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ANTIOXIDANT EFFECTS OF NATURAL BIOACTIVE COMPOUNDS

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Abstract

High concentrations of reactive oxygen species (ROS), have harmful effects which are expressed by living organisms' biomolecules damage, including lipids, proteins and nucleic acids. Numerous studies indicate that a reduced risk of various lifestyle diseases [2, 3, and 5], mainly cardiovascular diseases and cancer, as well as other disorders, is associated to a diet rich in fruits, vegetables and their products. Our research was focused on the principal roles of ROS in biological processes and diseases and how natural bioactive compounds of herbs determine their health-promoting properties [1]. Selected compound was Green tea.

During the experimental work, 300 g of gunpowder green tea leaves were taken for caffeine extraction, using dichloromethane and anhydrous sodium sulphate. The extracted caffeine was assessed by analysis with tannic acid. Total amount of phenolic compounds has been determined by using Folin-Ciocalteu and sodium carbonate solutions. 1 mL of aqueous solution for analysis, was treated with a Folin-Ciocalteu reagent, diluted before with water in 1 : 10 ratio). 5 mL of this diluted solution was poured into 1 mL of aqueous solution. Bluish color was obtained by the addition of 4 mL 7.5% of Na2SO4 solution in distilled water. Spectrophotometric determinations were performed after a 30 min. period of reaction, using a UV-Vis. spectrophotometer. A calibration curve was obtained from a series of gallic acid standard (25-150µg/mL of 50% methanol) used to express the concentrations of phenolic as g of gallic acid equivalents for one gram of dry weight. Also we performed a survey, using a survey method based in questionnaires with closed-ended questions, for 600 people belonging to different population groups, principally adults and people of the third age (60% adults, 30% elderly and 10% children's). Gained data, related with the tea consummation were processed mathematically using simple statistical analysis. Data were grouped and summarized and the mid-point of all them were determined (median).

The caffeine content was 3 - 4% and total polyphenols amounts were 25 - 35%, depending by selected samples. Determined percentage of the above compounds are responsible for the powerful antioxidant properties of selected green tea; most of his effects are associated also with flavonoids and their antioxidant potentiality. Our survey showed that only about 25% of adults were consumers of green tea, considering it a dietary product. About 5% of elderly were casual consumers with minimal knowledge's related with the product.

As conclusions, considerable % of caffeine and polyphenols were determined in specific varieties of green tea. A number of responders were regular consumers of green tea and some others were not familiarized with this product. A big difference between the consumers were discovered in their knowledge's about ROS effects and the antioxidant role of green tea and similar products. A very small part of them had sufficient knowledge's for its chemical compounds and therapeutic values.

***Key words**: Functional food, Natural bioactive compounds, Antioxidant, Green tea.*

1. Introduction

Tea (*Camellia sinensis*) contains all sorts of physiologically effective substances. Green tea is particularly known for its functional and healing effects. The composition of catechins in commercial teas varies based on the species, season, horticultural conditions, and most importantly, the degree of oxidation during the manufacturing process. There are four major varieties of teas: white, green, oolong, and black. Although all

teas are derived from the same *Camellia sinensis* plant, the processing methods for each tea are different (white tea is naturally dried using either sun drying or steaming methods before being minimally processed to prevent oxidation.) These processing methods protect the tea flavor and preserve the high catechin concentrations. Green tea represents about 20% of the total tea production.

The production process for green tea is very similar to white tea, and it also contains a relatively high concentration of catechins [4, 5, and 6].

Since the leaves have not been subjected to oxidation by enzymes, the polyphenols contained are mainly catechin gallate, including epigallacatechin gallate (EGCG), which is particularly powerful bioactive agent. The first thing we notice when enjoying a cup of tea is the stimulating effect. The caffeine it contains releases messenger substances in brain such as dopamine or serotonin which stimulate the central nervous system and considerably improve our mental capacity this effect was proven in the mid 1990's in extensive tests [2, 3, and 4].

Reactive oxygen species (ROS), play either harmful or beneficial role in biological systems. Beneficial effects of ROS include physiological roles in cellular responses against infectious agents and in several cellular signaling pathways. Harmful effects are due to high concentrations of ROS, which can damage biomolecules, including lipids, proteins and nucleic acids. Our cells contain the body's enzymes, which can neutralize the quantities of ROS that occur normally. The harmful effects of ROS are counterbalanced by the antioxidant action of both antioxidant enzymes and non-enzymatic antioxidants.

Selected compound was green tea, because what makes green tea special is its reported capacity to prevent cancer, strokes, heart attacks, high blood pressure and many other, often serious illnesses. These properties are related with its antioxidant potential, i.e. its reaction to the free radicals, which are constantly being created during metabolic processes, reducing their presence and their damages.

Our research was focused on the principal roles of ROS in biological processes and diseases and how natural bioactive compounds of herbs determine their health-promoting properties. Selected herbal teal was the green tea.

There are literally countless versions of green tea available. Although these are all different types of green tea, all of these different types share one common characteristic - they all come from the tea plant *Camellia sinensis*. What makes green tea special is its reported capacity to prevent: cancer, strokes, heart, attacks, high blood pressure and many other diseases, which is mainly due to its: antioxidant potential, and its reaction to the free radicals which are constantly being created during metabolic processes [4, 7, and 8]. Also known as Reactive Oxygen Species (ROS) these free radicals attack the human cell structures and damage DNA. Our cell contain body 'enzymes which can neutralize the quantitates of ROS as a result of stress, environmental pollution, diet and many others factors, but as a result of stress environmental pollution, diet and many other factors a surplus of free radicals can also build up. And this can have undesirable consequences - it can accelerate the aging process, damage blood vessels and modify the DNA which the main causes of cancer.

The different types of green tea emerge due to differing locations on where the tea is grown, slight changes in how the tea is cultivated, the differing climate in locations and a whole host of other factors.

Green teas mostly come from China and are known as: Gunpowder, Long Jing (Dragonwell), Pi Lo Chun (Green Snail Spring), Snowy Mountain Jian, Hyson Lucky Dragon. Some of the most known types of green tea from Japan, are: Gyokuro, Sencha, Bancha, Matcha, Houjicha, Kukicha and Genmaicha.

2. Materials and Methods

The gunpowder green tea leaves were taken for caffeine extraction, using dichloromethane and anhydrous sodium sulphate. This is one of the most popular types of Chinese green teas. This tea is still primarily grown in the Zhejiang province of China, although plantations for this tea are spreading elsewhere in China. One of the characteristics of this tea is that, once processed, the tea looks like tiny pellets. These pellets open up during the brewing process.

0.5 g of powdered green teal was weighted into a 50 mL volumetric flask. Then the sample was dissolved in 25 mL hot water (60 ^{0}C) using an ultrasonic bath within 10 minutes. The solution was cooled down and then it was added 5.0 mL solution of acetonitrile. The sample was then diluted in a volume of 50 mL distilled (in case of catechin calculation) with a stabilizing solution (containing an ethylenediaminetetraacetic acid (EDTA) solution, ascorbic acid-solution and acetonitrile). It was then centrifuged 13400 U/min. for 3 minutes. The supernatant was then measured with HPLC method.

A calibration curve was obtained from a series of gallic acid standard (25 - 150µg/mL of 50% methanol) used to express the concentrations of phenolic as g of gallic acid equivalents for one gram of dry weight. Catechin standards of epigallocatechin (EGC), epicatechin gallate (ECG), catechin, epicatechin (EC), epigallocatechin gallate (EGCG), gallocatechin (GC), and gallocatechin gallate (GCG) were prepared by accurately and was

separated by a Cosmocore-Cholester-column using an acetonitrile-water solution (acidified with phosphoric acid) gradient and detected using a UV-detector.

Also in this study, it was performed a survey, using a method based in questionnaires with closed-ended questions, for 600 people belonging to different population groups, principally adults and people of the third age (60% - adults, 30% - elderly and 10% - children's). Gained data, related with the consumed green tea, were processed mathematically determining median.

3. Results and Discussion

The results of the experimental work showed that the caffeine percentage of caffeine in the sample of the green tea corresponded to the value of 3 - 4% (Table 1) and total polyphenols 25 - 35%, whereas in the sample of the black tea total polyphenols were 30% (Table 2). Determined percentage of the above compounds are responsible for the powerful antioxidant properties of selected green tea; most of his effects are associated also with flavonoids and their antioxidant potentiality.

Table 1. Ingredients of green tea

Ingredients of green tea	On dry weight in %
Caffeine	3 - 4
Total Polyphenols	25 - 35
Flavon - 3-ole	17 - 30
Flavonols	3 - 4
Phenolic acid, Depside	5
Theaflavin	-
Thearubigen	-
Amnoacids	4
Organic acids	0.5
Polysacharides	13
Proteins	15
Lipides	3
Volative components	0.01 - 0.02

Table 2. Ingredients of black tea

Ingredients of Black tea	On dry weight in %
Caffeine	3 - 4
Total Polyphenols	30
Flavon - 3-ole	10
5	5
Phenolic acid, Depside	4
Theaflavin	0.3 - 1.8
Thearubigen	9 - 19
Amnoacids	4
Organic acids	0.5
Polysacharides	13
Proteins	15
Lipides	-
Volatile components	0.01

Referring to the survey, most of the respondents do not know the green tea plant and have lack of information that (black tea, pu-erh tea, green tea, Darjeeling tea, white tea) are produced from the same tea plant. 30% of elderly people used the green tea only for weight loss.

Figure 1 shows a chromatogram of a green tea containing the predominant catechins in tea. In addition, free gallic acid and moderate amounts of caffeine are naturally present in tea, and therefore were included in the mixed standard. The retention times of gallic acid, GC, EGC, caffeine, catechin, EC, EGCG, GCG, and ECG are show below in (min.), respectively.

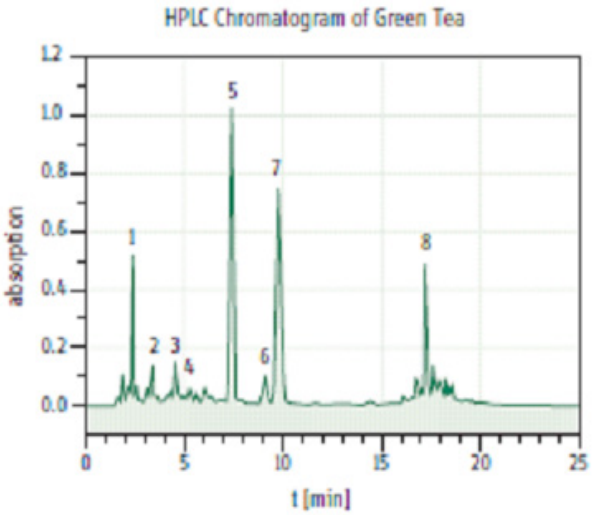

Figure 1. HPLC chromatogram of green tea

Legend: (1) Gallic acid, (2) theobromine, (3) epigallocatechin, (4) catechin, (5) caffeine, (6) epicatechin, (7) epigallocatechin gallate, (8) epicatechin gallate

The flavonol content of tea is minimally affected by processing, and flavonols are present in comparable quantities in oxidized and non-oxidized teas. Green teas contain more of the simple flavonoids called catechins, while the oxidization that the leaves undergo to make black tea converts these simple flavonoids to the more complex varieties called theaflavins and thearubigins.

Other bioactive compounds present in tea are: alkaloids, such as caffeine, theophylline and theobromine, amino acids, theanine (r-glutamylethylamide), proteins, carbohydrates, chlorophyll, volatile organic molecules, that is, compounds that easily produce vapours and contribute to the odour of the beverage, fluoride, aluminium, and trace elements.

Our survey showed that only about 25% of adults were consumers of green tea, considering it as a dietary product. About 5% of elderly were casual consumers were with minimal knowledge's related to the product.

4. Conclusions

- Considerable percentage of caffeine and polyphenols were determined in specific varieties of green tea and black tea. This percentage of antioxidants present in our healthy diet will help our organism to prevent the activity of ROS.

- A number of responders from our survey were regular consumers of green tea and some others were not familiarized with this product.

- A big difference between the consumers were discovered in their knowledge's about ROS effects and the antioxidant role of green tea and similar products. A very small part of them had sufficient knowledge's for its chemical compounds and therapeutic values. From all our survey we have check that only 40 from them know the really meaning of ROS. The rest consume products with antioxidant property but they don't know how they work in our organism.

5. References

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