QUANTITATIVE DETERMINATION OF METHYLXANTHINES AND POLYPHENOLS IN PLANT SUBSTANCES

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ABSTRACT: The use of legal psychoactive substances determined by the challenges of modern life is constantly growing. Caffeine-containing herbal substances possess a major role in this relation. The development of trade and the supply of exotic plant substances and products in recent years allow sale of a very broad range of products containing caffeine. The aim of our work is to determine the amount of total methylxanthines and caffeine as well as valuable polyphenols and flavonoids in five most commonly used caffeine-containing drugs: black tea, green tea, yerba mate, coffee and guarana berries.

Key words: legal psychoactive substances, polyphenols, flavonoids, caffeine, methylxanthines

Introduction

Caffeine is the world's most consumed psychoactive substance (Fig.1.). It acts as a central nervous system stimulant, and is legal and unregulated in nearly all parts of the world. Caffeine has pharmacological effects on heart, peripheral and central vasculature, renal, gastrointestinal and respiratory system. Beverages containing caffeine, such as coffee, tea and energy drinks enjoy great popularity. In North America, 90% of adults consume caffeine daily. There are numerous benefits from the use of caffeine containing drinks. It's proved that ordinary consumption of caffeine may be a protection against some diseases as Parkinson’s disease, and some types of cancer [9].

The most important sources of caffeine are coffee (Coffea spp.), tea (Camellia sinensis), guarana (Paullinia cupana) and Yerba Mate (Ilex paraguariensis). The amount of caffeine found in these products varies – the highest amounts are found in guarana (in the form of pasta) (4–7%), followed by tea leaves (3.5%), coffee beans (1.1–2.2%) and Mate tea leaves (0.89–1.73%) [4].

![Fig. 1. Chemical structure of Caffeine.](image)

Other valuable constituents in most used drugs containing caffeine are phenolics. A group of natural phenols called flavonoids are of very interest because they are powerful antioxidants, prevent and treat many diseases such as cardiovascular diseases and cancer. Catechins are the largest type of flavonoids in growing tea leaves. Catechins in tea include epigallocatechin-3-gallate (EGCG), epicatechin (EC), epicatechin-3-gallate (ECg), epigallocatechin (EGC), catechin, and gallatechin (GC). Among all the catechins in tea, EGCG is the main subject of scientific study with regard to its potential health effects [2], [7], [10], [13], [14]. Catechin monomer structures are metabolized into dimers theaflavins and they directly contribute to the bitterness and astringency of steeped black tea [11] (Fig. 2). Catechins constitute about 25%
of the dry weight of fresh tea leaf [1] although total catechin content varies widely depending on species, clonal variation, growing location, season, light variation, and altitude. They are present in nearly all teas made from *Camellia sinensis* (Theaceae) including white tea, green tea and black tea.

![Fig. 2. Chemical structure of Catechin.](image)

The mainly phenolic compounds in Guarana seed are hydroxybenzoic derivatives (acids, esters and others) and flavonoids [8]. The found phenols from Mate tea include caffeoyl derivatives of caffeic acid, chlorogenic acid, 3, 4-dicaffeoylquinic acid, 3, 5-dicaffeoylquinic acid, and 4, 5-dicaffeoylquinic acid. These caffeoyl derivatives are the primary constituents that account for the antioxidant capacity of Mate tea [6] (Fig. 3). The hydroxycinnamic acids - caffeic acid and its derivative chlorogenic acid (a caffeic acid ester of quinic acid) are the main phenols component in coffee. Coffee contains 70-350 mg of chlorogenic acid [3].

![Fig. 3. Chemical structure of 1) Caffeic acid and 2) Chlorogenic acid.](image)

The aim of this work is to establish the content of caffeine and plant phenols in plant substances from bulgarian markets, as well as the substance with the best correlation of these natural compounds.

**Material and methods**

As plant objects were use caffeine-containing drugs from bulgarian markets. They are leaves from *Camellia sinensis* (Green tea and Black tea from China), leaves from *Ilex paraguariensis* (or Yerba Mate tea from Paraguay), semen from *Caffeae arabica* from Ethiopia and semen from *Paullinia cupana* (or Guarana) from Brazil. To determine main components in our plant substances we used spectrophotometrical methods.

**Quantification of total phenols**

The determination of total phenols in the plant drugs was performed according to the European Pharmacopoeia [5] involving Folin-Chioalteu reagent and pyrogallol as standard. The analyses were carried out at 760 nm. The measurements were carried out using a Ultraspec 3300 pro UV/VIS spectrophotometer (USA). All determinations were performed in triplicate (n = 3).

**Quantification of flavonoids**

The content of the flavonoids in the plant drugs was spectrophotometrically determined at 430 nm by creating a complex with AlCl₃ according to the Russian Pharmacopoeia [12]. The content of flavonoids was calculated as quercetin. The measurements were carried out using a Ultraspec 3300 pro UV/VIS
spectrophotometer (USA). All determinations were performed in triplicate (n = 3).

**Quantification of total methylxantins and caffeine**

Accurately weighed amount of drug were boiled in water for 15 minutes. The combined aqueous extracts were acidified with sulfuric acid and concentrated. The solution was extracted with chloroform in separating funnel. Chloroform extract was washed with sodium hydroxide and then with water. After evaporation of a chloroform a mixture of methylxanthines was obtained. From this mixture the caffeine was prepared by sublimation then the percentage of caffeine in plant substances was calculated.

**Results and Discussion**

The amounts of the total polyphenols ranged from 7.32% to 3.01%. The highest level of polyphenols was found in Black tea (7.32%), followed by Yerba Mate (6.18%) and Green tea (5.97%). Coffe has the lowest content of total polyphenols (3.01%), preceded by Guarana (4.09%) (Fig. 4).

![Content of Polyphenols](image)

**Fig. 4.** Content of polyphenols in some plant drugs.

The highest content of flavonoids was also found in Black tea (1.72%) and the lowest amount was observed in Yerba Mate (only 0.04%). Green tea showed moderate content of flavonoids (1.12%), and Guarana and Coffe relatively low content (0.78% and 0.26%, respectively) (Fig. 5).

![Content of Flavonoids](image)

**Fig. 5.** Content of flavonoids in some plant drugs.
The data in Figure 6 revealed that the highest quantities of the methylxanthines were in Coffee (1.72%), followed by Yerba Mate (1.57%) and Guarana (1.57%) but the highest amount of caffeine was determined in the sample of Green tea (0.80%), followed by Black tea (0.67%). According to the presented data the content of Coffee in Yerba Mate and Guarana was approximately equal and didn’t exceed 0.40%.

Fig. 6. Compare of methylxantines and caffeine content in some plant drugs.

After the comparison of the caffeine and methylxanthines content in five plant drugs it was observed that in Green tea and Black tea were quantified the highest amounts of both substances. Green tea contained 1.31% methylxanthines and 0.80% caffeine and Black tea 1.15% and 0.67% respectively.

The obtained results demonstrated the benefit of consuming green tea and black tea, because the amount of valuable polyphenols and flavonoids compared with the stimulating effect of caffeine is highest. To comparison, the other substances also have high levels of total phenols, but low content of flavonoids. Most consumed in Bulgaria stimulating drink coffee showed the lowest caffeine content.

Conclusion

Tea is the richest source of polyphenols and flavonoids. This determines the benefits and the wide use of green and black tea as the main energizing drinks in the world.

References


12. Russian Pharmacopoeia. 1990. 11th ed., Moscow, Russia


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