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Antioxidant and antimicrobial activity of guarana seed extracts

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Abstract

The antioxidant and antibacterial activities of guarana (*Paullinia cupana*) seed extracts were determined. The seeds were extracted with water, methanol, 35% acetone and 60% ethanol at room (T_R) and at boiling (T_B) temperature of solvent.

Extracts were analyzed for the contents of caffeine and catechins, epicatechin (EC), catechin (C) and epicatechin gallate (ECG), by high performance liquid chromatography (HPLC). The contents of total phenols (according to the Folin–Ciocalteu procedure) and proanthocyanidins were analysed by UV spectrophotometry. The guarana seed water extract obtained at room temperature contained higher amounts of caffeine and catechins than did alcoholic guarana seed extracts.

The antioxidant and radical-scavenging activities of guarana seed extracts were evaluated using the β-carotene–linoleic acid emulsion system and the stable free radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$). All tested guarana seed extracts displayed strong antioxidant and radical-scavenging properties.

The guarana seed extracts were tested against three food-borne fungi: *Aspergillus niger, Trichoderma viride* and *Penicillium cyclopium*, and three health-damaging bacteria: *Escherichia coli, Pseudomonas fluorescens* and *Bacillus cereus* by the agar well diffusion and broth dilution assay. The alcoholic guarana seed extracts displayed stronger antimicrobial activity against all tested microorganisms than did water extracts.

Results presented here may suggest that seed extracts of guarana possess strong antimicrobial and antioxidant properties, and they can therefore be used as a natural additive in food, cosmetic and pharmaceutical industries.

Keywords: Guarana (*Paullinia cupana*); Seed extracts; Phenolics; Antioxidant activity; Antimicrobial activity

1. Introduction

Guarana (*Paullinia cupana*, Sapindaceae) is a Brazilian native plant, with seeds being the only part suitable for human consumption (Saldana, Zetzl, Mohamed, & Brunner, 2002). Guarana, a low-growing bush-type plant, is the richest vegetable sours of caffeine (Mehr, Biswal, Collins, & Cochran, 1996; Weckerle, Stutz, & Baumann, 2003). The seeds also contain theophylline, theobromine, xanthine derivatives and tannins and also (catechin, epicatechin, proanthocyanidins). They also contain saponins, starch, fats, choline and pigments (Bruneton, 1999; Edwards et al., 2005; Henman, 1982; Seidemann, 1998). These compounds have many favourable effects on human health, such as stimulative effects on the nervous and cardiovascular system. Guarana is used for a variety of therapeutic purposes, such as an antidiarrheic, diuretic and antineuralagic agent, as a painkiller, febrifuge and to treat migraine (Bruneton, 1999; Henman, 1982; Seidemann, 1998). Recently, the guarana seed products are being used in concentrates and soft drinks and as a diet product for slimming in the form of powder, tablets and jams (Basile et al., 2005; Henman, 1982; Saldana et al., 2002; Seidemann, 1998). Previous investigations also showed that guarana

Abbreviations: Ac, acetone; C, catechin; EC, epicatechin; ECG, epicatechin gallate; EtOH, ethanol; G, guarana seed extract; T_B, boiling temperature; T_R, room temperature; MeOH, methanol.

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seed extracts have antioxidant and antimicrobial properties (Basile et al., 2005; Espinola, Dias, Mattei, & Carlini, 1997; Mattei, Dias, Espinola, Carlini, & Barros, 1998).

It is reported (Basile et al., 2005; Baydar, Ozkan, & Sagdic, 2004; Dupont, Caffin, Bhandari, & Dykes, 2006; Nuutila, Puupponen-Pimia, Aarni, & Oksman-Caldentey, 2003; Proestos, Boziaris, Nychas, E, & Komaitis, 2006; Shon, Choi, Kahng, Nam, & Sung, 2004; Singh, Maurya, de Lampasona, & Catalan, 2006; Taguri, Tanaka, & Kouno, 2004; Tepe, Daferera, Sokmen, Sokmen, & Polissiou, 2005; Werlein, Kutemeyer, Schatton, Hubbermann, & Schwarz, 2005; Wong & Kitts, 2006) that phenolic compounds from herbs and spices are active against many bacteria and fungi and have antioxidant activity. Recently, there has been considerable interest in the use of such antioxidants and antimicrobial compounds from natural sources, not only for the preservation of foods and improving the shelf life of food products but also for increasing the stability of fats and oils and to control the human and plant diseases of microbial origin (Baratta et al., 1998; Basile et al., 2005; Benkeblia, 2004; Gulcin, Kufrevioglu, Oktay, & Buyukokuroglu, 2004; Sokmen et al., 2004; Tepe et al., 2005; Wong & Kitts, 2006). Evidently, the gurana seeds are such natural sources of phenolic-antioxidants and antimicrobial compounds and, since the use of guarana extract is interesting for the food, pharmaceutical and cosmetic industries, where such natural additives are required, systematic examinations of plant extracts for these properties have become important.

The aim of this study was to investigate antimicrobial activities of guarana seed extracts obtained with various extraction solvents and to evaluate antioxidant activity using two separate methods, namely β-carotene–linoleic acid emulsion systems and inhibition of free radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$). Extracts were analyzed for their contents of caffeine and phenolic components (total phenols, proanthocyanidins, and catechins) and a relationship between antimicrobial activity, antioxidant activities and the content of phenolic compounds was observed.

2. Materials and methods

2.1. Plant material

Seeds from guarana (*Paullinia cupana*, Sapindaceae) were purchased from a market in Brazil.

2.2. Chemicals and standards

All solvents and chemicals (analytical grade) used for extraction and for analytical purposes were purchased from Merck (Darmstadt, Germany) and from Fluka (Germany). Standards of (+)-catechin (Cat. No. C-1251) and (−)-epicatechin (Cat. No. E-1753) were supplied from Sigma–Aldrich (Germany) and (−)-epicatechin gallate (Cat. No. 54195) and caffeine (Cat. No. 27600) were supplied from Fluka (Germany). For the antifungal tests, potato dextrose agar (PDA; Merck, Cat. No. 1.10130.0500) was used and, for antimicrobial tests nutrient agar, based on meat, was used and was prepared from the following chemicals: sodium chloride (Cat. No. 1.06404.1000), meat extract (Cat. No. 1.03979.0500), peptone from meat (Cat. No. 1.07214.1000) which were purchased from Merck (Darmstadt, Germany) and D-(+)-glucose (Cat. No. G-5400) was supplied from Sigma (Germany).

2.3. Preparation of the extracts

Ten grams of powdered guarana seeds were extracted with selected solvents: distilled water, methanol, 35% acetone and 60% ethanol (volume of solvent per kg of raw material was 15 l, $R = 15$) for 2 h at room temperature and boiling temperature. The extraction system was composed of a round-bottomed flask with a three-necked top connected to the condenser, a magnetic stirrer and a boiler. After 2 h, the mixture was filtered, the solvent was evaporated under vacuum using a rotary evaporator and yields of extractions (%) were determined. The extracts were stored in a dry cool place.

2.4. Determination of phenolic compounds and caffeine

2.4.1. Analysis of total phenols

The concentration of total phenols in extracts was measured by a UV spectrophotometer (Varian-UV–VIS Spectrophotometer), based on a colorimetric oxidation/reduction reaction, as described by Škerget et al. (2005). The oxidizing reagent used was Folin–Ciocalteu reagent (AOAS, 1990). To 0.5 ml of diluted extract (20 mg in 10 ml distilled water) 2.5 ml of Folin–Ciocalteu reagent (diluted 10 times with water) and 2 ml of Na_2CO_3 (75 g/1 l) were added. The sample was incubated for 5 min at 50 °C and then cooled. For a control sample, 0.5 ml of distilled water was used. The absorbance was measured at 760 nm. The results were expressed as mg of gallic acid per g of extract (mg GA/g extract).

2.4.2. Analysis of proanthocyanidins

The content of extracted proanthocyanidins was analysed by a UV spectrophotometry method (Varian-UV–VIS Spectrophotometer), based on acid hydrolysis and colour formation, as described by Škerget et al. (2005). Fifty milligrams of the extract were dissolved in 25 ml of distilled water. To 2 ml of this solution, 20 ml of Fe sulphate solution (77 mg of $FeSO_4 \cdot 7H_2O$ in 500 ml of HCl: *n*-buthanol = 2:3) were added. For the control sample 2 ml of distilled water were used. The sample was incubated for 15 min at 95 °C. After incubation, the sample was cooled and analyzed by measuring absorbance at 540 nm. The results were presented as the amount of proanthocyanidins in the obtained extract (mg PAC/g extract).

2.4.3. Analysis of caffeine and catechins

The contents of caffeine and catechins, such as catechin (C), epicatechin (EC) and epicatechin gallate (ECG), in guarana seed extracts were determined by HPLC (HPLC, Agilent 1100, Germany) with modifications of a previously reported method (Gurbuz et al., 2005). Extract (20 mg) was dissolved in distilled water (10 ml) and filtered through a teflon membrane filter with pore size 0.45 μm. After filtration, aqueous extract was injected (20 μl) into an Eclipse XDB C-18 column (5 μm pore size, 4.6 mm × 150 mm). The mobile phase components were prepared as follows: to 1 l of 5% acetic acid were added 10 ml of acetonitrile (solvent A) and acetonitrile was solvent B. The analyses were preformed under gradient elution conditions. Elution with a flow rate of 1 ml/min was as follows: 0–20 min, 0–14% B in A; 20–28 min, 14–40% B in A; 28–30 min, 40–50% B in A; 30–35 min, 50–0% B in A. The caffeine and catechins were detected at 280 nm.

The concentrations of the caffeine and catechins in the extracts were calculated from the calibration curves.

2.5. Antioxidant activity

2.5.1. β-Carotene–linoleic acid assay

The antioxidant activity was elucidated on a heat-induced oxidation of an aqueous system of β-carotene and linoleic acid as described by Škerget et al. (2005). One milliliter of β-carotene/chloroformic solution (0.2 mg/ml) was placed in an Erlenmeyer flask containing linoleic acid (0.02 ml), Tween 40 (0.2 ml) and the corresponding guarana seed extract in methanol solution at a concentration of 10^{-3} M. Fifty milliliter of distilled water were saturated with oxygen for 15 min and placed in the flask. The mixture was shaken and stored for 2 h at 50 °C. The absorbance of the sample was measured on the Varian-UV–VIS Spectrophotometer at 470 nm at the beginning ($t = 0$ min) and after the experiment ($t = 120$ min). Antioxidant activity of the sample (s) was calculated as percent inhibition of oxidation versus control sample (c), using the equation

$$\%\ \text{Antioxidant activity}_{\text{emulsion system}} = 100 \times \left[1 - \frac{A_s^0 - A_s^{120}}{A_c^0 - A_{c120}}\right], \quad (1)$$

where A_s is the absorbance of sample at $t = 0$ min and $t = 120$ min, and A_c is the absorbance of control sample at $t = 0$ min and $t = 120$ min.

2.5.2. DPPH radical-scavenging system

The effect on antioxidants on DPPH radical-scavenging is thought to be due to their hydrogen-donating ability. DPPH· is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule (Gulcin et al., 2004). The reduction capability (on the DPPH radical) is determined by the decrease in its absorbance at its absorption maximum at 515 nm that is induced by antioxidant. This is visualised as a change in colour from violet to yellow (Gulcin et al., 2004; Miliauskas, Venskutonis, & van Beek, 2004). Gulcin et al. (2004) also reported that DPPH· is usually used as substrate to evaluate antioxidant activity. Radical-scavenging activity of guarana seed extracts against stable DPPH· (2,2-diphenyl-1-picrylhydrazyl) was determined spectrophotometrically, as described Miliauskas et al. (2004). Extract solutions were prepared by dissolving 0.01 g of dry extract in 10 ml of methanol. The solution of DPPH· in methanol (6×10^{-5} M) was prepared daily, before UV measurements. Three milliliters of this solution were mixed with 77 μl extract solution in the flask. The samples were kept in the dark for 15 min at room temperature and then absorbance was measured at 515 nm (changes in colour, from violet to light yellow). Radical-scavenging activity was calculated by the following formula:

$$\%\ \text{Inhibition} = [(A_B - A_A)/A_B] \times 100, \quad (2)$$

where A_B, absorption of blank sample ($t = 0$ min); A_A, absorption of tested extract solution ($t = 15$ min).

2.6. Antimicrobial tests

2.6.1. Microbial strains

Microorganisms were obtained as lyophilized cultures from the National Collection of Agricultural and Industrial Microorganisms (Hungary). Organisms were as follows: three species of moulds, *Aspergillus niger*, *Trichoderma viride* and *Penicillium cyclopium*, and two species of Gram negative bacteria, *Escherichia coli* and *Pseudomonas fluorescens* and one species of Gram positive bacteria, *Bacillus cereus.*

2.6.2. Preparation of test microorganisms

Agar cultures of fungi and bacteria for antimicrobial tests were prepared as described by Vagi, Simandi, Suhajda, and Hethelyi (2005).

The test fungi were maintained on PDA slopes and stored at 4 °C. Conidia was harvested in sterile distilled water containing approximately 10^5–10^7 conidia/ml. These conidial suspension were used immediately after preparation for determining the antifungal activities of the guarana seed extracts.

In the antibacterial experiments, test bacteria were grown on meat nutrient agar slopes for 24 h at 30 °C, except for *E. coli*, that was grown at 37 °C and then stored at 4 °C. Before the bacterial experiments were carried out, liquid medium was inoculated with freshly harvested bacteria and incubated for 24 h at the adequate temperature. These bacterial suspensions (approximately 10^7–10^8 cell/ml) were used to inoculate the test medium containing the guarana seed extracts.

2.6.3. Antifungal activity test

The antifungal tests were done as described by Benkeblia (2004), Ozcan and Boyraz (2000), Singh, Marimuthu, Catalan, and deLampasona (2004) and Vagi et al. (2005).

Each PDA sterile plate contained 19 ml of molten medium and 1 ml of an alcoholic mixture of guarana seed extracts (concentration 20 g/100 ml 96% ethanol). As alcoholic control, PDA plates containing the same amount of medium and 1 ml of 96% ethanol were prepared. For all test fungi, PDA plates without any materials were made and were used as control plates. The solid plates were inoculated with 0.1 ml of conidial suspension, measuring it into the holes (diameter 10 mm) in the centre of the medium. The plate was left undisturbed to allow diffusion of the sample into agar, and then the plate was incubated in the dark at 28 °C and the diameter of the mycelial growth was measured. The incubation was stopped when the mycelial mass of control plates had filled the plates (13–27 days). Each test was run in triplicate and averages were calculated. The antifungal activity was determined in terms of percentage mycelial inhibition calculated by the following formula:

$$I = [(C - T)/C] \times 100 \quad (3)$$

where I is inhibition (%), C is the colony diameter of the mycelium on the alcoholic control plate (mm), and T is the colony diameter of the mycelium on the test Petri plate (mm).

2.6.4. Antibacterial activity test

The antibacterial tests were done as described by Janssen, Scheffer, and Baerheim Svendsen (1987) and Vagi et al. (2005).

The growth of bacteria was followed by measuring the optical density of bacterial suspension using densitometry; 50 ml of nutrient liquid medium were measured into an Erlenmeyer flask; three flasks were prepared for each examined sample. The flask with the medium was sterilized in an autoclave at 121 °C for 15 min.

Into each sterile flask 1 ml of alcoholic guarana seed extract (concentration 20 g/100 ml 96% ethanol) was added and the mixture was inoculated with 1 ml of bacterial suspension. An alcoholic control was prepared by addition of 1 ml of 96% ethanol instead of guarana extract and then inoculated with 1 ml of bacterial suspension. Bacteria control in the sterile medium was applied without any added material. *E. coli* and *B. cereus* were incubated at 37 °C and 30 °C, respectively. *P. fluorescens* was grown on a shaking platform at 30 °C. For obtaining the initial bacterial concentration (x_0) samples were taken straight after inoculation. Afterwards samples were taken at 3, 5, 8, 24 and 27 h to follow the bacterial growth. Due to the strong colour of guarana seed extracts, 1–2 ml samples were taken before inoculation, and were used as control solution during the density measurements. The optical density of the samples was measured by densitometry (Varian-UV–VIS Spectrophotometer) at 550 nm. The growth of the bacteria is manifested in the turbidity of the suspension, and can be followed by the rise of optical density. The optical densities of control and alcoholic control bacteria suspensions were compared with the pure liquid media. The optical density of media containing guarana seed extracts were compared with the liquid medium containing only guarana seed extracts in the absence of bacteria (taken before inoculation). The inhibition per cent is determined as the optical density of the sample (OD_x) in the 27th hour of measurement compared with the optical density of alcoholic control (OD_{AC}) in the last hour of measurement

$$\text{Inhibition } (\%) = [(OD_{AC} - OD_x)/OD_{AC}] \times 100. \quad (4)$$

2.7. Statistical analysis

Experimental results were expressed as means ± standard deviation (SD) of three parallel measurements.

3. Results and discussion

3.1. Contents of phenolic compounds and caffeine in extracts

3.1.1. Total phenols in extracts

The contents of total phenols and yields (based on guarana seed extracts) are given in Table 1. The amount of total phenolic compounds extracted with the different solvent mixtures ranged from 119 to 186 mg GA/g extract. The guarana seed extracts contained the highest amounts of phenolic compounds when 35% acetone was used as extraction solvent at boiling temperature and the lowest amounts of phenolic compounds when water was used as extraction solvent at boiling temperature. Generally, higher amounts of total phenolic compounds in guarana seed extracts were determined in extracts obtained at boiling temperature of solvent, with exception of water, where a higher amount of total phenolic compounds in extract was obtained at room temperature.

3.1.2. Proanthocyanidins in extracts

The contents of proanthocyanidins in guarana seed extracts (Table 1) obtained with methanol, 35% acetone and 60% ethanol ranged from 38.9 mg PAC/g extract to 60.5 mg PAC/g extract. The lowest content of proanthocyanidins was obtained in water (13.1–29.4 mg/g extract) extract. Generally, for all tested guarana seed extracts,

Table 1
Extraction of guarana seed: yield and composition of extracts

Solvent	$T_{\text{of extraction}}$ (°C)	Extraction yield (%)	Total phenols (mg GA/g extract)	mg PAC/g extract
Water	T_R	11.7	181	29.4
	100*	23.8	119	13.1
Methanol	T_R	13.7	173	60.5
	62*	21.8	176	38.9
35% acetone	T_R	19.5	173	56.8
	85*	24.9	186	48.0
60% ethanol	T_R	18.4	166	54.1
	75*	24.8	172	49.3

* Boiling temperature; T_R, room temperature.

the content of proanthocyanidins was higher when they were obtained at room temperature.

3.1.3. Caffeine and catechins in extracts

The contents of caffeine and catechins, such as catechin (C), epicatechin (EC) and epicatechin gallate (ECG), in guarana seed extracts are presented in Figs. 1 and 2. As can be seen from the results, water at room temperature is the best solvent for extraction of caffeine and catechins from guarana seeds. The results also show 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).

Generally, all tested guarana seed extracts contained high amounts of EC and C, while on the other hand, the content of ECG was the lowest. The temperature of the extraction did not have a significant effect on the contents of caffeine and catechins in guarana seed extracts, except in the case when water at boiling temperature was used for extraction, where the lowest contents of caffeine and catechins were determined.

3.2. Antioxidant activity

The results of DPPH radical-scavenging activities of various guarana seed extracts are represented in Fig. 3.

Methanol, 35% acetone and 60% ethanol guarana seed extracts were the most effective DPPH radical-scavengers with inhibition being higher than 85%. Generally, temperature of the extraction solvent did not have a significant effect on radical-scavenging activity of extracts.

In the linoleic emulsion system, oxidation of β-carotene was effectively inhibited by all extracts of guarana seed (Fig. 3). The highest inhibitions of β-carotene oxidation were shown by methanol guarana seed extracts 87.8% (T_R) and 85.9% (T_B), respectively, while the lowest inhibition of β-carotene oxidation was shown by water guarana seed extract, 70.9% (T_R) and 67.8% (T_B), respectively. The temperature of the extraction did not have a significant effect on the inhibition of β-carotene oxidation.

As a result, guarana seed extracts possess excellent radical-scavenging and antioxidant activities analyzed by the DPPH$^{\cdot}$ assay and β-carotene–linoleic acid emulsion system. The lowest antioxidant and radical-scavenging activities were observed for the extracts obtained water. Extracts obtained with mixtures of organic solvent (acetone, and ethanol) and water generally showed higher radical-scavenging activity than did pure water extracts, while antioxidant activity was approximately the same. The highest antioxidant activity was observed for the pure methanol extract.

3.3. Antifungal activities of guarana seed extracts

Antifungal activities of guarana seed extracts against *T. viride*, *P. cyclopium* and *A. niger* are presented in Table 2, where the results of the last day of growing, applying three parallel measurements, are given. As can be seen from

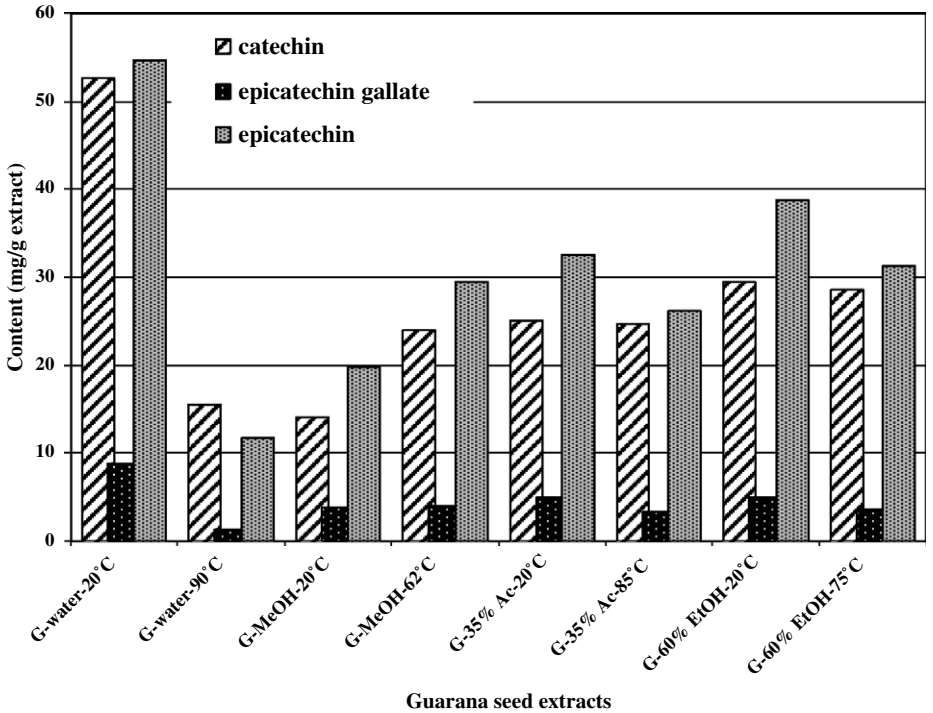

Fig. 1. Comparison of the catechin, epicatechin gallate and epicatechin contents (mg/g extract) for water, methanol, 35% acetone and 60% ethanol guarana seed extracts obtained at room (T_R) and boiling (T_B) temperature.

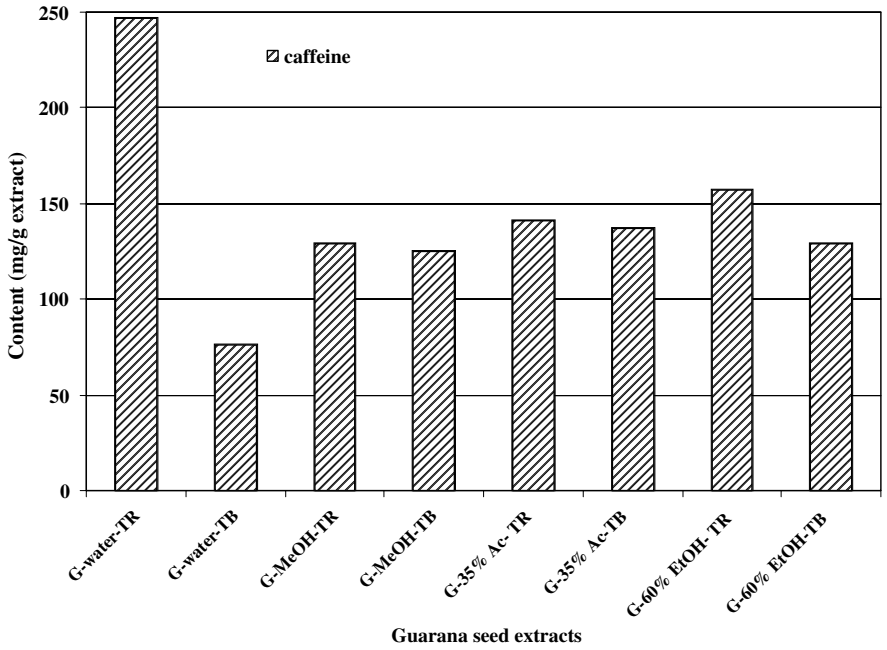

Fig. 2. The contents of caffeine (mg/g extract) for water, methanol, 35% acetone and 60% ethanol guarana seed extracts obtained at room (T_R) and boiling (T_B) temperature.

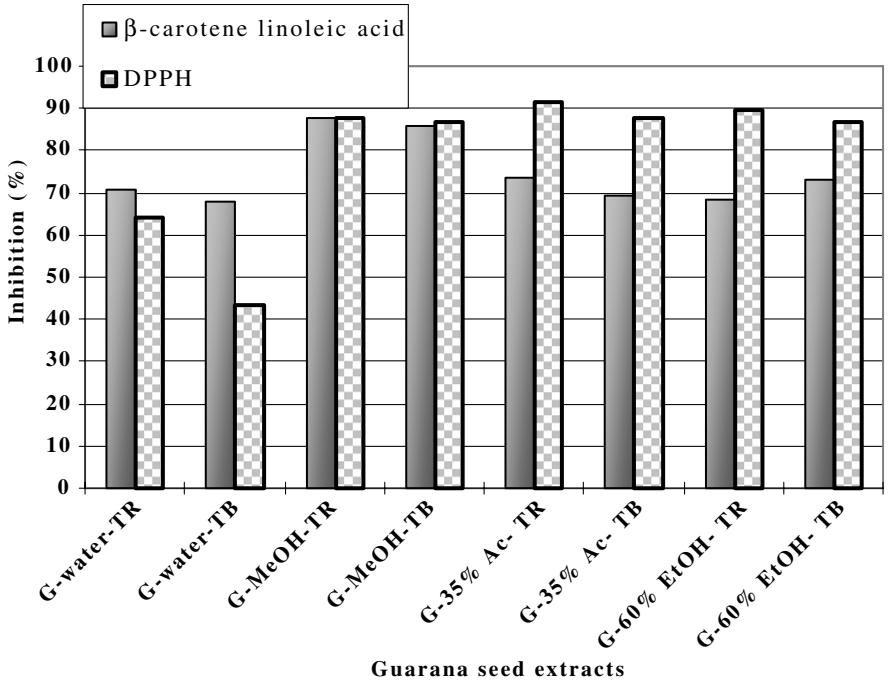

Fig. 3. The antioxidant activities for water, methanol, 35% acetone and 60% ethanol guarana seed extracts obtained at room (T_R) and boiling (T_B) temperature using DPPH· assay and β-carotene–linoleic acid emulsion systems.

Table 2, guarana seed extracts exhibited different inhibition levels against three tested mycelial fungi.

Generally, all tested guarana seed extracts exhibited highest antifungal activity against *A. niger*. An inhibitory effect against the spoilage fungus *A. niger* was similar for all tested guarana seed extracts, except for the boiling water extract where the lowest inhibitory effect was observed ($30.7 \pm 7.7\%$). Higher antifungal activities were generally observed for extracts obtained at room temperature.

The inhibitory activity of guarana seed extracts obtained at room temperature (T_R) against *A. niger* can be seen in Fig. 4.

Against fungus *T. viride*, methanol extracts obtained at room and boiling temperature displayed the highest

Table 2
Antifungal activities of guarana seed extract against three fungi in comparison with control of ethanol

Solvent	$T_{\text{of extraction}}$ (°C)	Inhibitory effect[a] (%) against test fungi		
		Trichodrma viride	*Penicillium cyclopium*	*Aspergillus niger*
Water	T_R	27.6 ± 10.0	39.4 ± 6.8	64.4 ± 2.4
	T_B	6.1 ± 2.4	13.4 ± 7.1	30.7 ± 7.7
Methanol	T_R	78.5 ± 7.3	46.9 ± 7.0	78.1 ± 7.5
	T_B	57.0 ± 2.3	44.5 ± 4.1	71.1 ± 2.4
35% acetone	T_R	55.6 ± 4.7	53.2 ± 3.1	74.8 ± 5.6
	T_B	34.7 ± 5.1	52.9 ± 1.9	61.1 ± 5.6
60% ethanol	T_R	46.8 ± 13.4	45.6 ± 1.5	70.7 ± 3.9
	T_B	29.6 ± 6.1	44.3 ± 5.4	69.6 ± 0.3

[a] Data are presented as the means ± standard deviation (SD) of triplicate determinations, T_R, room temperature; T_B, boiling temperature.

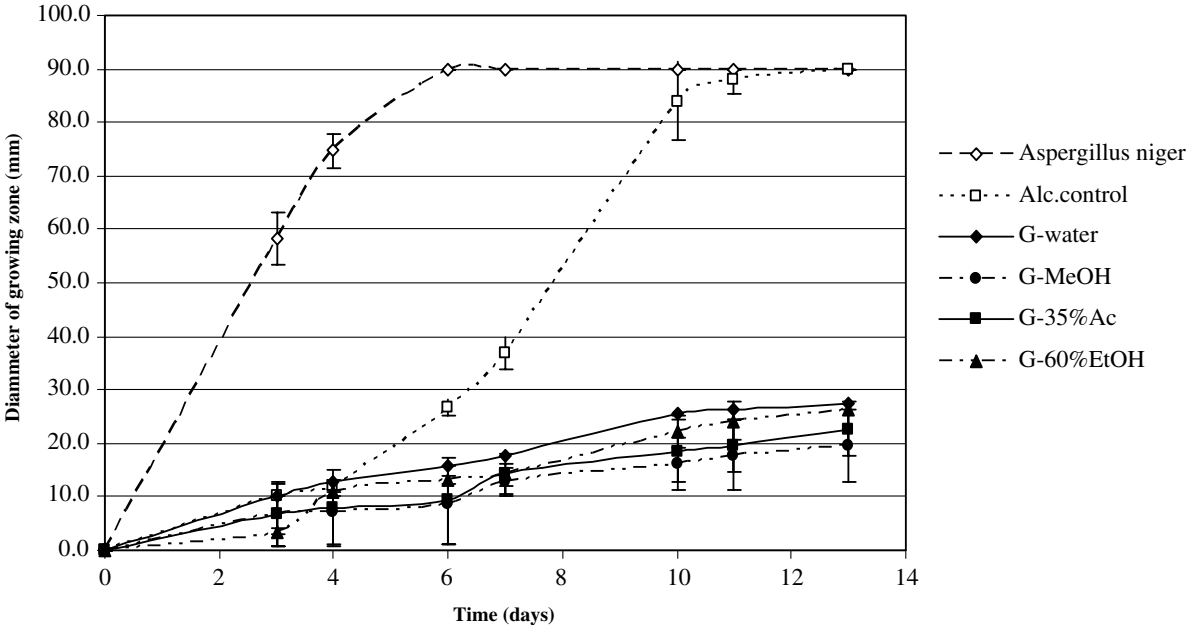

Fig. 4. Antifungal activities for water, methanol, 35% acetone and 60% ethanol guarana seed extracts obtained at room temperature (T_R) against *Aspergillus niger*.

antifungal activity (78.5 ± 7.3%, 57.0 ± 2.3%, respectively) while, in the case of the water extracts, only slight antifungal activity was observed (27.6 ± 10.0%, 6.1 ± 2.4%), respectively. However, high inhibitory effects against tested fungi were observed also for extracts obtained with organic solvent–water mixtures. For all experiments carried out with *T. viride*, the extracts obtained at room temperature had higher inhibitory effects. The inhibitory activity of guarana seed extracts obtained at boiling temperature (T_B) of solvent against *T. viride* can be seen in Fig. 5.

According to the results obtained by measuring the radial growth of *P. cyclopium* on plates, the antifungal activities of the methanol, 35% acetone and 60% ethanol guarana seed extracts were similar (from 44% to 53%), while low antifungal activities of water guarana seed extracts were observed. The temperature of extraction showed no effect on inhibitory activity of obtained extracts with exception of water extracts, where higher antifungal activity of extract obtained at room temperature was observed.

Generally, among the guarana seed extracts tested on three fungi, the methanol, 35% acetone and 60% ethanol extracts displayed higher antifungal activity than did the water extracts. It can be suggested that, for extraction of antifungal components from guarana seeds, methanol, 35% acetone and 60% ethanol should be used as solvents and extraction should be performed at room temperature.

3.4. Antibacterial activities of guarana seed extracts

Table 3 summarizes antimicrobial activities of guarana seed extracts in comparison with an alcoholic control after a 27 h growth.

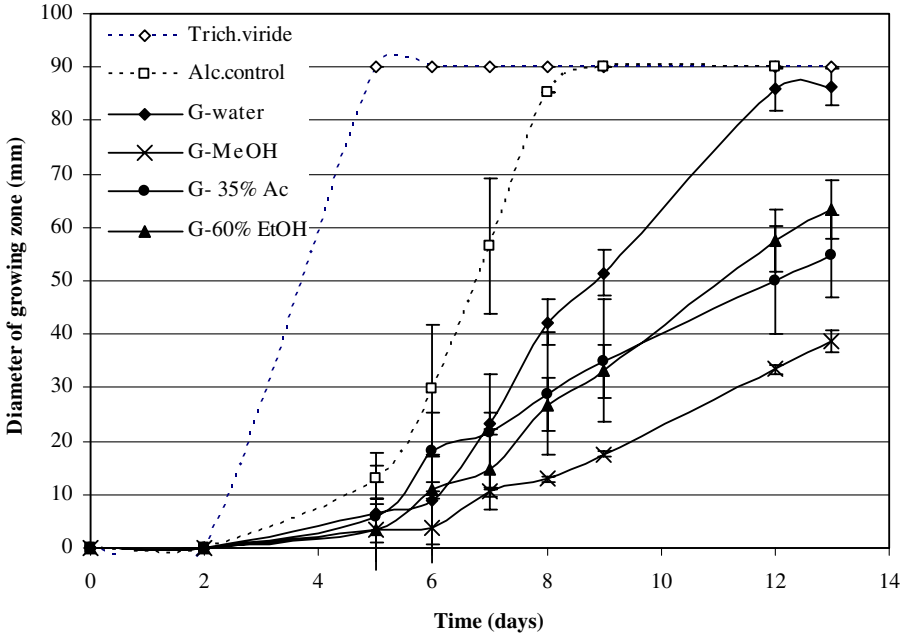

Fig. 5. Antifungal activities for water, methanol, 35% acetone and 60% ethanol guarana seed extracts obtained at boiling temperature (T_B) against *Trichoderma viride*.

Table 3
Antibacterial activities of guarana seed extract against three bacteria in comparison with control of ethanol

Solvent	$T_{of\ extraction}$ (°C)	Inhibitory effect[a] (%) against test bacteria		
		Bacillus cereus	*Escherichia coli*	*Pseudomonas fluorescens*
Water	T_R	2.1 ± 2.8	No inhibition	52.3 ± 2.0
	T_B	No inhibition	No inhibition	44.6 ± 11.3
Methanol	T_R	90.1 ± 0.3	100	97.3 ± 0.6
	T_B	97.6 ± 2.2	91 ± 0.9	97.1 ± 0.1
35% acetone	T_R	93.4 ± 3.3	80.2 ± 0.9	97.1± 0.9
	T_B	84.9 ± 3.4	83.5 ± 1.7	84.0 ± 3.7
60% ethanol	T_R	92.5 ± 1.6	92.7 ± 0.3	97.1 ± 0.9
	T_B	91.1 ± 0.6	94.9 ± 1.9	98.5 ± 0.3

[a] Data are presented as the means ± standard deviation (SD) of triplicate determinations, T_R, room temperature; T_B, boiling temperature.

Successful extraction of active botanical compounds from plant material is dependent on the type of solvent used in the extraction procedure (Parekh, Jadeja, & Chanda, 2005). In our studies, it was observed that plant extractions with organic solvents provide stronger antibacterial activity than do those extracted with water. As can been seen from Table 3 the methanol, 35% acetone and 60% ethanol guarana seed extracts showed strong antibacterial activity (>80%) against three health-damaging bacteria, and the range of antibacterial activity observed for these extracts is similar. These observations can be explained by different active compounds being extracted with each solvent. In addition, this study confirms the results from previous studies, which reported that water is not a suitable solvent for extraction of antibacterial substances from medical plants compared to other solvent, such as methanol, or ethanol (Karaman et al., 2003; Moniharapon & Hashinaga, 2004; Parekh et al., 2005). In Fig. 6, the grown curves of *E. coli* can be seen in comparison with ethanol control and guarana seed extract (T_R).

It can be concluded, that the guarana seed extract effectively inhibited the Gram positive bacterium *B. cereus* and the Gram negative bacteria *E. coli* and *P. fluorescens* when methanol, 35% acetone and 60% ethanol were used as solvents. This is in agreement with the previous study of Basile et al. (2005), which reported that the ethanol extract of guarana seeds inhibited both Gram positive and Gram negative bacteria. In the case of water, guarana seed extracts displayed no antibacterial activity against *E. coli* and low antibacterial activity against *B. cereus*; the inhibition activity was 2.1 ± 2.8%. The water guarana seed extract showed antibacterial activity only against the food-borne bacterium *P. fluorescens*. The inhibition activities were 52.3 ± 2.0% for water extract obtained at room temperature (T_R) and 44.6 ± 11.3% for extract obtained in boiling water (T_B). In Fig. 7, the growth curves of *P. fluorescens*

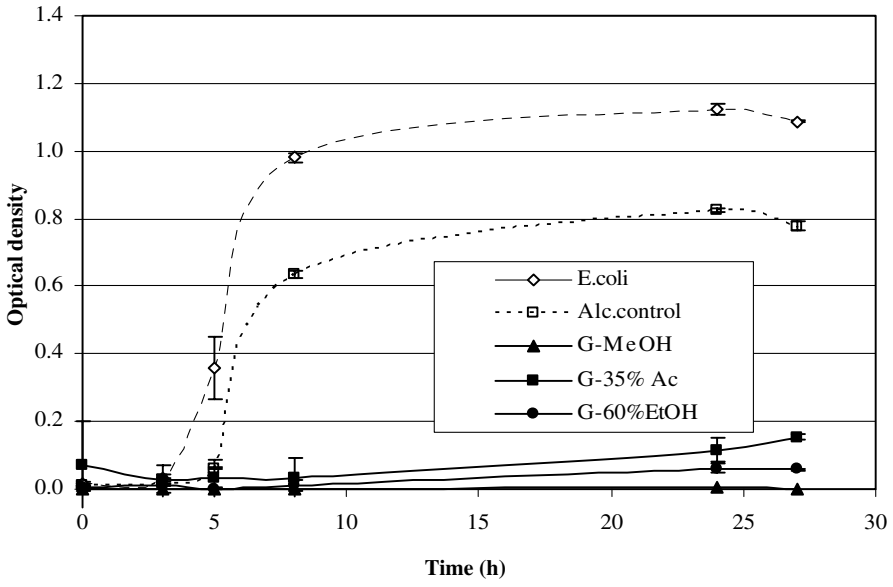

Fig. 6. The antibacterial activities for methanol, 35% acetone and 60% ethanol guarana seed extracts obtained at room temperature (T_R) against *Escherichia coli*.

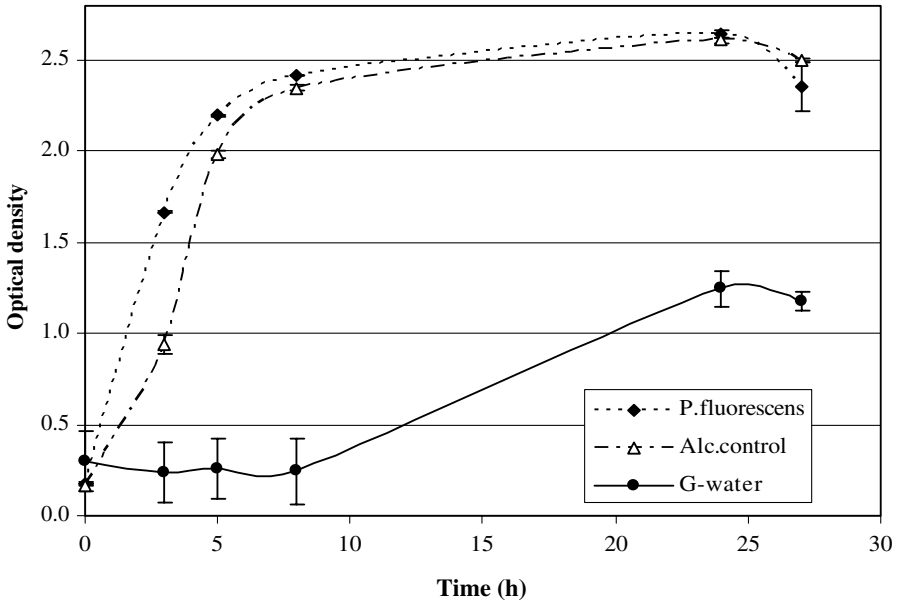

Fig. 7. The antibacterial activity for water guarana seed extract obtained at room temperature (T_R) against *Pseudomonas fluorescens*.

can be seen in comparison with those of ethanol control and water guarana seed extract (T_R).

Tests against food pathogenic bacteria showed that temperature of extraction did not affect the antibacterial activities of obtained extracts when methanol and 60% ethanol were used as extraction solvents.

4. Conclusions

Current work demonstrates that conventional organic solvent extracts of Brazilian native herb guarana have significant activity against the growth of food poisoning, spoilage bacteria, such as *E. coli*, *B. cereus*, *P. fluorescens* and spoilage fungi such as *A. niger*, *T. viride* and *P. cyclopium*. This is in agreement with the study of Basile et al. (2005), which showed that ethanol extracts from guarana seeds have antioxidant activity and inhibitory activity against both Gram-negative and Gram-positive bacteria. On the other hand, the present work demonstrates good radical-scavenging and antioxidative properties ($DPPH^{\cdot}$ and β-carotene method) for all extracts from guarana seeds.

The results show that type of extraction solvent had a big influence on the antioxidant and antimicrobial properties of obtained extracts, while temperature of extraction had no

significant influence on the results. The results showed that alcoholic extracts displayed the highest antimicrobial and antioxidant activity while water guarana seed extracts showed lower antioxidant activity and little or no antimicrobial activity against tested microorganisms. All tested guarana seed extracts contained high amounts of total phenolic compounds, while contents of catechins, such as EC, C, ECG and caffeine, were the highest in water guarana seed extract obtained at room temperature.

Future studies are required to determine the types of other bioactive compounds in seed extracts, as well as the efficiencies of individual phenolic compounds, caffeine and synergistic effects responsible for the antimicrobial and antioxidant activity of the guarana seed extract.

Due to their high antioxidant, antibacterial and antifungal activities the guarana seed extracts have promising potential as natural antioxidants in the food industries, in the preservation of foodstuffs against a range of food-related bacterial and fungal species or in the pharmaceutical and cosmetic industries.

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