

Vitamin C content (mg) = 100x ((V1x0,88x(I–T))/V2)) / A

Being:

V1 = Total volume of the solution made with the test sample

V2 = Volume of the solution under analysis used in the titration

A = Weight of the sample being analyzed used for the determination

I = Added volume of iodine solution 0,01 M

T = Volume of sodium thiosulfate solution 0,01 M spent on titration

0,88 = Amount of vitamin C, in mg, corresponding to 1 mL iodine solution 0,01 M

Lipid analysis

This analysis was based on the method described by Argandoña [15]. 1,5 g of fresh ora-pro-nóbis leaves were ground in a knife mill and weighed on an analytical scale with an accuracy of 0,001 gram. Then, they were placed in 3 tubes of 10 mL, where exactly 1,43 mL of chloroform was added, 2,86 mL of methanol and 1,14 mL of distilled water. The tubes were capped and placed in a centrifuge for 30 minutes. After this period, they were removed from the centrifuge and exactly 1,43 mL of chloroform and 1,43 mL of sodium sulfate solution 1,5% were added (w/v). Covered and shaken vigorously for two minutes. It was centrifuged at 1000 rpm for two minutes to accelerate separation and discarded the top layer and filtered quickly with filter paper number 15 in a 30 mL tube. Then, exactly 5 mL of the filtrate was measured and transferred to a 50 mL beaker previously tared and weighed. The 3 tubes already filtered were placed in an air circulation oven at 80 °C until the solvent had evaporated completely. Finally, they were cooled in a desiccator and weighed.

To obtain the percentage of lipids present in the ora-pro-nóbis leaves, the following calculation was performed:

Lipids (%) = ((M1x4)/M2)x100

Where:

M1 = Mass of lipids (g) contained in 5 mL

M2 = Mass of sample (g)

Ash content analysis

The ash content has been verified according to the technique proposed by the Institute Adolfo Lutz [14] in which, in 3 porcelain crucibles, 5 g of fresh ora-pro-nobis leaves were placed in the desiccator until the constant weight was obtained and then, slightly inclined, in porcelain triangles they were heated with a Bunsen burner equally on all sides of the crucible until the material was completely carbonized. Then the crucibles were transferred to a muffle at a temperature of 550 °C, where they remained until complete calcination for approximately 1 hour. Afterwards, the crucibles were transferred to a desiccator until they reached room temperature and weighed individually. At the end, the ash content was calculated for 100 g of fresh leaves, according to the following formula:

Ash content (g%) = ((100-U)xC)/A

Being:

U = Value of the moisture and volatile fraction of the sample used in g

C = Weight of crucible with ashes minus weight of empty crucible

A = Weight of crucible with dried material minus weight of empty crucible

Determination of potential of hydrogen (pH)

The methodology proposed by Argandoña [15] was used to determine the pH. The calibration was performed in a pH meter.

After calibrating the equipment, the electrode was left immersed in distilled water. The ora-pro-nóbis leaves were stored for 2 days under refrigeration at 5°C. After this period, 10 g of leaves were crushed in a knife mill and placed in a 150 mL beaker with 100 mL (1:10) of distilled water. Then, the electrode was dried with absorbent paper and immersed in the sample, where the reading was made at a temperature of 25 °C and in triplicate. After verifying the reading, the mean value of the three pH readings and the standard deviation were calculated.

Depending on the pH value, plant samples are subdivided into low-acid samples (pH above 4,5), acid samples (pH between 4,0 and 4,5) and very acid samples (pH below 4,0).

Obtaining the extract of ora-pro-nóbis leaves

To obtain the ora-pro-nóbis extract, 10 g of fresh leaves were ground in a knife mill and macerated with 50 mL 97,7% ethanol and 50 mL 99,5% acetone (1:10). They were kept under mechanical agitation in an agitator at 300 rpm for 4 hours. After this period, they were placed on an ultrasound for 20 minutes. Then the liquid was filtered with the aid of a glass funnel and filter paper number 15. After filtration, the extract was placed on a heating plate, maintained at a constant temperature of 30°C until reduction.

Analysis of antibacterial capacity

The antibacterial analysis of ora-pro-nóbis leaf extract was determined *in vitro* using the plate inhibition halo test, a method described by Fratianni [16] and adapted for this analysis. Standard strains of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) bacteria, representing one Gram-positive and one Gram-negative bacteria, respectively, were tested. The strains were supplied by Newprov™.

Each strain was pricked in Blood Agar until the third generation was obtained. Afterwards, the bacterial solution was performed based on the MacFarland scale of 0,5. Which was analyzed in a spectrophotometer at 625 nm. Afterwards, the bacterial mat was made in Muller-Hinton Agar medium, with the fusion discs already impregnated with the extract. The Petri dishes were then incubated at 37 °C for 18-24 hours under sterile conditions. A disc treated only with dimethyl sulphoxide (DMSO) as a negative control and the tetracycline disc as a positive control. The antibacterial activity present in the extract was evaluated from the observation of the presence of the inhibition halo and later the measurement was made so that it could be compared with the positive control of tetracycline with the measurement of the diameter (in mm) of the halo.

Results and Discussion

Ora-pro-nóbis leaves are increasingly being used for human consumption as an additional source of nutrients, especially for the low-income population, due to their easy cultivation. Then, this study analyzed vitamins B_6 and C, lipid, the ash content and pH of fresh leaves and the antibacterial activity of ora-pro-nóbis extract.

The fresh leaves of macerated ora-pro-nóbis showed a pH of 6,94. In the literature, the studies of fresh ora-pro-nóbis leaves, without the maceration process, showed values between 4,89 and 5,10 pH, also considered as low acidity [17,18]. The difference found in the pH of the analyzed sample and the samples from the literature can be due to the storage of these leaves for 2 days before the maceration process, which can decrease the acidity. This reduction in acidity may be related to the splitting of the starch present in the leaves into reducing sugars and this conversion generates pyruvic acid originating from cellular respiration, implying a reduction in acidity [19].

In this study, fresh ora-pro-nóbis leaves showed 145,62 mg of vitamin B_6/g. Vitamin B_6 is a collective term for pyridoxine, pyridoxal and pyridoxamine. Pyridoxine occurs mainly in foods of plant origin while pyridoxal and pyridoxamine are found in foods of animal origin [20]. This vitamin is of great importance for the nutrition of human beings, because besides being a powerful antioxidant, it is not synthesized in the organism [21]. In adults, the recommended daily intake of this vitamin is 1,3 mg [22], indicating that ora-pro-nóbis leaves are an important source of vitamin B_6.

The analysis of the vitamin C content of fresh ora-pro-nóbis leaves was 70,40 mg/100 g. Compared to another study carried out with fresh ora-pro-nóbis leaves, the result was 185,8 mg/100 g, but the titration method used for the determination was different, using 3% metaphosphoric acid for titration after a quantitative reduction of 2,6-dichlorophenol-indophenol dye by ascorbic acid [23]. But when the result of this study is compared with an analysis performed with dehydrated ora-pro-nóbis leaves in powder form, the vitamin C content was 42,35 mg/100 g [24], showing that the fresh leaves of this plant contain more vitamin C than the dehydrated powders. The dehydration process reduces the vitamin C content because it is considered a labile vitamin, that is, it is easily destroyed by the presence of oxygen, metallic ions, increased pH, heat and light [25].

When comparing the vitamin C content found in this study with the vitamin C content of natural orange juice, it can be seen that the ora-pro-nóbis leaves are much higher than the juice vitamin contents ranging from 17,96 to 25,86 mg/100 ml. However, when the vitamin C content is compared to the vitamin C content present in 100 mL of natural guava juice, the values are similar, ranging from 62,54 to 75,48 mg/100 mL [26]. But when compared to the value of vitamin C present in 100 mL of wholemeal pasteurized acerola juice (1000 mg/100 mL), the vitamin C content of the ora-pro-nóbis leaves (70,40 mg) is lower [27].

Vitamin C has a fundamental role in human nutrition because it is considered as an antioxidant and to provide its role in our bodies it is necessary to meet the daily intake recommendation for this vitamin of 75 mg for females gender and 90 mg for males gender [28]. An adult woman would needs 106,61 g and an adult man needs 127,93 g of ora-pro-nóbis leaves to achieve the recommendation. Because the amount of leaves is very large, daily consumption can be complemented with other sources of vitamin C throughout the day, however, in populations with low economic levels, where fruit and vegetable consumption is limited, the consumption of ora-pro-nóbis can be an important source of vitamins, and the leaves can even be used to prepare meals by adding to rice, sauces, and consumption as salad, among others.

The determination of the ash content in a food sample is quite important because it implies its nutritional value and represents the total mineral content and can therefore be used as a quality measure, and is often used as a criterion in the identification of foods [29].

In relation to the ash content found in this study, the mean ash content was 23,61 g% for 100 g of fresh ora-pro-nóbis leaves. This value is close to that found in another study that demonstrated an ash content of 16,1 g/100 g [23]. The leaves of fresh ora-pro-nóbis, when compared to the carrot leaf (10,5%) and dehydrated powdered ora-pro-nóbis (15,25 g) [24,30], has a higher ash content. Therefore, the ash content present in the fresh leaves is considerably high, indicating that the analyzed leaves have a high mineral content.

Most foods of vegetable origin are not considered a good source of lipids, just as in the ora-pro-nóbis leaves the lipid content was 1,07%. In a study carried out with lyophilized leaves and fresh ora-pro-nóbis leaves, the lipid content was determined by extraction with petroleum ether using the Soxhlet method, finding a value of 2,4 g/100 g and 0,3 g/100 g, respectively [17]. Thus, the ora-pro-nóbis leaves can be used for consumption in diets with lipid restriction, due to the low index of this nutrient [31].

The ora-pro-nóbis leaves can be used for everyday consumption in the population, not only in nutrient restriction diets or supplementation, further enriching human food and improving health.

The antibacterial activity present in extracts of various plant species can be used for the preservation of raw and processed foods, pharmaceutical products and natural therapies [6]. In this study, ora-pro-nóbis extract showed no inhibition against the bacteria *Staphylococcus aureus* and *Escherichia coli*. Similar results were found in another study, which evaluated the antibacterial activity of different extracts (total alcohol, hexane, dichloromethane, chloroform, ethyl acetate and methanol) of *Pereskia grandifolia* leaves by the microdilution method and also showed no inhibition against the same bacteria [32]. In a study that evaluated antibacterial action using petroleum ether extract from ora-pro-nóbis leaves by means of the halo inhibition test, it observed a great efficiency against several bacteria, including *Staphylococcus aureus* and *Escherichia coli* [33].

The petroleum ether is considered an organic solvent used to extract functional compounds present in plants, among them are the terpenes (monoterpenes and sesquiterpenes) that contains recognized antibacterial activity in its composition [34,35].

The absence of activity against the bacteria evaluated in this study indicates that the solubility of the plant extract can be considered an important interfering factor, because besides the solubilization of the plant extract being difficult to standardize for the tests of antibacterial activity, different solvents are also used that may or may not inhibit bacterial growth, thus often altering the results [36].

Gram-negative bacteria such as *Escherichia coli* have an extra layer of lipopolysaccharides and proteins, causing less sensitivity of this bacterium to plant extracts [37].

Conclusion

In view of the results obtained, it was possible to conclude that the fresh ora-pro-nóbis leaves present high levels of ash and vitamins B_6 and C, low lipid content and low acidity, which shows to be an adequate plant for consumption and an important source of nutrients for the human diet.

In addition, the leaves are viable in the preparation of a great diversity of food products, such as soups, salads, stews, among others, thus increasing the nutritional value of the daily diet of the population, especially those with greater need of nutrients. It is important to note that it is a plant of easy cultivation and can be cultivated in a simple way in gardens or pots.

No antibacterial activity was observed in ora-pro-nóbis leaf extract against bacteria *Staphyloccoccus aureus* and *Escherichia coli*. It is suggested that more research should be carried out using other extraction methodologies, in order to contribute to the development of new natural antibacterial agents, which may become therapeutic adjuvants applicable in the treatment of bacterial diseases and as food preservatives, with the purpose of reduce the use of synthetic preservatives, seeking the promotion of human health.

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