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ANTIOXIDANT ACTIVITY AND QUANTIFICATION OF CALCIUM, IRON, MAGNESIUM IN ETHANOLIC EXTRACT OF LEAVES OF *BETA vulgaris L.* (*beet*)

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Abstract: Nutritional studies such as that of legume sprouts can improve the nutritional value in the diet, an effect that is fundamental in the state of human health. The objective was to determine the impact of the germination process on the quantification of nutrients and antioxidant activity of leaves of BETA vulgaris L. (beterraga) grown in the province of Ica. Methodology: It is a basic and correlational study of explanatory analytical design. Results: The proximal chemical analysis showed a higher concentration of carbohydrates (54.53 g/100g) in ungerminated seeds, caloric value 310.59 Kcal/100g in ungerminated seeds, 325.37 Kcal/100g in germinated seeds, in the determination of minerals, zinc with 118.9 mg/100g in germinated, concerning the antioxidant activity by the method of free radical DPPH in non-germinated seeds presented an IC50 of 4.65 mg/ml and germinated IC50 of 20.91 mg/ml, For FRAP (TEAC) equivalent to mM of trolox, in non-germinated seeds 6.81 mg/ml and germinated seeds 28.86 mg/ml. Conclusion: Germination improved the nutritional quality of the leaves of BETA vulgaris L. (beet), of the proximal chemical composition, referring to the content of minerals calcium, magnesium, iron and zinc increased during the germination process, for the antioxidant activity germination did not show any benefit.

Keywords: *BETA vulgaris L.* chemical analysis, minerals, antioxidant activity.

INTRODUCTION

Currently in our environment there is a lot of talk about functional foods, due to their nutritional contribution that contribute to the maintenance of health, which has generated an increase in studies on the subject, which is becoming increasingly important.

In this case, the leaves of the beet were acquired in the Arenales market, where the roots are sold, since most people, when acquiring this tuber for consumption, ask for the leaves to be discarded. This causes an environmental and economic impact, which could be reduced with a proper use of these by-products that represent approximately fifty percent of the product and are known to be a valuable source of bioactive compounds, nutrients and minerals (Nutter, et. al., 2020). After being dried and ground, they were macerated to obtain an extract that was then taken to the rotary evaporator for its corresponding drying, joining all the extracts of the tested replicates, which were then stored in amber bottles until the corresponding analyses could be performed.

The *BETIS vinifera L. (beet),* also called "beet" is a very complex vegetable, because its intake helps regulate various functions in the body, especially in the digestive processes, due to its prebiotic effect, which helps improve the intestinal flora, highly recommended in cases of irritable bowel. Its content of calcium, iron, magnesium prevents anemia, hence the beet is considered one of the most effective fruits because it helps raise hemoglobin levels, helping to prevent and reduce the risk of neurodegenerative diseases. Thus we have, Concha & Guerra (2014) [((2) ())] "proved an increase of 87% hemoglobin levels in children from 2 to 4 years of age, with iron deficiency anemia, by the intake of beet jelly, for its high content of due folates, iron, vitamin C, vitamin A, among others"

Their versatility of uses is emphasized by their antioxidant action, which acts as anti--inflammatory. (Ortega, Carretero, Pascual, & Villar, 1996) [((3) () ())]That determines that anthocyanins are found in fruits and vegetables generate a wide color scheme ranging from bright red to purple and dark blue" (Kovarovič, Bystrická, Ján, & Lenková, 2017).[((4) () ())].

Barria, et.al., (2018) [(5)]aimed to demonstrate the influence of sugar beet (*Beta vulgaris L*) bioactive compounds on cardioprotective effects, general and specific studies on sugar beet were examined, they showed that the content of polyphenols varied from 218.00 mg.kg1 to 887.75 mg.kg1; anthocyanins ranged from 14, 8 ± 0, 0 mg.kg1 to 84.50 ± ,71 mg.kg1; and antioxidants was 8.37 ± 0.29, 1.83 ± 0.35 %, nitrate salt was 1800 mg NO3/kg fresh weight.

Guldiken, et.al. , (2016) [((6) ())]Investigated the antioxidant processing of fresh red beets, analyzing six different products, comparing their total phenolic content of flavonoids, antioxidants, handled by Folin Ciocalteu method mg GAE/100gr; they had high values of phenolic content to the flavonoids. Results in samples: fresh 255 mg, cooked 238 mg, dry 143 mg. And for the determination of antioxidant by the DPPH method the following samples were achieved: fresh 137 mg, cooked 131 mg, dry 143 mg.

Guine, et. al., (2018) [((7) ())]aimed to identify the antioxidant activity in beet, for this they executed three phases quantifying anthocyanins and antioxidant activity, the results of which indicated that the antioxidant activity was found by ABTS/ μmol TE / g method; 13.9-19.

Seijas, (2019) [((8) ())]Employment temperature levels of: 20, 40, 50 and 60 °C and time: 0, 40, 48 and 96 hours, setting average concentrations obtaining higher amount of pigment from the dry extract), likewise, these titrations were executed using ultraviolet visible spectroscopy. Concluding that it remained stable at 20 °C regardless of the accumulation time with an average value of 81.6 mg/L, indicating the instability of the degradation, then the degradation occurred between 0°C and 40 hours of accumulation, reducing the concentrations to an average of 0.72 0°C and 96 hours

Junco, Y. (2019)[((9) ())]**.** Determined the adsorption of calcium and magnesium present in groundwater, using the observational, correlational method and pre-experimental design, through the method of hydrochloric acid addition (HCL) optimized the adsorption capacity of magnesium and calcium; whose results were below the standard value of quality for water consumption, with a hardness of (500 mg/1), having low values, being able to reduce to 85% when comparing both concentrations.

MATERIAL AND METHODS

POPULATION

Sheets free of damage and/or deterioration

EXCLUSION CRITERIA

Damaged and/or mistreated leaves

DATA COLLECTION TECHNIQUES AND INSTRUMENTS

Sample

Dry plant matter from leaves of *BETA vulgaris L. (beet)*

Sampling

Sampling was intentional

VEGETABLE DRUG TREATMENT

The leaf samples were washed with distilled water and dried under shade, then the leaf samples were dried in the surrounding air at 40°C for 3 days; then the plant material was ground into 5 mm particles in a hammer mill.

OBTAINING EXTRACTS

It was prepared from 500 g of leaves according to the method described by Olga Lock de Ugaz (2010)[10]. The 500 kg will be crushed and left to macerate with 70° alcohol for approximately 7 days using 2 liters of said solvent. The same procedure was followed for the extract, except that the extraction solvent was distilled water.

a. Determination of antioxidants by the method: DPPH method

Kukoski, et.al., (2005).[(11) ()] "2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (DPPH) is a free radical of chromogenic compounds that can reach directly without prior preparation. The absorbance spectrum shows a peak at 515 nm."

The mechanism for the antioxidant activities of polyphenols originates when the polyphenol molecules inactivate the free radical by transferring an electron.

b. Determination of antioxidants by the FRAP method (TEAC).

To start this test, first the reactant for the activity is prepared, which is composed of a combination of 300 mM acetic buffer (Ph = 3.6), 1:1 (v: v: v: v). Thus we have that when adding 3 mL of the FRAP reactant (the previous preparation) in a container, measuring at 593 nm. the absorbance, 100 Ul of some oil solution was added mixing it with vortex for half a minute, after 360 seconds of latency at room temperature, a new reading is made at 593 nm, decreasing the values of the initial absorbances, where the sample was tested three times [((20)).]

DETERMINATION OF MINERALS: CALCIUM, IRON, MAGNESIUM

The ethanolic extract of leaves of *BETA vulgaris L. (beet)* will be determined the amount of minerals (calcium and magnesium), using atomic absorption spectrophotometry methods, from the ethanolic extract and its subsequent hydrochloric acid dissolution. NIH, (2018

- **Calcium determination**

The instrumental conditions for the measurements of the metals were: wavelengths for Ca: 422.7 nm, Mg: 285.2 nm and 766.5 nm for K. It is important to point out that the oxidizing gas mixture used for the atomization was air-acetylene (4/2), because this reaches the characteristic temperature to obtain the fog in the atomization of metals under study.

- **Iron determination**

Method by derivative spectrophotometry for the micro determination of iron at sub-mg/L levels. TPTZ (2,4,6-tripyridyl-1,3,5-triazine) was used as a chromophore reagent to form at pH = 5.0 a blue complex, which is retained and concentrated on a cation exchange sp sephadex C-25 resin.(Toral et.al.,2001)[12]

- **Magnesium determination**

Figueroa et. al., (2010) [13] "The ashes were diluted with 1 ml of HNO3 and it was gauged to 100 ml. After filtering, a 5 ml aliquot was taken, 10 ml of La 2O3 solution was added, to eliminate possible interferents, and it was gauged to 100 ml. The readings were taken at 285.2 nm in the atomic absorption spectrophotometry equipment".

RESULTS AND DISCUSSION

Weight of leaves (g)	Solvent volume	Dry weight	Percentage yield
52,3	500	4,3671	8,35
57,8	500	4,6282	8,01
51,9	500	4,2348	8,16
Average			8,17

Table 01. Yield of ethanolic extract of leaves of *BETA vulgaris L. (beet)*

* The extract was obtained by maceration first with 300 mL and after filtration, the frame was extracted again with 200 mL of the same solvent.

Parameters	Results	Units
Total solids	90,64	g/100g
Humidity	9,36 ± 0,96	g/100g
Ashes	18,7 ± 0,56	g/100g
pH	4,27 7 ± 0,27	..
Soluble solids	39,7	° Brix

Table 2. Characterization of the ethanolic extract of the leaves of *BETA vulgaris L. (beet).*

*Values are averages of three replicates.

Magnesium Standard (ppm)	Absorbance Repetition 1	Absorbance repetition 2	Average
0,05	0,059	0,058	0,059
0,1	0,109	0,113	0,111
0,3	0,301	0,325	0,309
0,5	0,559	0,526	0,548
0,8	0,898	0,823	0.87at3

Table 3. Values of magnesium standards

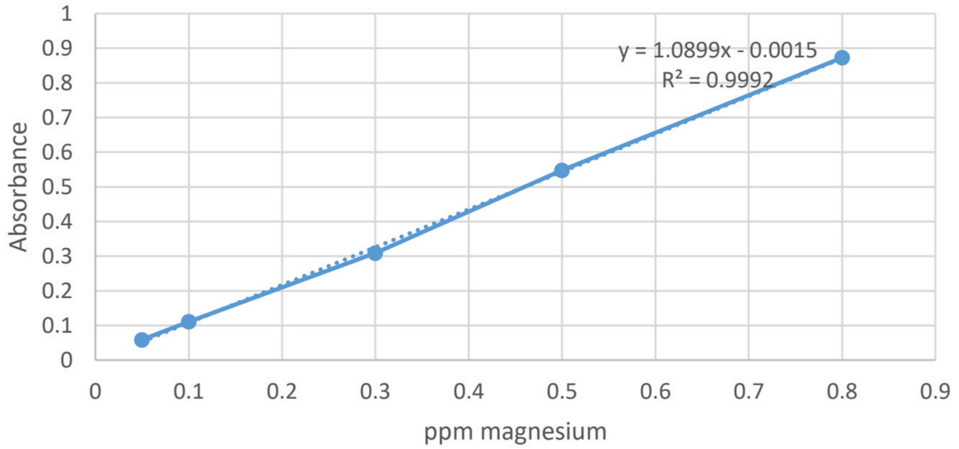

Figure 3. Calibration curve for magnesium

Calcium standard (ppm)	Absorbance Repetition 1	Absorbance repetition 2	Average
0,5	0,029	0,029	0,029
1	0,057	0,058	0,058
2	0,099	0,098	0,099
3	0,146	0,150	0,148
4	0,198	0,213	0,203

Table 4. Calcium standard values

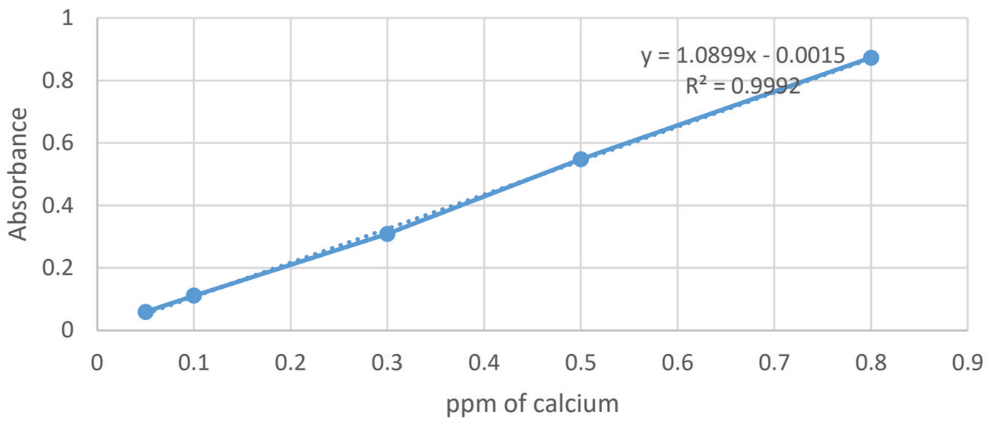

Figure 4. Calibration curve for calcitonic calcification.

Iron pattern (ppm)	Absorbance Repetition 1	Absorbance repetition 2	Average
0,5	0,024	0,028	0,026
1	0,047	0,051	0,049
3	0,125	0,113	0,119
5	0,215	0,213	0,219

Table 5. Values of iron standards

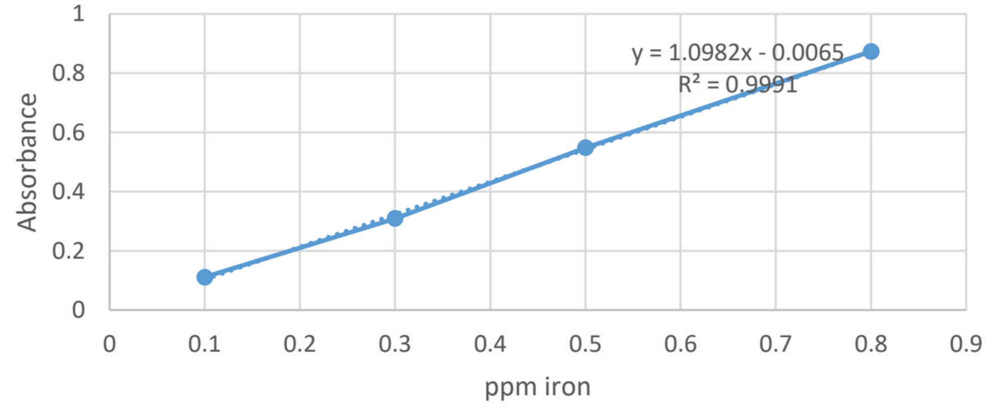

Figure 5. Calibration curve for iron

Zinc pattern (ppm)	Absorbance repetition 1	Absorbance repetition 2	Average
0,1	0,034	0,015	0,035
0,3	0,080	0,076	0,078
0,5	0,149	0,153	0,152
1	0,179	0,283	0,281

Table 6. Zinc standards values

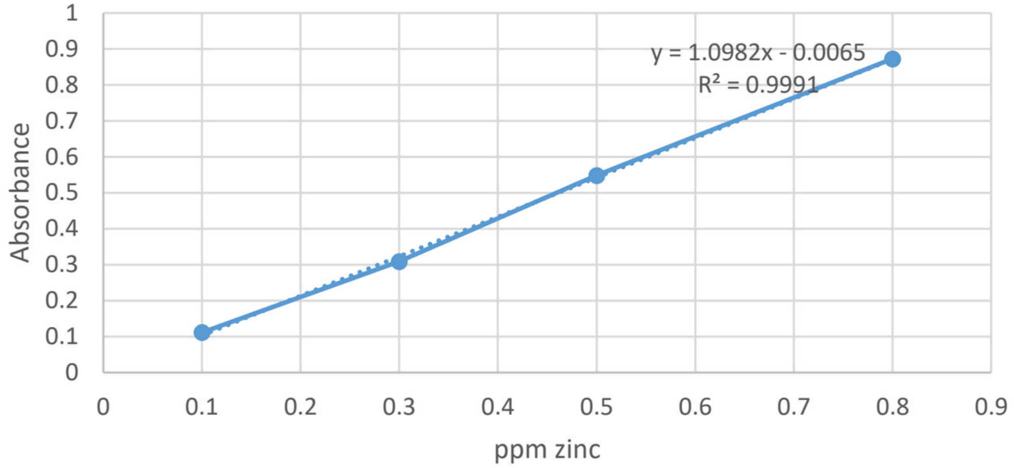

Figure 6. Calibration curve for zinc

Copper pattern (ppm)	Absorbance repetition 1	Absorbance repetition 2	Average
0,5	0,051	0,058	0,054
1	0,106	0,100	0,103
2	0,201	0,211	0,149
4	0,392	0,400	0,396

Table 7. Copper standard values

Reading: Values of copper standards

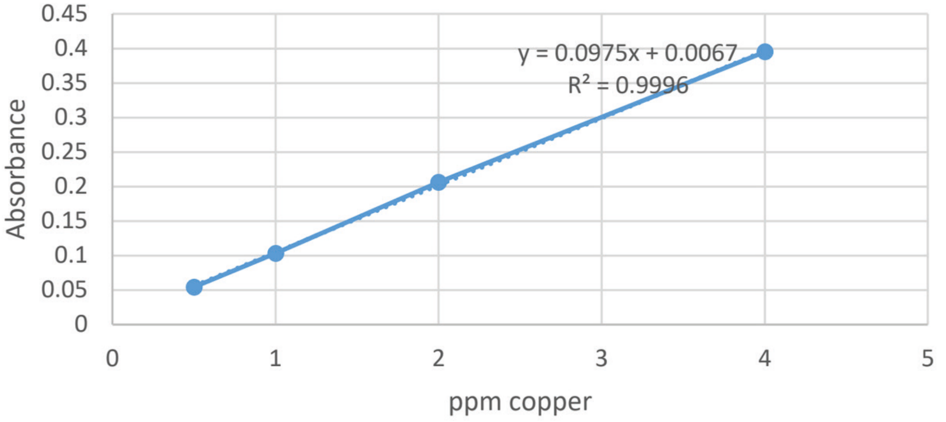

Figure 7. Calibration curve for copper

Manganese pattern (ppm)	Absorbance repetition 1	Absorbance repetition 2	Average
0,5	0,024	0,028	0,026
1	0,047	0,051	0,049
3	0,125	0,113	0,119
5	0,215	0,213	0,219

Table 8. Values of manganese standards

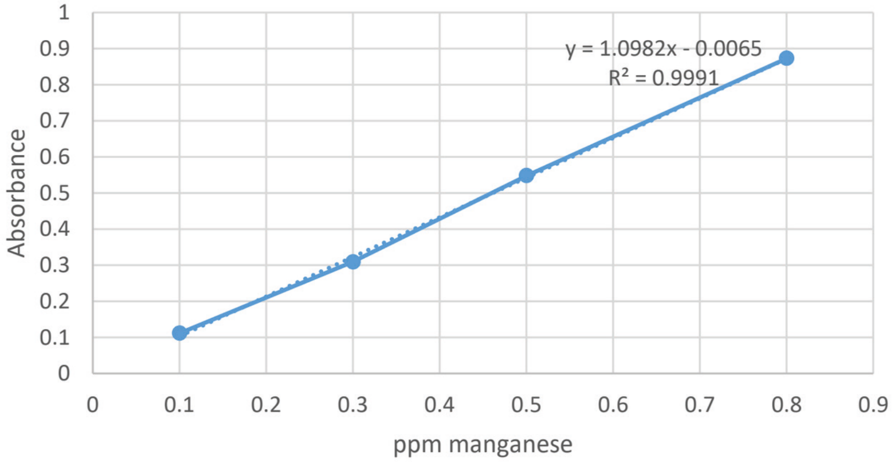

Figure 8. Calibration curve for manganese

Pattern (mgP2O5/ 100ML)	Absorbance repetition 1	Absorbance repetition 2	Average
0,0372	0,176	0,186	0,181
0,075	0,341	0,337	0,339
0,15	0,670	0,672	0,671
0,3	1,331	1,349	1,335
0,6	2,895	2,872	2,887

Table 9. pentoxide standard values

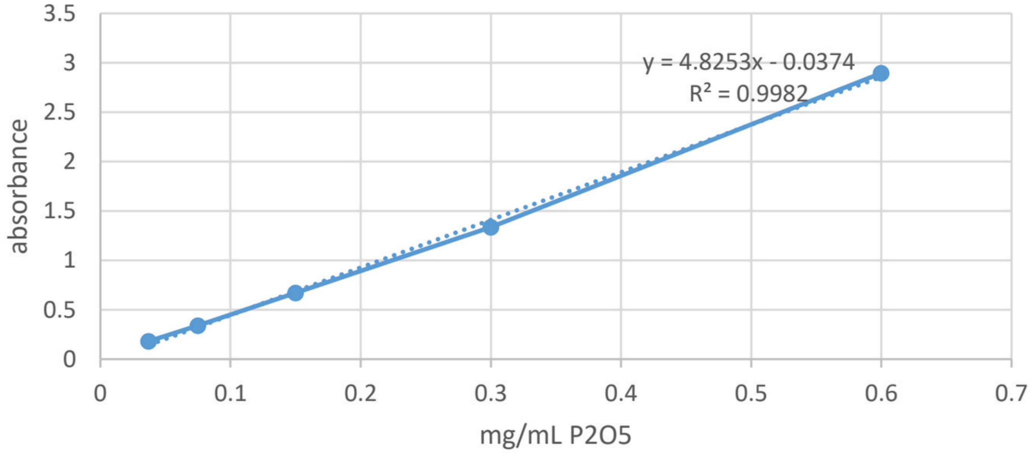

Figure 9. Quantification curve of phosphorus pentoxide standards.

Barium pattern (ppm)	Absorbance repetition 1	Absorbance repetition 2	Average
0,025	0,031	0,037	0,034
0,05	0,069	0,068	0,039
0,1	0,141	0,157	0,149
0,3	0,424	0,417	0,421

Table 10. Values of barium standards

Readout: Barium standard values

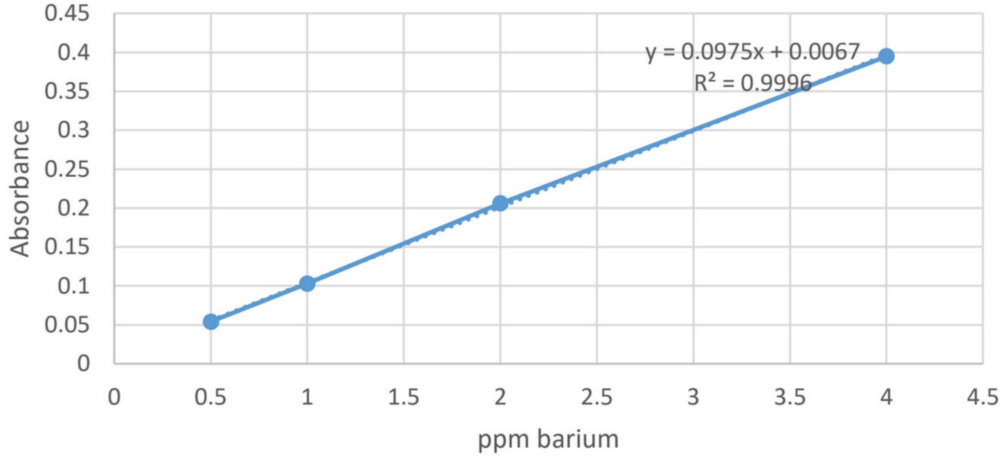

Figure 11. Calibration curve for barium.

Extract (mg/mL	Absorbance repetition 1	Absorbance repetition 2	Average	Percentage Inhibition
0,52	0,946	0,950	0,948	2,07
1,05	0,919	0,9234	0,921	4,85
2,10	0,859	0,873	0,866	10,54
4,19	0,777	0,781	0,779	19,52
8,38	0,628	0,624	0,626	35,33
white	0,968			

Table 11. Absorbance values of the DPPH radical of the extract *(beet)*

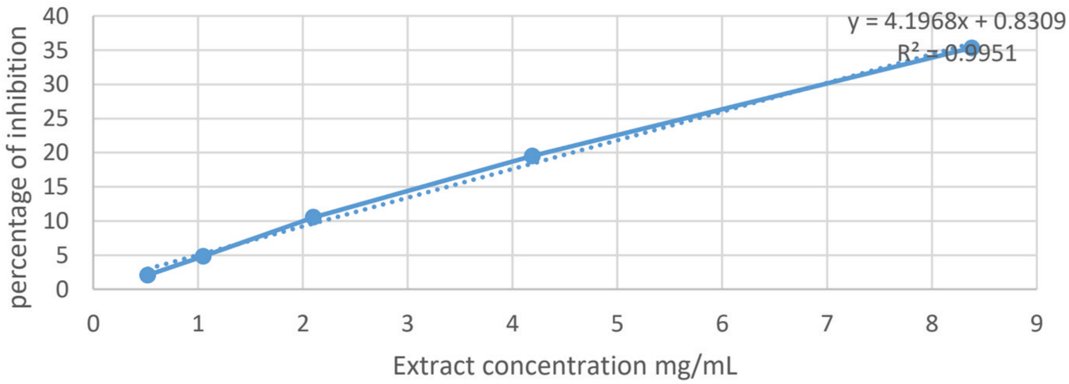

Figure 11. Correlation curve of extract concentration vs. percentage inhibition of DPPH radical **IC 50 = 11.72 mg/mL.**

Extract (mg/mL	Absorbance Repeat 1	Absorbance repetition 2	Average	Mimoles Equivalents of trolox
0,47	0,075	0.084	0,078	--
0,95	0,194	0,176	0,189	0,051
1,89	0,362	0,310	0,378	0,193
3,79	0,778	0,786	0,782	0,497
7,57	1,456	1,481	1,476	1,02

Table 12. Absorbance values by the FRAP method (TEAC) *of beet* leaf extract solutions.

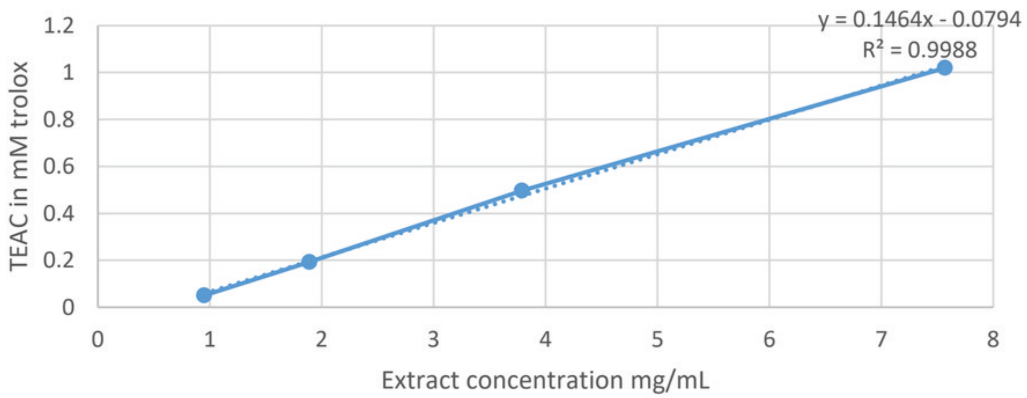

Figure 12. Correlation between concentration vs trolox equivalents by FRAC method **1 mg of extract equals 0.067 mM trolox.**

DISCUSSION

BETA vulgaris L. known as : beet, a vegetable beet cultivated for its leaves, which are very long, white and fleshy petiole; according to the study carried out by the Food and Agriculture Organization of the United Nations (FAO, 2011) in its global initiative for the reduction of food losses and waste, one third of the world production of food intended for human consumption is lost or wasted each year (Gustavsson et al., 2011) [(14]

Table 1. We can observe the yield of dry extract from the dried leaves, this in order to technically and economically evaluate the process, taking into account the amount of biomass that is generated in the markets as waste.

Table 2. The parameters we have chosen for the characterization of the extract considering that these value ranges must always be reached in order to obtain a good product. Thus, the humidity range cannot be higher than

10%, which guarantees its preservation and avoids the possible growth of microorganisms, as well as the ash content, which should be between 17 and 19 percent, representing the mineral salt content of the extract.

Table 3, 4, 5. Among the elements such as calcium, iron, magnesium, zinc, it can be observed that the extract of the leaves contain appreciable amounts for health, especially with regard to zinc, since deficiency of this mineral is quite frequent in malnutrition, as well as deficiency of other micronutrients. In children it reduces the incidence and severity of diarrhea, pneumonia and possibly malaria, increases the absorption of water and electrolytes, improves immunological mechanisms, including cellular immunity and produces high levels of antibodies, improves appetite (Velásquez et al 2014)[(15)]Magnesium which plays a role in more than 300 enzymatic reactions besides being involved in energy metabolism, it is also involved in the maintenance of cellular ionic balance through its association with sodium, potassium and calcium (Baca et al, 2015)[(16)] It is observed that the contribution of iron is considerable in the leaves even being considered a waste product and the immense amount of biomass produced, Iron is an essential element of hemoglobin and among its functions mainly is to carry oxygen to all tissue cells through the blood and it is well known the consequences of its deficiency or deficiency. As for manganese intake, with a value of 6.03 mg/100 it can be considered a rich source of this element considering that the maximum requirements are in the order of 2.8 mg per day (NIH 2018)[(17.]

Table 9. It represents the values of the phosphorus standards that allow us to establish the quantification curve visualized in graph 9, and with which the phosphorus content expressed as phosphorus pentoxide in the extract was found. Although dietary phosphorus deficiency is not commonly reported, it is only observed in rare hereditary disorders involving renal phosphorus loss. The recommended daily intake is 700 mg/day of phosphorus in healthy adults (Heaney 2012, Martin et al 2012)[(18]

Table 10 Figure 10. The conditions for the determination of barium, found in nature in various forms: as barium compounds, naturally occurring in drinking water and in food, but generally not high enough to be a matter of health concern. Also, one study showed that people who drank water containing up to 10 ppm barium for 4 weeks did not suffer from alterations in pressure or heartbeat (ATSDR 2016)[(19]

In the determination of antioxidant activity by the DPPH method, it can be seen that this capacity in the extract is low, since we need an amount of 11 mg to be able to inhibit 50% of the absorbance of this radical). In the determination of the antioxidant activity by the DPPH method, it can be appreciated that the result of the extract is low; since in order to inhibit 50% of the absorbance of this radical, an amount greater than 11 mg/ml is needed, even more so when it comes to crude plant extracts, results similar to those of (Televiciute D; et al, 2020)[(20))]For the FRAP method, method based on the electron transfer mechanism, it can be said that the concentration found is also not related to the antioxidant capacity, as well as; (Megat Rusydi, M.R., et al.2011)[(21)]reports decrease or shortage of some secondary metabolites such as tannins, phenolic compounds and flavonoids.

CONCLUSIONS

Beet plants grown and marketed for consumption of their tubers generate a large amount of waste that could be used to improve the profitability of the agri-food chain;

- The dry extract yield is considered appreciable and a rich source of bioactive compounds and nutrients to be evaluated.

- The ethanol extract of *Beta vulgaris* (beet) leaves could be considered as a valuable and accessible source of mineral nutrients, mainly due to its high content of zinc, copper, iron and magnesium.

- The antioxidant activity of the ethanol extract of *Beta vulgaris* (beet) leaves, determined by DPPH and FRAP methods, can be considered poor.

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