Capillary Gas Chromatography-Mass Spectrometry (CGC-MS) Analysis and Antioxidant Activities of Phenolic and Components of Guarana and Derivatives

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Abstract: The GC-MS analysis of phenolic compounds present in guarana (*Paullinia cupana*), an important product of the Amazonian forest consumed in supplements or in soft drinks has been the subject of study. The therapeutic properties and possible protective effects reported for guarana and derivative products could be associated with the antioxidant activity of their phenolics content. The purpose of this study is i) to provide molecular structural information about the composition of guarana in phenolics; ii) to ascertain the effect of solvent type on the extraction procedure; and iii) to determine the antioxidant activity of powders, pericarp, pulp seeds, capsules and bar from *Paullinia cupana*, as oxygen radical absorbance capacity with fluorescein ($ORAC_{FL}$). Three more phenolic compounds, Quercetin, (+)-Catechin and (-)-Epicatechin have been identified in this report as trimethylsilyl (TMS) derivatives. The amount of total phenolics found in plant materials containing guarana varied from 25.10 to 124.99 mg of gallic acid/g dry sample whereas that the antioxidant activity ranged from 441.5 to 1468.3 μmol TEAC/g dry sample. A high correlation was found between the estimated phenolic contents and the TEAC values ($r = 0.937$, $P < 0.01$) for all the types of guarana samples tested.

Keywords: Guarana (*Paullinia cupana*), phenolic compounds, capillary gas chromatography-mass spectrometry, antioxidant activity.

1. INTRODUCTION

A number of studies have shown that chronic diseases such as cancer, cardiovascular, inflammatory, and neurodegenerative pathologies, and aging, are associated [1-3] with oxidative stress, a metabolic condition that causes cell degeneration. Consumption of phytochemicals in the diet, especially phenolics, has been closely connected [4-6] with the reduced risk of diseases. Fruits and vegetables, as well as plant beverages such as tea and coffee contributes [7, 8] to the dietary intake of antioxidants.

The guarana, a plant *Paullinia cupana* native species belonging to the family of the Sapindaceas, from the central Amazonian, exists in two varieties: sorbilis and typical. It produces a fruit that it is spherical, blackish and brilliant, assuming a form of capsule in whose interior there is only a seed that when it matures changes from green to red-orange [9]. Once the complete maturity is reached, the "white of the eye", botanically an aril of mealy consistency, is chosen. Then it is rubbed of manually and the seeds are roasted to facility the removal of the glossy, tough and dark brow seed coat (pericarp) and the later grinding of the kernels in a hardwood mortar [10-12].

Paullinia cupana (guarana) has been the focus of considerable attention due to its high caffeine content and pharmacological activity and behaves as a tonic and stimulant of the nervous system [13] an aphrodisiac [14] and beneficial in memory performance [15]. Guarana has been used to treat chronic diarrhea, neuralgia and dysentery [16], and to reduce the body weight [17]. Guarana have indications in cases of depression, in arteriosclerosis, as tonic of the heart, as anti-inflammatory, showing also antioxidant action (inhibitor of lipid peroxidation process) [9]. It is also reputed to be a cardiovascular drug and to prevent atherosclerosis (guarana extracts inhibited platelet aggregation in rabbits following either intravenous or oral administration) [9]. Some guarana studies have been undertaken with the purpose [18, 19] of to determine adulterations and to monitorize the quality control of guarana products in the phytopharmaceutical industry. It has been shown that guarana seed extracts have antioxidant and antimicrobial properties [20, 21].

In Latin America, mainly in Brazil, a great interest in the field of food science has aroused because of the use of guarana as a flavouring agent in beverages such as "guarana soda", of an elevated consumption in the population. More recently, guarana has been employed in the form of powder, tablets and jams, either pure or in association, as a dietetic product for slimming. The capsules and powders added to

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juices and other drinks are also products of great consumption [9].

Many separation techniques such as gas–liquid chromatography (GLC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) have been proposed to separate and identify phenolic compounds [22, 23]. HPLC and CE, especially coupled a with photodiode array detector, do not require derivatization prior to qualitative and quantitative analysis. Hence, they have become the most commonly used techniques for the analysis of phenolic compounds in plants. Nevertheless, they do not often provide satisfactory performance, and the UV–visible spectrum does not supply [24] sufficient evidence for unambiguous identification. For these reasons, capillary gas chromatography combined with mass spectrometry (CGC–MS) is a useful alternative technique, which can provide sufficient data for full structural analysis. More generally, it may be used to determine molecular masses thus establishing the respective substitution pattern on the phenolic ring(s). Trimethylsilyl (TMS) derivatives of phenolic compounds were prepared prior to CGC–MS analysis [25].

In the present study the method of CGC-MS was used for the analysis of major phenolic substances present in seeds of the guarana. The effectiveness of different solvents in extracting phenolics from guarana toasted seed powder has also been studied. Finally, the antioxidant capacities of powders, pericarp, pulp seeds, capsules and bar from *Paullinia cupana* (guarana) have been evaluated.

2. MATERIALS AND METHODS

2.1. Reagents

All solvents and reagents from various suppliers were of the highest purity needed for each application. The Folin-Ciocalteu reagent and sodium carbonate were purchased from Sigma® (St. Louis, MO). Gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Fluorescein and 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) were purchased from Aldrich Chem. Co. (Dorset, UK).

2.2. Plant Material

The guarana (seeds, pericarp, seed powder, capsules and bar) were obtained from different sources. The seeds and seed powdered dry were obtained from Brazil Amazonic producers in Ariquemes, RO (63º 02' 27" W and 9º 54' 48" S), Alta Floresta, MT (56º 05' 10" W and 9º 52' 32" S) and Sinop, MT (55º 30' 09" W and 11º 51' 51" S). The bar was obtained from Maués, AM (57º 43' 07" W and 3º 23' 01" S). The guarana capsules were obtained at pharmacy shops in Florianópolis, Brazil. The samples were transported to the University of Seville and stored frozen until used. The seeds were separated from the pericarp and pulp seed. Processing steps described above were applied to three replicates (n=3) in all samples studied (thirty). All samples were dried previous analysis and resulted were expressed en dry weight (DW).

2.3. Sample Preparation and Derivatization

Guarana seed powder dry was de-oiled with hexane (one part powder to 10 parts of hexane, w/v). After shaking the mixture for 10 min at room temperature, the liquid was separated from the solid by vacuum filtration through a sintered glass filter (Pyrex® 10–15M). The solid residue was evenly distributed over a tray and kept under the hood in the dark to evaporate the hexane. The extraction method used for dried samples is described in that follows: Twenty milliliters of 60% aqueous methanol containing BHT (1 g L^{-1}) was added to 2.0 g of dried sample. Then 5 mL of 6 M HCl were added. The mixture was stirred carefully. In each sample nitrogen was bubbled for ca. 40–60 s. The extraction mixture was then sonicated for 15 min and refluxed in a water bath at 90 °C for 2 h. The mixture was then extracted with 30 mL (3 × 10 mL) ethyl acetate. The organic layer was collected and reduced to 10 mL by rotary evaporation (37 °C) and centrifuged for 10 min. Anhydrous Na_2SO_4 was then added to remove residual moisture. Phenolic extracts were evaporated to dryness under a nitrogen stream and immediately derivatized with 100 μL of a mixture of HMDS+DMCS in pyridine (3:1:9) and aliquots of 3 microliters were injected on split mode.

2.4. GC-MS and GC-SIM-MS Instrumental Analysis

The GC–Ion trap-MS experiments were performed using a Trace GC 2000 gas chromatograph coupled to a Polaris-Q Ion trap mass spectrometer (ThermoFinnigan, Austin, TX, USA) equipped with an AS 2000 autosampler operating in full scan mode and in selective ion monitoring (SIM) mode only for identification purposes. The column used was a Zebron ZB-5ms (Phenomenex, Torrance, CA, USA) fused silica capillary column (30m long x 0.25mm I.D. x 0.25 film thickness). The oven temperature was programmed as follow: the initial temperature was held for 5 min at 150ºC and then from 150 to 295ºC at 3ºC/min and maintained for 18 min. Injector temperature was set to 300ºC. Carrier gas was Helium at 1 mL/min in constant flow mode. The MS operating conditions were the following: ion source and transfer line temperatures 200 and 290ºC, respectively. The instrument was tuned in EI positive mode using perfluorotributylamine (FC-43) according to manufacturer's recommendations in order to achieve the best sensitivity. Parameters such as automatic gain control (AGC) and multiplier (1150V, 105 gain) were set by automatic tuning. The electron energy was 70 eV and the emission current 250 A. Samples were analyzed as TMS ether derivatives. Xcalibur version 1.4 software was used for data acquisition and processing of the results. Each determination was carried out in duplicate.

2.5. Extraction of Polyphenolic Compounds from Guarana Seeds

Solvents containing different volumes of de-ionized water methanol or acetone were used to determine the effectiveness of solvent type on the extraction of phenolics from guarana seed powder. Known weights of guarana seed powder were mixed with solvent at a ratio of 1:10 (w/v). The mixture was sonicated for 15 min, and shaken for 30, 60 min and 24 h at room temperature. Then, the mixture was centrifuged at 4 °C for 20 min at 5000 rpm. Supernatants were filtered through a funnel with glass wool, which was washed with 3-4 mL of solvent. The volumes of filtered supernatants were recorded to calculate total phenolics.

2.6. Determination of Total Phenolics Content

Total phenol content of guarana ethanol extracts was determined using the Folin–Ciocalteu assay [26]. Briefly, a 100 µL aliquot of ethanol extract was assayed with 500 µL Folin reagent and 1.5 mL sodium carbonate (20%, w/v). The mixture was vortexed and diluted with water to a final volume of 10 mL. After incubation for 30 min at room temperature, the absorbance was read at 765 nm in a cuvette of 1 cm and total phenols in the guarana extract were expressed as gallic acid equivalents (GAE), using a calibration curve of a freshly prepared gallic acid solution. For the gallic acid, the absorbance versus concentration curve is described by the equation $y = 0.0013\ x - 0.0074$ ($R^2 = 0.9986$).

2.7. Determination of Catechol Content

The quantitative analysis of catechol in guarana seeds was performed by a spectrophotometric method [27] based on the reaction of *p*-aminophenol with catechol giving rise to indophenol dye-like species. For the calibration curve, the *p*-aminophenol solution was prepared at 1 mM using 0.1 M HCl aqueous solution. Catechol standard solution was prepared at 0.01 M in 95% ethanol. The calibration curve was prepared by loading, separately, in six 10 mL volumetric flasks 3.0 mL of 2% (w/v) NaOH solution, 3.0 mL of p-aminophenol solution and 0.0, 10.0, 25.0, 50.0, 75.0 and 100 µL of catechol standard solution. The final volume was completed with ethanol and the absorbance was measured [27] at 586 nm 1 min after adding catechol. As for catechol determination, we used the standard addition method because of the interference of the matrix. Three mL of 2% (w/v) NaOH solution and 3.0 mL of p-aminophenol were added to six 10 mL volumetric flasks. Then 1 mL of guarana ethanol extract and the adequate catechol standard solution volume 0.0, 10.0, 25.0, 50.0, 75.0 and 100 µL were added. The final volume was completed with ethanol and the absorbance was measured [27] at 586 nm run 1 min after the addition of catechol.

2.8. Antioxidant Activities. ORAC Assay

The method of Davalos, Gómez-Cordovés and Bartolomé [28] and Prior *et al*., [29] was modified as follows: The reaction was carried out in 75 mM phosphate buffer (pH 7.4), and the final reaction mixture was 600 μL. Sample (60 μL) and fluorescein (360 μL; 14 µM, final concentration) solutions were placed in the well of the microcell (1 mL). The mixture was preincubated for 15 min at 37 ºC. AAPH solution (180 μL; 4,8 mM, final concentration) was added rapidly. The microcell was immediately placed in the reader and the fluorescence recorded every minute for 80 min. A blank (FL + AAPH) using phosphate buffer instead of the antioxidant solution and eight calibration solutions using Trolox (1-8 μM, final concentration) as antioxidant were also carried out in each assay. All the reaction mixtures were prepared in duplicate, and at least three independent assays were performed for each sample. The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the FL decay curve and were expressed as Trolox equivalents as micromole per liter or per gram. The area under curve (AUC) was calculated as:

$$AUC = (0.5 + f_5/f_0 + f_{10}/f_0 + f_{15}/f_0 + f_{20}/f_0 + \ldots.. + f_{75}/f_0 + f_{80}/f_0) \times 5$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i. The relative ORAC value (Trolox equivalents) was calculated as relative ORAC value(μM) = [$20K$ ($AUC_{sample} - AUC_{Blank}$)/($AUC_{Trolox} - AUC_{Blank}$)]. Were K = sample dilution factor. Raw data were exported from the Fluostar Galaxy software to an Excel (Microsoft, Roselle, IL) spreadsheet.

2.9. Statistical Analyses

All tests were carried out in duplicate. Data were analyzed using the STATISTICA´99® version software package. Data on antioxidant activity and phenolic content underwent a correlation test. Anova/Manova comparison test was performed to determine significant differences at P=0.05. Regression analysis [30-31] to determine ORAC values was performed by Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1. Extraction of Phenolic Compounds from Guarana Seed

Ethanol alone or de-ionized water alone was ineffective as a solvent for the extraction of phenolic compounds from guarana seed powder. Total phenol content of ethanol extracts from guarana seed powder when extracted with solvent containing 50%, 60% or 70% ethanol (95% v/v) in water was about 72 mg gallic acid equivalent (GAE)/g seed powder (P>0.05) (Fig. **1**). The trend of absorbance at 280 nm for extracts with ethanol was similar to that of the total phenols, which indicates that the absorbance of the extract is directly related to the extractable polyphenols in solution.

High and significant correlation coefficients were observed among total phenol content, absorbance at 280 nm and antioxidant activity of the ethanol supernatants. Correlation coefficient between total phenol content and absorbance at 280 nm was 0.940 (P<0.01). Supernatants from a solvent containing 50%, 60% or 70% methanol in water yielded about 63 mg GAE per g guarana seed. This was the highest concentration in the extract compared with the other methanol–water mixtures (Fig. **2**). Increasing water in the mixture from 70% to 100% significantly reduced the amount of phenolic compounds extracted (P<0.05). Methanol only was insufficient for the extraction of phenolic compounds from guarana seed powder. Extracts using solvent containing 50% or 60% methanol have the highest absorbance at 280 nm (P<0.05) (Fig. **2**). Unlike ethanol–water solvents, methanol–water solvents appear to show differences in solvent concentration optimum when evaluated as total phenolics and absorbance at 280 nm. Total phenol contents of methanol–water extracts were correlated with absorbance at 280 nm (r=0.958, P<0.01).

The effects of shaking time and solvent on the extraction of phenolic compounds from guarana seed were also studied. Results (Fig. **3**) indicated that shaking the mixture of guarana seed powder in 70% methanol (v/v) at room temperature for 30 min or 60 min had no significant effect on the total phenol contents of the extracts from guarana seed (P>0.05).

Among acetone–water mixtures as solvents, those containing 50%, 60% and 70% acetone by volume showed

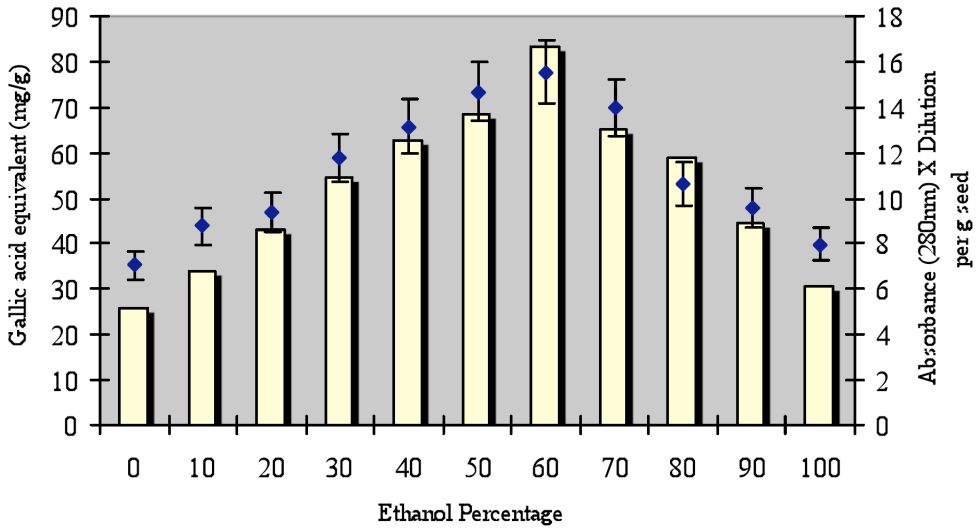

Fig. (1). Effect of ethanol (95% v/v) on the total phenol contents and absorbances at 280 nm of supernatants from dried and ground guarana seeds.

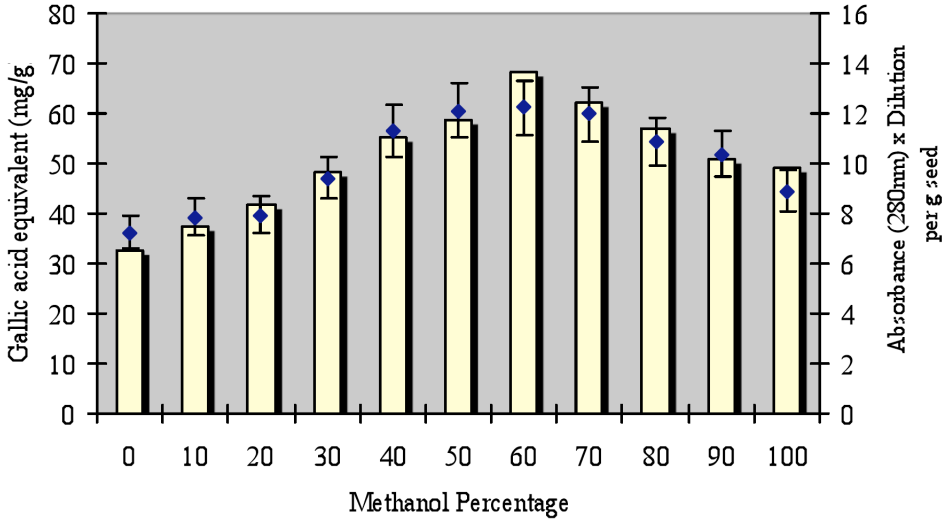

Fig. (2). Effect of methanol on the total phenol contents and absorbances at 280 nm of supernatants from dried guarana seeds.

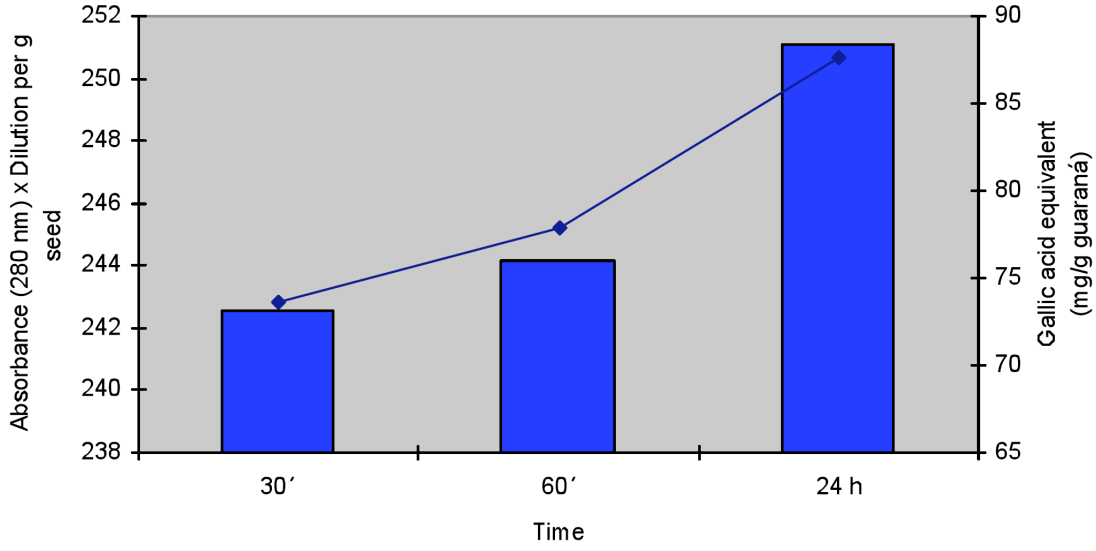

Fig. (3). Effect of the time in extraction of the total phenol contents and absorbances at 280 nm from dried guarana seeds.

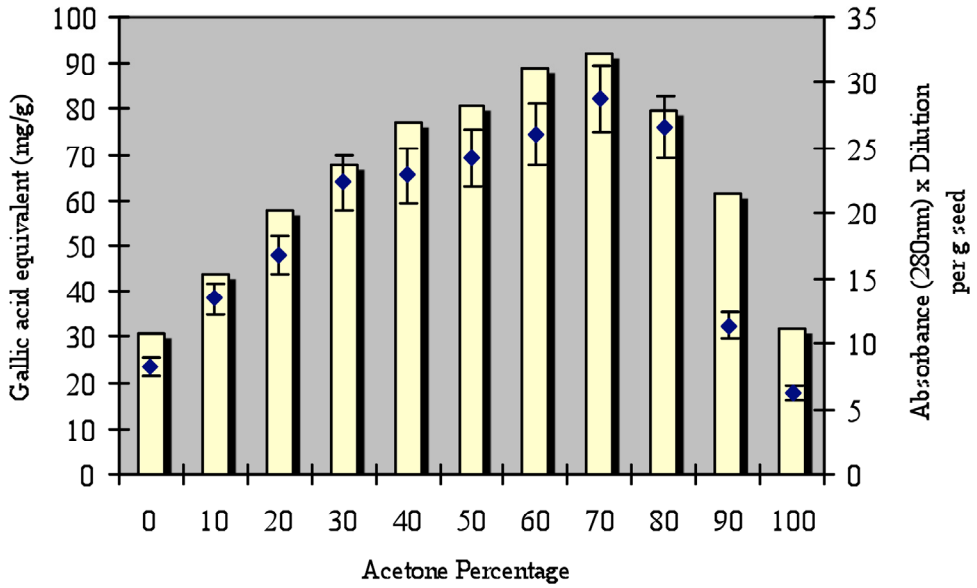

Fig. (4). Effect of acetone on the total phenol contents and absorbances at 280 nm of supernatants from dried guarana seeds.

the highest total phenol content (about 87.3 mg GAE/g dry guarana seed powder) in the extracts (Fig. **4**) (P<0.05). Neither acetone nor de-ionized water alone was as effective as the 50% or 75% acetone solvents. The antioxidant activity of extracts when using 70% acetone solvent was the highest among the acetone solvents mixtures essayed (P<0.05). The extracts from solvents containing 50% acetone or more, in general, had higher absorbance at 280 nm, meaning that tannin extraction was effective when the extraction solvent contained 50% or more acetone.

Most catechins have a maximum absorption at around 280 nm, and (+)-catechin was reported to be the major catechin monomer in guarana seeds [32]. The correlation between total phenol contents and total tannins (absorbance at 280 nm) in guarana seeds extracts were significant (r^2=0.905, P<0.01). In a study with grape skin presented by Yilmaz and Toledo [33] showed significant correlations (r^2 = 0.814, P < 0.01) between total phenol contents in the grape skin extracts and the total tannins measured as absorbance at 280 nm.

A mono-component solvent system (water, ethanol, acetone or methanol) was not as efficient in extracting phenolic constituents of guarana seeds as an aqueous solution containing at least 50% water. Thus, resulted obtained of IPT and antioxidant activities showed that a select mixture of the water: acetone was as efficient solvent extractor. Results obtained above indicate that an acetone-water mixture is an effective solvent. This is in agreement with the study of Majhenic *et al*., [21], which reported that solvents mixed with water (such as 60% ethanol and 35% acetone) are better for extraction of caffeine and catechins from guarana seed than are pure solvents (such as methanol).

3.2. GC–MS Analysis

The isolation and quantification of phenolic compounds in plant material is a difficult task because of their chemical complexity (Fig. **5**). The sensitivity and resolving power of capillary GC–MS make this technique particularly suitable for unambiguous detection of phenolic compounds in the hydrolysed samples. Silylation is the most widely used derivatization procedure for sample analysis by GC. The derivatives are generally less polar, more volatile and more thermally stable. The silylation reaction is a nucleophilic substitution reaction. Chu *et al*., [22] and Proestos *et al*., [25] propound the use of a mixture of TMCS and BSTFA (water bath at 80°C for 45 min) to complete the derivatization. Several variables should be examined to determine its role in the derivatization procedure: 1) temperature; 2) reaction time; and 3) the amount of the silylating reagent required to complete the derivatization. In this study, the mixture HMDS: DMCS in pyridine was used because the derivatization step is carried out at ambient temperature in order to avoid the enolization process observed when BSTFA is used, due to the need to employ high temperature reactions (80-90 ºC). Carlson and Thompson [34] had extracted methylxanthines and polyphenols from guarana matrix samples with a heated phosphate buffer-methanol solution, and identified the methylxanthines theobromine, theophylline, and caffeine, and the polyphenols (+)-catechin and (-)-epicatechin, in the liquid chromatographic (LC) system, with a Nova-Pak C18 column eluted with phosphate buffer-methanol mobile phase (pH = 3.50), monitored at 272 nm.

The GC oven temperature program, as well as the injector and detector temperatures used, was based on previous experience with the analysis of phenolic compounds in olives extract [35]. Data obtained showed excellent resolution between all compounds of interest. Molecular weights (MWs) and important ions present in the mass spectra of silylated phenolic compounds in the guaraná extracts examined are presented in Table **1**. Three more phenolic compounds, Quercetin, (+)-Catechin and (-)-Epicatechin were identified by the present method as TMS derivatives and the structures were confirmed by matching with the Wiley and NIST libraries. With the proposed method we identify nine phenolic compounds as well as fructose, glucose, citric acid and caffeine (Table **1**).

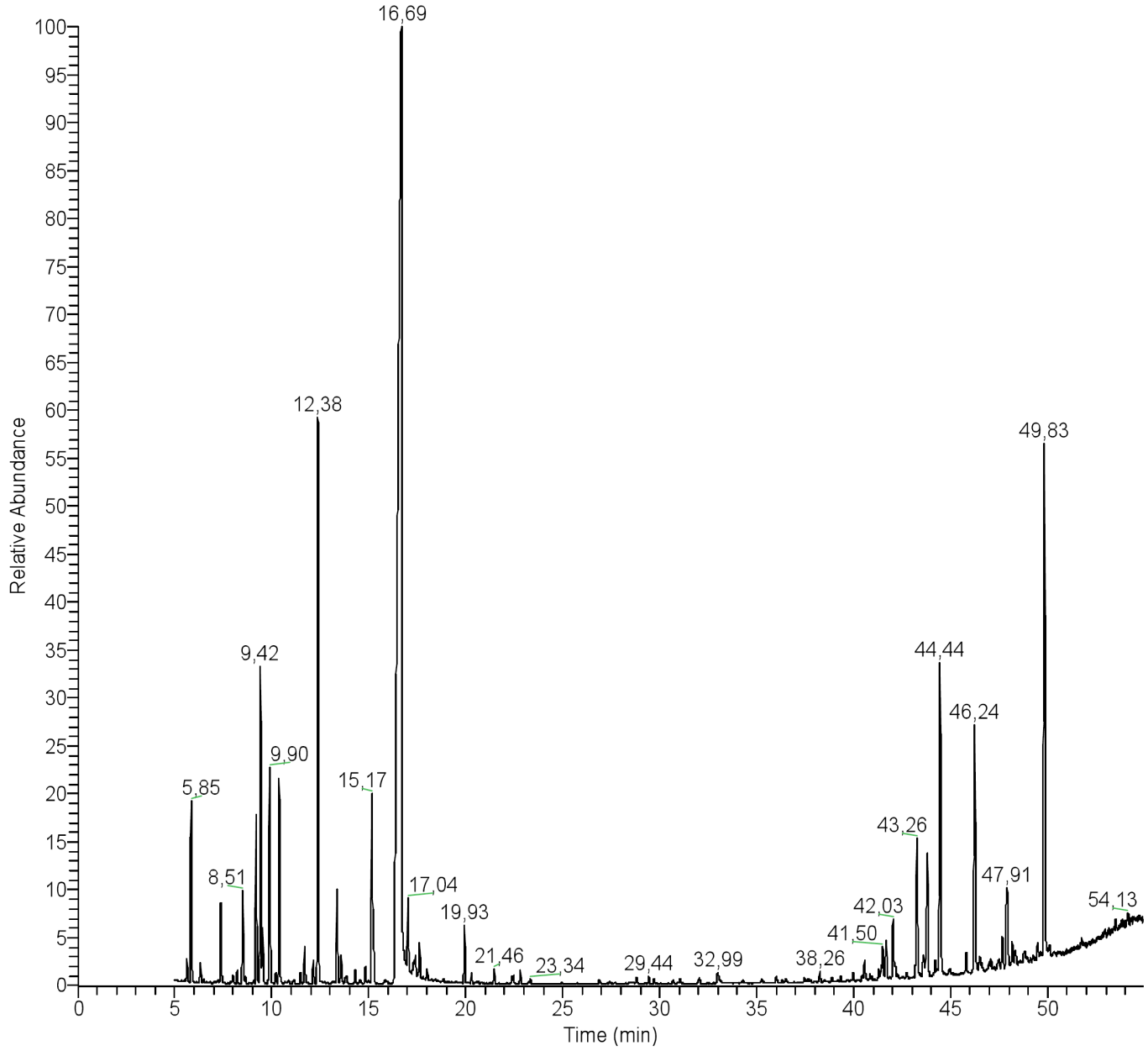

Fig. (5). CGC-MS analysis of phenolic compounds of an extract of guarana

3.3. Total Phenolic Content and Total Antioxidant Capacity

The total phenolic content of the guarana samples studied (powders, pericarp, pulp seeds, capsules and bar) is shown in Table **2**. Though the origin of the different samples is completely different, the comparison of results is made only with indicative purposes. The phenolic content ranged from 30.77 to 124.12 mg of TAE/g of DW. Seed pericarp exhibited the lowest total phenolic level (30.77 mg of TAE/g), whereas seeds powder and bar contained the highest one (105.56 and 124.12 of TAE/g of DW, respectively). Basile *et al*., [20] reported in fresh seeds obtained 8.43 mg GAE/g as the content of the phenolic in ethanol extract of the guarana from Brisighello (Verona, Italy) and Majhenic *et al*., [21] reported the contents of total phenols (based on guarana seed extracts) ranged from 119 to 186 mg GAE/g extract. Phenolics are aromatic compounds that are hardly soluble in water, but easy soluble in ethanol, the increasing solubility of these compounds in hydroalcoholic solutions may affect the rate and the amount of its absorption. In fresh fruits and vegetables usually have very high moisture contents, and so typical processing of these fresh materials into juice or purees extracts primarily the water-soluble fractions [33]. However, a significant amount of the antioxidant are left in the residue after the aqueous fraction has been separated, being thus a second extraction necessary in order to get the total antioxidant capacity of the sample.

Following the analytical procedure described above we found values of catechol equivalent ranging from 0.35 to 2.23 mg/g. By applying the ANOVA assay, we note that the commercialized capsule samples shown the greater variability, due possibly to the fact that these samples are

Table 1. Molecular Weights (MWs) and Important Ions Present in the Mass Spectra of Silylated Phenolic Compounds in the Examined Guarana Extracts by GC-MS

Compound	RT	MW*	Identified Ions (m/z)
3-hydroxybenzoic methyl ester	6.33	224	209(100) 224(79) 177(60) 89(56) 149(33)
Benzoic acid, 4 [trimethylsilyl) oxy-trimethylsilyl ester	9.20	282	73 (100) 267(98) 193(59) 223(57) 186(29) 282(28)
1,3,5 trihydroxybenzene, 3,4,5-trihydroxybenzoic acid (gallic acid)	9.90	342	342(100) 327(74) 73(65) 328 (24) 344(16)
3,5 dimethoxy-4-hydroxybenzoic acid (syringic acid)	10.38	342	342(100) 327(56) 73(70) 237 (14) 344(16) 310 (10)
Isovanillic acid	13.37	312	297(100) 312(83) 267(65) 223(51) 282(41)
Fructose	13.58	540	73(100) 204(46) 217(30) 147(24) 437(22)
Citric acid	15.17	480	73(100) 147(29) 75(25) 273(20) 375(70)
3,4 dihydroxybenzoic acid (protocatechuic)	15.90	370	73(100) 193(84) 370(38) 45(21) 355(22) 194(31) 311(11)
Caffeine (1,3,7-trimethylxanthine)	16.59	194	194(100) 109(38) 55(30) 67(24) 193(29)
Glucose	17.04	540	73(100) 204(86) 147(39) 191(38) 217(38) 361(24)
Quercetin (3´, 4´, 5,7-tetrahydroxyflavonol)	42.03	662	575 (100), 647 (32), 487(28) 662(5)
(+)-Catechin (5,7,3´,4´-tetrahydroxyflavane)	43.26	650	368(100), 355, 650, 267, 383, 179, 310
(-)-Epicatechin	44.44	650	368(100), 355, 267, 147, 650

*As sylilated compounds.

subjected to various elaboration procedures. However, there is no statistical difference in the phenolic content of bar samples as well as in the capsule and power samples.

Table 2. Total Phenolics, Catechol and Antioxidant Activity of the Guaraná Products[a]

Guarana Sample	TPH[b] (mg of TAE/g)	[c]CT (mg/g)	$ORAC_{FL}$ (μmol TEAC/g)
Bar	124.1 ± 6.4	2.20 ± 0.1	1079.6 ± 36.6
Seed pericarp	30.7 ± 1.0	0.48 ± 0.03	356.2 ± 12.1
Seed powder	105.6 ± 4.5	1.83 ± 0.2	987.3 ± 33.8
capsules	57.6 ± 5.4	1.74 ± 0.1	558 ± 38
Powder	73.2 ± 3.3	1.15 ± 0.05	906.1 ± 39.5

[a]The results are presented as a mean standard deviation (SD) for triplicate analysis.
[b]TPH results are expressed in gallic acid equivalents (TAE).
[c]CT results are expressed in catechin equivalents and $ORAC_{FL}$ results are expressed in Trolox equivalents (TEAC).

The antioxidant capacity (expressed as TEAC values) of guarana extracts is presented in Table **2**. It ranged from 356.22 to 1079.62 μmol TEAC/g of DW. Though usually the seeds have the highest amount of total phenolics, an appreciable quantity is obtained in the cases of capsules, bars and powders. The highest value of TEAC found corresponds to the seed (sum pericarp seed more powder seed): 1343.53 ± 45.9 μmol TEAC/g of DW (mean ± SD). The order of antioxidant activity in the different type guarana type samples tested was seeds > bar > powder > capsules. The results of the antioxidant assay shown that the acetone 70% extract from guarana presents a considerably antioxidant scavenging activity against peroxyl radical (Table **2**).

Results obtained in this study indicated an interesting antioxidant activity for all extracts from guarana. This is in agreement with the previous study of Majhenic *et al*., [21] which reported that the guarana seed extracts possess excellent radical-scavenging and antioxidant activities analyzed by $DPPH^{\cdot}$ assay and β-carotene-linoleic acid emulsion system. This is probably due to the phenolic compounds present in the extract, well documented in the case of other products as coffee, cocoa and tea [36].

A linear relationship was observed between TEAC and total phenolics or cathecol content. High correlation coefficients were found between the total phenolic content and antioxidant activity (r: 0.937, $P < 0.01$) for all the types of guarana tested measured by $ORAC_{FL}$ assay, and total phenolics and cathecol contents (r: 0.835, $P < 0.01$).

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CONFLICT OF INTEREST

Declared none.

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