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Review

# *Paullinia cupana*: a multipurpose plant – a review

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ABSTRACT

Seeds of guarana (*Paullinia cupana* Kunth, Sapindaceae) feature diverse pharmacological functions, for example, antimicrobial, antioxidant, anticarcinogenic, stimulating, and cognitive functions, as well as liver protection and weight loss. Many of these actions are probably due to the high content of methylxanthines and tannins in its seeds. In Brazil, the world's largest producer of guarana, the plant material is predominantly used in the soft drinks industry, although it is also used in the cosmetic and pharmaceutical industries. Although the Amazon region has the largest cropping area, the state of Bahia is the main guarana producer in Brazil (71%). This review focuses mainly on the possible pharmacological actions of guarana that have been investigated. Moreover, it discusses less-considered topics, such as the toxicology and quality control of seeds and extractives of guarana that will ultimately influence the safety of its use. In addition, it presents a detailed discussion of the methods used to prepare herbal drugs and their extracts, focusing on the importance of standardization and on the direct impact of preparatory factors, on the pharmacological properties of guarana extracts.



## Introduction

The guarana (*Paullinia cupana* Kunth, Sapindaceae) plant is associated with a remarkably wide range of pharmacological actions (Roncon et al., 2011; Bittencourt et al., 2013; del Giglio et al., 2013; Portella et al., 2013; Rangel et al., 2013; Bittencourt et al., 2014; Hertz et al., 2015; Machado et al., 2015; Matsuura et al., 2015; Kober et al., 2016). For hundreds of years, guarana has been grown and widely used by Brazilian Indians. Commonly, guarana seeds are used by simply dissolving the powder of the toasted and ground seed in water, either alone or in combination with other commercially available herbal drugs. Nowadays, it is commercially exploited, mainly by the soft drinks industry, although it is also highly valued by the cosmetic and pharmaceutical industries.

The pharmacological properties of guarana have been the main focus on two recent reviews (Hamerski et al., 2013; Schimpl et al., 2013). Although there has been a great deal of interest in studying caffeine from guarana including its benefits and harms (Nawrot et al., 2003; Beck, 2005; Nyska et al., 2005; Tfouni et al., 2007), the most diverse pharmacological effects of guarana are associated with the tannins present in the plant seeds, which represent about 16% of the seed composition (Yamaguti-Sasaki et al., 2007; Pelozo et al., 2008; Sousa et al., 2010; Dalonso and Petkowicz, 2012).

Ultimately, using the plant bioactives, either as pure compounds or as standardized extracts, requires extraction, pharmacological screening, isolation and characterization of the biological compound, as well as toxicological and clinical evaluation. Often, one or more of these primary steps is overlooked in most scientific papers. Furthermore, the varied preparatory methods used in the extraction and isolation, for instance, creates chemical diversity among the extracts or purified compounds, which affects their pharmacological properties (Marques et al., 2016). Moreover, combining bioactives from various sources creates a variety of pharmacological effects that are still far from being exhausted.

The aim of this review is to present a state of the art assessment of guarana, particularly the chemical compounds that have been identified and characterized in its seeds. The main focus is to review the possible pharmacological actions of guarana, based on an exhaustive and intense appraisal of the relevant literature. In addition, it intends to introduce less-considered yet pivotal topics, such as toxicology and quality control of herbal drugs and extractives, as well as a broad discussion on the method of preparation

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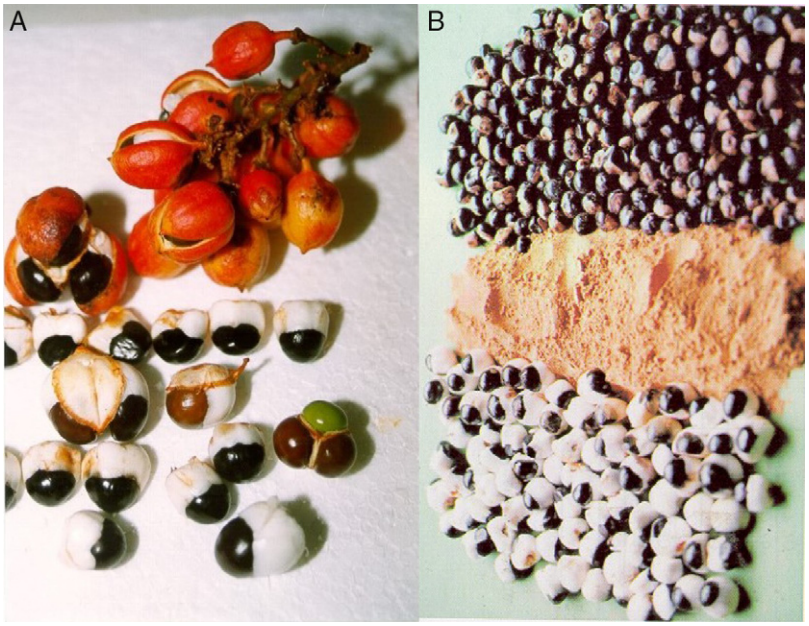



**Fig. 1.** Fruits and seeds of guarana. (A) Orange fruits with red capsules containing black seeds partially covered by white arils; (B) from bottom to top: newly-collected seeds partially covered by arils, dried and toasted powdered seeds; toasted, still undamaged seeds.

of extracts and their standardization. In this context, we searched the Web of Science, Scopus, and PubMed databases, as well as the Brazilian scientific databases, such as Scielo.

## History

The guarana plant has a well-established history that started before the conquest of America. It has been domesticated in interfluvial forests between the lower Tapajós River and the lower Madeira river in the Brazilian Amazon (Smith and Atroch, 2007). The Maués Indians in Brazil discovered and named the guarana fruit. They were the first consumers of the guarana beverage (Kuri, 2008). The fruits of guarana are orange-red capsules that contain black seeds, partially covered by white arils (Fig. 1). The contrasting colors of the partially open fruit, creates the appearance of eyeballs, thus, giving credence to the legend (Fig. 1A) about the origin of the domestication of guarana. This myth, which is attributed to the Maués Indians, has it that a malevolent god attracted a beloved male child of the village into the jungle and killed him out of jealousy. The people of the village found the child dead, lying in the forest. A benevolent God consoles the village with a present in the form of guarana. He plucked out the left eye of the child and planted it in the forest, where it became the wild variety of guarana. The right eye was planted in the village, and it sprouted and produced fruits that resembled a child's eye (Beck, 2005).

The first written description of guarana was made by a Jesuit missionary named Johannes Philippus Bettendorf (1625–1698), in 1669. As a missionary in the Amazon region, he observed that the Indians used to consume a drink made of guarana, which they reported as having diuretic properties and being effective against headaches, fevers, and cramps. In the mid-18th century, other reports described the use of guarana against diarrhea and its ability to mitigate the risks of heat stress (Henman, 1982; Smith and Atroch, 2007). Ever since, many features of this plant have begun to be explored including an increasing amount of research on its chemical composition (Henman, 1982). Due to the widespread use of guarana, mainly as a result of its stimulating effect on the central nervous system, it was officially described in the 1977 Brazilian Pharmacopoeia (Farmacopeia Brasileira, 1977).

The first substance of guarana was isolated in 1826 and named guaranine, a tetramethylxanthine identical to caffeine. With further studies, researchers started to attribute the medicinal properties of guarana to several xanthines (caffeine, theophylline, and theobromine, for example) and the numerous tannins present in the plant (Henman, 1982). The stimulating effects of guarana are apparently more lasting than the effects of coffee due to the tannins present in the guarana plant.

## Cultivation and processing

Brazil is virtually the sole producer of guarana in the world. Guarana is originally from the Amazon, found primarily in the southeast region of the state of Amazonas, in the towns of Maués and Parintins (Machado, 1946; Corrêa, 1984) (Fig. 2). Guarana plants are abundant in the region of Maués, 250 km away from Manaus. They can also be found in small areas of the Venezuelan Amazon. In recent decades, the cultivation of guarana has been encouraged in other areas, particularly in the valleys of the rivers Purus and Tapajós (Amazonas), in the states of Pará, Acre, and Rondônia, in the cocoa-producing region of Bahia between the cities of Salvador and Ilhéus, in the Ribeira Valley in the state of São Paulo, and in the region of Alta Floresta, Mato Grosso (Henman, 1982; Corrêa, 1984; Duke, 1987; Suframa, 2003).

In 1974, the national guarana production, in Maués and other production areas of the state of Amazonas, was calculated at around 180–200 tons of dried seeds, while, in 1977, a study reported the production had increased to 300 tons of dried seeds (Nazaré and Figueiredo, 1982). In 2003, the production was estimated at approximately 4300 tons per year (Suframa, 2003). However, because of the significant economic exploitation, the production did not meet the demand, which posed risks of tampering.

Until the 1980s, the township of Maués was the undisputed leader in the production of guarana, representing 90% of the small farm production in Brazil. However, the expansion of the commercial use of the guarana seeds, in soft drinks and by pharmaceutical and cosmetic manufacturers, led thousands of farmers in southern Bahia, known as an area of cocoa cultivation, to grow the guarana plant (Table 1).


**Fig. 2.** The painted areas refer to the main Brazilian states envolved in guarana crop. The markings refer to the townships cities of Maués and Parintins, Amazonas state. *Source: Google Maps.*

**Table 1**
Area, production, and yield in guarana crops in the year 2016.

| State | Planted area (ha) | Harvested area (ha) | Production (t) | Average yield (kg ha$^{-1}$) |
|---|---|---|---|---|
| Acre | 6 | 4 | 2 | 500 |
| Amazonas | 8113 | 4912 | 855 | 174 |
| Bahia | 6500 | 6500 | 2600 | 400 |
| Mato Grosso | 353 | 317 | 180 | 568 |
| Pará | 92 | 24 | 12 | 500 |
| Rondônia | 92 | 91 | 37 | 407 |
| Total | 15,156 | 11,848 | 3686 | 424 |

*Source*: IBGE (2017).

Alta Floresta has an ideal hot and humid climate and soil properties for the cultivation of this fruit and is home to about 40 of the leading producers of guarana in Brazil. It is reported that in 2008, guarana plantations in the state of Mato Grosso, occupied approximately 89.6 ha, of which 46.8 ha were west of Alta Floresta town (Gouveia et al., 2012).

According to data from the Brazilian Institute of Geography and Statistics (IBGE, 2017), the plantations of guarana in Brazil occupied approximately 15,156 ha in 2016, with the state of Amazonas (8113 ha) and Bahia (6500 ha) the major contributors, together accounting for 96% of the cultivation area in Brazil, followed by 353 ha in Mato Grosso (Fig. 2). Although the state of Amazonas has the largest cultivated area, the largest production comes from Bahia (70%) because of its high yield (Table 1).

There has been increased interest in the guarana plant for its medicinal and stimulant properties by the food, pharmaceutical and cosmetic industries. Consequently, over the last decade, there has been a significant increase in the area of plantations. However, the average yield values, which consider the production by planted area, have remained constant (Table 2).

Guarana is widely used in the food industry in the form of syrups, extracts, and distillates, primarily as a flavoring agent and as a source of caffeine by soft drink manufacturers (Henman, 1982; Duke, 1987). The greatest economic value of guarana is currently in the manufacture of beverages. The American Beverage Company (Ambev) alone uses 70% of the guarana seeds produced annually in Maués. The remaining production (30%) is destined for the phytochemical industry and exportation, mainly to Japan and the United States (Suframa, 2003). In 1972 the Law 5823, termed the Law of Juices, was enacted. This law established quantitative limits of guarana at 0.2–2 g l$^{-1}$ soda and 1–10 g l$^{-1}$ syrup (Homma, 2014). This led to a huge demand for the product because there was an increasing production of guarana soft drinks. The consistent increase in the demand for guarana has encouraged cultivation of guarana plants and agricultural techniques to improve production, rendering it are a promising market for farmers.

A limiting factor to production and expansion of guarana crops in Amazonas is anthracnose, a disease caused by the fungus *Colletotrichum guaranicola* Albuq. Several studies have assessed the genetic diversity of *C. guaranicola* (Duarte et al., 1995; Bentes and Matsuoka, 2002; Bentes and Neto, 2011), providing useful information regarding disease management and crop improvement. Moreover, because of the presence of various types of lesions and the evolution of the disease in infected leaflets, a study was performed to clarify whether there were different population types of the pathogen. Consequently, eight types of pathogens were found in monosporic isolates in lesions formed in young leaflets of different guarana plants (Duarte et al., 1995). Genetic variability of twenty isolates obtained from infected plants in guarana crops was reported (2011).

Anthracnose can restructure endophytic bacterial communities by selecting certain strains in the phyllosphere of *P. cupana* (Bogas et al., 2015). The understanding of these interactions is important for the development of strategies for biocontrol of *Colletotrichum*. Silva et al. (2018) isolated and identified endophytic fungal communities from the roots and seeds of guarana genotypes susceptible and tolerant to anthracnose that grow in two sites of the Brazilian Amazonia forest. Another study (Silva et al., 2016) isolated endophytic bacteria from guarana seeds and tested the antagonistic activity of these bacteria against *Colletotrichum gloeosporioides*. The same authors suggested that these bacteria could be applied, in the future, to increase plant growth and disease resistance to anthracnose.

Additionally, varieties of guarana are being researched by the Brazilian Agricultural Research Corporation (Embrapa), to increase production and disease resistance. Varieties resistant to

**Table 2**
Area, production and average yield of guarana in the Decade (2007–2016).

| Year | Planted area (ha) | Harvested area (ha) | Production (t) | Average yield (kg ha$^{-1}$) |
|---|---|---|---|---|
| 2007 | 13,210 | 13,144 | 3388 | 258 |
| 2008 | 15,321 | 14,904 | 3056 | 205 |
| 2009 | 15,278 | 15,271 | 4604 | 301 |
| 2010 | 13,980 | 10,552 | 3739 | 354 |
| 2011 | 14,382 | 10,989 | 4151 | 378 |
| 2012 | 13,998 | 11,489 | 3794 | 330 |
| 2013 | 13,916 | 11,269 | 3662 | 325 |
| 2014 | 13,278 | 11,348 | 3588 | 316 |
| 2015 | 11,381 | 11,361 | 3596 | 317 |
| 2016 | 15,156 | 11,848 | 3686 | 311 |

*Source*: IBGE (2017).

anthracnose (for example, cultivars BRS Marabitana and guarana BRS Saterê) were released in 2013. In the future, the production of guarana in the Amazon is expected to rise by up to 40%, without the need for increased forest deforestation. Also, a pulping machine has been developed for guarana fruits, eliminating the need for fermentation (Santos, 2014).

An alternative to reduce the use of fertilizers is to provide plants with better soil nutrient absorption conditions. Arbuscular mycorrhizal fungi (AMF) fit in this context because they increase the root absorption area of the plants, allowing them to explore the soil more efficiently, thereby, rendering them less dependent on chemical fertilizers and, simultaneously, providing greater productive capacity from soil (Bona et al., 2017). Accordingly, the AMF are an important component of the microflora in natural ecosystems. A study performed by Oliveira and Oliveira (2005) reported the seasonal dynamics of AMF in plants of *P. cupana*. The maximum mycorrhizal colonization percentages and the highest numbers of spores were reached during February and May 2000 (rainy season). Rainfall, moisture content, and soil nutrient levels were significantly and positively correlated with colonization and with the number of spores. Soil moisture content was positively correlated with the number of spores.

The domestic industry and export demands for guarana, used for various purposes, along with factors, such as the development of new strains and modernization of machinery for the processing and knowledge of endophytic microorganisms, will gradually boost guarana cultivation.

## Botanical characteristics

The official name currently accepted for guarana is *P. cupana* Kunth, Sapindaceae, according to The Plant List (2013) *Paullinia sorbilis* Mart. is also accepted as a synonym (Funk et al., 2007; Forzza, 2010). The scientific name comes from Christian Franz Paullini, a German botanist and physician who lived in the 18th century (1643–1712). He was the first to scientifically classify guarana, although the plant had already been cultivated for hundreds of years in the Brazilian Amazon (Kuri, 2008).

The guarana plant is a lowland, tropical, woody, climbing shrub, which is adapted to a hot and humid climate (Lunguinho, 2007). There are between 4 and 5 deep grooves in the main stem and the different branches. The branches are pilose at the end but glabrous at the base, measuring 4–8 mm in diameter. The skin is very dark and the woody body is simple. The leaves measure 40 cm in length and width and have partitions, in a distichous arrangement, pinnately compound, with 5 leaflets. The inflorescences are of two types including those whose branches develop in the axils of the leaves and those with branched peduncles, which develop in the tendrils (Corrêa, 1984); the inflorescences may be longer than 30 cm (de Menezes-Júnior, 1942). The flowers are partially single-sexed, zygomorphic and small, with an approximate length between 1.5 and 2 cm from the stem (Escobar et al., 1984). The fruits are ellipsoidal or spherical, apiculate capsules that are red when ripe and measure 2–3 cm (Fig. 1). They have 1 or 2 egg-shaped seeds, of approximately 12 mm in length, with an abundant aril before maturity. The seed is unevenly convex on both sides, sometimes surmounted by a short, glabrous, glossy, brown-purple, or brown-black apical tip, and it features a wide hilum, which is surrounded by a fleshy, membranous and whitish aril. The embryo has no endosperm, has a short lower root-stem axis and thick, unequal, fleshy, firm, plano-convex cotyledons (de Menezes-Júnior, 1942; Corrêa, 1984).

## Chemical aspects

Guarana is derived from the seeds of *P. cupana*, known for its stimulant properties. The seeds are the commercially useful part of the plant because of the large amounts of caffeine, theobromine, and theophylline, as well as the high concentration of tannins and other compounds, such as saponins, polysaccharides, proteins, fatty acids (Table 3) (Angelucci et al., 1978; Henman, 1982; Spoladore et al., 1987; Baumann et al., 1995; Nazaré, 1998; Ushirobira et al., 2004; Yamaguti-Sasaki et al., 2007; Higgins et al., 2010; Schimpl et al., 2014), and trace elements, such as manganese, rubidium, nickel and strontium (de Gois et al., 2016). Although the concentration of caffeine can vary widely in the preparation of guarana,

**Table 3**
Chemical composition of seeds of guarana (*Paullinia cupana*) and pharmacopoeial standards.

| Substance | Content (%) | References | Pharmacopoeia values (%) |
|---|---|---|---|
| Caffeine | 2.41–5.07 | (Henman, 1982; Spoladore et al., 1987; Baumann et al., 1995; Andrade et al., 1999; Zeidan-Chulia et al., 2013; Bittencourt et al., 2014) | >5 |
| Total tannins | 5.0–14.1 | (Marx, 1990; Ushirobira et al., 2004; Fukumasu et al., 2006a; Yamaguti-Sasaki et al., 2007) | >4 |
| Proteins | 7.0–8.0 | (Angelucci et al., 1978; Nazaré, 1998) | – |
| Polysaccharides | 30–47 | (Angelucci et al., 1978; Nazaré, 1998) | – |
| Sugars | 6.0–8.0 | (Angelucci et al., 1978) | – |
| Fibers | 3.0 | (Angelucci et al., 1978) | – |
| Fatty acids | 0.16 | (Angelucci et al., 1978) | – |
| Total ashes | 1.06–2.88 | (Angelucci et al., 1978; Mattei et al., 1998; Nazaré, 1998) | <3.0 |
| Moisture | 4.3–10.5 | (Angelucci et al., 1978; Mattei et al., 1998; Nazaré, 1998) | <9.5 |

guarana provides about 50 mg caffeine per gram. The effects of ingestion of guarana are similar to those of caffeine. However, the duration of action may be considerably different due to possible interactions between the caffeine and saponins and tannins in guarana (Babu et al., 2008).

Table 3 shows the values chemical composition established by the Brazilian Pharmacopoeia (Anvisa, 2010) for guarana samples. The main chemical constituents of guarana (Box 1 ), caffeine, theobromine, and theophylline, are designated as methylxanthines. These compounds are often classified as purine alkaloids, as a result of their remarkable biological activity, restricted distribution, as well as the structural presence of heterocyclic nitrogen. However, because of their biogenetic origins (from purine bases rather than amino acids), in addition to their amphoteric nature, methylxanthines are more accurately classified as pseudo-alkaloids (Moraes et al., 2003).

Various methods to extract and analyze the methylxanthines in guarana seeds have been reported in the literature. A study conducted by Brenelli (2003) found less than 1.4% caffeine was extracted from samples of guarana powder using the Soxhlet extraction method. Other studies have found 4.8% (Saldaña et al., 2002) and 4.1% (Mehr et al., 1996) caffeine in guarana seeds, by applying supercritical fluid extraction. Several procedures have also been described in the literature for the identification and quantification of these components (methylxanthines and total tannins) in guarana. The most common techniques used are spectrophotometry (Andrade et al., 1999; Ushirobira et al., 2004; Pelozo et al., 2008; Sousa et al., 2011; Ribeiro and Coelho, 2012; Roggia et al., 2016), Raman spectroscopy (Edwards et al., 2005), capillary electrophoresis (CZE) (Sombra et al., 2005; Kofink et al., 2007), high-performance liquid chromatography (HPLC) (Marx and Maia, 1990; Ushirobira et al., 2004; Klein et al., 2012), and ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry UPLC-MS (da Silva et al., 2017).

As above-mentioned, caffeine, a 1,3,7-trimethylxanthine, is found in large quantities in guarana seeds. The "Instituto Agronômico de São Paulo" (Agronomical Institute of São Paulo), in Brazil, conducted a study to characterize the caffeine content in the tegument, kernel, and seeds of forty mother plants of guarana. They found mean caffeine levels of 2.33% in the kernel, 1.09% in the tegument, and 2.15% in the integral seed (Spoladore et al., 1987). The composition of various commercial samples of guarana seeds has been analyzed by gas chromatography (GC). A caffeine content ranging from 2.41 to 4.07% was documented (Pagliarussi et al., 2002), which was below the minimum required concentration of caffeine (5%) by the Brazilian Pharmacopoeia (Anvisa, 2010). Conversely, studies using spectrophotometric methods reported 4.88–6.20% methylxanthines and 3.0–5.5% total tannins (Ushirobira et al., 2004; Yamaguti-Sasaki et al., 2007; Sousa et al., 2011). This difference is probably due to a harvest performed without standardized procedures, at various times, a collection of immature fruits, and, mainly, different drying procedures. For example, different levels of methylxanthines and tannins associated with different methods of seed drying were shown (Ushirobira et al., 2004).

Methylxanthines and catechins were identified in various preparations containing guarana including dried seeds, powders, tablets and capsules (Carlson and Thompson, 1998). The content of purine alkaloids in three samples of guarana and 39 commercial products was investigated (Meurer-Grimes et al., 1998). These authors found that the majority of the samples of guarana showed caffeine (2.95–5.12%) as the main alkaloid, with traces of theobromine and theophylline. Another study, with the objective of determining the caffeine levels in various brands of commercially available guarana powder, reported a wide variation in the levels of caffeine, ranging from 0.95 to 3.67% (Tfouni et al., 2007). Sousa et al. (2010) determined 3.95% caffeine and 0.87% tannins in guarana seeds. This variability among literature studies is possibly due to differences in the origin of the raw material, as well as genetic and environmental aspects, and the drying process that the raw materials have undergone.

The guarana derivatives by CZE and HPLC analysis were compared (Sombra et al., 2005). The caffeine results were similar to those of previous studies, ranging from 1.3 to 3.3%. Chiral CZE has been used to separate the enantiomers, catechin and *ent*-catechin, and epicatechin and *ent*-epicatechin, in guarana samples (Kofink et al., 2007).

A study on the composition of the fraction of tannins present in samples of guarana seeds showed that the total tannin content was considerably high, at around 12–14.1% of the dry matter, consisting mainly of condensed tannins, such as proanthocyanidins (10.7%), catechin (5.98%) and epicatechin (3.78%) (Marx, 1990). In addition to the studies cited above, which have quantified the constituents in the herbal drugs, others have analyzed guarana seed extracts. A chemical evaluation of the semipurified extract of guarana seeds showed the presence of caffeine, catechin, epicatechin, and procyanidins B2, B3, and B4 (Ushirobira et al., 2007), as well as *ent*-epicatechin, and procyanidins A2 and C1 (Yamaguti-Sasaki et al., 2007), by means of nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (Box 1).

Some of the constituents of the previous study (Ushirobira et al., 2007) were identified and quantified (Klein et al., 2012). These authors reported that the semipurified guarana extract (mg/EPA) contained condensed tannins, such as 180.75 μg catechin, 278.875 μg epicatechin, and 300.875 μg caffeine. The method of micellar electrokinetic chromatography has been recently developed for separation and validation of EPA. It has proved to be efficient for the chiral separation of catechin, epicatechin, procyanidins B1, B2, and B4, as well as caffeine (Mello and Ito, 2012).

Other studies, using both aqueous extracts (Barbosa and Mello, 2004; Campos et al., 2011) and hydroalcoholic extracts (Lima et al., 2005; Bittencourt et al., 2013; Portella et al., 2013) revealed quantities of constituents similar. The caffeine ranged from 1.2 to 7.97% and the tannins from 1.5 to 12%. These differences are probably due to genetic and environmental factors, as well as the extraction conditions.

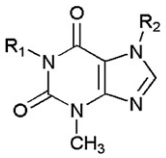
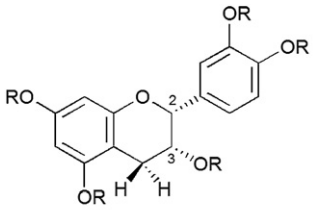
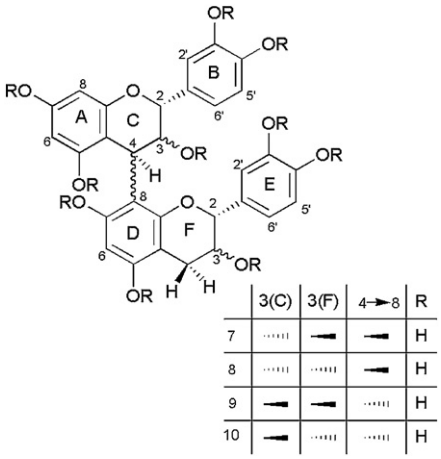
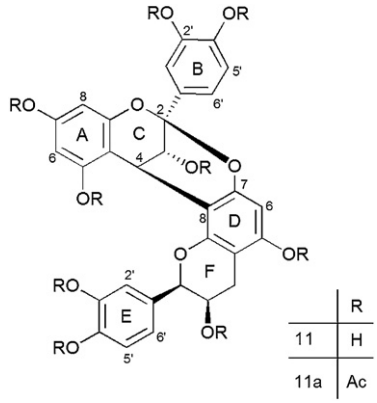
Guarana seeds also contain acylglycerol and cyanolipids, a class of lipids found in some families, for example, Sapindaceae and Boraginaceae. The chemical composition of the total oil extracted from guarana seeds has indicated the presence of cyanolipids (3%) and acylglycerols (28%). NMR analysis indicated the presence of type I cyanolipids, *cis*-vaccenic acid (30.4%) and *cis*-11-eicosenoic acid (38.7%), as the main fatty acids. Paullinic acid (7.0%) was also an abundant component and oleic acid (37.4%) was the predominant fatty acid in the acyl chain of the acylglycerols (Avato et al., 2003). The presence of high molecular weight polysaccharides in samples of guarana was investigated (Dalonso and Petkowicz, 2012). Pectin and a group of polysaccharides called hemicelluloses, such as xylans were identified in these samples.

Guarana methylxanthines have been extensively studied over the years. However, for many other classes of compounds, with possibly interesting pharmacological effects, investigations have been scarce. Examples include the saponins and the fatty acid types. Also, although tannins have been isolated from guarana, there is still much to be explored about this class of substances.

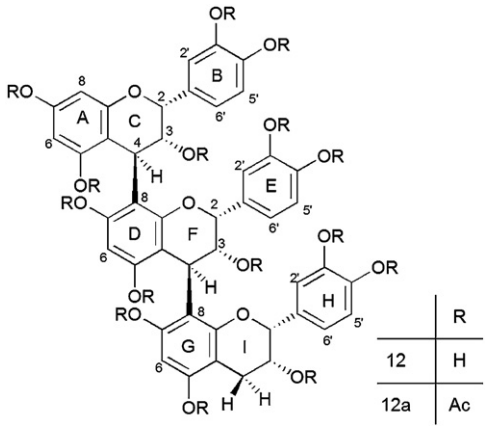
## Pharmacological properties

Certain plant and herbal products, sold as food supplements, are popular medicines that are often perceived as safe, namely, non-toxic. This is not necessarily true, particularly if taken with prescription drugs, over-the-counter medicines or used in combi-

**Box 1: Identification of the main chemical constituents in samples of guarana.**

| Name of substance | Chemical structure | References |
|---|---|---|
| *Methylxanthines*<br>Caffeine (**1**)<br>(1,3,7-Trimethylxanthine)<br>Theobromine (**2**)<br>(3,7-dimethylxanthine)<br>Theophylline (**3**)<br>(1,3-dimethylxanthine) |  <br>R1 = CH3, R2 = CH3 (1)<br>R1 = H, R2 = CH3 (2)<br>R1 = CH3, R2 = H (3) | (Marx and Maia, 1990; Baumann et al., 1995; Meurer-Grimes et al., 1998; Andrade et al., 1999; Pagliarussi et al., 2002; Weckerle et al., 2003; Ushirobira et al., 2004; Sombra et al., 2005; Pagliarussi et al., 2006; Tfouni et al., 2007; Ushirobira et al., 2007; Yamaguti-Sasaki et al., 2007; Sousa et al., 2010; Klein et al., 2012; Bittencourt et al., 2013; Schimpl et al., 2014) |
| **Tannins**<br>*Condensed tannins*<br>Epicatechin (**4**)<br>Catechin (**5**)<br>*ent*-epicatechin (**6**) |  <br>2 = 3 = R = H (4)<br>2 = R = H, 3 = OR (5)<br>2 = 3 = OR (6) | (Marx, 1990; Ushirobira et al., 2004; Kofink et al., 2007; Ushirobira et al., 2007; Yamaguti-Sasaki et al., 2007; Sousa et al., 2010; Klein et al., 2012; da Silva et al., 2017) |
| Procyanidin B1 (**7**)<br>Procyanidin B2 (**8**)<br>Procyanidin B3 (**9**)<br>Procyanidin B4 (**10**) |  <br>3(C) = 3(F) = 4→8 = R = H (7)<br>3(C) = 3(F) = R = H, 4→8 = OR (8)<br>3(C) = 4→8 = R = H, 3(F) = OR (9)<br>3(C) = R = H, 3(F) = 4→8 = OR (10) | (Ushirobira et al., 2004; Ushirobira et al., 2007; Yamaguti-Sasaki et al., 2007; Klein et al., 2012; da Silva et al., 2017) |
| Procyanidin A2 (**11**) |  <br>R = H (11)<br>R = Ac (11a) | (Yamaguti-Sasaki et al., 2007; da Silva et al., 2017) |

Procyanidin C1 (**12**)



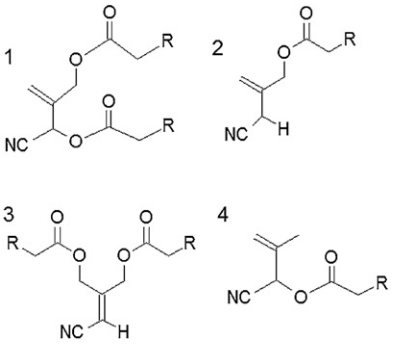

| | R |
|---|---|
| 12 | H |
| 12a | Ac |

(Yamaguti-Sasaki et al., 2007; da Silva et al., 2017)

*Cyanolipids and acylglycerols*

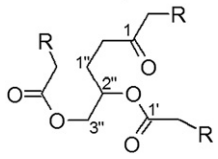
Cyanolipids

1.1-cyano-2-hydroxymethylprop-2-en-1-ol-diester
2.1-cyano-2-methylprop-1-en-3-ol-ester
3.1-cyano-2-hydroxymethylprop-1-en-3-ol-ester
4.1-cyano-2-methylprop-2-en-1-ol-ester




(Avato et al., 2003)

Acylglycerols




(Avato et al., 2003)

nation with other herbs. Moreover, they may have adverse side effects, such as stimulation and hallucinogenic properties (Carlini, 2003). Nevertheless, such products are readily available and have widespread use. A wide variety of dietary supplements for weight loss are marketed with claims of efficacy (Andersen and Fogh, 2001; Armstrong et al., 2001; Boozer et al., 2001; Opala et al., 2006; Onakpoya and Ernst, 2012). The lack of data on the toxicity and/or efficacy of many ingredients found in these products, even the predominant ingredients, is a cause for concern (Baghkhani and Jafari, 2002; Moaddeb et al., 2011; Lude et al., 2016).

Guarana powder is a product easily available both in natural product stores and over the internet. It is either marketed alone or in combination with other herbal drugs, creating the likelihood of additive or synergistic effects (Spinella, 2001). It is also included in a variety of energy drinks. The latter are easily found in gyms and supermarkets but they contain stimulants and/or additives. Guarana has also gained popularity because it is regarded as a "functional food". Guarana seeds, as above-mentioned, contain large amounts of caffeine (40–80 mg caffeine per gram guarana extract), as well as minor amounts of the related compounds, theobromine and theophylline (Henman, 1982), which are stimulating substances (Mottram and Chester, 2015). The drink with the highest natural content of caffeine in the world is made from toasted guarana seeds, possessing at least 5% methylxanthines, expressed as caffeine (Prance and Nesbitt, 2005; Anvisa, 2010). Long-term intake of the various components of these energy drinks can result in significant changes in the cardiovascular system (Higgins et al., 2010), and even convulsions (Iyadurai and Chung, 2007). When guarana is added to energy drinks, it increases the amount of metabolized caffeine (McGuire, 2014). A series of adverse events are associated with the consumption of guarana including irritability, heart palpitations, anxiety, disorders of the central nervous system and myoglobinuria (Galduróz and Carlini, 1994; Donadio et al., 2000; Haller et al., 2005; Pittler et al., 2005; Sharpe et al., 2006; Richardson et al., 2007). Guarana can also exacerbate epileptic seizures, lowering the seizure threshold or increasing the duration of seizures (Spinella, 2001). Despite these reports, when taken alone, guarana has few adverse effects and the majority of them are similar to those observed after the consumption of products containing a high caffeine content (Ravi Subbiah, 2005). The daily dose of caffeine recognized as safe for adults is 400 mg (Nawrot et al., 2003).

Aphrodisiac effects in rabbits were reported after administration of a combination of commercially available plant extracts containing guarana (Antunes et al., 2001). In another study (de Aquino et al., 2016), male Mediterranean fruit flies were fed diets containing guarana powder (3%). These flies are pests of global importance for horticulture that can be controlled by the sterile insect technique (SIT), which depends on the sexual performance of lab-reared males when they are released into the field. The experiments indicated that the males fed diets enriched with guarana showed greater success in mating, representing a new and viable option to increase the efficiency of SIT (de Aquino et al., 2016).

Guarana was placed alongside the plants with psychoanaleptic activity (stimulants), with emphasis on anorexigenic or weight reduction properties. Although the consumption of guarana can induce changes in lipid metabolism, these effects have been associated with the methylxanthine content of the extract (Lima et al., 2005). Guaraná showed anti-adipogenic potential due to its ability to modulate miRNAs and genes related to this process (Lima et al., 2017) or an increase in energetic metabolism and stimulation of mitochondrial biogenesis, contributing to control of weight gain, even when associated with high-fat diet (Lima et al., 2018).

Preparations containing guarana in association with other herbal drugs, are widely used for weight loss in humans (Andersen and Fogh, 2001; Boozer et al., 2001; Bérubé-Parent et al., 2005; Opala et al., 2006; Ruxton et al., 2007; Bulku et al., 2010), with positive results. As a result of its methylxanthine content, the guarana extract can block adenosine and phosphodiesterase inhibitors, thereby, increasing noradrenaline activity (Carlini, 2003). Considering the effects of caffeine on blood pressure elevation, guarana should be avoided by hypertensive individuals. It is also strongly recommended that the combination of guarana and supplements containing ephedra, such as Ma Hung and products with ephedrine alkaloids, should be avoided because it can increase the risk of myocardial infarction and sudden death (Boozer et al., 2001; Nyska et al., 2005). Also, antiarrhythmic medications, such as amiodarone, should not be consumed with guarana because such an association may decrease plasma amiodarone concentration, particularly in the heart (Rodrigues et al., 2012).

Literature studies have associated guarana with an impressive array of pharmacological functions (Box ). For instance, guarana has gastroprotective properties and offers much better therapeutic benefits than caffeine in gastrointestinal disorders (Campos et al., 2003). A hepatoprotective effect of guarana powder seeds was demonstrated in rats (Kober et al., 2016). Other studies have highlighted the cytoprotective effects of guarana (de Oliveira et al., 2002; Freitas et al., 2007; Leite et al., 2011, 2013; Oliveira et al., 2011; Bonadiman et al., 2017) including neuroprotection (Bittencourt et al., 2014) and acetylcholinesterase inhibition (Trevisan and Macedo, 2003). Consequently, guarana has been suggested as a promising source of phytochemicals that can be used as an adjuvant therapy in the management of cognitive disorders, such as Alzheimer's disease (Bittencourt et al., 2014; Ruchel et al., 2017), although this disease has multiple etiologies. Although some authors (Mingori et al., 2017) suggest that ingestion of guarana powder (21 mg of guarana powder (body weight, kg/day)) in middle-aged male Wistar rats does not improve cognitive development, they claim that this treatment can modify the machinery of oxidative stress and the neurodegenerative-signaling pathway by inhibiting pro-survival in the hippocampus and striatum. These results may contribute to the development of unfavorable microenvironments in the brain and neurodegenerative disorders.

Additionally, the aqueous solution of guarana seed powder has shown antigenotoxic activity in animals chemically treated to induce DNA damage (Fukumasu et al., 2006a; Kober et al., 2016).

The dry extract of guarana (ESG) has been used in topical formulations for the prevention and treatment of gynoid lipodystrophy because it increases the number of blood vessels in the dermis when used at 50% (Chorilli et al., 2004). The simultaneous transdermal delivery of the main substances present in guarana extracts was established, with penetration rates being highly dependent on the concentration and the vehicle (Heard et al., 2006).

Studies in our group have shown that the crude and semipurified extracts of guarana have antidepressant effects in chronic treatment that are comparable to the antidepressant, imipramine (Audi and Mello, 2000). These effects cannot be linked to the methylxanthines because the results found with this substance alone are different from those found in the administration of the extracts (Otobone et al., 2007). Thus, some condensed tannins, which were isolated from the semipurified fraction, could be responsible for this activity because they can cross the blood-brain barrier and thereby act on the central nervous system (Youdim et al., 2004). It is suggested that a mechanism other than the adenosine receptor antagonist, cyclopentyl adenosine (CPA), is involved in the antidepressant activity of guarana, because it is due to caffeine, rather than guarana (Campos et al., 2005).

The efficacy of guarana extract against chemotherapy-induced fatigue and depression symptoms in patients with solid tumors (Miranda et al., 2008) and at post-radiation (Miranda et al., 2009), revealed that in both cases, the patients showed no significant difference in the effects of fatigue and depression compared to the control group. However, in two other studies conducted by different research groups, the standardized guarana extract proved to be effective, with low toxicity, in the treatment of chemotherapy-related fatigue in patients with breast cancer (Campos et al., 2011) and in patients with solid tumors (del Giglio et al., 2013).

Guarana contains various substances, for example, methylxanthines and polyphenols with chemopreventive and antineoplastic properties. Thus, guarana may act as a chemopreventive agent in carcinogenesis, demonstrating a potentially valuable health benefit. It can reduce cellular expansion of neoplastic cells, decrease the incidence and multiplicity of macroscopic lesions, and reduce the proliferation of tumor cells and increase tumor cell apoptosis, consequently, reducing the area of the tumor (Fukumasu et al., 2006b, 2008, 2011; Mingori et al., 2017).

A study conducted to monitor the acute effects of guarana powder (2.10% caffeine; 16% tannin) on cognition, anxiety and sleep in normal volunteers had negative results, suggesting the need for studies with chronic treatments (Galduróz and Carlini, 1994). In another study that assessed the chronic administration of guarana on cognition in 15 normal elderly volunteers, no significant changes were observed (Galduróz and Carlini, 1996). However, the authors explained the negative results could have been due to the insufficient treatment duration (150 d) or, moreover, that the tests used were not sensitive enough to detect the expected cognitive alterations (Galduróz and Carlini, 1996).

Guarana has been used for a long time by the Indians as a stimulant and this effect is greatly associated with the presence of a large amount of caffeine in guarana seeds as above-mentioned. The psychoactive properties of the guarana extract were first observed by Kennedy et al. (2004). The results showed that doses of guarana (75 mg) and ginseng alone, or the combination of the two plants, led to an improvement in cognitive performance in humans. Nevertheless, in a study by the same research group, the effects of various doses of guarana (37.5, 75, 150, and 300 mg) were evaluated for the first time in humans and the same result was found, mainly at the lower doses, at a maximum of 4.5–8.4 mg caffeine (Haskell et al., 2007). Both studies used a hydroalcoholic extract of guarana prepared by exhaustive percolation (Kennedy et al., 2004; Haskell et al., 2007). The authors suggested that the improvement in cognitive performance promoted by the standardized guarana extract could not be attributed solely to the caffeine content (11–12%) because at the lower doses, the level of caffeine was not considered to be sufficient to produce positive effects (Kennedy et al., 2004; Haskell et al., 2007). Improvement in cognitive performance was also obtained by investigating the influence of mouth rinsing of guarana and ginseng ($0.4\,g\,25\,ml^{-1}$) (Pomportes et al., 2017).

Another study also showed positive results at a low dose ($0.3\,mg$ guarana $ml^{-1}$), which contained $6.2\,\mu g\,ml^{-1}$ caffeine (a quantity around 16 times lower than the caffeine used as a reference drug) (Espinola et al., 1997). Better results were found for cognitive capacity, including stability in the parasympathetic modulation in individuals who consumed a vitamin-mineral-guarana extract supplement, compared with the equivalent dose of caffeine

**Box 2**
Pharmacological activities of the seeds of *P. cupana* or their associations described in the literature.

| Pharmacological activity | Details of the extract or dosage form | *In vivo*/*in vitro*/*ex vivo* test | Objective | Control used | Dose tested for drugs | Findings | Ref. |
|---|---|---|---|---|---|---|---|
| 1. Tonic action | Aqueous extract (40 °C, 2 h, 2×) | *In vivo*: blood samples from mice | To evaluate the tonic action of guarana on normal mice and trained mice on and epinephrine-induced glycogenolytics | Control: without treatment | 20, 100, and 500 mg $kg^{-1}$ | Aqueous extract with suppressive activity of exercise-induced hypoglycemia in mice, which confirms its traditional use as a tonic | (Miura et al., 1998) |
| 2. Controlling hot flushes | It is not clear. The extract contained 7.97% caffeine and 1.47% tannins | *In vivo*: women | To assess whether guarana decreases the number and severity of hot flushes in women after diagnosis of breast cancer | Placebo | 50 mg of dry extract use by mouth twice a day for 6 weeks | There was a reduction in the number and severity of hot flushes | (Oliveira et al., 2013) |
| 3. Relaxation of corpora cavernosa (aphrodisiac effect) | Association: Catuama®. Mixture of hydroalcoholic extracts of 4 plants: 5% guarana, 1% *Zingiber officinalis* (ginger), 5% *Trichiliacatigua* (catuaba), and 5% *Ptychopetalum olacoides* (muirapuama). Ethanol extracts (1:1) were also extracted for 7 days at 25 °C for each plant. | *Ex vivo*: isolated corpus cavernosum of rabbits | To investigate the effects of Catuama® and its constituent plants isolated in the tissue of the corpora cavernosa of rabbits using a cascade bioassay | Positive control: acetylcholine (0.6 nmol) or glyceryl trinitrate (1.3 nmol) | Bolus injections of Catuama®: 1, 3 and 10 mg, and individual doses of guarana (0.5–5 mg), *Z. officinalis* (1–10 mg), *P. olacoides* (2–20 mg) and *T. catigua* (1–10 mg) | Catuama® caused short-term and dose-dependent relaxation. Out of the 4 extracts tested individually, the guarana plant was the most effective, indicating that it is the main responsible factor for the effect of relaxation of the corpora cavernosa of rabbits, allegedly attributed to Catuama® | (Antunes et al., 2001) |
| 4. Weight loss and delay in gastric emptying | Association: preparation of extracts of herbal drugs (YGD) (capsules), containing: 112 mg yerba mate, 95 mg guarana, and 36 mg of damiana, administered with 420 ml of apple juice | *In vivo*: humans | To determine delayed gastric emptying and weight loss over 10 and 45 days and weight maintenance over 12 months | Placebo capsules (lactose) administered with 420 ml of apple juice | 3 capsules of YGD per day | There was a significant delay in gastric emptying; it reduced the time of perception of gastric fullness and led to significant weight loss over 45 days, in overweight patients treated in a context of primary health care | (Andersen and Fogh, 2001) |
| 5. Weight loss | Association: commercial mixture Metabolife-356® containing Ma Huang and guarana as main active ingredients. Each tablet contained 12 mg ephedrine alkaloids and 40 mg caffeine | *In vivo*: humans (25–55 years) with BMI ≥ 29 and ≤35 kg $m^{-2}$ | To examine, in overweight people, short-term safety and effectiveness in weight loss, of a herbal supplement that contains Ma Huang, guarana and other ingredients | Placebo: a tablet identical in appearance, but without the mixture of plants | 2 tablets, 30 min before meals, 3 times per day for 2 months. Daily: 72 mg – ephedrine alkaloids; 240 mg – caffeine | The study reported weight loss, fat loss, reduction of waist circumference and hip circumference and of triglyceride levels; there were adverse effects with potential risks (self-reported palpitations, increase of serum glucose and transient increases in systolic blood pressure) | (Boozer et al., 2001) |
| 6. Weight loss | Association: the 4 mixtures (capsules) contained varying doses of green tea (in which epigallocatechin-3-gallate (EGCG) represented 45% of the dry weight) and white tea (with a fixed dose of caffeine). They also contained unknown amounts of catechins | *In vivo*: men (20–50 years) with BMI between 20 and 27 kg $m^{-2}$ | To compare the effect of the mixture of extracts of green tea and guarana (fixed dose of caffeine and variable doses of EGCG), within 24 h, on energy expenditure and fat oxidation. To determine if there is a dose-dependent effect of EGCG and, if so, what dose produces better effect without inducing significant cardio stimulation | Placebo: cellulose | 3 capsules per day. Each capsule has a fixed dose of caffeine (200 mg) and variable amounts of EGCG (90, 200, 300, and 400 mg) | The mixture EGCG and caffeine must be considered as a good complement to a weight loss program and has potential for the treatment of obesity. Some authors suggest that a dose of 90 mg of EGCG (3 times a day) represents the optimum concentration to produce an effect on nutrient oxidation | (Bérubé-Parent et al., 2005) |

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| 7. Ergogenic effect and "fat-burning" effect | Two extracts were tested: A. Extract obtained by percolation into ethanol:water solvent (6.6:3.4 v/v), with 0.153 g $g^{-1}$ of caffeine. B. Extract decaffeinated by extraction with chloroform (tannins and catechins were not removed) | *In vivo*: Wistar rats | To evaluate the effect of supplementation with guarana (14 days) on aspects of lipid metabolism in rats with a sedentary lifestyle and trained rats | Non-supplemented sedentary and trained rats | 0.130 and 0.325 g $kg^{-1}$-dry extract per BW (body weight) | Intake of guarana is able to induce alterations in lipid metabolism, because of the methylxanthine content of the extract | (Lima et al., 2005) |
| 8. Weight loss and change in body composition | Association of 2 tablets A. Tablets with extracts of asparagus, green tea, black tea, guarana, yerba mate and purple beans; B. extracts of purple pods, *Garcinia cambogia* and chromium in the form of yeast | *In vivo*: humans (21–55 years, with BMI between 25.2 and 39.6 kg $m^{-2}$) | To evaluate the efficacy and safety of plant extracts for weight reduction and changes in body composition | Placebo | Two tablet per meal, at two main meals. Tablet A, 1 h before a meal Tablet B, half an hour after a meal | Reduction of fat and increase in lean body mass. There were no significant differences in weight and BMI measurements | (Opala et al., 2006) |
| 9. Ergogenic and "fat burning" effect | Two extracts were tested: A. Extract obtained by percolation in ethanol: water (6.6:3.4, v/v), with caffeine content of 0.153 g $g^{-1}$ of extract. B. Decaffeinated extract by extraction with chloroform (eliminated all the methylxantines; tannins and catechins were not removed) | *In vivo:* male Wistar rats | To evaluate the effect of guarana (14 days) supplementation on aspects of lipid metabolism in sedentary and trained rats | Sedentary and trained rats not supplemented | 0.130 and 0.325 g $kg^{-1}$ (dry extract per BW) | The consumption of guarana is able to induce changes in lipid metabolism, but the predominant element seems to be the methylxanthine content of the extract | (Lima et al., 2005) |
| 10. Weight loss | Association: Zotrim®, tablets containing extracts of yerba mate, guarana and damiana (YGD). Each tablet of YGD contains: guarana (95 mg); yerba mate (112 mg); damiana (35 mg), with a total of approximately 11.2 mg caffeine | *In vivo*: humans | To evaluate the effect of the administration of YGD on the decrease in weight, BMI, waist circumference, hunger and satiety, in a group of health professionals | Placebo: lactose tablet | 2 tablets 15 min before meals for 1 week; then increasing to 3 tablets 15 min before meals for 5 weeks. Total: 6 weeks, 3 meals per day | Significant reduction in self-reported weight, waist circumference and hip circumference. 22% of individuals had clinically significant weight loss | (Ruxton et al., 2007) |
| 11. Weight loss and antioxidant activity | Association: extract powders of *Salvia officinalis*, *Camellia sinensis*, guarana, and two vitamins (thiamine and niacin) (STG). The amount of each component was not informed | *In vivo*: Fischer rats-344 | To test a new diet that provides nutritional support for speeding up metabolism and maintaining healthy weight and energy, as well as to evaluate the safety and efficacy of STG | Control: Group 1 that received normal feed | Group 2: 1× STG; Group 3: 7× STG; 1×: normal feed with 192 mg of STG per kg | The administration of STG has not reduced weight gain drastically. However, it has helped to maintain healthy body weight as well as antioxidant capacity of vital target organs (liver, heart and kidneys) | (Bulku et al., 2010) |
| 12. Anti-adipogenic effect | Guarana: 2.42% of flavonoids, 9.18% of total phenolics and 12.4% of caffeine | *In vitro:* 3T3-L1 cell line | To evaluate the effects of guarana on genes and miRNAs related to adipogenesis in 3T3L1 cells | Control: without treatment | 50, 100, 150, 200 and 300 μg $ml^{-1}$ | The results showed that guarana modulates the expression of several genes and miRNAs associated with adipogenesis, as well as an increase of β-catenin nuclear translocation, which might contribute to adipogenesis inhibition. The effect of guarana on the reduction of triglycerides was dose dependent of 100–300 μg $ml^{-1}$ (12%, 20%, 24% and 40%, respectively) | (Lima et al., 2017) |

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| 13. Protection against gastric lesions | Dried seed extract. There is no information about the method of extract preparation, neither about the quantification of the main components | *In vivo*: rats and mice | To analyze the effects of the guarana extract on gastric lesions induced by indomethacin and ethanol, gastric secretion and gastrointestinal transit and to compare them with the effects of pure caffeine | Control: tap water | Guarana extract 50 and 100 mg $kg^{-1}$ Caffeine 20 and 30 mg $kg^{-1}$ | Guarana seems to offer far better therapeutic benefits than caffeine in gastrointestinal disorders | (Campos et al., 2003) |
| 14. Hepatoprotective effect | Guarana seed powder diluted in water (12.240 mg $g^{-1}$ of caffeine, 6.733 mg $g^{-1}$ of theobromine, 4.336 mg $g^{-1}$ of total catechins and 16 mg $g^{-1}$ of condensed tannins) | *In vivo*: male Wistar rats | To evaluate the hepatoprotective effect of guarana seed powder on $CCl_4$-induced liver injury (carbon tetrachloride) in rats | Hepatoprotective agent: silymarin (100 mg $kg^{-1}$). $CCl_4$ (1 ml $kg^{-1}$, 50 percent $CCl_4$ in olive oil) for induction of liver toxicity. Control: water | 100, 300, and 600 mg $kg^{-1}$, daily for 14 days | The results indicate that guarana has hepatoprotective activity in $CCl_4$-induced liver injury in rats, preventing the break of strands of cellular DNA | (Kober et al., 2016) |
| 15. Antiallergic effect | Hydroethanolic extract 30% (reflux for 2 h). The dry extract was dissolved in DMSO and diluted in saline or buffer | *In vivo*: mice | To investigate the effects of the hydroethanolic extract of guarana seeds (GSE) on the increase of IgE-stimulated vascular permeability and the effects on IgE-induced mast cell degranulation | Control: saline | 0.1, 0.3, or 1.0 g $kg^{-1}$ | GSE administered orally inhibited the reaction of anti-dinitrophenol IgE-induced passive cutaneous anaphylaxis. GSE also inhibited β-hexisosaminidase release in RBL-2H3 cells induced by IgE receptor-mediated pathways. These results indicate that GSE had an inhibitory effect on the allergic reaction and may have therapeutic application in inflammatory allergic diseases | (Jippo et al., 2009) |
| 16. Immunomodulatory activity | Crude extract (CE) of guarana was prepared using acetone:water (7:3, v/v). The CE was partitioned with ethyl acetate, and removed the organic solvent to yield the EAF. | *In vitro:* splenocytes and cytokines | To evaluate immunomodulatory activity of guarana seeds crude extract (CE) and ethyl-acetate fraction (EAF) | Untreated cells and cells treated with methylprednisolone (100 μM) | CE and EAF: 5, 10, 50, and 100 μg $ml^{-1}$ | All cytokines evaluated had their levels reduced after treatment, following dose-response model | (Carvalho et al., 2016) |
| 17. Antagonist action | Aqueous extract of *P. cupana* and 4 TLC-separated fractions | *In vitro*: blood of humans and rabbits and *In vivo*: rabbits | To evaluate the antagonist action of guarana extract in platelet aggregation induced by (adenosine diphosphate) or arachidonate, but not by collagen | Control: without treatment | There are no reports of the concentrations used for the study, only the volume used for the *in vitro* and *in vivo* tests | Extracts of guarana inhibit platelet aggregation in rabbits, after administration both intravenously and orally | (Bydlowski et al., 1988) |
| 18. Antagonist action | Aqueous extract of *P. cupana* and 4 TLC-separated fractions | *In vitro*: blood of rabbits | To evaluate the effect of guarana extract on platelet aggregation by studying its effects on platelet synthesis of thromboxane in rabbits | Control: no treatment | 100 mg $ml^{-1}$ | Guarana has an antiplatelet action and this may be partly due to reduced thromboxane synthesis. The authors suggest that one of the fractions containing mainly caffeine would be partly responsible for this action, as well as other compounds that may be present | (Bydlowski et al., 1991) |

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| 19. Effect on blood vessels in the papillary dermis | Dry guarana extract (ESG) added to self-emulsifying bases. There is no information about the method of extract preparation | *In vivo*: Wistar rats (topical use) | To study the effects of the emulsion with different concentrations of extract, mixed with two chemicals promoters of cutaneous permeation (oleic acid or isopropyl myristate), on the blood vessels in the papillary dermis of rats | The control group did not receive formulation | ESG 20% or 50% | The rats were not good experimental models to study hypodermis.<br>DGE 20% did not cause significant changes in the papillary dermis of rats. DGE 50% increased cutaneous microcirculation, and the chemical promoters of absorption did not potentiate the effects | (Chorilli et al., 2004) |
| 20. Cognitive effect and muscle strength | Guarana powder added to a complex consisting of creatine, dispersed in 500 ml of water | *In vivo*: humans | To compare the effects of ingestion of creatine + guarana (G+CRE) supplement on muscle strength and cognitive performance | Placebo: sugar-free, flavored carbonated water | Complex consisting of: 1 g of creatine; 1.5 g guarana; 150 mg of taurine; 133 mg of caffeine; 120 mg L-glutamine; 106.7 mg of vitamin C; 100 mg L-arginine and 1.1 mg of vitamin B1. Ingested in two doses, 60 and 30 min before exercise | The creatine + guarana supplement seems to have a beneficial effect on muscle strength and cognitive performance for decision-making. Thus, it may be interesting to improve the performance of athletes in sports with high cognitive constraints | (Pomportes et al., 2015b) |
| 21. Cognitive and mood effects | *Berocca® boost*, multivitamin and mineral salts with 222.2 mg guarana (40 mg caffeine per tablet) | *In vivo:* humans | To investigate the impact of Berocca® Boost consumed before exercise on cognitive performance and mood measured before and after exercise, and substrate metabolism | Placebo | The effervescent tablet for each group (supplement with and without guarana) was randomly distributed. It was dissolved in 250 ml of water | The consumption of a complex of vitamins and minerals containing guarana before exercise can positively impact the performance of posterior memory and reduce effort during moderate intensity exercise in active men | (Veasey et al., 2015) |
| 22. Improvement of overall performance of the body | Suspension of guarana seed powder of *P. cupana* in water: Tween-80. The powder contained 2.1% caffeine and 16% tannins | *In vivo*: Swiss mice and Wistar rats | To evaluate the action of guarana on the overall performance of the body *in vivo*: physical performance (forced swimming), learning and memory (active and passive avoidance) and Lashley maze III and longevity tests. To compare the effects of anti-fatigue with ginseng | Control suspension: water/Tween 80. Drug Reference: caffeine ($0.1\ mg\ ml^{-1}$) | Guarana: 0.3 and $3.0\ mg\ ml^{-1}$; Ginseng: $5\ mg\ ml^{-1}$ | The animals treated with $0.3\ mg\ ml^{-1}$ of guarana showed improved physical performance. It was useful for maintenance of previously acquired memory | (Espinola et al., 1997) |
| 23. Cognitive effect | Guarana powder (capsules). The powder contained 2.1% caffeine and 16% tannins | *In vivo*: humans (above 60 years) | To evaluate the effects of long-term administration of guarana on cognition of normal elderly volunteers | Placebo: brown sugar capsules.<br>Drug reference: caffeine (12.5 mg) | Two guarana capsules per day (500 mg each) for 5 months | There were no cognitive differences in volunteers. The length of treatment may have been insufficient and the neuropsychological tests employed were not sensitive enough to test the expected changes | (Galduróz and Carlini, 1996) |

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| 24. Improvement in cognitive performance | Ethanolic dry guarana extract; *Panax ginseng*, and their combination. Both standardized. Ginseng: exhaustive percolation (40% Ethanol-60% water at temperatures <40 °C), 4% ginsenolides. Guarana: exhaustive percolation (50% ethanol-50% water at temperatures <50 °C), methylxanthines (11–13%) | *In vivo*: humans | To analyze the cognitive effects and mood in the treatment with guarana. To evaluate the potential for additive effects or synergistic effects after the common combination, available commercially, of guarana with *Panax ginseng* | Gelatin capsules: without plant extracts | 2 capsules per day: 75 mg of guarana (≈12% caffeine), 200 mg of ginseng, or a combination of them (75 mg 200 $mg^{-1}$) | Single doses of either one (guarana and ginseng), and a combination of both of them, improved cognitive performance in comparison with the placebo in young and healthy subjects | (Kennedy et al., 2004) |
| 25. Cognitive effects | Lyophilized crude extract (EBPC) and the semipurified constituents (EPA and EPB). There is no information about the extraction methodology | *In vivo*: Male Wistar rats | To investigate the effects of chronic treatment of EBPC or EPA and EPB of guarana seeds in the cognitive behavior of rats | Control, Caffeine (10.0 mg $kg^{-1}$) or scopolamine (2.0 mg $kg^{-1}$). Control: treated with NaCl 0.9% and 0.2% Tween 80) | EBPC (30.0 or 60.0 mg $kg^{-1}$) and EPA (2.0 or 4.0 mg $kg^{-1}$), and EPB (2.0 or 4.0 mg $kg^{-1}$) | EPBC and EPA of guarana seed extracts were active by oral administration and showed significant nootropic effect. The chronic treatment showed the same increase in body weight and average life time, indicating low toxicity of the extracts | (Otobone et al., 2005) |
| 26. Cognitive effect | Standardized extract (Kennedy et al., 2004) of guarana seeds (PC-102) (11–12% caffeine) | *In vivo*: humans | To assess the acute effects of dose-dependent behavior of guarana extract | Placebo: without the guarana extract | 1 capsule per day which contains: 37.5 mg, 75 mg, 150 mg, or 300 mg of a guarana extract | Guarana has improved the performance of secondary memory and increased the alert and mood ratings. The two lower doses produced more positive cognitive effects that the higher doses | (Haskell et al., 2007) |
| 27. Cognitive performance, mood, and functional activation of the brain | Used 2 commercially available supplements: (A) Berocca® boost, multivitamin and mineral salts with 222.2 mg guarana (40 mg caffeine per tablet); (B) Berocca® performance (no guarana in its composition and the highest levels of B complex vitamins and vitamin C) | *In vivo*: humans | To determine if Berocca® boost and Berocca® performance could differentially affect the mood and mental performance when compared with the placebo. To examine neural substrates using functional magnetic resonance imaging (fMRI) to determine such effects | Placebo: 330 ml effervescent drink with similar color | 1 tablet per day for each group (supplement with and without guarana) | fMRI revealed that both multivitamin treatments increased activation in areas associated with working memory and processing of attention, with the effect being greater in the group treated with the supplement containing guarana. Moreover, they showed an increase in cerebral activation in the groups treated with the supplements containing guarana or not | (Scholey et al., 2013) |
| 28. Cognitive performance | 0.4 g guarana complex (GUA: 37.5 mg of guarana + 12.5 mg ginseng + 22.5 mg vitamins C, Isoxan Actiflash® Booster | *In vivo*: humans | To investigate the influence of serial mouth rinsing (MR) with nutritional supplements on cognitive control and time perception during a 40 min submaximal exercise | Placebo | Guarana complex 0.4 g 25 $ml^{-1}$ (GUAc); caffeine 67 mg 25 $ml^{-1}$ (CAF); carbohydrate 1.6 g 25 $ml^{-1}$ (CHO) | The results suggest that the serial administration of CHO, CAF and GUAc MR improves cognitive performance and decreases subjective perception of effort | (Pomportes et al., 2017) |

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| 29. Cognitive effects | Effervescent drink prepared from two commercially available supplements: (MV1) Berocca® boost, multivitamin and mineral salts with 222.2 mg guarana (40 mg caffeine per tablet); (MV2) Berocca® performance (no guarana in its composition and the highest levels of B complex vitamins and vitamin C) | *In vivo*: humans | To investigate the acute brain electrophysiological changes associated with multivitamin and mineral supplementation, with and without guaraná, using the steady-state visually evoked potential (SSVEP) | Placebo | Effervescent drink prepared with and without guarana, MV1 and MV2, respectively | The authors suggest that single doses of multivitamin and mineral preparations, both with and without guaraná, influence functional brain activity in healthy younger adults. In contrast, multivitamin and mineral treatment with guaraná showed a tonic shift toward greater excitatory processes after a single treatment, consistent with the known actions of caffeine | (White et al., 2017) |
| 30. Cognitive function and oxidative stress | Power guaraná containing caffeine (34.19 mg $g^{-1}$), theobromine (0.14 mg $g^{-1}$), catechin (3.76 mg $g^{-1}$), epicatechin (4.05 mg $g^{-1}$) | *In vivo*: middle-aged male Wistar rats | To investigate the effect of a commercial guarana extract (CGE) on cognitive function, oxidative stress, and brain homeostasis proteins related to cognitive injury and senescence | Control group (saline): gavage of 1 ml of 0.9% saline/BW (kg)/day | Guarana-treated group: gavage of 21 mg of guarana powder/BW (kg $day^{-1}$); Caffeine-treated group: gavage of 0.84 mg of caffeine powder/BW (kg $day^{-1}$) | The chronic supplementation with guarana extract was not effective against oxidative stress and did not provide any cognitive benefit during the 6 months aging of the Wistar rat model. The authors suggest that CGE intake does not improve cognitive development, but modifies the oxidative stress machinery and neurodegenerative-signaling pathway, inhibiting pro-survival pathway molecules in the hippocampus and striatum | (Mingori et al., 2017) |
| 31. Cognitive effect and heart rate variability | Commercial mineral vitamin supplement containing guarana and ginseng, effervescent tablet Isoxan Actiflash®. Each tablet: 300 mg guarana and 100 mg ginseng. In addition to Natrol® commercial caffeine supplement: 100 mg caffeine | *In vivo*: humans | To valuate cognitive performance and heart rate variability after ingestion: commercial supplement with multi-vitamin-mineral preparation supplemented with 300 mg guarana; caffeine supplement or placebo supplement | Placebo | 1 tablet per day for each group (supplement with guarana, with caffeine or placebo) | The results suggest that the intake of a mineral multivitamin supplement containing added guarana improves decision-making performance and is accompanied by a regulation of the stable autonomic nervous system in the first hour | (Pomportes et al., 2015a) |
| 32. Effects on cognition, anxiety and sleep | Guarana powder (capsules). The powder contained 2.1% caffeine and 16% tannins | *In vivo*: humans | To verify the eventual acute effects of guarana on cognition, anxiety and sleep in normal volunteers | Group 1: placebo; Group 2: caffeine (12.5 mg) | 2 capsules per day per (500 mg each) for three consecutive days | The authors could not demonstrate any significant change in the researched effects with the treatment with guarana powder | (Galduróz and Carlini, 1994) |
| 33. Anxiolytic effects | The extract was prepared (1 kg) by turbolysis (acetone:water) (7:3, v/v). 158 g lyophilized extract (EBPC) (patented process) was partitioned with ethyl acetate: EPA (ethyl acetate fraction) (44 g) and FAQ (aqueous fraction) (114 g) | *In vivo*: male Wistar rats | To investigate the effects of chronic administration of semipurified extract (EPA) of guarana in rats submitted to the elevated t maze model (ETM) of generalized anxiety disorder and panic disorder | Positive control: paroxetine (3 mg $kg^{-1}$); Negative Control: vehicle (0.9% NaCl; 2% Tween 80) | EPA; 4, 8, or 16 mg $kg^{-1}$. EPA has 34.95% caffeine and 17.53% tannins | EPA is administered orally; produced a panicolytic effect in rats in the ETM test; and the serotonergic and dopaminergic neurotransmitter systems are involved in this effect. It is suggested that EPA can be a useful drug in the treatment of mood disorders | (Roncon et al., 2011) |

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| 34. Anxiolytic and panicolytic effect | The aqueous fraction (FAQ) of guarana (Roncon et al., 2011) | *In vivo*: male Wistar rats | To evaluate the anxiolytic and panicolytic effect of FAQ in rats (ETM) and if the serotoninergic, dopaminergic and glutamatergic neurotransmitters are involved in this effect | Positive control: Paroxetine (3 mg $kg^{-1}$) Control: vehicle (0.9% NaCl, 2% Tween 80) | FAQ 8 mg $kg^{-1}$ | The FAQ is effective orally, produces anxiolytic and panicolytic activity in rats in the ETM test. The serotoninergic, dopaminergic and glutamatergic neurotransmitters are involved in the anxiolytic effect and the serotoninergic and dopaminergic neurotransmitters, in the panicolytic effect | (Rangel et al., 2013) |
| 35. Psychological well-being, anxiety and mood | Commercial product containing guarana extract containing 2.5% (m/m) of caffeine | *In vivo*: humans | To evaluate the effects on psychological well-being (PWB), anxiety and mood of a commercially available guarana preparation used according to the labeled dosages and instructions | Placebo (corn starch) | Capsules (360 mg), 3 times a day for 5 consecutive days | There were no significant differences between the placebo and guarana in any of the 6 areas of PWB on SAS (state anxiety scale) or in any of the 16 mood scales. Therefore, these results did not show any significant effects on psychological well-being, anxiety or mood | (Silvestrini et al., 2013) |
| 36. Antidepressant effect | Catuama® (commercial formulation already cited) (Antunes et al., 2001). The dry extract contains 40.31% guarana, 28.23% *T. catigua*, 28.23% *P. olcaloides* and 3.26% *Z. Officinallis* | *In vivo*: mice and rats *In vitro*: synapto-somal membrane | To assess the possible antidepressant-like effects of this product by means of pharmacological and neurochemical *in vivo* and *in vitro* procedures | Negative control for all tests: saline (10 ml $kg^{-1}$ po); *in vivo* tests: positive control, imipramine (10 or 15 mg $kg^{-1}$ ip, 6 h), d) *In vitro*: fluoxetine, cocaine, or desipramine (35, 3.4; 30 μg $ml^{-1}$), respectively | Forced swimming: (150 to 300 mg $kg^{-1}$ po), 6 h or (200 mg $kg^{-1}$) for 7 days. Tail suspension test: Catuama® (150–300 mg $kg^{-}$ po), 6 h. Open field test: Catuama® (300 mg $kg^{-}$). *In vitro*: Catuama® (10–1000 μg $ml^{-1}$ or 200 mg $kg^{-1}$ po) once a day for 42 days | The results show pharmacological and neurochemical evidence of the antidepressant activity of Catuama®. The product might be useful for the clinical management of moderate and mild depressive states, alone or in association with current antidepressant drugs | (Campos et al., 2004) |
| 37. Antidepressant effect | Guarana extract, there are no reports about the extraction. It is a "short communication" | *In vivo*: mice | To analyze the effects of the guarana extract compared with caffeine in the behavior of the mouse in forced swimming and open field tests | Distilled water (vehicle): 10 ml $kg^{-1}$ | Guarana extract (25; 50 and 100 mg $kg^{-1}$). Caffeine (10, 20, and 30 mg $kg^{-1}$) | The results suggest possible antidepressant effects | (Campos et al., 2005) |
| 38. Anxiolytic, antidepressant and motor stimulant effects | EBPC, EPA, and EPB (Roncon et al., 2011) | *In vivo*: Wistar rats | To investigate the pharmacological properties of EBPC and their EPA and EPB fractions, after acute and chronic oral administration in rats | Control: NaCl 0.9% 0.2% Tween 80 Imipramine HCl (20.0 mg $kg^{-1}$, ip), caffeine (10.0 mg $kg^{-1}$, ip) and diazepan (2.0 mg $kg^{-1}$, ip) | EBPC (3.0; 30.0; or 60.0 mg $kg^{-1}$). EPA (2.0 or 4.0 mg $kg^{-1}$). EPB (2.0 or 4.0 mg $kg^{-1}$) once a day for 40 days | Results suggest that the extract EBPC and the EPA statement produced an antidepressant effect after long-term administration | (Otobone et al., 2007) |
| 39. Change in chemotherapy-induced fatigue and depressive symptoms | Capsules of guarana extract (there is no information about extract preparation) | *In vivo*: patients | To evaluate the effect of the guarana extract on chemotherapy-induced symptoms of fatigue and depression in patients with solid tumors | Placebo tablet | 75 mg orally for 21 days | Results suggest that, guarana was not effective in preventing chemotherapy-related symptoms of depression and fatigue | (Miranda et al., 2008) |

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| 40. Postradiation depression and fatigue | Capsules of extract of guarana (there is no information about extract preparation) | *In vivo*: patients | Evaluate the effectiveness of guarana in the treatment of fatigue and depression post-radiation | Placebo tablet | 75 mg orally on a daily basis | Results suggest that have no statistically significant differences between guarana and placebo groups. No statistical decrease of post-radiation effects of fatigue and depression | (Miranda et al., 2009) |
| 41. Improvement of fatigue in patients with breast cancer | Capsules of standardized dried guarana extract (50 mg). The guarana preparation had a pH of 4.83 (10% solution in water), water content of 3.9%, 1.7% tannins, and 6.46% caffeine | *In vivo*: a patient with breast cancer | To evaluate the efficacy of the guarana extract on the fatigue, sleep quality, anxiety, depression symptoms and menopause in a group of patients with breast cancer, submitted to chemotherapy | Placebo: cellulose capsules identical to guarana capsules | 50 mg orally twice a day for 21 days | Guarana has proved to be an alternative non-toxic, effective, low-cost for short-term treatment of fatigue in patients with breast cancer receiving chemotherapy systematically | (Campos et al., 2011) |
| 42. Fatigue related to chemotherapy | Extraction in ethanol 70%. Standardized dried extract (PC-18) (0.096% theobromine) | *In vivo*: patients with solid tumors | Evaluate the effectiveness of an extract of guarana in patients with different solid tumors treated with chemotherapy | Placebo | 37.5 mg orally twice per day starting after 7 days of beginning of chemotherapy, for 3 weeks | The extract of guarana can be effective for the treatment of chemotherapy-related fatigue in patients with a variety of solid tumors with acceptable toxicity | (del Giglio et al., 2013) |
| 43. Alteration in radioactive marking and cell morphology | The commercial guarana powder was diluted in a solution with 0.9% NaCl | *In vivo*: Wistar rats<br>*In vitro*: Heparinized whole blood | To evaluate the influence of the guarana on process of marking using technetium-99m ($Tc^{-99m}$) | NaCl 0.9% | 20.0, 30.0, 50.0, 100.0, and 200.0 $\mu g\ ml^{-1}$ prepared in NaCl 0.9% solution. | The results showed a significant reduction in the uptake of radioactivity for RBC because of the guarana. Moreover, the addition of the dug caused a change in the morphology of these cells | (de Oliveira et al., 2002) |
| 44. Alteration in radiopharmaceutical binding of blood components | 2 g guarana powder has been diluted with 10 ml 0.9% NaCl. After centrifugation and discard of the supernatant, a salt solution of guarana (200 $mg\ ml^{-1}$) was obtained, which was used in all subsequent dilutions | *In vivo*: Wistar rats<br>*In vitro*: Red blood cells (RBC) | To study the influence of commercial guarana extract on the binding of radiopharmaceutical technetium-99m-dimercaptosuccinic acid ($^{99m}$ Tc-DMSA) on blood constituents using 2 precipitating agents: trichloroacetic acid (TCA) and ammonium sulphate (AS) | NaCl 0.9% | 200 $mg\ ml^{-1}$ prepared in NaCl 0.9% solution | Guarana has a relevant effect on the binding of $^{99m}$Tc-DMSA with insoluble fractions of the proteins of blood cells. It is suggested that this extract can affect the sites of action of AS and TCA. The presence of different metabolites with redox properties because of the metabolism of the guarana extract, could be competing for the same binding sites of $^{99m}$Tc-DMSA in plasma proteins, and cell proteins | (Freitas et al., 2007) |
| 45. Anti-aging and antioxidant activity | A. Aqueous extract of guarana seeds (GE): Caffeine = 102.8 $mg\ g^{-1}$, theophylline = 2.3 $mg\ g^{-1}$, theobromine = 1.0 $mg\ g^{-1}$. B. Alkaloid extract of guarana obtained through standard alkaline dichloromethane extraction (AlkE) | A. antioxidant activity. *In vitro*: DPPH and *in vivo*: *C. elegans*. B. Anti-aging. *In vivo*: *C. elegans* | To investigate the anti-aging and antioxidant activity of guarana using the model organism *Caenorhabditis elegans* | Control: without guarana | Antioxidant activity: 100, 200, and 300 $mg\ ml^{-1}$ of GE and AlkE. Anti-aging: 300 $mg\ ml^{-1}$ GE. PolyQ40: 100, 200, and 300 $mg\ ml^{-1}$ of GE. | This study demonstrated substantial antioxidant *in vivo* and anti-aging activity of guarana in *C. elegans*. Aqueous extract of guarana can extend the lifespan and attenuate markers of aging, such as the age-related muscle function decline and polyQ40 aggregation. | (Peixoto et al., 2017) |

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| 46. Antioxidant activity | The standardized guarana powder had 8.80% of moisture, 1.51% ash, 2.10% caffeine and 16% of tannins. An ethanolic extract at 50% was prepared for the *in vitro* assay | *In vitro*: Lipid peroxidation reaction | To investigate the *in vitro* antioxidant activity of guarana powder by measuring spontaneous lipid peroxidation inhibition in rat brain homogenates | Without guarana (water 0.1% tween 80) | 0.8 1.6, 3.3, and 6.6 mg $ml^{-1}$ of final concentration mid-reaction | Guarana exerted a clear antioxidant effect by inhibiting the process of spontaneous peroxidation, a fact that might be related to the high concentrations of tannins present in guarana and may suggest a possible adaptogen effect of the plant | (Mattei et al., 1998) |
| 47. Antioxidant activity | Ethanolic extract (dynamic solid–liquid extraction: 8 h at 8 atm, ambient temperature) | *In vitro*: 3T3-L1 cells | To evaluate the antioxidant activity of ethanolic guarana extract on 3T3-L1 cells after induced cellular damage by using ferric ammonium citrate | Inducer: ferric ammonium citrate | 0.5, 1.0, and 2.0 mg $ml^{-1}$ | Lipid peroxidation reduction was 62.5%, using the guarana extract at 2 mg $ml^{-1}$. This effect was dose-dependent | (Basile et al., 2005) |
| 49. Antioxidant activity | Aqueous extract (AqE); acetone-water EBPC (crude extract) and two subfractions: EPB and EPA | *In vitro*: Phospho-molybdate complex (RAC) and DPPH | To determine the antioxidant activity of different guarana extracts (AqE, EBPC, EPA, and EPB) by the RAC (relative antioxidant activity) and DPPH | DPPH: DPPH + methanol (control). BHT + DPPH + methanol (blank); (CAR): ascorbic acid (standard) | RAC: 0.3 ml of the sample for 3 ml of reagent DPPH: 2.0 for 20.0 mg $ml^{-1}$ | The semipurified fraction (EPA) presented the highest content of polyphenols in total, reflecting the analysis for antioxidants, with a low $IC_{50}$ and a higher RAC compared with the other extracts | (Yamaguti-Sasaki et al., 2007) |
| 50. Antioxidant activity | The seeds were degreased with toluene:ethanol (2:1, v/v) in a Soxhlet extractor (48 h). The dried material was treated with methanol:water (4:1, v/v) under reflux. The residue was dried in an oven and used for extraction of polysaccharides. Sequence of extraction: DMSO, water, NaOH | *In vitro*: DPPH | To investigate the antioxidant activity of the methanolic extract and the peptic fraction of guarana seeds | Butyl hydroxyanisol (BHA) and ascorbic acid as positive controls | 0.1, 0.5, 1.0, and 10.0 mg $ml^{-1}$ | The methanolic extract exhibited a strong ability to capture the DPPH radical (90.9% and 10 mg $ml^{-1}$). In the same concentration, the polysaccharide showed an antioxidant activity of 68.4%. For a higher concentration, the methanolic extract and the polysaccharide exhibited effects of removal of similar hydroxyl radicals (70%) | (Dalonso and Petkowicz, 2012) |
| 51. Antioxidant activity | Hydroalcoholic guarana extract (70:30) (300 mg $ml^{-1}$). The lyophilized extract was diluted in distilled water and prepared at the concentration of 200 mg $ml^{-1}$ Caffeine = 12.240 mg $g^{-1}$, theobromine = 6.733 mg $g^{-1}$ total catechins = 4.336 mg $g^{-1}$, and condensed tannins = 22 mg $g^{-1}$ | *In vitro*: Samples of LDL, human serum and TRAP | To investigate *in vitro* the potential effects of guarana on the oxidation of LDL, human serum and TRAP | Control: without guarana | 0.05, 0.1, 0.5, 1, and 5 μg $ml^{-1}$. TRAP: 0.01–10 μg $ml^{-1}$ | Guarana has shown a high antioxidant activity *in vitro*, especially at concentrations of 1 and 5 μg $ml^{-1}$, shown by the suppression of conjugated dienes and TBARS production, tryptophan destruction and high TRAP activity | (Portella et al., 2013) |
| 52. Protective/antioxidant effect | Hydroalcoholic guarana extract (70:30) (300 mg $ml^{-1}$). The lyophilized extract was diluted in distilled water and prepared (200 mg $ml^{-1}$). Caffeine (12.240 mg $g^{-1}$), theobromine (6.733 mg $g^{-1}$), total catechins (4.336 mg $g^{-1}$) and condensed tannins (16 mg $g^{-1}$) | *In vitro*: culture of embryonic fibroblasts (NIH-3T3 cells) | To investigate the protective potential of guarana on cytotoxicity caused by sodium nitroprusside (SNP), which releases cyanide and/or nitric oxide (NO) | Negative control: cell culture without SNP and guarana. Positive control of toxicity: sample with 10 μM SNP | 0.5, 1, 5, 10, and 20 mg $ml^{-1}$ (aqueous solution) | Guarana has antioxidant effects on NO metabolism, mainly in situations where increases NO levels occur | (Bittencourt et al., 2013) |

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| 53. Protective effect | Guarana powder (Bittencourt et al., 2013). Hydroalcoholic guarana extract (70:30). Caffeine = 12.240 mg $g^{-1}$, theobromine = 6.733 mg $g^{-1}$ total catechins = 4.336 mg $g^{-1}$ | *In vitro*: worm strains (*Caenorhabditis elegans*) | To investigate whether guarana demonstrates protective effects against methylmercury-induced toxicity, as well as the mechanisms involved | Control: without extract | 100, 500, and 1000 μg $ml^{-1}$ | The guarana extract GEE afforded a protective effect in skn-1 (ok2315) worms (exposed to methylmercury for 6 h), an effect likely modulated by upregulation of genes involved in metal transport, detoxification and antioxidant response | (Arantes et al., 2016) |
| 54. Oxidative Stress | Extra fine guarana powder. Phytochemical composition and nutrient composition of the powder, for example: total phenolic compounds 151.8 mg $g^{-1}$; catechin 30.0 mg $g^{-1}$; proanthocyanidin B1 3.72 mg $g^{-1}$; caffeine 39.8 mg $g^{-1}$, among others | *In vivo*: overweight humans; *Ex vivo*: oxidation of LDL and total plasma antioxidant capacity | To evaluate the effects of guarana on antioxidant markers and antioxidant activity of phase II enzymes in healthy overweight individuals with after individual and daily intakes | Each participant acted as control after the 15th day of treatment interruption, for a further 15 days | 3 g of the powder diluted in 300 ml of water before intake, daily for 15 days before breakfast | The treatment has reduced oxidative stress of clinically healthy overweight individuals, by means of the direct antioxidant action of absorbed catechins and growing regulation of antioxidant/detoxifying enzymes | (Yonekura et al., 2016) |
| 55. Oxidative stress and metabolic disorders (effects on the oxidation of LDL) | Hydroalcoholic guarana extract – alcohol and water (70:30) (300 mg $ml^{-1}$). Caffeine = 12.240 mg $g^{-1}$, theobromine = 6.733 mg $g^{-1}$ total catechins = 4.336 mg $g^{-1}$, and condensed tannins = 22 mg $g^{-}$1. Extract obtained and lyophilized was diluted in distilled water (200 mg $ml^{-1}$), infused by 7 min, and centrifuged | *In vivo*: Human blood samples; *In vitro*: samples of isolated LDL | To investigate the potential effects of guarana in elderly people in the oxidation of serum | Control: without guarana | 0.05, 0.1, 0.5, 1, and 5 μg $ml^{-1}$; 2 groups: those who ingest guarana (at least 5 times per week) and those who had never ingested it | Regular intake of guarana or its possible inclusion in the diet may produce certain health benefits and potential defense against oxidative stress and metabolic changes. A reduction of 27% in LDL oxidation | (Portella et al., 2013) |
| 56. Effects on metabolic comorbidities | Usually guarana power is mixed with water and sugar | *In vivo*: humans | To evaluate the association of metabolic disorders, anthropometry, oxidative metabolism and the habitual intake of guarana in the elderly | Those who have never ingested guarana | Variable: according to consumption before treatment, at least twice a week or more often | The group that consumed guarana showed a lower prevalence of arterial hypertension, obesity and metabolic syndrome than the control group. A protective effect potential of guarana is suggested against metabolic disorders in the elderly | (Krewer et al., 2011) |

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| 57. Hypercholesterolemic and anti-inflammatory effects | Guarana powder (Caffeine = 3.754 mg $g^{-1}$; theobromine = 2.065 mg $g^{-1}$, total catechins = 1.330 mg $g^{-1}$; and condensed tannins = 6.747 mg $g^{-1}$). | *In vivo*: adult male Wistar rats | To evaluate the effects of guarana on the metabolism of adenine nucleotides in lymphocytes and biochemical parameters of rats with induced hypercholesterolemia | Saline | Guarana powder 12.5, 25, or 50 mg $kg^{-1}$ per day. Caffeine 0.2 mg $kg^{-1}$. Oral administration for 30 days | There was an increase in the hydrolysis of adenosine triphosphate in the lymphocytes of rats with hypercholesterolemia and treated with 25 or 50 mg $kg^{-1}$ per day, when compared with the other groups. The cholesterolemic group treated with guarana (50 mg $kg^{-1}$) showed a decrease in the activity of ecto-adenosine deaminase, in comparison with the groups with normal diet. Guarana has been able to reduce total cholesterol and LDL to basal levels in rats with hypercholesterolemia. High concentrations of guarana associated with a hypercholesterolemic diet probably contributed to the reduction of the inflammatory process | (Ruchel et al., 2016) |
| 58. Hyperlipidemia and cognitive disorders | Guarana powder (Caffeine = 3.754 mg $g^{-1}$; theobromine = 2.065 mg $g^{-1}$, total catechins = 1.330 mg $g^{-1}$; and condensed tannins = 6.747 mg $g^{-1}$). | *In vivo:* adult male Wistar rats | To determine the possible preventive effect of guarana powder on memory impairment and acetylcholinesterase (AChE) activity in the brain structures of rats with Poloxamer-407-induced hyperlipidemia | Saline | 12.5, 25, and 50 mg $kg^{-1}$, administered by oral gavage once a day for a period of 30 days. Caffeine 0.2 mg $kg^{-1}$; Sinvastatin human equivalent dose; To induce hyperlipidemia: 500 mg/kg of Poloxamer-407 | Guarana powder was able to reduce the levels of total cholesterol and LDL in a manner similar to simvastatin and partially reduced the liver damage caused by hyperlipidemia. It also was able to prevent changes in the activity of AChE and improve memory impairment due to hyperlipidemia. The authors suggests these results may be due to the presence of methyxanthines in guarana, and it may be a source of promising phytochemicals that can be used as adjuvant therapy in the management of hyperlipidemia and cognitive disorders | (Ruchel et al., 2017) |
| 59. Cytoprotective/spermatogenic effect | Guarana extract diluted in water at the time of administration | *In vivo*: male Wistar rats | To evaluate the potential effect of guarana in the prevention or attenuating of cadmium-induced damage in rats testis | Water | 2 mg $g^{-1}$, BW diluted on water, once a day for 56 days | The guarana was effective in attenuating morphological changes in Leydig cells, as well reducing the inflammatory response. The animals treated only with guarana showed a significant increase in testosterone levels in plasma and in the proportions of volumetric seminiferous tubules, which are indicative of spermatogenic process stimulation | (Leite et al., 2011) |

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| 60. Cytoprotective effect | Commercial dry guarana seed powder (125 mg) has been diluted in DMSO (1 ml) for 24 h, centrifuged; supernatant was filtered | *In vitro*: SH-SY5Y Cells | To evaluate whether guarana could protect the dopaminergic human cell line SH-SY5Y against rotenone-induced cytotoxicity | DMSO | 9.7–625.0 g $ml^{-1}$ diluted in DMSO | The guarana has significantly increased cell viability of SH-SY5Y cells treated with rotenone, in a dose-dependent manner | (de Oliveira et al., 2011) |
| 61. Cytoprotective effect | Guarana extract diluted in water at the time of administration | *In vivo*: male Wistar rats | To evaluate if guarana is capable of reducing cadmium-induced morphological damage in rats testis | Water | 2 mg $g^{-1}$ BW, diluted on water, once a day for 56 days | After exposure to cadmium, the animals supplemented with guarana showed a significant decrease in the proportion of damaged seminiferous tubules. Also, guarana supplementation has been effective in keeping the number of Leydig cells per testis in animals exposed to cadmium | (Leite et al., 2013) |
| 62. Anticholinesterase activity | Ethanolic extract (exhaustive extraction) 1.5 mg $ml^{-1}$ | *In vitro*: liophilized anticholinesterase enzyme | To evaluate extracts of various plants, including guarana, which could inhibit the activity of the enzyme acetylcholinesterase. The inhibitors of this enzyme showed greater efficiency in the clinical treatment in Alzheimer's Disease | In microplates: blank (10% methanol in 50 mMTris buffer, HCl pH 8) | 1.5 mg $ml^{-1}$ | Guarana proved to be quite promising for the isolation and characterization of compounds that inhibit acetylcholinesterase, because its extract inhibited the enzyme by 65% | (Trevisan and Macedo, 2003) |
| 63. Neuroprotective (prevents cell cytotoxicity) | Guarana powder. Caffeine (34.19 mg $g^{-1}$), theobromine (0.14 mg $g^{-1}$), catechins (3.76 mg $g^{-1}$) and epicatechin (4.05 mg $g^{-1}$). A stock solution of guarana (10 mg $ml^{-1}$) is prepared for subsequent dilution | *In vitro*: neuronal cells (SH-SY5Y) | To evaluate the potential effect of the guarana extract against aggregation β-amyloid 1–42, glycation of proteins, as well as cytotoxicity induced by methylglyoxal (MGO), glyoxal (GO), and acrolein (ACR) in SH-SY5Y cells | Negative control: cells without treatment | Guarana powder 10, 100, and 1000 μg $ml^{-1}$ dissolved in a culture medium. Caffeine 40 μg $ml^{-1}$ dissolved in a DMEM-F12 medium | The guarana is capable of inhibiting albumin glycation mediated by glucose/fructose, MGO, and GO | (Bittencourt et al., 2014) |
| 64. Protective effect against DNA damage | Guarana powder diluted in water at the time of use:(total tannins: 13.0%, condensed tannins: 5.72%) | *In vivo*: mice | To investigate the cytotoxic/anti-genotoxic properties of guarana in rat hepatocytes injected with N-nitrosodiethylamine (NDE) | Water | Guarana powder 2.0 mg $g^{-1}$ BW, for 16 days | The treatment with guarana showed a reduction by 52.54% in comet image length of animals exposed to NDE. Guarana has a potential protective effect against NDE-induced DNA damage in rat liver | (Fukumasu et al., 2006a) |
| 65. Neuroprotective effect | Guarana powder seeds. The administered solution was prepared on the day of use by dilution in water of guarana powder (12.240 mg $g^{-1}$ caffeine, 6.733 mg $g^{-1}$ theobromine, 4.336 mg $g^{-1}$ total catechins, and 16 mg $g^{-1}$ condensed tannins | *In vivo*: male Wistar rats | To evaluate the neuroprotective effects of guarana seed powder on DNA damage induced by $CCl_4$ (carbon tetrachloride) in rats | Normal control: water. $CCl_4$ control: (1 ml $kg^{-1}$, 50% in olive oil) to induce DNA damage. | 100, 300, and 600 mg $kg^{-1}$, daily for a period of 14 days | Guarana prevents $CCl_4$-induced breakage of DNA strands in lesions in rats | (Kober et al., 2016) |

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| 66. Chemopreventive effect | Guarana powder (Fukumasu et al., 2006a) was mixed with commercial food powder with the same granulation | *In vivo*: BALB/c female mice | To check the effects of guarana on hepatocarcinogenesis in mice | Control group: only commercial food | 0.1, 1.0, or 2.0 mg $g^{-1}$ BW for 25 weeks | The incidence and multiplicity of macroscopic lesions were reduced by the treatment with guarana. According to these results, guarana showed inhibitory effects on DEN-induced hepatocarcinogenesis in mice. *P. cupana* can act as a chemopreventive on carcinogenesis, reducing cellular expansion of preneoplastic cells. | (Fukumasu et al., 2006b) |
| 67. Antineoplastic activity | Crude extract (CE) of guarana was prepared using acetone:water (7:3, v/v). The CE was partitioned with ethyl acetate, and removed the organic solvent to yield the EAF | *In vitro*: six human tumor cell lines | To evaluate antineoplastic, activity of guarana seeds crude extract (CE) and ethyl-acetate fraction (EAF) | Control groups were treated with the same amount of dimethyl sulfoxide (0.1%) | CE and EAF: 10–200 μg $ml^{-1}$ | CE and EAF fractions presented $IC_{50}$ values of 70.25 μg $ml^{-1}$ and 61.18 μg $ml^{-1}$ in HL-60 leukemia cell line, respectively | (Carvalho et al., 2016) |
| 68. Anticancer effect | Guarana powder diluted in water | *In vivo*: female C57Bl/6 mice | To evaluate the effect of growth inhibition of daily administration of guarana in the experimental model of metastasis of B16F10 melanoma cells | Control: water | 2.0 mg $g^{-1}$ BW diluted in water, daily until 21 days | The treatment of guarana has decreased proliferation and increased apoptosis of tumor cells, thereby reducing the area of the tumor (68.6%) | (Fukumasu et al., 2008) |
| 69. Anticancer effect | Hydroethanolic extract of guarana (70:30) at 300 mg $ml^{-1}$ (Bittencourt et al., 2013): Caffeine (12.240 mg $g^{-1}$), theobromine (6.733 mg $g^{-1}$), total catechins (4.336 mg $g^{-1}$) and condensed tannins (16 mg $g^{-1}$). The *in vitro* tests were performed using lyophilized extract diluted directly in culture medium | *In vitro:* human cancer colorectal HT-29 cell line | To investigate effect of guarana and its metabolites (caffeine, theobromine and catechin) on HT-29 cytotoxicity and cell proliferation on colorectal cancer (CRC) and on oxaliplatin sensitivity. Also to evaluate the potential apoptosis induction by guarana with and without concomitant oxaliplatin exposure, considering late and early apoptotic HT-29 cells as well as by the differential modulation of genes related to apoptosis pathway. All protocols evaluated the cytotoxicity (24 h exposition) and anti-proliferative effect (72 h exposition) by MTT assay | Cells untreated | Concentrations of guarana extract (0, 5, 10, 30, 100, 300, and 1000 μg $ml^{-1}$)with or without the LD50 oxaliplatin | Cells exposed to guarana at a concentration of 100 μg $ml^{-1}$ presented a similar cytotoxic effect as HT-29 cells treated with oxaliplatin and did not affect the sensitivity of the drug. Guarana was able to induce apoptosis and up-regulate the p53 and Bax/Bcl-2 genes. The result suggests that beverage foods rich in caffeine, other than coffee and teas, have an antitumor effect against CRC cancer. However, the chemical association caffeine-catechin is probably more plausible to explain the antitumor effect of these foods, such as guarana investigated here, rather than only caffeine | (Cadona et al., 2016) |
| 70. Anticancer effect | Guarana powder (Fukumasu et al., 2006a) diluted in ethanol | *In vivo*: female BALB/c mice | To report the antiproliferative effect of treatment with guarana in Ehrlich Ascitic Carcinoma (EAC) in mice | Control: water | 100, 1000, and 2000 mg $kg^{-1}$ BW for 28 days | The treatment with guarana for 21 days increased survival of mice. The guarana acts directly on cells and the pre-treatment performed by the model proposed in this study is not necessary | (Fukumasu et al., 2011) |

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| 71. Antiproliferative effect | Hydroethanolic extract of guarana (70:30). (Bittencourt et al., 2013): Caffeine (12.240 mg $g^{-1}$), theobromine (6.733 mg $g^{-1}$), total catechins (4.336 mg $g^{-1}$) and condensed tannins (16 mg $g^{-1}$). The lyophilized extract was diluted in water (200 mg $ml^{-1}$), boiled for 7 min, centrifuged, and filtered for later dilution in water | *In vitro*: breast cancer cells MCF-7 | To evaluate the effects of guarana in the response of breast cancer cells to 7 chemotherapy agents currently used in the treatment of breast cancer | Untreated cells | Guarana extract 1, 5, and 10 μg $ml^{-1}$ diluted in distilled water and added to cell culture medium | The main results showed an antiproliferative effect of guarana at concentrations of 5 and 10 μg $ml^{-1}$, and a significant effect on chemotherapeutic drug action. Guarana improved the antiproliferative effect of chemotherapeutic agents, causing a decrease of >40% in cell growth after 72 h of exposure. The results suggest an interaction of guarana with chemotherapeutic drugs | (Hertz et al., 2015) |
| 72. Proliferative effect (decrease in the process of senescence) | Guarana powder (Bittencourt et al., 2013). Hydroethanolic guarana extract (70:30) at 300 mg $ml^{-1}$ | *In vitro*: senescent adipocyte-mesenchymal cells (ASCs) | To investigate whether supplementation with guarana in culture medium with SMA cells can help decrease the process of senescence, as well as reduce the potential damage caused by oxidative stress | Untreated senescent cells | Guarana extract 1, 5, 10, and 20 mg $ml^{-1}$ | In senescent cells exposed to guarana at 5 mg $g^{-1}$ concentration increased cellular proliferation occurred compared to untreated senescent cells. The results suggest that supplementation of guarana could reverse the processes of early senescence in ASCs. These results have potential for application in regenerative medicine | (Machado et al., 2015) |
| 73. Herb–drug interaction | Guarana extract containing 12% caffeine obtained commercially. There are no reports on extract preparation | *In vitro*: male Wistar rats | To investigate if a commercial standardized (certified) extract of guarana seeds may influence the pharmacokinetics of amiodarone in rats following their simultaneous oral co-administration and after a 14-day guarana pre-treatment period | Control: Vehicle 0.5% aqueous solution of carboxymethyl cellulose | A. Single dose of Guarana (821 mg $kg^{-1}$) and amiodarone (50 mg $kg^{-1}$) diluted on vehicle;<br>B. 14 days with Guarana (821 mg $kg^{-1}$ per day) and amiodarone (50 mg $kg^{-1}$) only on 15th day diluted on vehicle | The decrease in plasma concentrations of amiodarone was also accomplished by a significant reduction in the tissue concentrations of amiodarone and MDEA (a metabolite of amiodarone), particularly in the heart. It is suggested that the guarana extract should not be ingested with amiodarone | (Rodrigues et al., 2012) |
| 74. Against oral diseases | Extraction of guarana, acetone/water (70:30, v/v), obtaining the EBPC (patent). EBPC was partitioned with ethyl acetate, which resulted in aqueous and ethyl acetate fractions | *In vitro*: buccal epithelial cells | To evaluate the effect of guarana on cell surface hydrophobicity (CSH), biofilm formation and adhesion of *C. albicans* to polystirene, composite resins, and buccal epithelial cells (BEC) | Positive control: chlorhexidine gluconate 2%; negative control: phosphate buffered saline | Aqueous fraction from guarana extract 10 mg $ml^{-1}$ | The guarana extract showed no antifungal activity, nor reduced adhesion of *C. albicans* to the surface of nanoparticle composites. However, it reduced adhesion of *C. albicans* to BEC and to polystyrene. These results indicate that this extract has potential for use in the prevention of oral diseases | (Matsuura et al., 2015) |

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| 75. Control of dental plaque bacteria | Aqueous guarana extract produced by turbolization. Total tannins: 5.78% | *In vivo*: humans | To test guarana extracts in different concentrations and in the form of mouth wash in the activity against dental plaque bacteria | Positive control: chlorhexidine-gluconate 0.12% | Mouthwashes with 10 ml of guarana extract at 5 and 7% for 1 min for 4 times per day | The guarana extracts in the concentrations in use were efficient in comparison to the positive control | (Barbosa and Mello, 2004) |
| 76. Prevention of dental plaque bacteria | AqE, EBPC, EPA, and EPB | *In vitro*: Adhesion test and MIC | To analyze, on a preliminary basis, the antibacterial potential of different guarana extracts of (AqE, EBPC, EPA and EPB) against *Streptococcus mutans* | Negative control: without treatment; positive control: chlorhexidine-gluconate 1.2 μg $ml^{-1}$ | Extracts with a final tannins concentration of 750 μg $ml^{-1}$: AqE 4.64 mg $ml^{-1}$; EBPC 2.41 mg $ml^{-1}$; EPA 2.50 mg $ml^{-1}$; and EPB 4.39 mg $ml^{-1}$ | EBPC gave the best result; even with a lower concentration, it showed the best action on the adherence of *S. mutans*, with 79.69% inhibition. It is suggested that this extract can be used for the prevention of dental plaque bacteria | (Yamaguti-Sasaki et al., 2007) |
| 77. Antimicrobial activity | Ethanolic extract (dynamic solid-liquid extraction: 8 h at 8 atm, ambient temperature), evaporation of the solvent to a dry material | *In vitro*: MIC | To evaluate the antibacterial activity of the guarana extract against Gram-positive and Gram-negative bacteria | Control cultures containing only buffer. Positive control: standard antibiotics | Guarana extracts 16–128 μg $ml^{-1}$ | *Pseudomonas aeruginosa* (27853) (MIC = 16 μg $ml^{-1}$), *Proteus mirabilis* (7002) (MIC = 32 μg $ml^{-1}$), *Proteus vulgaris* (12454) (MIC = 32 μg $ml^{-1}$), and *Escherichia coli* (11229) (MIC = 32 μg $ml^{-1}$) were the most inhibited | (Basile et al., 2005) |
| 78. Antimicrobial activity | EBPC, FAQ, EPA, and subfractions of EPA, and isolated compounds | *In vitro*: MIC | To evaluate the antibacterial activity of extracts and isolated compounds of guarana against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* | Positive control: standard antibiotics | All tested substances at 2 mg $ml^{-1}$ | Even in concentrations above 1000 μg $ml^{-1}$, the guarana extract showed no activity against these organisms: *Staphylococcus aureus* (25923), *Bacillus subtilis* (6623), *Escherichia coli* (25922) and *Pseudomonas aeruginosa* (15442) | (Antonelli-Ushirobira et al., 2007) |
| 79. Antimicrobial activity | The different extracts were prepared with these solvents: distilled water, methanol, 35% acetone and 60% ethanol at room (TR) and at boiling (TB) temperature of solvent. The main substances of the extract were quantified | *In vitro*: antimicro-bial activity | Investigate the antimicrobial activity of extracts of guarana against three food-borne fungi: *Aspergillus niger, Trichoderma viride* and *Penicilliumcyclopium*, and three health-damaging bacteria: *Escherichia coli, Pseudomonas fluorescens* and *Bacillus cereus* by the agar well diffusion and broth dilution assay | Control: ethanol at 96% | 0.2 g $ml^{-1}$ at 96% ethanol | The guarana extracts have significant activity against the growth of deteriorating bacteria that cause food poisoning, such as *E. coli*, *B. cereus*, *P. fluorescens* and deteriorating fungi, such as *A. niger*, *T. viride* and *P. cyclopium*. Ethanolic extracts showed greater antimicrobial activity than aqueous extracts | (Majhenic et al., 2007) |
| 80. Antimicrobial activity | Crude extract (CE) of guarana was prepared using acetone:water (7:3, v/v). The CE was partitioned with ethyl acetate and removed the organic solvent to yield the EAF | *In vitro*: MIC and MBC | To evaluate antibacterial activity of guarana seeds crude extract (CE) and ethyl-acetate fraction (EAF) | Without extract | CE and EAF: 0.5–250 μg $ml^{-1}$ | CE and EAF fractions showed a bacteriostatic activity (MIC = 250 μg $ml^{-1}$). However they do not show bactericidal activity (MBC > 250) | (Carvalho et al., 2016) |

(100 mg) alone (Pomportes et al., 2015a). In summary, evidence suggests that various components, such as flavonoids (Scholey and Haskell, 2008), saponins, and tannins (Espinola et al., 1997; Mattei et al., 1998; Otobone et al., 2005) can contribute to this psychoactive effect. This effect can also be attributed to the synergetic interactions between these various substances and/or other psychoactive substances present in the guarana extract. It is suggested that the biological activities of guarana go beyond the extensively reported central nervous system stimulation (Peixoto et al., 2017).

Recent studies by our group (Audi et al., 2010; Roncon et al., 2011; Rangel et al., 2013) showed that the semipurified guarana extract has both anxiolytic and panicolytic effects. The serotonergic, dopaminergic and glutamatergic neurotransmitters are involved in the anxiolytic effect, whereas serotonergic and dopaminergic neurotransmitters are involved in the panicolytic effect (Rangel et al., 2013).

There is great interest in the substitution of synthetic antioxidants by natural counterparts in food, encouraging a search for natural sources of antioxidants. This extends to other perishable goods, such as cosmetics, pharmaceutical products, and plastics. In addition, other biological properties are associated with antioxidants, for example, anticarcinogenicity, antimutagenicity, antiallergenicity, and anti-aging activities (Moure et al., 2001). Several studies have shown that guarana has antioxidant activities (Mattei et al., 1998; Basile et al., 2005; Majhenic et al., 2007; Yamaguti-Sasaki et al., 2007; Dalonso and Petkowicz, 2012; Bittencourt et al., 2013; Portella et al., 2013), which have been largely attributed to the polyphenols (particularly tannins). However, the polysaccharides in guarana powder have also shown antioxidant activity *in vitro* (Dalonso and Petkowicz, 2012). The antioxidant effect is reported to be dose-dependent and present even at low concentrations in animals (1.2 $\mu g\,ml^{-1}$) (Mattei et al., 1998; Basile et al., 2005). In another study testing crude and semipurified guarana extracts, it was found higher content of polyphenols, lower $IC_{50}$ 0 value and higher relative antioxidant capacity (RAC) for the semipurified extract (Yamaguti-Sasaki et al., 2007).

Another promising feature of guarana is its antimicrobial activity, with possible use in industry product conservation or, for example, to prevent diseases caused by microorganisms. The aqueous extract of guarana, in the form of mouthwashes, has been evaluated in human individuals free of cavities and periodontal diseases. The antiplaque activity, determined according to the method of Greene and Vermillion (1964) through the Simplified Oral Hygiene Index, revealed the guarana extract was statistically efficient compared to the positive control and, therefore, a potential alternative in the control of dental plaque (Barbosa and Mello, 2004). The *in vitro* antibacterial activity of a semipurified guarana extract against *Streptococcus mutans*, a bacterial species associated with cariogenic activity was demonstrated (Yamaguti-Sasaki et al., 2007). The antibacterial activity against *S. mutans* was directly proportional to the polyphenol content present in the extract.

Guarana extracts have also displayed activity against several strains of bacteria and fungi (*Pseudomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, Escherichia coli, Bacillus cereus, Pseudomonas fluorescens, Aspergillus niger, Trichoderma viride* and *Penicillium cyclopium*) (Basile et al., 2005; Majhenic et al., 2007). According to the results, the alcoholic extracts possessed greater antimicrobial activity than aqueous extracts of guarana seeds, which had little or no antimicrobial activity against the microorganisms tested (Majhenic et al., 2007). Crude, semipurified fractions of guarana (up to 1000 $mg\,ml^{-1}$) were tested against strains of *Staphylococcus aureus, Bacillus subtilis, E. coli* and *P. aeruginosa*, but no activity of these fractions against these organisms was obtained (Ushirobira et al., 2007). In contrast, extracts of guarana seeds obtained by supercritical technology, using 40% cosolvent, showed antibacterial activity against a methicillin-resistant strain of S. aureus (Marques et al., 2016). These conflicting results may be due to the form of preparation of the extract, method of seed drying, location the raw material was collected from, among other factors, as will be discussed later.

Some drug interactions have been attributed to guarana, such as potentiation of the action of chemotherapy drugs, causing an antiproliferative effect (Hertz et al., 2015). When administered with anticoagulants, guarana may inhibit platelet aggregation by increasing the risk of bleeding (Nicoletti et al., 2007). Conversely, guarana extract can be potentially useful in the prevention of diseases, such as thrombosis and other vascular problems, and there are studies demonstrating its antagonist action on platelets (Bydlowski et al., 1988, 1991; Ushirobira et al., 2007).

Guarana has also been used for its adaptogenic effect and, therefore, it is very useful in cases of drug addiction, particularly to relieve the hangover from the abuse of alcoholic beverages (Carlini et al., 2006).

All previously cited researchers used guarana seeds for their studies. However, a moderate antiplasmodial activity of chloroform extracts of branches and fruits of guarana have been shown (Lima et al., 2015). In this study, the methanolic extracts and aqueous extracts of the same plant parts and also chloroform, methanolic and aqueous extracts of the leaf, did not show antiplasmodial activity.

The reports show that guarana displays countless health benefits in cognitive disorders, such as depression and panic disorder or Alzheimer. Also, it is very promising as an antibacterial for oral diseases, such as plaque and periodontal diseases, and against bacterial species associated with cariogenic activity. Thus, the pharmacological activities should be fully explored with in-depth *in vitro* and *in vivo* tests, and, ultimately, clinical trials to prove these activities in humans.

## Toxicity

Herbal drugs used in the preparation of medications for therapeutic purposes are foreign to the human body. Therefore, like any foreign substance, the products of their biotransformation may lead to reactions in the human body. For this reason, popular and even traditional use, are insufficient to validate herbal drugs as effective and safe medications. Safety should be assessed with pre-clinical and clinical pharmacological and toxicological studies (Lapa et al., 2010). Preclinical toxicological studies are conducted according to the internationally accepted protocols, although legal requirements vary from country to country.

*In vivo* and *in vitro* studies have been conducted to evaluate the possible toxicity of guarana extracts or their association with other plants (Box ). Fluid extracts of guarana were used in association with other plants, to test the toxicity of these formulations *in vivo*, and demonstrated to be safe (Oliveira et al., 2005; Mello et al., 2010). Namely, they did not present any observable toxic effects with a 28 or 30 days treatment, with rabbits and human being. Other *in vivo* and *in vitro* studies (Espinola et al., 1997; Mattei et al., 1998) have described absence or low toxicity to aqueous extracts of guarana, even after 23 months of treatment. The cytotoxicity of the aqueous extract of guarana at 10, 20, 30, and 40 $mg\,ml^{-1}$ in Chinese hamster ovary (CHO) cells was investigated (Santa Maria et al., 1998). At the lowest dose tested, the extract was harmless, but the authors warn that a prolonged use or high doses might be harmful to human health. In contrast, a semipurified fraction of guarana presented a possible toxic effect to the liver, with greater biological susceptibility in male rats at doses of 150 and 300 $mg\,kg^{-1}$, after 90 d of treatment (Antonelli-Ushirobira et al., 2010). In another study, human neuronal SH-SY5Y cells treated with guarana, developed

**Box 3:**
Toxicology of seeds of *P. cupana* or their associations described in the literature.

| Details of the extract or dosage form | *In vivo*/*In vitro* | Objective | Control used | Dose tested for drugs | Findings | Ref. |
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| a. Association: Catuama®. Mixture of hydroalcoholic extracts of 4 plants: 5% guarana, 1% *Zingiber officinalis* (ginger), 5% *Trichilia catigua* (catuaba), and 5% *Ptychopetalum olacoides* (muirapuama) | *In vivo:* humans, 18–45 years, BMI 19–27 | To investigate whether chronic administration (28 days) of Catuama® displays any observable toxic effects in human volunteers (both males and females) | No control was used | 25 ml Catuama®, twice a day for 28 days | Administration of Catuaba® extract has caused no observable toxic effects in male and female human volunteers | (Oliveira et al., 2005) |
| b. Association: extracts of fluids from *Anemopaegma mirandum* (catuaba), *Cola nitida* (kola nut), *Passiflora alata* (passion fruit), *Paullinia cupana* (guarana) (1%), *Ptychopetalum olacoides* (Sceletium tortuosum), and thiamin chlorhydrate (Nerviton®) | *In vivo*: Adult New Zealand rabbits | To evaluate the potential toxicological effect of the phytomedicinal product, when administered orally for 30 days in New Zealand rabbits (males and females) in a daily oral dose ten times as big as the one prescribed for humans | Control: data collected from each animal immediately before treatment | 4.3 ml $kg^{-1}$ of the phytomedicinal product | The results confirm the relative safety of this product, based on the aspects of toxicological analysis | (Mello et al., 2010) |
| c. Standardized guarana powder with 8.80% moisture, 1.51% ash, 2.10% caffeine and 16% tannins. An ethanolic extract at 50% was prepared for the *in vitro* assay. | *In vivo:* Male Swiss mice and male Wistar rats | To assess the possible toxic effects of guarana. *In vitro* and *in vivo* experiments (acute and chronic) were carried out in lab animals and were compared with those produced by *Panax ginseng* | Without guarana (water and Tween 80) | Observation screening: 2000 mg $kg^{-1}$ (o.a.), 1000, and 2000 mg $kg^{-1}$ (iv). The potentiation of sleep time and chronic effects: 0.3 and 3.0 mg $ml^{-1}$. Anatomy of pathology: 3.0 mg $ml^{-1}$ | The guarana plant, just like ginseng, has no toxic effects. This was demonstrated after acute administration of high doses of these products, as well as in the chronic treatment with lower doses. No alterations were found in animals for body weight, mortality, or even at the histopathological level | (Mattei et al., 1998) |
| d. Suspension of seed powder of *P. cupana*in water: Tween-80. The powder contained 2.1% caffeine and 16% of tannin | *In vivo:* Swiss mice and Wistar rats | To evaluate possible toxicity of guarana in mice and rats | Control: water/Tween 80. Reference drug: caffeine 0.1 mg $ml^{-1}$ | 0.3 and 3.0 mg $ml^{-1}$ | The animals had the same average life time, indicating a low toxicity of guarana, even after 23 months of treatment | (Espinola et al., 1997) |
| e. Semipurified guarana extract (EPA). Acetone:water (7:3; v/v), and then partitioned with ethyl acetate (10×, 5 l) (EPA) | *In vivo:* male Swiss mice and Wistar rats of both sexes | To evaluate the toxicity of EPA fraction (containing caffeine and various flavan-3-ols and proanthocyanidins) of guarana in rodents | Control: water | Evaluation of acute toxicology. A single dose of EPA: po, 5.0, 2.5, or 1.0 g $kg^{-1}$; ip, 2.5, 1.5, 1.0, 0.5, or 0.1 g $kg^{-1}$. Evaluation of the subchronic toxicology. Once daily for 90 days, orally: 30, 150, or 300 mg $kg^{-1}$ | A $DL_{50}$ was 1,769 g $kg^{-1}$ (po) and 0.593 g $kg^{-1}$ (ip). After 90 days, the animals presented biochemical alterations that indicated that the liver is the target organ, in case of possible toxicity of EPA, especially in males, in doses of 30, 150, and 300 mg $kg^{-1}$ | (Antonelli-Ushirobira et al., 2010) |

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| f. Aqueous extract was prepared at a concentration of 200 mg ml$^{-1}$ (infusion for 7 min, and centrifugation) | *In vitro:* Chinese hamster ovary (CHO) cellsand bacterial cells of *Photobacterium phosphoreum* | To evaluate the cytoxicity of aqueous guarana extracts | Control: absorbance determined before the addition of the extract | Plant extracts added to the culture medium at 4 concentrations: 10, 20, 30, and 40 mg ml$^{-1}$ | The results indicate that low concentrations of guarana are safe, while higher doses of this product may have cytotoxic effects | (Santa Maria et al., 1998) |
| g. Guarana powder (4% caffeine) diluted | *In vitro*: neuronal human SH-SY5Y cells | To clarify the morphological and biochemical abnormalities caused by caffeine, taurine and guarana, alone or in combination, as they are the main components in energy drinks | Control: untreated cells | 3.125, 12.5, and 50 mg ml$^{-1}$ | Cells treated with 12.5–50 mg ml$^{-1}$ of guarana developed signs of degenerative neuritis in the form of swelling in various segments. The treated cells also showed qualitative signs of apoptosis, including formation of vesicles in the membrane, cell retraction and cleaved caspase-3 positive cells | (Zeidan-Chulia et al., 2013) |
| h. Crude extract (CE) of guarana was prepared using acetone:water (7:3, v/v). The CE was partitioned with ethyl acetate, and removed the organic solvent to yield the EAF | *In vitro:* peripheral blood mononuclear cells (PBMC) and splenocytes | To evaluate the cytotoxicity of CE and EAF (MTT assay) | Control: untreated cells | PBMC-5, 10, 50, 100, and 200 μg ml$^{-1}$. Splenocytes-EAF and CE: 10–200 μg ml$^{-1}$ | After 48 h, both EAF and CE were not toxic at 200 μg ml$^{-1}$ dose in PBMC. However, at 200 μg ml$^{-1}$ both EAF and CE were toxic to splenocytes | (Carvalho et al., 2016) |
| i. Power guaraná containing caffeine (34.19 mg g$^{-1}$), theobromine (0.14 mg g$^{-1}$), catechin (3.76 mg g$^{-1}$), epicatechin (4.05 mg g$^{-1}$) | *In vivo*: middle-aged male Wistar rats | To investigate the effects of a chronic administration of CGE to middle age Wistar rats on parameters related to oxidative stress, cognitive injury, senescence, and behavior in the striatum and hippocampus | Control group (saline): gavage of 1 ml of 0.9% saline/BW (kg)/day | Guarana-treated group: gavage of 21 mg of guarana powder/BW (kg/day) (CGE); Caffeine-treated group: gavage of 0.84 mg of caffeine powder/BW (kg/day) for 6 months | The results showed that CGE and caffeine treatments did not cause any renal or hepatic toxicity | (Mingori et al., 2017) |

signs of neurite and apoptotic degeneration (Zeidan-Chulia et al., 2013). The authors suggested that excessive removal of intracellular reactive oxygen species to non-physiological levels could be a cause of guarana-induced *in vitro* toxicity. Moreover, the guarana extracts were considered to be genotoxic as assessed by lysogenic induction in *E. coli* (da Fonseca et al., 1994). These extracts were also able to induce mutagenesis in *Salmonella* Typhimurium. This genotoxicity was attributed to the presence of a molecular complex formed by caffeine and flavonoid (catechin and epicatechin) in the presence of potassium.

Therefore, in toxicity testing, it is important to determine the dose of the drug, bearing in mind the form of pharmaceutical preparation of its extract and to specify the amount administered, particularly in *in vivo* tests, where the weight of the animal is taken into account. The lack of standardization of these factors may raise doubt about the definition of a safe dose, administered both in acute or in chronic regimen.

In addition to the numerous health benefits and useful therapeutic indications for humans, guarana has demonstrated low toxicological potential, as evidenced by various *in vitro* and *in vivo* studies. Thus, it can be safely used by patients when in pharmaceutical formulations.

## Quality control

The quality of herbal drugs is determined mainly by the content of the bioactives responsible for the therapeutic effects and by the absence of contaminants. Each stage of production, from cultivation to extraction of raw materials, have an impact on the quality and quantity of the active compounds present in plants (Carvalho et al., 2010). The poor quality of the raw plant is a cause of concern to health care professionals and the scientific community because it may interfere with the efficacy and safety of the product (Boullata and Nace, 2000).

The analysis of commercial samples shows that these medications often do not meet pharmacological specifications of quality. This is indicative of the need to implement quantitative techniques to control the physical and chemical quality of raw plant materials. Furthermore, pharmaceutical companies that purchase these products must have greater discretion for proper use, storage and manipulation of these products, while performing appropriate quality control (Bara et al., 2006).

After a medicinal plant is harvested, it may lose quality in subsequent stages of processing, which makes the drying process fundamental for the quality of the final product (Borgo et al., 2010). Guarana seeds can be dried by several distinct methods and the choice of a particular method strongly influences the quality of the product. If drying is not performed properly, it can enable the degradation of bioactives, allow the infestation and growth of microorganisms, compromising the content of active ingredients (Carvalho et al., 2010). A physical chemical evaluation of guarana seeds submitted to different drying methods was conducted (Ushirobira et al., 2004). These authors reported the highest content of methylxanthines obtained with seeds dried in a metal pot for 4 h, with the addition of water. In contrast, the highest content of total tannins was found under the same conditions, but without the addition of water. Another relevant point is the temperatures used in these drying processes. The temperature 120 °C was determined as optimal for drying plant extracts using the fluidized bed drying technique (Pagliarussi et al., 2006).

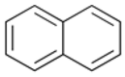
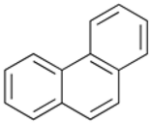
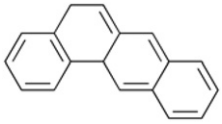
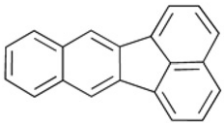
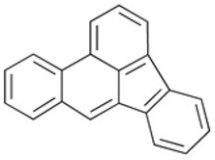
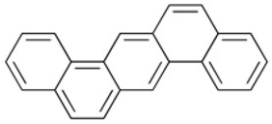
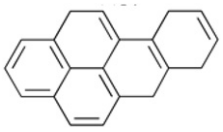
Some of the processing steps for guarana, such as its storage, are also fundamental in quality control of the final product. The incorrect storage of seeds can lead to loss of material whether for physical or biological reasons. One concern about the quality of natural products is the potential for contamination by fungi, with the risk of the presence of mycotoxins (Kneifel et al., 2001). This makes a product unfit for consumption, even when in its original package (Carvalho et al., 2010). Considering the increased use of herbal products as alternative medications, standards have to be established for herbal drugs to reduce risks to consumer health. Fungal contamination can occur in the process of planting and harvesting, as well as in the manipulation of seeds, if performed improperly (Kneifel et al., 2001). This contamination can lead to deterioration and affect organoleptic characteristics.

There are no specification limits for fungal contamination of guarana in the Brazilian legislation, nor is there any mention of how it should be marketed or packaged. The Brazilian legislation (Anvisa, 2001) only establishes maximum limits for coliforms at 45 °C (10 CFU $g^{-1}$), *Staphylococcus* coagulase positive (500 CFU $g^{-1}$) and *Salmonella* sp. (absence in 25 g) for guarana (powder, capsules, tablets or similar forms) alone, or in combination with other drugs.

The chemical composition of guarana (Table 3) is stamped by the package by its energy content. In this way, there may be contamination with fungal strains as a result of the high content of lipids and carbohydrates in guarana seeds. Additional factors, such as water activity, moisture, substrate composition, and insect-caused damage also influence fungal growth and mycotoxin production (Aquino et al., 2007). The presence of mycotoxigenic strains in samples of guarana has been reported (Bugno et al., 2006). In another study, it was found the presence of toxigenic strains in 2% of the samples analyzed and identified *Aspergillus* sp. and *Penicillium* sp., which are both mycotoxin-producing, affecting food safety (Martins et al., 2014). One strategy to decrease fungal contamination could be radiation by ionization (gamma rays) of these plant materials, avoiding the risk of contamination of consumers and manufacturers. This could be useful in attesting the sanitary quality of the product (Aquino et al., 2007). A microbiological study by Aquino et al. (2007), showed that 90% of the samples of powder guarana, purchased in open-air markets, showed fungal growth above the limit set by the World Health Organization (WHO) (1998) which is $10^3$ CFU $g^{-1}$ in raw materials for internal use. The predominant flora was composed of *Aspergillus* (82%) and *Penicillium* (15%) (Aquino et al., 2007). A total of 70% of the vegetal raw material from factories and pharmacies also exceeded the limit established by WHO (Aquino et al., 2007). The treatment of these samples with 5 kGy of irradiation reduced 85% of the contamination, remaining within the limits established. With the highest dose (10 kGy), gamma irradiation completely eliminated the contamination of guarana (Aquino et al., 2007).

Another type of contamination produced in the processing of guarana seeds that may affect their quality is polycyclic aromatic hydrocarbons (PAH). PAH are a family of compounds characterized by having two or more condensed aromatic rings (Box 4). They represent an important class of chemical formed during the incomplete combustion of organic material and are considered to be carcinogenic and genotoxic. They occur as contaminants in various types of food, mainly as a result of environmental pollution and some types of processes, such as smoking, drying and roasting. During the processing of guarana seeds, these substances could be formed as chemical contaminants. The presence of five PAH compounds was analyzed in thirteen brands of guarana powder selected (Camargo et al., 2006). At least 1 of the 5 contaminants was present in 81% of the samples, and in 35% of samples, all of the PAH were detected. This study (Camargo et al., 2006) showed a wide variation in the average levels of PAH (0.05–13.95 μg $kg^{-1}$), among the evaluated brands. Another study reported the concentration of PAH found in various brands of guarana powder ranged from 0.39 to 1.60 μg $kg^{-1}$ (Veiga et al., 2014). These results indicate that this wide concentration range probably results from the various forms of guarana processing, leading to the presence of these contaminants in the final product. However, the quantities of PAHs found were below

**Box 4: Chemical structures of polycyclic aromatic hydrocarbons that may be present as contaminants of guarana seeds, other sources and their effects on humans.**

| Compounds | Sources | Associated with: |
|---|---|---|
| Naphthalene  | Black walnut, in many essential oils, cigarette smoke, and mothballs | irritating to the eyes and skin, hemolytic anemia, damage to the liver and neurological system, cataracts and retinal hemorrhage, potentially carcinogenic |
| Phenanthrene  | Cigarette smoke | irritant, photosensitizing skin to light |
| Benzo(a)anthracene  | Gasoline and diesel exhaust, tobacco and cigarette smoke, charcoal-broiled foods, asphalt and mineral oils | potentially carcinogenic |
| Benzo(k)fluoranthene  | Gasoline exhaust, cigarette smoke, coal tar, coal and oil combustion emissions, lubricating oils | potentially carcinogenic |
| Benzo(b)fluoranthene  | Gasoline exhaust, tobacco and cigarette smoke, coal tar, soot, amino acids and fatty acid pyrolysis products | potentially carcinogenic |
| Dibenzo(a,h)anthracene  | Gasoline exhaust, tobacco smoke, coal tar, soot and certain food products, especially smoked and barbecued foods. | mutagen and potentially carcinogenic |
| Benzo(a)pyrene  | Environment pollution and cigarette smoke | potent mutagen and carcinogen |

*Source: Pubchem. https://pubchem.ncbi.nlm.nih.gov/.*

the values set by European legislation (EC 835/2011) for other food types, as there is no specific legislation in Brazil for the safe limit of these compounds in guarana.

There is a need to implement analytical tests of quality control that are accurate, sensitive, reproducible, easy to implement, and low-cost, for both the analysis of a medicinal plant, as well as for the analysis of its extracts and derivatives. There are several physico-chemical and analytical tests used to characterize a medicinal plant, for example, loss on drying, level of extractives, dry matter content, level of methylxanthines, total tannins, moisture, and ash. Thermal analysis, for example, thermogravimetry, is a potential tool for measuring technological parameters, in quality control, and in the analysis of moisture and ash contents (Araújo et al., 2006). Spectrophotometric methods have been used in samples of guarana seeds to determine methylxanthines and total tannins (Ushirobira et al., 2004; Yamaguti-Sasaki et al., 2007; Sousa et al., 2011).

UV-visible spectrophotometry, chromatographic analysis by thin-layer chromatography, HPLC (Marx and Maia, 1990; Klein et al., 2012; Machado et al., 2018), CZE (Sombra et al., 2005; Kofink et al., 2007), and micellar electrokinetic chromatography (Mello and Ito, 2012) are techniques used in the separation of substances present in the guarana extract. This is an important step to establish a chromatographic profile of the extracts and consequently, their standardization.

A simple and rapid HPLC-PDA method was developed and validated for the simultaneous quantification of seven chemical markers in dry guaraná seed powder: theobromine, theophylline, caffeine, catechin, epicatechin, procyanidins A2 and B2 (Machado et al., 2018). The extraction method developed employed liquid-solid maceration using a solvent mixture of ethanol:water (8:2, v/v) with diluted acid ($H_3PO_4$ 0.1% in water, v/v) with three successive extractions in 10 min each.

## Preparation of extracts, standardization and pharmaceutical forms

The choice of extraction method is the most important part of the extraction process because the composition of bioactives will be heavily dependent on this step and, consequently, it will have an impact on the expected pharmacological action. This can occur in both alternative ("green") extractions, such as supercritical extractions (by microwave or ultrasound) and in conventional extractions, namely, liquid extractions based on organic solvents or mechanical pressing. Supercritical extraction, for example, can be selective, and can obtain more or less polar compounds, according to the cosolvent added to the system, or by defining other conditions of extraction, for example, temperature (Marques et al., 2016). In addition, supercritical extracts of the same plant can show a greater concentration of phenolic compounds and antiradical activity than extracts resulting from solid extraction-liquid extraction (Pinelo et al., 2007). They can even be more effective in extracting substances with antimicrobial activity compared with extracts obtained from methanolic extraction (Liu et al., 2007). A similar behavior occurs in conventional extractions, that is, they may present more or less pronounced biological activities, or even absence of activity, depending on the conditions of extraction used for the same plant (Kalia et al., 2008; Chiste et al., 2014; Murugan and Parimelazhagan, 2014; Bektas et al., 2016; Nguyen et al., 2016). Therefore, it is still surprising that many studies do not consider these aspects in the preparation of herbal drugs and do not clarify how the relevant extract was prepared.

Some authors indicate the treatment dose based on guarana seed powder (Galduróz and Carlini, 1994, 1996; Fukumasu et al., 2006b; Bulku et al., 2010), often bought commercially and previously ground, in the form of capsules or tablets (Campos et al., 2011; Silvestrini et al., 2013). In other studies, the treatment involves simple dilution of the powder in water or another solvent of the toasted and ground seed alone (Espinola et al., 1997; de Oliveira et al., 2002; Fukumasu et al., 2006a; Freitas et al., 2007; Fukumasu et al., 2008, 2011; Krewer et al., 2011; Leite et al., 2011; Oliveira et al., 2011; Leite et al., 2013; Kober et al., 2016; Yonekura et al., 2016). In addition, ground guarana seeds or their extracts may be used in association with other herbal drugs, usually in commercially available formulations (Antunes et al., 2001; Boozer et al., 2001; Campos et al., 2004; Bérubé-Parent et al., 2005; Opala et al., 2006; Ruxton et al., 2007; Kennedy et al., 2008; Bulku et al., 2010; Pomportes et al., 2015a, 2017). Several studies have reported on an extract obtained using a controlled temperature, defined extraction time and standardized amount of solvent (Bydlowski et al., 1988; Bydlowski et al., 1991; Miura et al., 1998; Barbosa and Mello, 2004; Basile et al., 2005; Lima et al., 2005; Haskell et al., 2007; Jippo et al., 2009; Portella et al., 2013; Machado et al., 2015). Other authors grind the intact seed and subsequently extract it with organic solvents and then semipurify these extracts, commonly by means of partitions with different solvents (Otobone et al., 2005, 2007; Antonelli-Ushirobira et al., 2007; Yamaguti-Sasaki et al., 2007; Roncon et al., 2011; Dalonso and Petkowicz, 2012; Rangel et al., 2013; Matsuura et al., 2015) or other types of extraction, for example, supercritical extraction (Mehr et al., 1996; Saldaña et al., 2002; Marques et al., 2016).

All of these methods of extraction or preparation of the samples are valid, provided that researchers perform quality control of herbal drugs and standardization of the extract by means of chromatographic or spectroscopic techniques (Ushirobira et al., 2004; Edwards et al., 2005; Sombra et al., 2005; Kofink et al., 2007; Pelozo et al., 2008; Klein et al., 2012; Roggia et al., 2016; Mingori et al., 2017). However, most studies to date have not quoted if quality control of medications was performed. Often, there is also a lack of essential information about the process, for example, the type of extraction performed, the solvent used, temperature, time of extraction or other relevant and important information necessary for the standardization.

An efficient way that some authors have found to give greater reliability to their results, as well as provide specific information about the steps of extraction, is the standardization of analytical methods to evaluate and quantify the major components of their extracts (Kennedy et al., 2004; Haskell et al., 2007; Majhenic et al., 2007; Yamaguti-Sasaki et al., 2007; Campos et al., 2011; Fukumasu et al., 2011; Roncon et al., 2011; Bittencourt et al., 2013, 2014; Portella et al., 2013; Hertz et al., 2015; Kober et al., 2016), for example, by HPLC (Klein et al., 2012; Cadona et al., 2016; Machado et al., 2018), CZE (Kofink et al., 2007), and NMR (Yamaguti-Sasaki et al., 2007). The key substances present in the guarana plant can be used as chemical markers (Funasaki et al., 2016). The intake of ultrafine guarana powder was used in overweight humans, by diluting this powder at the time of consumption (Yonekura et al., 2016). These authors searched the nutrients of the powder (proteins, lipids, carbohydrates, ashes, humidity, and calories) and phytochemical composition of guarana seeds (total polyphenols, catechins, proanthocyanidins, and methylxanthines) after extraction, and investigated the individual flavonoids by HPLC with an electrochemical detector.

Other authors have prepared dosage forms of these fractionated and standardized extracts. The development of a pharmaceutical form comprises several steps including studies on pre-formulation and formulation themselves, which consist of the physical, chemical, physicochemical, and biological characterization of all raw materials including the drug used in the preparation of the product, as well as the anatomical and physiological characterization of the route of administration and absorption and, finally, the preparation of the dosage form (Wanczinski et al., 2002).

From a pharmaceutical technology perspective, the drying of plant extracts is a crucial step to developing a product suitable for industrial use and therapeutic application (Couto et al., 2013). Spray drying is a promising approach for the development of phytopharmaceutical intermediate products. It is a method of preparation of microparticles that is widely used in the fields of pharmaceutics and biochemistry and in the food industry due to the wide availability of the equipment and ease of industrialization. A UV–vis method of validation was developed for the quantification of caffeine and total polyphenols using the granulated form of the extract of guarana seeds (Pelozo et al., 2008). The method showed a good performance in the quantification of caffeine and total polyphenols. Microspheres containing semipurified guarana extract were obtained by spray-drying, using a combination of maltodextrin and gum arabic, which provided a satisfactory encapsulation efficiency (80–110%) and product efficiency (55–60%), thus, demonstrating the viability of producing these microspheres by spray-drying (Klein et al., 2015).

In another study, the same group of researchers evaluated the technical feasibility of producing a semipurified extract of guarana in tablet form, using a process of direct compression (Klein et al., 2013). Using method provided in pharmacopoeia, technological and physical-chemical assays were performed. They obtained tablets with quality features that meet pharmacological specifications and are suitable and safe for administration (Klein et al., 2013). Although several authors are concerned about standardizing extracts to investigate their biological activity or pharmacological effect, there are still gaps to be filled regarding the form of preparation of the medicinal plant, in numerous studies which use guarana seeds.

The dissolution behavior of various herbal medicines in the form of capsules and pills containing guarana obtained from different locations was evaluated (Sousa et al., 2011). These authors found that 100% of the herbal drugs examined, were in disagree-

ment about the presence of 4 markers, showing that 60% had 3 markers (caffeine, catechin, and epicatechin), while 40% had just caffeine. Only the capsules had at least 80% of the markers. The fourth marker, theophylline, was not found in any of these herbal medicines. These results highlight the need for rigorous quality control, starting with the medicinal plant, thus, ensuring the therapeutic action of these drugs.

Some authors carefully report the implementation of quality control of the drug. However, values of caffeine and total tannins of guarana are discrepant from those already reported in the literature on the chemical composition of this medicinal plant (Table 3) or do not meet previously established pharmacopoeial standards (Galduróz and Carlini, 1994, 1996; Espinola et al., 1997; Mattei et al., 1998; Oliveira et al., 2013).

From this discussion, it is evident that studies are primarily focused on the results of the research; however, it is crucial that previous measures should be taken. The form of preparation of the extract can select a specific group of compounds and that can often provide conflicting results for the same pharmacological action being investigated. Many environmental factors have an impact on the synthesis of secondary metabolites, both for total contents and relative proportions. Some of these include UV radiation, water availability, seasonality, atmospheric composition, altitude, temperature, and soil composition (Gobbo-Neto and Lopes, 2007). These factors, combined with the genetic factor and the form of extraction, increase the chance of an extract being unique.

Therefore, when there is a lack of standardization and even lack of concern about how the extract is obtained, questions arise as to the reliability of the results. When searching the same pharmacological effect, authors using the guarana extract can present variable results for numerous reasons, namely, the method itself or the test used to measure that effect. Additionally, the comparison of studies is often not feasible because the forms of preparation of the extract are distinct or often unknown.

## Conclusions

Although guarana has been the focus of many scientific studies, there are still gaps to be filled. This literature review described the botanical characteristics, presented recent data on the cropping and production of guarana, and highlighted all the substances that have currently been identified in this plant. Studies that showed the full range of pharmacological actions already searched for guarana seeds were covered. In addition, the importance of quality control of herbal drugs was emphasized, followed by the required standardization of their extract, due to consequent impacts on pharmacological action.

It is known that the pharmacological activities of plants are due to the distinct and diverse compounds existing in their composition and their proportion can be changed depending on the way the extract is prepared. These differences will be resolved through quality control of the medicinal plant and the standardization of its extract. The quality control of herbal drugs is essential to ensure the pharmacological standard of quality of guarana by means of analysis required for this plant. Another crucial point is the standardization of the extract that will be used for both *in vitro* and *in vivo* tests, by identifying and quantifying the main compounds present in guarana seeds. With this data set and knowledge of the low potential for toxicity of the extract, the results and conclusions can have greater reliability concerning guarana, as well as provide a reference for future scientific studies.

## Conflicts of interest

The authors declare no conflicts of interest.

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