CENTER FOR ADVANCED FOOD TECHNOLOGY
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POLYPHENOLIC CONTENT OF BOROJO

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Background

Borojo (*Borojoa patinoi*) grows in the Amazon and Central America. The fresh fruit weighs an average of 740 g, of which 88% is pulp and 12% seeds and shell. The pulp of this fruit has a high content of carbohydrates, fiber, calcium and phosphorus. The health benefits of Borojo have been known for centuries by the indigenous people of Colombia and elsewhere in South/Central America. People who included Borojo in their diet believe it has a number of beneficial properties that include:

- Stabilizes blood sugar levels
- Natural aphrodisiac
- High phosphorus content stimulates cellular activity promoting hormone generation
- Helps to Maintains normal blood pressure
- Fortifies DNA and RNA structures and helps cellular regeneration
- High energy source
- Helps with Cholesterol problems

A review of the scientific literature did not yield any published support for the health benefits of Borojo, due to the fact that Borojo has not yet been studied by the medical or scientific communities. The information about the nutrient content of Borojo is limited but indicates that Borojo is a good source of protein, vitamins and minerals, such as calcium and phosphorous. The purpose of this study was to explore if Borojo contained polyphenolic compounds similar to those found in tea and fruits and vegetables, which are responsible for the health promoting properties of these foods. Polypenols are strong antioxidants and are believed to protect cells against damage. In addition to polyphenolics, Borojo could contain other health promoting substances, but screening for all the possible health promoting compounds will be the subject of ongoing study.

Material

A sample of dried Borojo pulp was supplied by Nutropical LLC. The material was a brown granular powder and labeled as Pulpa de Frutas Deshidratado. No fresh fruit from which the pulp was derived was available.

Approach and Results

Little is known about the composition of Borojo, therefore it was decided to determine if the fruit contained polyphenolic compounds and if so, in what quantity. Most fruits contain polyphenolic compounds thus this was seen as a good starting point. The presence of polyphenolics could also provide a rationale for further research to elucidate the health promoting properties of Borojo.

The methods of analysis employed are similar to those used for the analysis of polyphenolic in tea and other fruits and vegetables. Dr. Ho's laboratory conducted all the analysis and is recognized as a leading authority on polyphenol analysis in foods. The Folin-Ciocalteu polyphenol test was used to determine the polyphenol content. This is a standard test and is used

routinely to determine the polyphenol content of tea. In brief, polyphenols react with the Folin reagent and produce a blue color that is measured spectrophotometrically. A copy of the method is attached.

The Bojoro fruit powder had relatively high content of polyphenols as measured by Folin-Ciocalteu polyphenol test. The value was 0.323 g gallic acid equivalent per 100 g of dried fruit sample. Gallic acid equivalents is the unit of measurement used to express the polyphenol content because gallic acid is a common component of many polyphenols. It is difficult to make an exact comparison against other fruits and vegetable rich in polyphenols due to differences in serving size, but it is fair to say that the polyphenol content of Borojo is high and significant.

After it was determined that Borojo contained a significant amount of polyphenols, the next step was to identify the exact chemical species of polyphenol. If it was the same type of polyphenol as that found in other plant material such as tea, coffee or fruits and vegetables, then a parallel regarding health benefits could easily be drawn.

Ethyl acetate was used to extract the polyphenols from the fruit powder. Surprisingly, when it subjected to HPLC analysis, it contains only one major compound (see Figure 1). This is quite unusual because most other plant material contain many polyphenolic compounds. The compound was purified and about 25 mg of this pure compound was obtained by using reverse phase open column chromatography. Another attached figure is the HPLC-MS spectra of this compound. This compound gave a molecular weight of 388 by negative ESI mode. (see bottom spectra in the Figure 2).

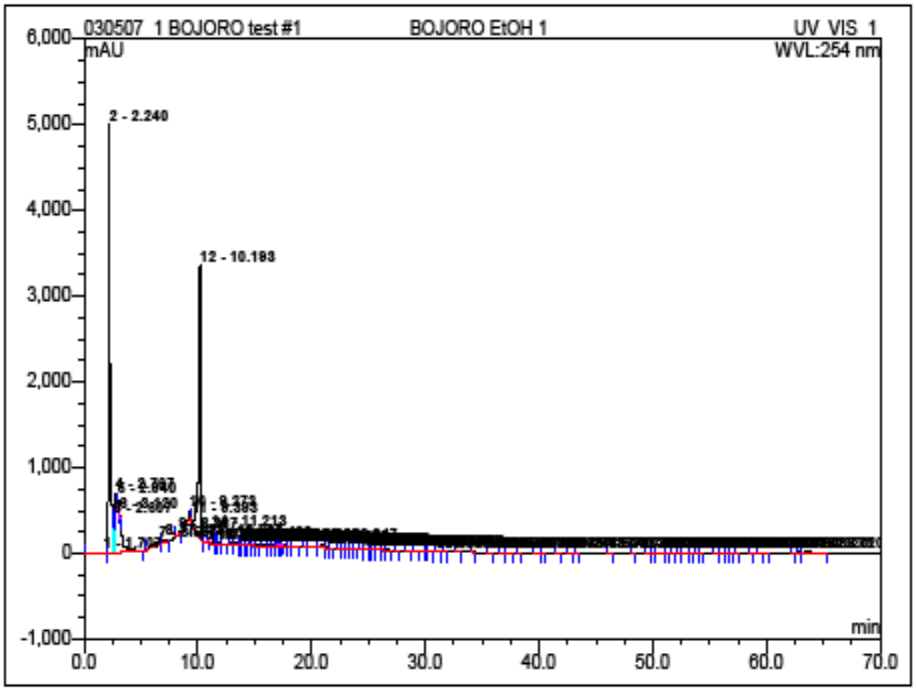
The compound was then subject to further analysis by NMR (Nuclear Magnetic Resonance) in an attempt to identify it. The NMR data are very complicated and we believe it may be a novel polyphenol. Work continues to identify the compound and/or elucidate the chemical structure of this novel compound.

Conclusion

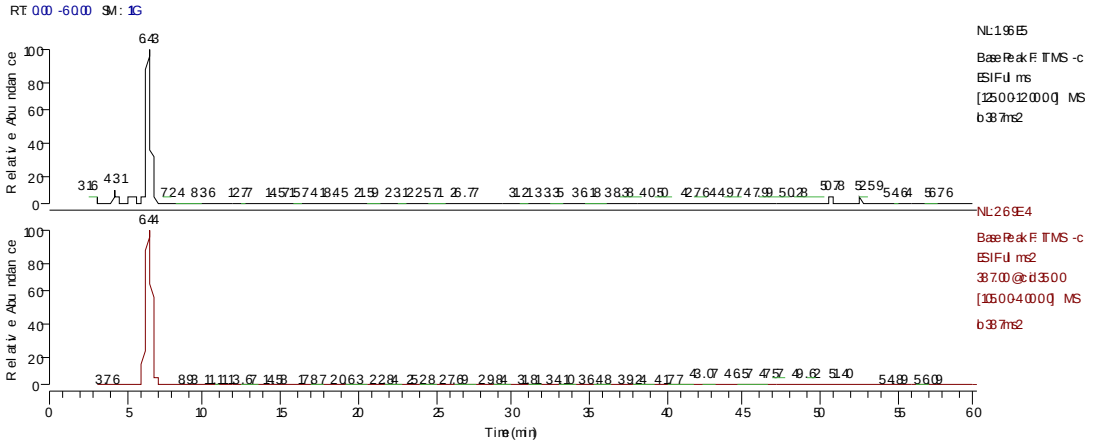
Borojo is rich in a novel polyphenolic compound which may be partly responsible for the health benefits attributed to Borojo. The polyphenol content was determined by the Folin- Ciocalteu test and confirmed by High Pressure Liquid Chromatography and Mass Spectrometry. Most plant materials contain a large number of different polyphenolic compounds, but Borojo appears to be unique in that it contains only one polyphenol in relatively high concentration. Future work is planned to identify this novel polyphenol and elucidate its structure. In addition to the dried pulp, the fresh fruit from which it was derived should be analyzed.

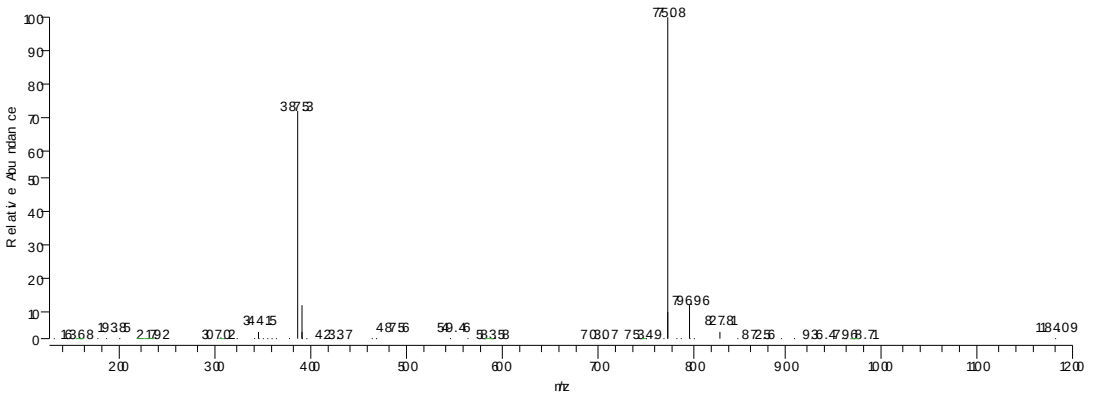
1 BOJORO EtOH 1

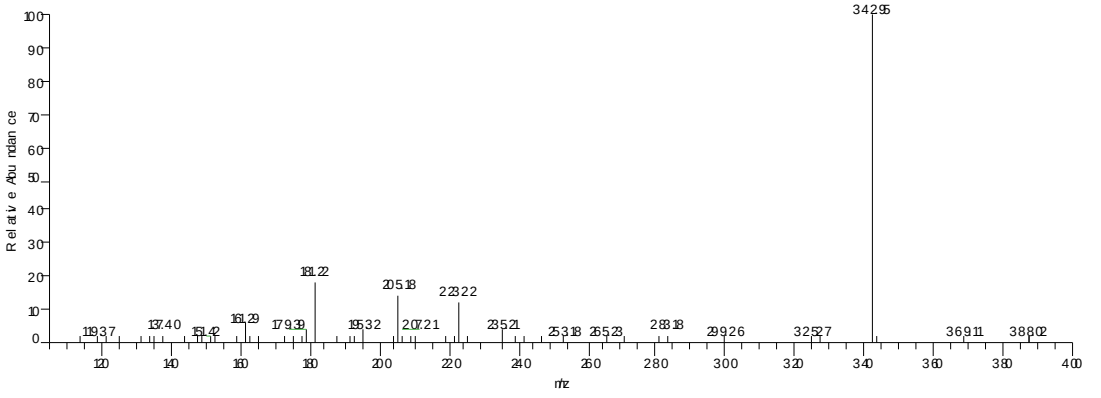
Sample Name:	BOJORO EtOH 1	*Injection Volume:*	90.0
Vial Number:	RA1	*Channel:*	UV_VIS_1
Sample Type:	unknown	*Wavelength:*	254
Control Program:	020107 polyphenol test	*Bandwidth:*	n.a.
Quantif. Method:	Default	*Dilution Factor:*	1.0000
Recording Time:	3/5/2007 13:04	*Sample Weight:*	1.0000
Run Time (min):	70.05	*Sample Amount:*	1.0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.71	n.a.	1.397	1.420	0.07	n.a.	BMb
2	2.24	n.a.	4998.282	933.818	43.97	n.a.	bM
3	2.61	n.a.	426.580	38.918	1.83	n.a.	M
4	2.77	n.a.	696.835	384.986	18.13	n.a.	MB
5	2.94	n.a.	124.568	13.418	0.63	n.a.	Rd
6	3.12	n.a.	39.184	1.768	0.08	n.a.	Rd
7	6.57	n.a.	42.450	36.922	1.74	n.a.	BM
8	7.13	n.a.	28.163	13.176	0.62	n.a.	MB
9	8.37	n.a.	30.546	2.958	0.14	n.a.	BMB
10	9.27	n.a.	108.151	18.405	0.87	n.a.	BMb
11	9.39	n.a.	49.501	2.887	0.14	n.a.	bMB







Total Polyphenols by Folin-Ciocaltheu Assay

Background

Folin-Ciocalteu Reagent

The Folin reagent is sensitive to reducing compounds, in this case polyphenols, thereby producing a blue color upon reaction. This blue color is measured spectrophotometrically. The procedure described herein is used to measure the relative polyphenol content of tea extracts and tea solutions, using Gallic Acid as a standard. The tea extract or solution should be prepared to an appropriate concentrartion so that the Absorbance readings are within the range of the standards. A reagent blank should be prepared at the same time. The samples are diluted and reagents are added as specified in the procedure. Full reaction and color development is complete in **ONE** hour. The absorbance of each solution is measured at 725nm along with the reagent blank and all standards. A regression analysis is performed on the standards (Absorbance vs. Concentration) and the results for the samples determined from the regression.

The Folin & Ciocalteu reagent can be obtained from Sigma (Product #F9252).

A Beckman DU650 spectrophotometer is typically used. This method is available as an ISO method and is can be found in the literature.

Materials and Methods:A

Tea Leaf samples (Green or Black):
Approximately 210g boiling Deionized (DI) water is added to 2.00g sample of loose tea leaf in a beaker . Brew the tea for 4 to 5 mins with stirring. After 4 –5 minutes the leaf is filtered through teabag paper into a tared beaker. Squeeze the tea bag filter bag to express the remaining liquid into the beaker. The beaker with the tea extract is placed on a balance and the net weight brought to 200grams with DI water if needed.

Reagents and Materials

Folin & Ciocalteu's Phenol Reagent (*Sigma) Cat.item F-9252*

Gallic Acid *(Fisher Scientific) Cat.item A122-500*

Sodium Carbonate *(Aldrich) Cat.item 22353-0*

Spectrophotometer (A Beckman DU650 is an example of the type of unit), with methacrylate cells having a 1cm pathlength.

Pipettes of various sizes e.g. Brand Tech Dispensette III Bottle-top dispensers and Eppendorf adjustable vol. pipettes.

Test Tubes – 16 X 100mm (Fisher 14-961-29)

Prepare a saturated Sodium Carbonate Solution by adding 50g Sodium Carbonate to 200ml Deionized (DI) water in a 600ml beaker. The solution is stirred and heated until the Sodium Carbonate is completely dissolved. The solution is filtered and stored at Room Temperature until crystals precipitate. A seed crystal may be added to induce precipitation. If this does not work, place the beaker in a refrigerator for about 1 hour or until crystals appear.

Standards

Use Class-A volumetric glassware for preparing the standards.

Prepare a 1000ppm stock solution of Gallic Acid by weighing 1.0050g Gallic Acid (adjusted for 2% moisture this will give 1000ppm solution) and dissolving in 1L deionised distilled water (1 ml Ethanol and/or sonicating will help in the dissolution). Store this Stock Solution in an amber glass container in a refrigerator and use it to prepare fresh 'working standards' each time the analysis is run, remember to bring a portion of the Stock Solution to Room Temp. before pipetting..
Working Standards are prepared as follows:

200ppm	20mls stock solution to 100mls with DI water in a vol. flask.1
150ppm	15mls stock solution to 100mls with DI water in a vol. flask.
100ppm	10mls stock solution to 100ml with DI water in a vol. flask.
50ppm	5mls stock solution to 100mls with DI water in a vol.flask

25ppm 5mls stock solution to 200mls with DI water in a vol. flask

Method:

Place a series of test tubes in Row 3 of the rack and label --- Blank, 25ppm std., 50ppm std., 100ppm std., Final sample 1, Final sample 2, Final sample 3, etc.
Place a series of test tubes in Row 1 directly behind the 'sample' tubes and label --- 1:10 dil. #1, 1:10 dil. #2, 1:10 dil. #3 etc.

Prepare the 1:10 dilutions by pipetting 0.5ml of each sample extract into the corresponding test tube in Row 1, add 4.5ml DI to each and vortex.

Pipette 0.5ml of DI water into the "blank test tube"

Pipette 0.5ml of each "Working Standard" into the corresponding marked test tube in Row 3

Pipette 0.5ml from each '1:10 dil' test tube in Row 1 into the corresponding 'Final sample' test tube in Row 3. (There should now be 0.5 ml in each test tube in Row 3.)

Starting with the "blank test tube" add 4.5mls DI water, 0.2ml Folin Reagent, 0.5ml Saturated Sodium Carbonate to each test tube and vortex (these additions should be completed within 15 seconds and in the order described.)
Finally, add 4.3mls DI water to each and invert to mix (place gloved thumb over the test tube and invert, wipe thumb on damp paper towel and proceed to next sample). Repeat this procedure for all the test tubes in the rack.

Allow samples to sit for a minimum of 1 hour at room temperature**.** (Allowing the samples to sit for more than an hour is acceptable, as long as each sample and all standards are treated exactly the same).

Read the absorbance of each tube on a spectrophotometer at 725nm. Using a 1cm cell.

If the spectrophotometer is not capable of reading at 725nm, a wavelength of 765nm is acceptable but use the same wavelength for all measurements.

All test tubes and cells should be emptied into a container clearly labeled for hazardous waste, they should also be rinsed and the rinse solution added to that container.
Place a series of test tubes in Row 1 directly behind the 'sample' tubes and label --- 1:10 dil. #1, 1:10 dil. #2, 1:10 dil. #3 etc.

Prepare the 1:10 dilutions by pipetting 0.5ml of each sample extract into the corresponding test tube in Row 1, add 4.5ml DI to each and vortex.

Calculations:

If the Spectrophotometer you are using performs the regression analysis and gives you the concentration then all you need to do is (X10) to correct for the dilution you did earlier.
If the Spectrophotometer does not perform the regression analysis then the next step is:

Perform a linear regression of the absorbance data for the Gallic Acid standards vs. concentration of Gallic Acid and use the resulting graph and the absorbance data for the diluted samples to calculate the concentration of polyphenols in ppm for each sample read. Correct for dilution.

Concentration polyphenols (A) in ppm = Absorbance ÷ the x coefficient from the regression analysis.

Concentration in ppm in sample (B) = Concentration in ppm (A) X 10 (i.e.correcting for dilution made earlier)

To convert ppm polyphenols (B) to mg polyphenols per 2g sample (C) divide (B) by 5

To convert (C) to mg of flavonoids in 2 gram sample, multiply (C) X 0.84,

(this is a correction factor for nonflavonoid material that is detected up by the Folin Reagent)