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RESEARCH ARTICLE **(Open Access)**

Chemical composition and heavy metals in *Camellia sinensis L.*

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Abstract

Green tea (*Camellia sinensis L.*) is widely used as a medicinal plant. Researchers have made significant attempts to verify the value of this plant by scientific pharmacological screens in response to the different traditional assertions about its utility in treating a variety of diseases. As a stimulant, diuretic, astringent, and to promote heart health, green tea was employed. Green tea is also traditionally used to relieve flatulence (gas), control blood sugar and body temperature, aid in digestion, and enhance cognitive functions. According to reports, green tea, which is brewed from unfermented leaves, has the highest concentration of potent antioxidants called polyphenols. The purpose of this study was to identify the chemical composition of green tea and the presence of heavy metals compared to standards. The essential oil was extracted from the leaves of *Camellia sinensis L.* by steam distillation and chemically analyzed using GC/FID. The main components obtained in *Camellia sinensis L.* oil were: Bornyl acetate (2.670%), Thymol (2-isopropyl-5-methyl phenol) (4.092%) aromandrene (2.252%), and α-Humulene (2.432%). These components are reported to have anti-inflammatory, analgesic, antibiotic, also sedative properties and antiseptic, antibacterial, and antifungal actions. This study is designed to evaluate also four heavy metals by ICP-MS technics as bellow: Cd (0.15 mg/kg), Sn (<0.01mg/kg), Hg(0.001mg/kg), and Pb (1.8mg/kg). The amount of lead is somewhat high compared to other metals but within standards determined by WHO. This study provides reliable evidence on the chemical composition of green tea and that special care should be taken in the factors involved in the cultivation, processing, treatment, and packaging of teas in general. These contaminants have the potential to harm the environment irreparably even in low concentrations. Special importance should be given to the presence of heavy metals on purpose to protect the environment, the flora, and human health.

Keywords: *Camellia sinensis L*; Green Tea; chemical composition; heavy metals.

1. Introduction

Tea has a lengthy history; it was first used in China and has since spread, either directly or indirectly, throughout the world. According to the various definition techniques used in various nations, tea can be divided into a wide variety of types. The first tea to be found was green tea, which is a non-fermented tea. The presence of chemical components in green tea that are intimately related to human health has been proven by a significant number of researches.

The components that are isolated and separated from green tea, such as tea polyphenols, caffeine, theanine, and polysaccharides, have pharmacological effects on health, including anti-cancer [1], anti-oxidation [2], nervous system protection [3], and blood sugar reduction [4]. According to research, green tea is safe for those with hypertension, high cholesterol, coronary heart disease, arteriosclerosis and diabetes. Tea has many medicinally beneficial and active substances. The key chemicals that have been identified and their chemical structures are mentioned below.

1.1. Chemical components

The term "tea polyphenols" refers to all polyphenols found in tea. Over 30 different types of compounds exist, with catechins, flavonoids, anthocyanins, and phenolic acids being the majority of them [5]. Green tea has the highest concentration of polyphenols (20–30%), which makes it a good natural antioxidant. The majority of the alkaloids in tea are purine alkaloids. Most of them (2%) have caffeine in them. Moreover, it has very little theophylline and theobromine in it [6]. The main chemical constituents behind these three alkaloids are what give tea its cooling effect [7]. One of the key elements impacting tea quality is the type and concentration of amino acids. Amounts of amino

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acids in tea range from 1% to 4%. Twenty protein amino acids and six non-protein amino acids have so far been identified in tea, totaling 26 amino acids. Theanine, glutamic acid, arginine, serine, and aspartic acid have the largest concentrations [8]. Tea includes a little number of monosaccharides and disaccharides, including glucose, fructose, galactose, sucrose, and others, which accounts for the tea soup's modest sweetness. The majority of the carbohydrates in tea are polysaccharides, which are insoluble in water and include cellulose, starch, and pectin [5]. Green tea's scent is mostly composed of volatile aromatic compounds. The fragrance components make up only around 0.005% to 0.020% of the chemical composition of green tea, however, the varieties are very diverse [9]. There have been numerous publications on the examination of the volatile substances found in green tea, and new substances have also been found and recognized. The substances that create the aroma of green tea are primarily volatile aromatic substances. The aroma components make up only about 0.005% to 0.020% of the chemical composition of green tea, but the varieties are very diverse [9]. There have been numerous accounts on the analysis of the volatile substances found in green tea, and new substances have also been found and identified. Ash, the inorganic component in tea, is primarily made up of a few natural elements and their oxides. P and K are the two mineral elements that are found in the highest concentrations, followed by Ca, Mg, Fe, Mn, Al, S, Si, and minor elements like Zn, Cu, and F [10,11]. Scientists have focused a lot of attention on the valuable impact that minerals have on how the tea plant and the human body operate physiologically[12].

2. Materials and Methods

2.1. Materials

All the following reagents used for microwave digestion and oil extraction: nitric acid, hydrogen peroxide, and hexane were from Merck. Digestion microwave (BERGHOF model Speedwave XPERT), Analytical balance (Ohaus Corporation, USA), ICP-MS (Agilent technologies model 7800). Clevenger equipment (Isolab Labogerate GmbH). Nexis Gas Chromatograph GC-FID -2030.

2.2. Methods

The selection of sample was taken from the random pharmacies in Tirana. The origin of Green Tea (*Camellia sinensis L.)* used in this experimental work is from Bangladesh. Part of the plant chosen to be analyzed were aerial parts (leaves). Collected samples were shed and dried in absence of sunlight. It was then powdered using a grinding machine. The powdered samples were kept in a dry, air tight container at room temperature prior to analysis.

2.2.1. Analysis of sample preparation by microwave digestion.

This analysis was based on the digestion of 0.5g of sample (plant leaves) after grinding and placing in a container to which 6 ml of concentrated HNO_3 and 1 ml of H_2O_2 (30%) were added. Then the container was placed in the microwave where the heating plan was carried out with three running steps.

The digested samples were transferred to PTFE tubes and then diluted to a final volume of 50 ml with HNO_3 2% [13].

Nitric acid is most frequently employed since it is the best oxidant for arsenic during microwave digestion; nevertheless, some substances are compatible with hydrochloric acid rather than nitric acid. The indirect method involves first a closed vessel microwave digestion step, which is then followed by an analysis of the instrument. For this reason, we have used nitric acid for the digestion process by microwave.

2.2.2. Oil extraction

Clevenger apparatus was used to extract the oil from the leaves. Before extraction, the leaves of the Green Tea (*Camellia sinensis L.)* plant was cleaned of various impurities such as soil, stones, or other foreign plants. From the plant taken for analysis, 50 g were weighed and then dried at 40^0C to constant weight. After drying the plant underwent a grinding process before coming into contact with steam. Steam distillation extraction was carried out in a Clevenger apparatus using a ratio of 10:1 (ml/g) water/dry herb, which means 50g of sample was placed in a 500ml volumetric flask and filled with water until in the sign.

After building the Clevenger apparatus equipped with a condenser, they were placed in the round flask, which would be heated in a heating bowl. The Clevenger system was connected to a continuous water source so that the cooling process is done as well as possible. The heat is controlled so that the oil with water vapor enters the graduated distillation tube and the excess water returns to the flask. The water-plant mixture was then subjected to distillation for an optimal number of hours which was determined to be 3 hours. In the first 30 minutes after the oil had begun to collect in the Clevenger column, about 1 mL of hexane was introduced into the condenser to prevent any polyphenols or other essential oil components from contacting the water.

The obtained essential oils were dried with anhydrous sodium sulfate and stored at 4 - 5^{0}C for further analysis.

2.2.3. The Instrument Optimization for GC-FID and ICP-MS

Analysis of essential oil was carried out in Nexis Gas Chromatograph GC-2030 with a data handling software Lab solution, equipped with a Split/Split less injector SPL 2030, On – Column injector unit OCI 2030 and Flame ionization detector FID-2030. The essential oil was extracted from the leaves of Green Tea (*Camellia sinensis L.)* by steam distillation and chemically analyzed using GC/FID. 10µl of essential oil, was accurately weighed in a vial and 1000µl of Hexane. Temperature program of the column: 60^{0}C for 1 min, 4^{0}C/1min till 180^{0}C and 20^{0}C/1min till 250^{0}C, total 40 min. A carrier gas, Helium column flow 1ml/min constant speed, total flow 14 ml/min split ratio 100:1. Injection volume 1µl (10µl syringe).

For heavy metals analysis, a conventional Agilent 7800 ICP-MS was employed. An Agilent SPS 4 autosampler was used for sampling. The typical sample introduction system was set up in the 7800 ICP-MS (Inductively coupled plasma mass spectrometry).

The optimization of the instrument is done every day according to the conditions recommended by the manufacturer.

3. Results and Discussion

3.1. Data analytical methods for profile analyses with GC-FID

The identification of chemical compounds was made by a comparison of their relative retention time and mass spectra with those of compounds published in literature and databases. The identified components were listed according to their retention time. The percentage composition of compounds was calculated according to their chromatographic peak area.

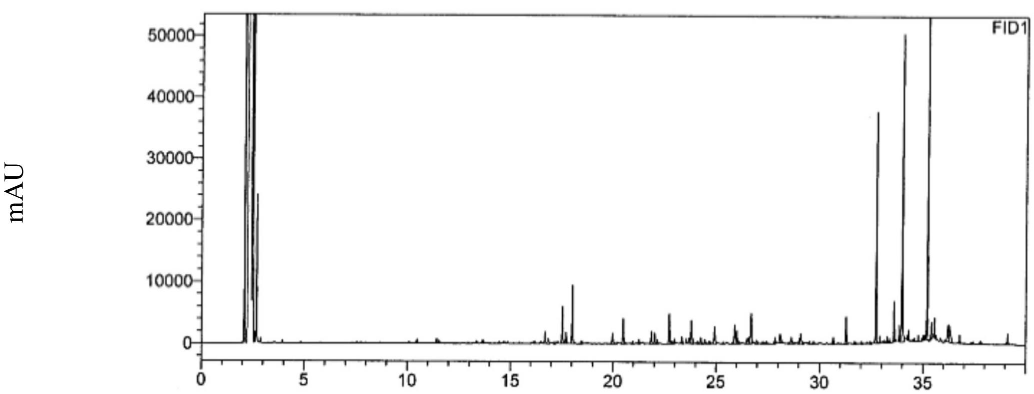

Figure 1.GC-FID Chromatogram of Green Tea profile

The essential oil was extracted from the leaves of Green Tea (*Camellia sinensis L.*) by steam distillation and chemically analyzed using GC/FID. The main components obtained in extracted oil were: Thymol (4.092%), Bornyl acetate (2.670%), α-Humulene (2.432%), aromandrene (2.252%), Caryophyllene- (E.) (0.981%), Myrtenyl acetate (0.97%), Caryophyllene oxide (0.381%), cis-Beta-Terpineol (0.312%), neo-iso-3 Thujanol (0.288%), Ledol (0.269%), Myrtenol (0.114%), Camphor (0.114%), E-Beta-Ocimene (0.108%), alpha-terpineol (0.104%), Borneol (0.058%), Terpinen-4-ol (0.041%). These components are reported to have antioxidant properties, anti-inflammatory, analgesic, antibiotic, also sedative properties and antiseptic, antibacterial, and antifungal actions.

3.2. Data analytical methods for heavy metal analyses with ICP-MS.

Four toxic heavy metals, including Pb, Hg, Sn, and Cd, were examined in total. These days, therapeutic plants are thought to be a source of heavy metal toxicity in both humans and animals. Tin, lead, cadmium, and mercury are the heavy metals most frequently linked to human harm. The World Health Organization (WHO) establishes boundaries for the presence of heavy metals in unprocessed medicinal plants. However, the majority of users do not check for heavy metal accumulation before using this plant. Heavy metals of the sample Green Tea (*Camellia sinensis L.*) were analyzed using extracted oil samples by ICP-MS. The percentage of oil after extraction was 0.2 %.

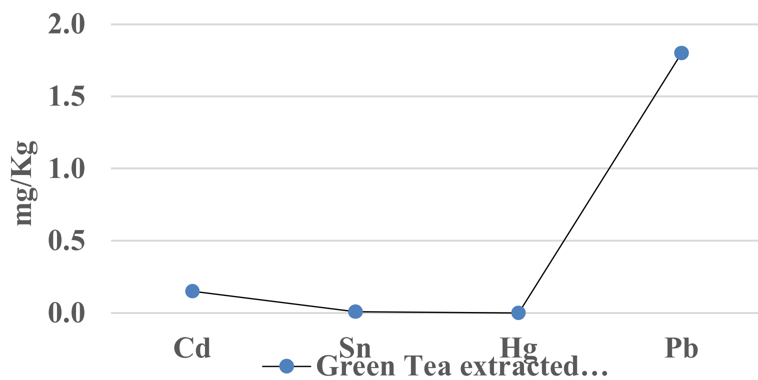

Figure 2. Distribution of the presence of heavy metals in Green Tea extracted oil.

This study is designed to evaluate also four heavy metals by ICP-MS technics as bellow: Cd (0.15 mg/kg), Sn (<0.01mg/kg), Hg(0.001mg/kg), and Pb (1.8mg/kg). The amount of lead is somewhat high compared to other metals but all values are within standards determined by FAO/WHO.

4. Conclusions

This study provides evidence of the dependability of relative amounts of compounds in tea types on factors involved in the cultivation, processing, handling, and packaging of teas that lead to the commercially available subtypes.

The consumption of different types of tea derived from Green Tea (*Camellia sinensis L.*) is most often associated with their antioxidant properties. However, more research is needed to elucidate the properties and mechanisms of action of phenolic compounds in this drink enjoyed worldwide. Heavy metals accumulate in plant parts as a result of environmental pollution, which ultimately makes their way into the human food chain. Before using plant parts and extracts for human ingestion, it is necessary to regularly test raw materials for the presence of pollutants.

5. Acknowledgements

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