

BRAND VALUE VERSUS THE QUALITY OF PHYTOTERAPEUTIC PRODUCTS WITH ANTIOXIDANT BIOCOMPONENTS FROM THE GREEN TEA

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ABSTRACT. A rich source of compounds with antioxidant effect is the tea, especially the green tea. Antioxidants activities including those contained by the green tea, depend not only on their structural properties, their chemical reactivity to radical peroxy- acids or other active species, for example but they also depend on many factors, such as concentration, temperature, light, substrate type, physical state of the system, as well as the many microorganisms and minerals that act as pro-oxidant or synergists. This study aims to verify a correlation between the image in the consumer's mind, the image of the brand for a phytotherapeutic product from herbal tea, and its actual quality, particularly by the presence of a suitable antioxidant activity that corresponds to the antioxidant concentration within the necessary components. This correlation was checked with various quality control techniques, including HPLC.

Keywords: antioxidants, green tea, quality, brand value

INTRODUCTION

Natural antioxidants are found in almost all the plants, microorganisms, fungi and even in animal tissues. Most natural antioxidants are phenolic compounds, the most important group of natural antioxidants are tocopherols, phenolic acids and flavonoids.

Flavonoids are a large group of natural phenols contained by plants.

They are characterized by C6–C3–C6 Carbon skeleton. The basic structure of these compounds is the existence of two aromatic rings linked by an aliphatic chain of three carbons, which was normally condensed to form a ring of Piran, or, more unusually, a furan ring.

Flavonoids, including flavones, flavonols, isoflavonols, flavonons and chalcones appear in all higher plant tissues.

Flavonols and flavones are found in almost any plant, especially leaves and petals, flavonons appearing more often than flavones (Voss, 1992).

The most common flavonoids are apigenin, quercetin, miricetina, morine and kaempferol. Approximately 90% of the flavonoids contained by plants occur as glycosides.

The ability of flavonoids to inhibit lipid oxidation is well documented, both for the byproducts of natural lipids and other lipid models.

Flavonoids can act as antioxidants by eliminating the radicals that include superoxide anion, hydroxyl radical and lipid radical peroxyacids.

Other mechanisms of action of some flavonoids include the annihilation of singlet oxygen, metal chelating and lipooxygenizing inhibition.

The presence of these antioxidants in a variety of teas is well known and studied in many publications.

Tea composition field attracts further interest in phytochemistry and pharmacognosy researchers. Relatively recent work indicates the presence of compounds belonging to polyphenol category in green tea and black tea.

Antioxidant properties of tea are often attributed to these polyphenols. Latest news in the field is linked to the discovery of dimmers or oligomers of some polyphenols, previously known in a monomer form.

For the tea components study, high–pressure liquid chromatography, HPLC is highly affordable.

Efficiency of chromatographic columns, available today, enables an advanced separation of a significant number of components. In addition, the development of specific and selective detection methods opens a new dimension for the use of this technique in phytochemical analysis bill in general and particularly for the study and characterization of tea.

Herbal products consumers generally expect that a product name, product brand, has a high quality component.

This is because a brand is a real estate property, that means the product is not limited, but assumes the existence of a mental space, a fraction in their minds) and at the same time, it is intangible (Cooper, 2010).

Basically one of the brand's core missions is to differentiate the product–in this case a green tea leading brands–from other similar products on the market, no matter how many they are.

Differentiation is based on the concept of singularity, which creates in the mind of a potential consumer market the perception that there is no similar product brand product (Kaufer, 2010).

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As such, the sale is the brand. In the era of multimedia, the guarantee of a product is the brand name rather than just recommendations of a professional. The power of a brand lies in its ability to influence buying behavior (Temple, 2010).

Thus, the advanced technology described above enables us the control to observe the extent to which brand value lies in product quality and Green Tea in the form of bags made by one of the market leaders in this field.

Therefore, this study runs polyphenolic extract dominant components of the variety of green tea and their separation by using high pressure liquid chromatography (HPLC).

Development and testing of HPLC methodology is also part of the original study.

Within this study information is collected on general plant biocomponents of sachets of tea and on this potential of impurities given that plants can extract and set different types of compounds out of air and soil. This way, variations in chemical composition depend on the stage of plant growth, soil conditions, climatic factors and analyzed species. (Ragaei et al., 2006).

MATERIALS AND METHODS

The purpose of the study was an assortment of green tea, a brand of one of the market leaders in this field, delivered in bags with doses of 1.30 g.

According to the importer company statement, the dose of a bag contains 30 mg flavonoids.

Chromatographic analysis

Solvents used for extraction and elution (methanol and hexane) were of chromatographic purity, coming from "Merck" company. Extraction was done by recirculation of methanol over raw material in a Soxhlet extraction installation, belonging to "QuickFind" company. Analytical HPLC separation was performed with a column "Nucleosil" with a stationary reverse-phase (C18) of size 25x0, 46 cm. Mobile phase pumping unit, mixer scheduled degassing unit and the ultraviolet detector and visible absorption were manufactured by "Jasco".

Separation process, executed in an isocratic manner was controlled by specialized software "Borwin", also provided by "Jasco".

Mobile phase (mixture 70% methanol and 30% aqueous solution containing 0.5% formic acid) was passed through the chromatographic column with a constant flow of 1 ml / minute.

The absorption spectrum of methanolic extract was recorded with a double beam spectrophotometer operating in 190-900 nm, a product of the firm "Pye Unicam" model "Spectron 300".

The studied extract and the reference solvent were placed in vats made of fused quartz, transparent in the

ultraviolet spectrum, the optical path of 0.5 cm, product of "Hellma" company.

Extraction of components of interest was done in two stages. During the first phase, the quantity of 13.0 g of original material (content of 10 small bags) was washed three times for 10 minutes with 30 ml of hexane in order to remove pigments and non-polar compounds.

After filtration, the plant mass was dried in nitrogen stream and placed in the plant extraction (Soxhlet). By recirculating a volume of 250 ml methanol for one hour, the mixture of interest was extracted.

To prevent oxidation of polyphenolic compounds, Soxhlet system was filled with nitrogen during the extraction.

The obtained extract was diluted with methanol in order to obtain a suitable concentration for recording and monitoring the absorption spectrum of the eluted detector from the chromatographic column.

Chemical analysis

The chemical composition was determined by Official Methods of Analyses (AACC 2000 and AOAC 2006).

Analysis to determine sample composition was performed using standard procedure.

Water content was determined after air drying, fat content using by Soxhlet extraction apparatus, carbohydrates were determined using the differentiation, incineration ash, crude fiber by incineration after digestion with acid / base after acid digestion of samples Kjeldahl method and protein.

Determination of dry matter by drying in oven at 105°C.

Determination of crude ash by igniting the sample at 450°C.

Protein determination was made by determining the total amount of nitrogen (N) by Kjeldahl method, total nitrogen value x factor = value Nx6 protein, 25 (1 / 0.16=6.25).

The determination of lipids was done by gravimetric method.

Determination of available carbohydrates was done by difference (total carbohydrates, dietary fiber).

Total carbohydrates (AOAC, 2000)

100-(weight in grams [protein + fat + water + ash + alcohol] in 100 g).

Available Carbohydrates

100 - (weight in grams [protein + fat + water + ash + alcohol + dietary fiber] in 100g).

Ascorbic acid (vitamin C) is determined based on the fact that titration of ascorbic acid is a strong reducing agent, which readily loses hydrogen atoms, turning into dehydroascorbic acid which also has a vitamin action.

Vitamin activity is lost when the lactone cycle of dehydroascorbic acid is hydrolyzed forming dicetogulonic acid.

The method used is based on titration of ascorbic acid in plant extracts with 2,6-dichlorindofenolul to onset of persistent pink color at least 5 seconds.

Determination of 2,6-dichlorindofenol antibody solution is achieved with a solution of the exact concentration of vitamin C freshly prepared and titrated in the same conditions as samples.

RESULTS AND DISCUSSIONS

As far as the results of chromatographic analysis are concerned, Figure 1 represents the optical absorption spectrum recorded in the field of ultraviolet domain.

It appears that the methanolic extract solution has a well defined absorption band at 274 nm with maximum

local, band attributable to an electronic transition of $n-\pi^*$ type, so a transition in which the phenolic oxygen electrons are largely involved.

Another maximum of absorption, well below 240 nm, probably involves transitions that are less specific to OH phenolic group.

In addition, spectral range at wavelengths below 240 nm is likely to interference with other secondary components of interest for this paper.

Therefore, based on the absorption spectrum, it was decided to monitor the chromatographic elute of 274 nm.

Figure 2 renders the chromatograms obtained in the previously mentioned working conditions.

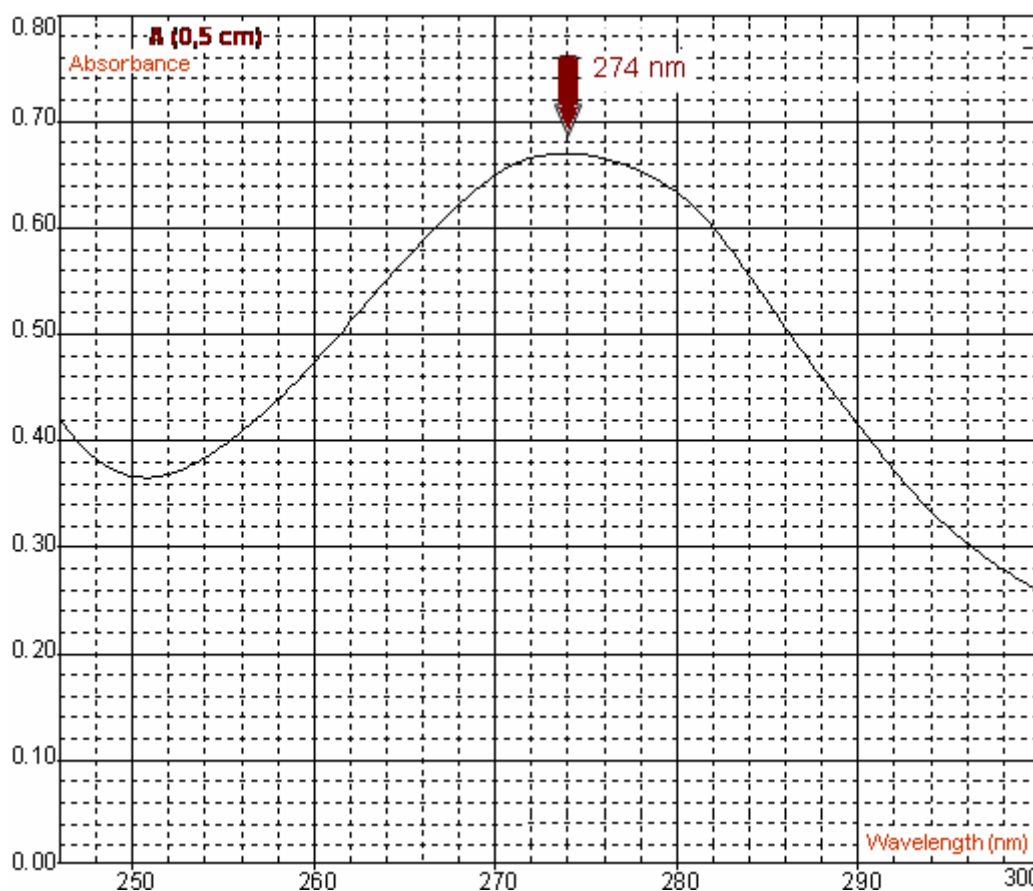


Fig. 1 The absorption spectrum of the methanolic extract from the green tea (brand mark)

The two “peak” sites chromatography, located at 11.79 minutes and 12.92 minutes, corresponding to dominant components in methanolic extract of green tea. Based on comparison with published data in the literature (Li Ma et al., 2002), higher mobility component can be attributed (lower retention time) to epigallocatechine (EGC) and component with the higher retention time to the epigallocatechin–gallate (EGCG).

Chromatographic separation conditions of the authors quoted, EGC and EGCG components had

retention times of 12.6 and 14.0 minutes (similar values to those obtained in this paper).

Figure 3 reproduces the authors cited above chromatogram, obtained under very similar to those adopted in this study (C18 column, eluent acetonitrile–water 60:40, 0.1% aqueous phase containing phosphoric acid, flow to ml / min, detection 279 nm).

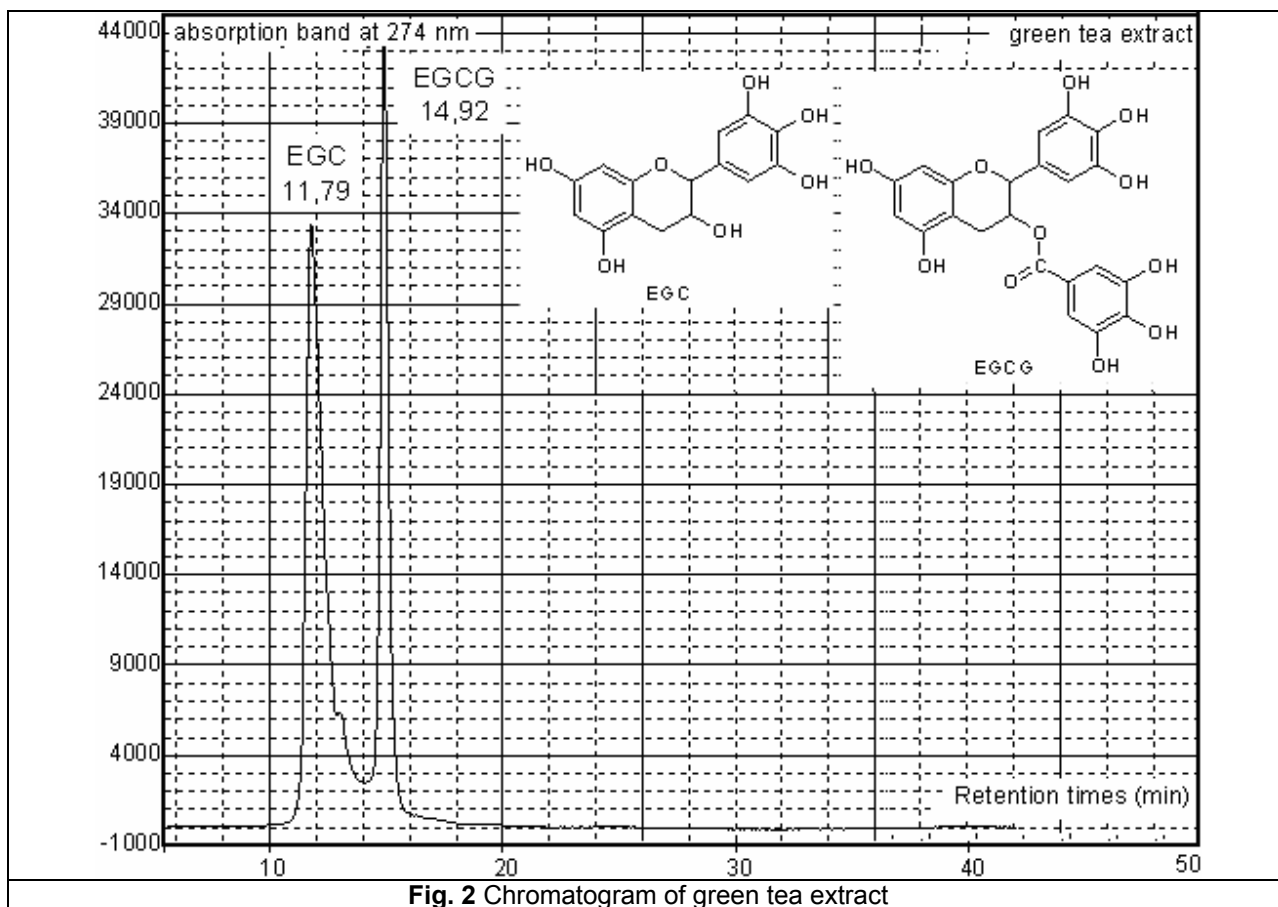


Fig. 2 Chromatogram of green tea extract

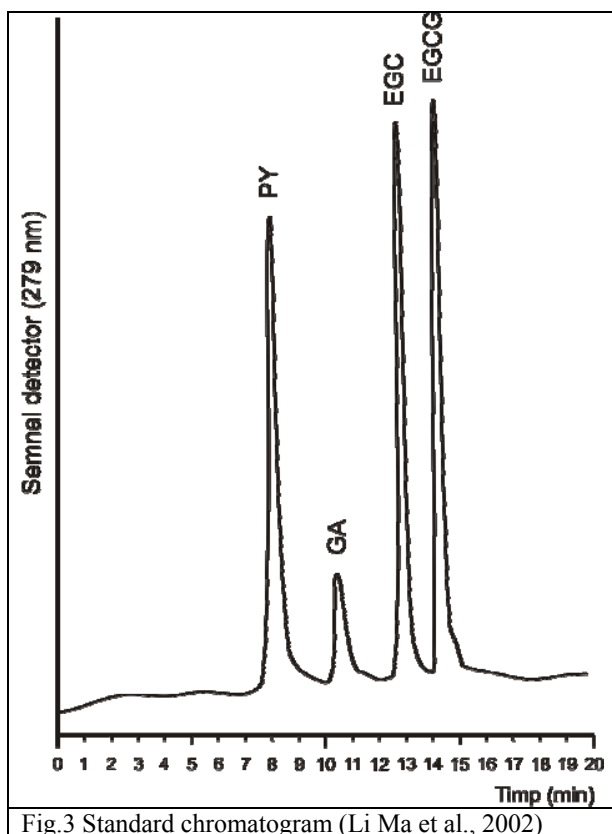


Fig.3 Standard chromatogram (Li Ma et al., 2002)

The authors analyzed a standard mixture of known identity components. Retention times close to the similar values of this work support the hypothesis on the identity of separate components of green tea extract.

As for chemical analysis some results were obtained about some minerals fixed by plants from soil or air.

Thus, the sulfur is absorbed by plants from soil in the form of SO_4 ions through roots and leaves a lesser amount in the form of SO_2 in the air. (Imelouane, et al. 2010)

The results of physic-chemical plant ash are presented in Table 1.

Plants use the sulfur for the biosynthesis of organic sulfur compounds (cysteine).

Insufficient sulfur in plant nutrition produces slow and then stops growing. Leaves turn yellow or weakly appear a red color, there is a premature aging.

Sulphur deficiency leads to an increase in soluble nitrogen (ammonia, nitrites, amines, amides) and to a reduced formation of protein substances.

Chlorine in the soil is taken up by plants through the root system and the gaseous atmosphere by leaf stomata. Chlorine is important activator of enzyme (cytochrome oxidase).

Oxidation of C cytochrome reduced by coenzyme I is accelerated by the presence of chlorine.

Table 1

Physical analysis of Green Tea plants						
Plant	Physical status	Color	pH ash	Conductibility ash soil (MS/cm)	Chlorine mg / l	Sulfur mg / l
Green Tea	Dry dust	White	12.02±1.03	721±6.09	12.03±0.02	8.02±0.06

Data are presented as mean±SD

Table 2

Chemical composition of Green Tea plants							
% Lost when drying of Green Tea plants	% ash content	% of ash					
		alcohol		water		acid	
		Soluble Subst.	Insoluble Subst.	Soluble Subst.	Insoluble Subst.	Subst. soluble	Subst. insoluble
0.139±0.04	6.1±0.01	31.12±1.03	65.62±2.03	23.10±3.01	78.20±1.03	96.27±1.05	3.03±0.02

Data are presented as mean ±SD

The ash was identified as a fine powder (Santos 2010).

Determinations carried out on soluble active substances show that 40% ethanol extract the greatest amount of active ingredients in products. The total amount of ash insoluble in HCl and 100 g / L within the limits set by FR X, up to 11% and 3%. (Dimitriya S et.al. 2010)

Given the environment the plants live, too much ash would insoluble dust (sand) deposited on plant products, therefore inappropriate products because of pollution. Please note that the materials collected by us corresponded to this point.

Analyses conducted to determine the organic nitrogen content (converted into proteins) show that the analyzed plants were efficient in absorbing nitrogen from the soil, and other mineral elements (Piozzi et al., 2011).

Chemical composition of Green Tea plants	
Chemical compounds	Green Tea plants
Dry substance %	75.5±0.2
Lipids %	8.1±0.02
Protein %	13.4±0.02
Total fibres (g/100g)	20.6±0.03
Soluble fibres (g/100g)	5.0±0.02
Insoluble fibres (g/100g)	15.6±0.01
Carbohydrates %	32.6±0.27
Energy (kcal)	4.75±0.06

Data are presented as mean ± SD

In terms of chemical substances existing in the studied green tea plants, belong to several groups of active ingredients: polysaccharide compounds, vitamins, minerals, as shown in Table 4.

Plant-derived vitamins are of great interest because of their impact on human health.

They are essential for metabolism because of their redox chemistry and role as enzymatic cofactors,

not only in animals but also in plants. Several vitamins have strong antioxidant potential, including both water-soluble (vitamins B and C) and lipid-soluble (vitamins E) compounds. (Buțu et al., 2010)

Ascorbic acid or vitamin C is a monosaccharide oxidation-reduction (redox) catalyst found in both animals and plants. Ascorbic acid is redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide. (Amany et al., 2010)

Table 4

The quantity of active substances for the studied of Green Tea plants	
The compound	Concentration in the of Green Tea plants (mg/100g)
Vitamin E	1.60±0.01
Vitamin B ₁	0.43±0.02
Vitamin B ₂	2.5±0.05
Nicotinic acid	10.0±0.01
Vitamin B ₆	1.0±0.04
Biotin	0.05±0.07
Folic acid	0.30±0.02
Vitamin C	4.82±0.03

Data are presented as mean ±SD

In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants. Ascorbic acid is present at high levels in all parts of plants and can reach concentrations of 20 millimolar in chloroplasts.

CONCLUSIONS

In possession of EGC and EGCG gauges, chromatographic methods described in this study, preceded by the extraction process commented, is suitable to developing the methodology for quantitative



determination of two components, the comparative characterization and standardization sortments of green tea.

Moreover, one can see both qualitative and quantitative characteristics of the sample taken for a work as appropriate with high quality.

Thus we can see that the value of the product brand Green Tea is found in a considerate quality of our product in terms of contained antioxidant biocomponents.

Plant chemical composition analysis showed a high solid content, due to more intense photosynthesis which accumulates a larger amount of carbohydrates. A review of current literature suggests that the primary role of vitamin within the body is to function as an antioxidant.

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