# Some questions about Teae folia (Thea sinensis L. seu Camellia sinensis L. Kuntze) as a medicinal raw material

**Olena V. Grechana [1*], Anatoly G. Serbin [2], Sergiy D. Trshecinskiy [1], Olexandr I. Panasenko [1], Lina Yu. Klimenko [2], Tetyana V. Oproshanska [3], Olena O. Saliy [4]**

[1] Faculty of Pharmacy, Zaporizzhya State Medical University, Zaporizzhya UKRAINE
[2] Faculty of Pharmacy, National University Pharmacy, Kharkiv, UKRAINE
[3] Institute of Advanced Training of Pharmacy Specialists, Kharkiv, UKRAINE
[4] Kyiv National University of Technology and Design, Kyiv, UKRAINE
*Corresponding author: 1310grechanaya@ukr.net

**Abstract**
Background: One of the most popular plant and beverage is a *Thea sinensis* or *Camellia sinensis* (Theaceae) also known as Theae Folia or Green Tea. There are some reasons for it, such that the sensory properties, prices are relatively cheap, stimulant effects, and their potential health benefits. Motherland of this plant is considered the China. This is where people have paid attention to the ability of a decoction of the leaves have a tonic effect on the human body and the first to grow this plant. Tea folia as a medicine are made from unfermented tea leaves and young shoots. This process is fast in special drying machines. Objective: European Pharmacopoeia have monograph Green Tea and the herb that we know as a food drink have as medicine herb now. The objective of research of herbal medicine is the quality and safety of natural products. Materials and Methods: Macroscopical and microscopical features of the leaf and shoots have been analysis using an optical microscope. Phytochemical and physico-chemical analysis were evaluated. The observation of the spectrum profiles is done by interpreting the typical peak that appears. Results: Theae Folia have actinocytic stomata, unicellular trichomes, heterogenous mesophyll which is characterized by the presence of calcium oxalate crystals and sclereid cells. Phytochemical analysis indicated resources the presence of purine alkaloids caffeine and theabromine and many other drugs. The content of caffeine from Teae Folia ethanolic and water extract is differ to time. Conclusion: Pharmacognostic and phytochemicals features established in this study may be used as part of the pharmacopoeial standard, which can play an important role in its standardization.

**Keywords:** medicinal plant, microscopic, caffeine

## INTRODUCTION

The first mention of tea found in Chinese manuscripts dating back about 5 000 years. From the VIII century, we have famous "Treatise on the case", where we are knew that the first appreciated the properties of tea leaves by the Chinese Shen Nong. He studied a variety of plants and took many poisons, and the tea was for him the antidote. In ancient times, the Chinese believed it cure for 72 poisons and used only for the treatment of patients. In the Buddhism period, tea became popular among ordinary Chinese. Drink was loved all over China. Tea tree was grown on plantations. Later, as the result of the selection humankind got bush (Elfahmi at al. 2014; Malongane at al. 2017).

Chinese began to monitor the color change of tea leaves during production. This caused improvements in the tea production. In the XVIII century, it was known more than a hundred sorts and it has become the most popular drink in China (Chaturvedula and Prakash 2011; Kilel at al. 2013; Mofokeng, & Mashingaidze, 2018).

The list of herbal medicines for health purposes in the Ukraine at this time is to increase rapidly. The herbal medicines are used to improve health (promotion), restoring health (rehabilitative), disease prevention (preventive) and healing (curative) and is expected to support the development of public health. This condition supported by the potential of Ukrainian's natural resources, which is consisting of 25 000 plants species. More than 2 000 species of plants have been knew as a medicine that has been used as folk medicine.

Nowadays tea is one of the most popular plant and drinks in the world because of the sensory properties,
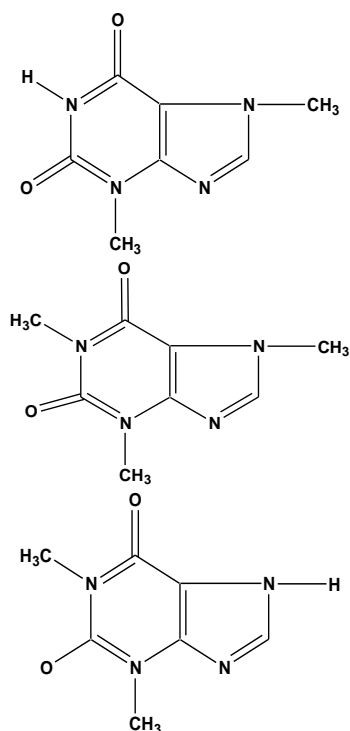
**Fig. 1.** The structure of purine alkaloids (caffeine, theophylline, theobromine)

prices are relatively cheap, the stimulant effects and their potential health benefits. Tea or also known as *Thea sinensis* L. seu *Camellia sinensis* L. Kuntze belongs to the family Teaceae (Camelliaceae), including more than 45 varieties. Of these, only two species are widely cultivated commercially - subtropical Chinese and tropical Indian.

The lead role is assigned the oxidative processes intensivity of the processing of raw materials due to which different types of tea are obtained from the same raw materials: unfermented (white and green), semi-fermented (oolong), and fermented (black tea). There is produced more black tea (70%), green - 25%, other varieties of tea account about 5%. Green tea leaf is taken from the young shoots, tea leaves and buds. It has a simplest process by withering and drying.

Tea (*Camellia sinensis* L.) is very diverse plant in terms of chemical composition. It's contined about 2 000 chemical components (N.B.: the chemical composition freshly picked green tea leaf and dry tea is not the same). In dry tea is had more complex chemical composition, because it has processing. Given that the tea comes in the human body in the drink form, great importance is the chemical composition of water and ethanol tea extracts.

Despite the long history of tea investigation and its widespread use (Alcazar at al. 2007; Dias at al. 2013), the chemical composition tea is not fully understood, but existing data sometimes are contradictory. The tea leaf has three main phytochemical groups:

1. Purine alkaloids - caffeine, theophylline, theobromine;
2. Flavonoids. The most significant are: catechin, epicatechin, gallocatechin, epigallocatechin, etc;
3. Tannins. Predominate catechin dimers.

Alkaloids are nitrogen-containing heterocyclic compounds with high functional activity. There are three main compounds from tea alkaloids: caffeine, theobromine and theophylline (**Fig. 1**). Tea contains more caffeine than coffee or cocoa, but it effect is softer and it is an important component of tea, comprising 3 - 5% of the total weight. This is due to associated tea caffeine with tannin (theine or caffeine tannate). Theine gives a bitterness to tea, tonic effect on the organism, improves mental performance, increases activity, and stimulates cardiovascular and central nervous system work (Ahmed at al. 2019).

In addition, tea is showed antiseptic and antioxidant properties and various types of bioactivity (Unachukwu at al. 2010; Ekayanti at al. 2017). The antioxidant properties can prevent free radicals and inhibit oxidative stress and inflammation (Mahmood at al. 2010; Elya at al. 2015) Oxidative stress and inflammation associated with various diseases including obesity disease, dyslipidemia, diabetes, cardiovascular, neurodegenerative and cancer (Wang at al. 2011).

The studies are reported that ethanol extract of tea has antidiabetic activity by inhibition of alpha-amylase enzyme activity, alpha-glycosidase and dipeptidyl peptidase IV in vitro (Gondoin at al. 2010; Wang at al. 2011; Yang at al. 2016).

That why it is necessary to develop and further study tea leaves.

The objective of research and development of herbal medicine is to improve the quality and safety of natural products. Therefore, it is necessary to standardize raw materials and extracts to maintain the uniformity of quality, safety and efficacy (Grechana 2015; Ekayanti at al. 2017). Raw material quality parameters include moisture content, ash content, acid insoluble ash, water soluble extract content, content of ethanol soluble extract and concentration of substances identity. It also conducted organoleptic, microscopic, macroscopic, chemical content identification, fingerprint profiles, and contamination analysis (Mourya at al. 2019; European Pharmacopoeia 9th Edition).

## SUBJECTS, MATERIALS AND METHODS

### Objects

*Tea sinensis* L. *(Camellia sinensis* L. Kuntze)* are not growing in Ukrainian territory. We took the raw materials from the Ukrainian manufacture «Ліктрави» Zhitomir, Ukraine. Fresh plant we obtained from the Botany Children Park, Zaporizzhya, Ukraine. Tea leaves were sorted, collected, and then dried under sunlight. Furthermore, the tea leaves are withered with a dryer.

The green tea leaves powder is made by grinding dried green tea leaves by using a grinder.

### Micropreparation

Micropreparations for the study of the stem and leaf blade anatomical structure were prepared from freshly picked and after that fixed in mixture alcohol-glycerol-water (1: 1: 1 v/v) or from dried and then soaked the same mixture raw materials.

### Methods of evaluation

Identification of raw materials is conducted by observation of organoleptic, macroscopic and microscopic identification. Macroscopic identification of green tea leaves was observed based on the length and width of leaves, color and description of the leaves and stems. Leaf color was identified before and after the drying process by using a light Microscope «БІОЛАМ ЛОМО» (Russia). Microscopic identification is done by using cut crosswise and lengthwise of leaf, petiole and stem and dried powder green tea leaves. A microscope was used for magnification 80, 120, 160, 400, 600 and 800 times. The obtained data were recorded by a digital camera «OLYMPUS SH - 21». Photos were processed using the «Adobe Photoshop CS3» computer software.

### Drug preparation

Ground tea (1.000 g) was extracted with boiling water (50 ml) in a 250-ml conical, flat-bottomed laboratory flask with a narrow neck. After each periods (10, 20, 30 min and 1 day) the extracts were filtered. These extracts were analyzed through GC-MS for identification of phytocompounds.

An Agilent 7890B gas chromatograph equipped with a mass-spectrometric detector 5977B, a glass column DB-5ms (30 m x 250 mcm x 0.25 mcm) and injection cooling system Gerstel CIS 4 was used. The operating conditions of the instrument were as follows: oven temperature, 200°C; injector temperature, 230°C; detector temperature, 150°C; carrier gas (helium) flow-rate, 1.3 ml/min; injection volume, 0,5mcl.

### Data Processing

The NIST data bank, database NIST 14 library was utilized for figuring out name, molecular weight and structure of the test materials components. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

### RESULTS

The characterization of organoleptic observation is including shape, smell, color and taste of *Camellia sinensis* L. Kuntze leaves. Color was determined in daylight. The smell was established by rubbing the leaf between the fingers. The taste was determined in dry raw materials and its infusion. Green tea has a distinctive aroma and astringent taste as same as the other tea.
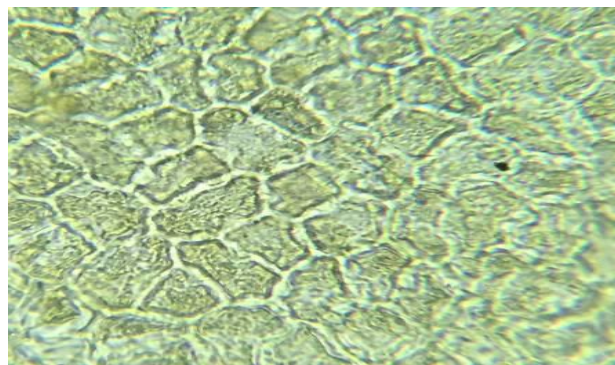


**Fig. 2.** The observation of upper leaf epidermis (magnituding (40x15) by 600 times)

The macroscopic examination of the leaf is consisted of botanicals characteristic observation, such as shape and size of the leaf blade, shape and length of the petiole, nature of the nervation and extremity of the leaf, pubescence, and presence of secretors cells. The dimensions of the leaf plate was studied using a ruler.

The microscopic examination was carried out with C-section observation of leaf and stem or specific part fragments of tea powder. The purpose of this observation is to examine specific parts contained in the organs of plants used.

Macroscopic characteristic of *Thea sinensis* L. (*Camellia sinensis* (L.) Kuntze) showed that the leaves are glossy dark green, alternate, short-petiole, coriaceous, lanceolate or elongated-ovate, and roughly serrate. The leaf blade is 6-18 cm long, 2-6 cm wide, short-stemmed, alternate arrangement, stiff leaves, elongated ellipse-shape with finely toothed edges, and pinnately netted venation. Mature leaves are bright green colored, smooth, and leathery. The underside of young leaves is covering of short downy hair and appears silver. Young leaves *Camellia sinensis* (L.) Kuntze rolled up, oval, with a length of 1.5-3 cm, diameter 1-2 mm.

The cells of the upper (**Fig. 2**) and lower leaves epidermis (**Fig. 3**) are thickened-membrane and parenchymal.

Upper epidermal cells are large, with undulating walls, withaut any trichomes. The lower epidermis cells are larger than those of the upper surface, with slightly undulating walls (**Fig. 3**). Stomatas on the upper epidermis are absent, on the lower epidermis they are very frequent (**Fig. 3**), surrounded by 3 (very rarely by 4) round-stomata cells, which tangentially-elongated and samed size. They're locating radially around to the stomata (**Fig. 3**). The shape and size of the stomata cells are different from the basal epiderma cells. The type of stomata apparatus is actinocytic (**Fig. 3**). The pubescence is represented by simple long unicellular hairs on the lower epidermis and it is absent on the upper epidermis (**Fig. 4**).
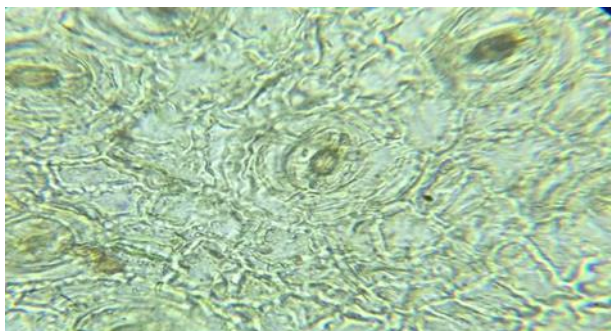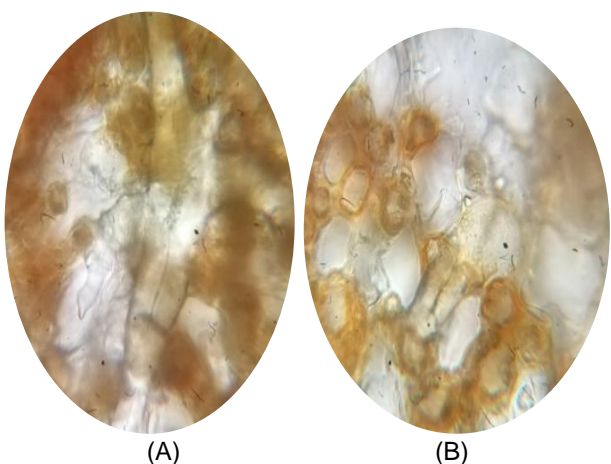
**Fig. 3.** The observation of lower leaf epidermis (magnituding (40x15) by 600 times)
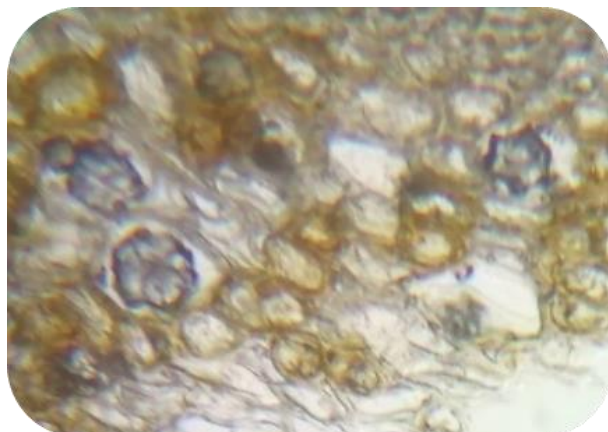


**Fig. 4.** The observation of lower leaf epidermis (magnituding (8x15) by 120 times)



**Fig. 6.** The observation of leaf-mesophyll sclereids in cross sections (magnituding (40x15) by 600 times)



**Fig. 7.** The observation of central leaf streak in cross sections (magnituding (8x15) by 120 times)
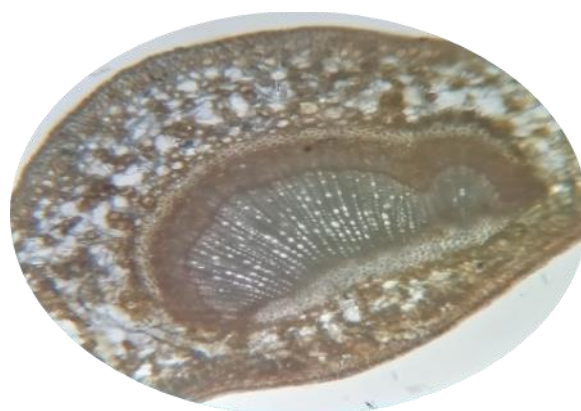


(A)　　　　　　　　　　(B)

**Fig. 5.** The observation of lower leaf epidermis (magnituding (40x15) by 600 times)

The observation of cross sections of *Camellia sinensis* (L.) Kuntze leaves under a microscope, found the cuticle, epidermis, mesophyll, crystals of Calcium oxalate, sclereid cell (**Figs. 5** and **6**).

The mesophyll is heterogeneous and asymmetrical, it characterize by the presence of sclereid cells and crystalles of Calcium oxalate. There are present sclereids in mesophyll at cross-section of the leaf: with branched-shape - astrosclereids (**Fig. 5a**) and unbranched-shape - brachysclereids (**Fig. 5b**). Sclereids are pointe, irregular.

In addition, also in the mesophyll are founded cells-idioblasts with crystals of calcium oxalate (druses) (**Fig. 6A**). Parenchymal cells similar to those of most other leaves, and not very distinctive.

The central leaf streak has rounded shape in cross section (**Fig. 7**). Above the streaks epidermal cells are parenchymal quadrangular shape, with thick rectangular membranes. The pubescence is rare and represented by simple unicellular hairs with a warty cuticle. Under the epidermis above the bundle is 2-3 layers of lamella - angular collenchyma. Leading bundle is collateral, and surrounded by sclerenchyma.

Microscopic observation of the *Teae Folia* powder found any fragments of leaves and a layer quiet lot of fine hairs.
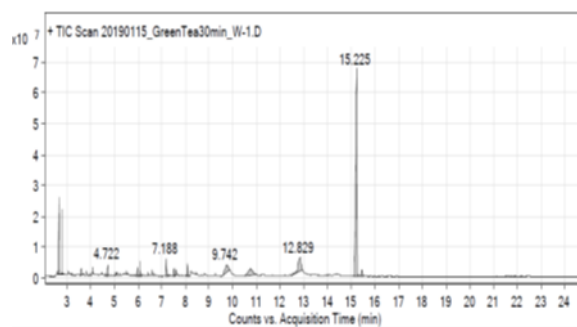
Extracts of tea leaves were obtained using boiling water with extraction method. The crude extracts obtained were red-brownish with a distinctive aroma of tea. The extracts examination were conducted by phytochemical examinations. Phytochemical analysis of the extracts showed the presence of alkaloids, flavonoids, tannins, glycosides, saponins and other compounds.

**Table 1.** Constituent elements of *Thea Folia* water extract

| No | Compound Label | 10 min | 20 min | 30 min | 24 hours |
|----|---------------|--------|--------|--------|----------|
| 1 | Methylacetate 2C | + | - | - | - |
| 2 | Acetic acid | + | + | + | + |
| 3 | Methylacetate | + | + | + | + |
| 4 | Malic acid | + | - | - | - |
| 5 | L-Lactic acid | + | + | + | + |
| 6 | Dihydroxyacetone | - | + | - | - |
| 7 | Acetoacetic acid | + | + | + | + |
| 8 | Tiglic acid | + | - | + | - |
| 9 | Valeraldehyde | - | + | - | + |
| 10 | 2-Acetyl-1-pyrroline | + | + | + | - |
| 11 | Hydroquinone | + | + | + | - |
| 12 | 4-fluorophenylacetylene | + | + | + | - |
| 13 | Dimethyl fumarate | - | - | - | + |
| 14 | Zingerone | - | - | - | + |
| 15 | 5-Hydroxymethylfurfural | + | + | + | + |
| 16 | Ethyl maltol | + | + | + | - |
| 17 | Pyrogallol | + | + | + | - |
| 18 | Sucrose | + | + | + | + |
| 19 | d-Mannose | + | - | - | + |
| 20 | Pantoic acid | + | + | + | - |
| 21 | Quinic acid | - | - | - | + |
| 22 | Laminitol | + | - | - | + |
| 23 | Caffeine | + | + | + | + |
| 24 | Theobromine | + | + | + | + |
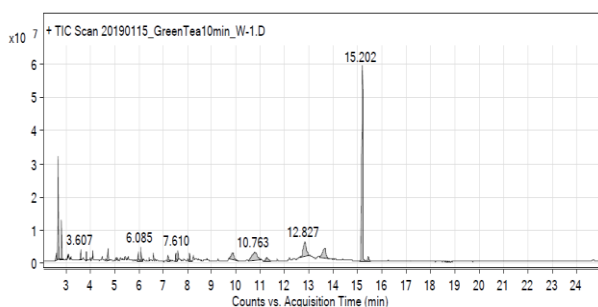


**Fig. 8.** The observation of GC-MS spectra profile (water extraction, 10 min)
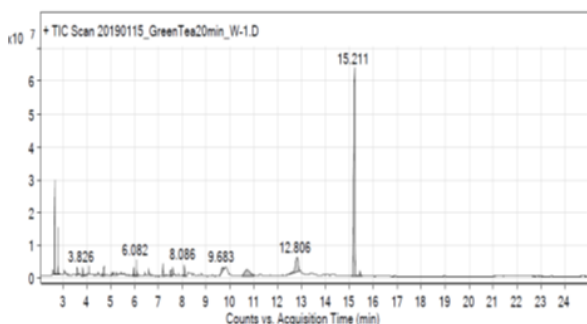


**Fig. 9.** The observation of GC-MS spectra profile (water extraction, 20 min)



**Fig. 10.** The observation of GC-MS spectra profile (water extraction, 30 min)
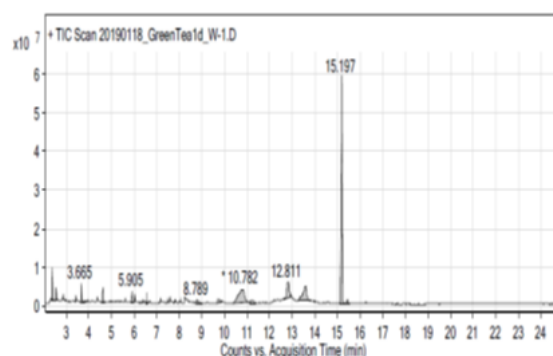


**Fig. 11.** The observation of GC-MS spectra profile (water extraction, 24 hours)

material and their speed yield to the extractant (**Fig. 8-11**).

Observation of the GC-MS spectrum profiles is done by interpreting the typical peak that appears. The interpretation of GC-MS spectra profiles showed organic compounds which mostly containing functional groups O-H from phenolic or alcohol, C-H aromatic, N-H amine or amide, C=O carbonyl, C=C alkene, and C-O ester in the green tea water extracts (**Table 1**). We observed the release of chemical components from dried raw materials to extractant is different in time (10, 20, 30 minutes and 24 hourse). We were especially interested in the amounts of caffeine and theobromine in the raw

## DISCUSSION AND CONCLUSIONS

The chemical analysis is the help information about behavior of plant extracts. In addition, the different leaf ages produce different tea qualities since their chemical compositions are different. Microscopic and macroscopic characters are one of the important criteria for identification. The results of the stomata distribution showed that between stomata and distribution in the leaves are hypostomatic. While stomata is the entrance of $CO_2$ for photosynthesis, the food is produced from this raw materials in the process of photosynthesis by plants. Stomata associated to photosynthesis to produce food for plants. Photosynthesis is correlating to the weather, and it is altered the synthesis of secondary metabolites in tea plant (for example: purine alkaloids).

The presence of unicellular trichomes on the Theae Folia underside creates a microenvironment of water vapor here.

Calcium oxalate crystals are one of the characteristic of the Theae Folia and the presence of the crystalline also associated with the photosynthesis rate. The result of macroscopic and microscopic characterization of the leaves can determine the anatomical structures of plant metabolite storage.

The quality of herbal medicines relies on their bioactive constituents. Therefore, content of purine alkaloids is an important and powerful tool for standardization and determination bioactive compounds. Theae Folia have a simple processing without any fermentation process and high content of purine alkaloids especially caffeine. It has been confirmed by the determination of the caffeine, theobromine and others compounds.

We observed a decrease in the total amount of components in the extracts. Two ingredients were specific to a 10-minute water extract of Theae Folia (methylacetate 2C and malic acid). The 20-minute extract was differed with dihydroxyacetone content. The oldest extract contained specific ingredients too: dimethyl fumarate, zingerone, and quinic acid. The 30-minute extract was not any specific ingredients. All extracts were including the acetic acid, L-lactic acid, 5-hydroxymethylfurfural, sucrose, acetoacetic acid but their containing was various. Ordering to results of the phytochemical analysis the presence of methylacetate was increased into 30 min extract; in the 24 hours-extract it quantity was decreasing. Sucrose behaved in an interesting way: being present in all extracts, it amount increased sharply at the day. Obviously, this was the result of the glucosides breakdown during the day. We can see the cycled appearance and disappearance of tiglic acid, and vice versa, the cycled absence and then the appearance of valeraldehyde. A sufficiently large number of substances are disappearance that characterize fresh infusions of Teae Folia: 2-acetyl-1-pyrroline, hydroquinone, 4-fluorophenylacetylene, ethyl maltol, pyrogallol, pantoic

acid from the 24-hour infusion. The behavior of the d-mannose and laminitol is the same: they are appeared in very fresh and very old infusions

Purine's compounds Theae Folia are the powerful analeptic and energetic agent and positive effect on health. Main purine alkaloids found in Teae Folia are caffeine and theobromine, which in half-hour extract higher than others. That is why tea should be infused for better invigorating and breath-enhancing effects during at least half an hour. And vice versa, in order to get the least amount of caffeine, the infusion should be consumed in the first ten minutes of infusion.

The current study revealed that the result of macroscopic and microscopic characterization of the Theae Folia could determine the anatomical structures of plant metabolite storage. The present work can be used to provide data about the pharmacognostic characteristics of the Theae Folia (*Thea sinensis* L. seu *Camellia sinensis* L. Kuntze, Theaceae) that proves the importance of these results. This study will be helpful in the pharmacognostic and phytochemical identification of Theae Folia aqueous extract for the proper obtaining of crude drugs from green tea leaves and provide pharmacological preparation in the future.

## ACKNOWLEDGEMENTS

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