**ISSN 2063-5346** *Section A-Research paper*

# THE GC-FID INVESTIGATION OF THE CHEMICAL COMPOSITION OF ALBANIANS TEA PRESENT IN PHARMACIES AND HERBAL STORES IN ALBANIA

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**Abstract**

The main reason for launching this study is the lack of information that should be provided on the labels of tea packaging, sold in herbal shops and Albanian pharmacies. This paper aims to investigate and study the chemical profile using the GC-FID method of teas sold in Albanian pharmacies such as black tea, *Althaea officinalis*, *Melissa officinalis,* and *Salvia rosmarinus*.

4 different plants were taken for analysis: black tea was collected in China, while *Althea officinalis*, *Melissa officinalis,* and *Salvia rosmarinus* were collected in Albania. The plants were washed and their essential oils extracted: black tea and Althea officinalis were extracted by Clevenger apparatus. *Melissa officinalis* and *Salvia rosmarinus* were extracted by supercritical carbon dioxide (SC - $CO_2$). For the presence of heavy metals such as Cd, Pb, Sn, and Hg, two methods were used: ultraviolet-visible (UV-VIS) spectrophotometers for plants after $CO_2$ extraction, and inductively coupled plasma mass spectrometry (ICP-MS) was used for the essential oils obtained from the Clevenger apparatus.

The data collected for these essential oils showed the high content of terpenes and monoterpenes in these essential oils as follows: 5.17% in black tea, 26.26% in *Althaea officinalis*, 40.4% in *Salvia Rosmarinus*, and 50% in *Melissa officinalis*. The presence of heavy metals was a second analysis carried out and the results obtained showed that the most abundant metal is lead compared to the other metals investigated. From the results, we conclude that using $CO_2$ extraction was a more efficient method than Clevenger extraction because it was possible to capture some unstable components - highly volatile components referred to in other studies are difficult to capture with the Clevenger method. Pb remains one of the heavy metals which is found in large quantities.

**KEYWORDS:** Medicinal plants, chemical profile, SC- $CO_2$ extraction, heavy metals

## INTRODUCTION

Albania, due to its geographical location, is a sunny country with an interesting plant population, most of whom are medicinally used. Albania is confronted with numerous health and environmental issues. One of the most serious issues is label placement on the many teas available in pharmacies or herbalists/pharmacies. One of the reasons for this paper is the package information they show.
Four teas were examined, Camellia Sinensis - Black tea, Althaea officinalis, Melissa officinalis, and Salvia Rosmarinus and various studies have revealed that they have numerous benefits. These teas have been examined for their chemical profile, as well as for the presence of heavy metals.

**Figure 1.** The Black tea.

**Figure 2.** Althaea officinalis

**Thea sinensis / Black Tea (Camellia Sinensis):** Fights fever and illness while revitalizing and toning the body. It protects against poisoning, encourages weight reduction, and causes blood fats to melt. People with tension and nervous exhilaration should avoid using it. Drinking black tea on a regular basis has been shown in studies to help prevent the onset of cardiovascular disorders [ 1].

**Althaea officinalis** - The medicinal plant marshmallow, Althaea officinalis L. (A. officinalis), has been used to cure cough for centuries. Applying medicinal extracts of marshmallow roots produces instant results, such as a protective film on inflamed mucosa. [2]
The other two plants were obtained from the herbal pharmacy (As they are collected from nature, unprocessed, and only packaged), while the herbs for black tea and althea officinalis were purchased from the pharmacy.

**Figure 3**. The Salvia rosmarinus

**Figure 4**. Melissa officinalis

**Melissa Officinalis-** The herb is used as an infusion for its digestive, diaphoretic, sedative, antispasmodic, anti-inflammatory, antimicrobial, and sedative properties. Recent research suggests that the plant extract improves cognition and may be useful in the treatment of amnesia and Alzheimer's disease. These effects could be ascribed to AchE inhibition or nicotinic receptor activity. [3] [4]. Melissa officinalis includes essential oil (citroneral, citral, linalool, geraniol, and aldehydes), flavonoids, apigenin, luteolin, tannins, sesquiterpenes, caffeic acid, chlorogenic acid, rosmarinic acid, polyphenolics, and sesquiterpenes.

**Salvia Rosmarinus** –The entire plant has tonic, anticonvulsive, invigorating, and antiseptic qualities. Intestinal infections, diarrhea, colitis, flatulence, liver problems, influenza, colds, rheumatism, indigestion, and oral cavity wounds are among the conditions it is used to address.

The herbal extract boosts blood flow in the vessel and strengthens the circulatory system. [5, 6].

These plants went under investigation for their chemical composition and for the presence of heavy metals.

Heavy metals have numerous negative environmental consequences; for example, the change of mercury into methylmercury in the presence of water results in highly toxic sediments [7]. Chromium exists in the natural environment in two stable forms: Cr (VI) and Cr (VII).

Cr(III) is less toxic and insoluble, whereas Cr(VI) is exceedingly toxic and highly soluble. Chromium is used in many industrial uses, but it is harmful to the local environment. The effluents and solid wastes from the mining, chrome-plating, leather tanning, and dye-manufacturing industries are high in chromium concentration and have been recognized as significant health hazards due to pollution to the environment. Chromium has the potential to cause cancer. [8].

However, some heavy metals are involved in the regulation of certain physiologic bodily processes. Natural heavy metals enter the body through food, air, and water, where they control a variety of biological activities. [9, 10]. Most toxic heavy metals, such as lead, thallium, cadmium, and antimony, are prevalent in industrial operations and are significant polluters of the environment. Heavy metals are distinguished by their large atomic mass and toxicity to living organisms. The majority of heavy metals pollute the environment and the atmosphere, and some are toxic to people. Heavy metals can become highly toxic when mixed with various environmental components such as water, soil, and air, and people and other living organisms can be exposed to them as a result.

Black tea and althea officinalis were cleaned, disintegrated in a microwave, and then the Clevenger technique was used to extract the essential oil from them. The essential oil was acquired, and then it underwent a chemical study. GC-FID was used to identify their chemical composition, and ICP-MS was used to determine the presence of heavy metals. Salvia Rosmarinus and Melissa officinalis were collected, cleaned (of physical waste), and then put through a supercritical $CO_2$ extraction procedure. After the extract was produced, it was diluted with hexane in a 2:1 ratio, and the essential oil was examined for the presence of heavy metals using the UV-VIS spectrophotometric technique and the GC-FID to determine its chemical profile.

Analyzes performed with GC-FID showed that these teas had a high content of monoterpenes and terpenes: 5.17% in black tea, 26.26% in Althaea officinalis, 40.4% in Salvia Rosmarinus, and 50% in Melissa officinalis.

The analyzes performed with the ICP-MS method for black tea and Althea officinalis respectively showed that the highest presence of heavy metals appeared with that of lead. In black tea, the lead had a presence of 0.19 mg/kg, and its presence in althea officinalis was 0.41 mg/kg.

While the analyses performed with UV-VIS spectrophotometer showed that: the presence of lead was detected in higher concentrations 0.3 ppm compared to the other heavy metals detected in the essential oil, in the same range was even its concentration present in Melissa officinalis, 0.27 ppm. Lead is the more frequent and abundant heavy metal in these plants.

## METHOD AND MATERIALS

### *2.1 Materials*

- 4 different plants,
- SC – $CO_2$ apparatus,
- Clevenger apparatus,

- The elemental calibration standard was prepared from 10 LG mL-1 of a multi-element stock standard solution. $HNO_3$ (Suprapure grade, 65%) and $H_2O_2$ (30%) were bought from Merck.

***2.2 Equipment and Apparatus***

- ☐ Technical scale, Shimadzu Corporation, UW 4200HV, max 4200 g, Min 0.5g
- ☐ Technical scale, Constant, 14192 -478-f Max 40 kg (kg), accuracy 2g
- ☐ Technical scale, Shimadzu Corporation, UW 220HV, max 220 g, Min 1g.
- ☐ Memmert thermostat, 105 degrees Celsius
- ☐ Thermal heater for the distillation process,
- ☐ Clevenger system
- ☐ Extraction apparatus (autoclave)
- ☐ Thermometer up to 0 - 100 C
- ☐ Micropipette, Eppendorf, 0.5 - 10ul
- ☐ Micropipettes, Eppendorf, 100 - 1000 ul
- ☐ Bathroom 8 (for warm water), Ultrasonic Cleaner USC 600 TH
- ☐ Water cooler with continuous circulation (for cold water)
- ☐ Shredder for plant parts
- ☐ Laboratory sieves 0.7mm, 0.8mm, and 1mm
- ☐ Chemical glasses
- ☐ Cylinders
- ☐ Flask with round bottom 1000ml.
- ☐ Glass vial
- ☐ Pasteur pipette.

***2.3 Reagents:***

1. hexane
2. sodium sulfate
3. water,
4. analytical acetone.

Four different plants (two from the Administrative Unit of the Elbasan Municipality, *Melissa officinalis*, and *Salvia rosmarinus*) and two from a herbal store in Tirana ( black tea was collected in China, while *Althea officinalis – origin from Albania),* were analyzed with DC -FID for the chemical profile and using the ISO Spectrophotometer and ICP-MS for the detection of heavy metal.

Melissa Officinalis and Salvia Rosmarinus were cleaned before being subjected to $CO_2$ extraction. Each of these plants' essential oils is extracted using the supercritical $CO_2$ extraction technique.
Plants are selected and milled in a controlled, dry, and ventilated atmosphere. The plant's integrity and health are evaluated using a humidity sensor and a microscope. Regarding the present state of production: The plant particle size used is 0.3 mm, with a maximum humidity of 6%, according to SCFE (supercritical fluid extraction) technology standards. Extraction of solids from ground materials (or pellets, granulates) is commonly done in batch mode using food carbon dioxide as a solvent.
The apparatus consists of two separators that separate $CO_2$ from the extract before recycling it, as well as two or more extractors that operate above the $CO_2$ critical pressure. The temperature and pressure of the carbon dioxide gas are raised until they reach the supercritical state as the first step in the supercritical $CO_2$ extraction process. At 31.1 °C and 1071 psi, carbon dioxide enters a supercritical state. (72.87723 atm). To achieve this, a heater and a high-pressure pump are used. A considerable amount of organic raw is present in the extractors through which the supercritical $CO_2$ passes.

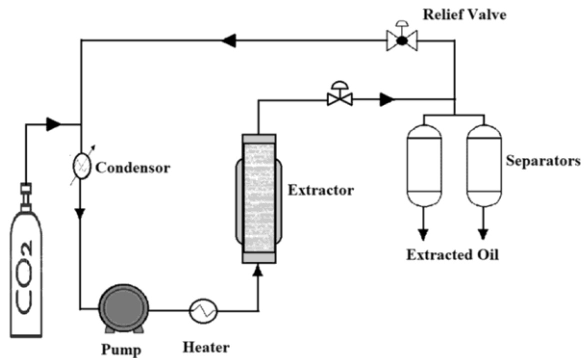


**Figure 5.** The Supercritical $CO_2$ extraction apparatus.

For the other 2 plants, the black tea and althea officinalis, the method source were used:

- European Pharmacopeia,6th edition 2008. Council of Europe, Strasbourg, France.
- AOAC Official Method 962.17, Volatile Oil in Spices, Journal of AOAC International vol. 80, No.4, 1997.
- FSSAI, Manual of Methods of Analysis of Foods, Spices and Condiments, 2015.

### *2.3 Microwave digestion study of sample preparation*

This experiment needed 0.5 g of material, which was then transferred to a PTFE disaggregation vial. 6 mL of trace metal HNO3 and 1 mL of trace metal H2O2 were introduced. The sample was prepped for disaggregation by weighing it and then disintegrating it in a microwave for 30 minutes. (The heating program for the microwave oven was executed in three running steps.)
The disaggregated sample was then put in a 50 ml vial and diluted with 2% HNO3 to the appropriate concentration. The ICP-MS injection operation is then finished.

### *2.4 Oil extraction*

The dry leaves (from their natural packaging) were cleaned of impurities and other solid matter as part of the process. The samples were comminuted before hydrodistillation extraction, and the hydrodistillation process started. A sample holding 50 grammes of tea was submerged in 500 mL of water and boiled for 3 hours at boiling point temperature using a distillation flask heater and a Clevenger-type apparatus. (figure shown below).

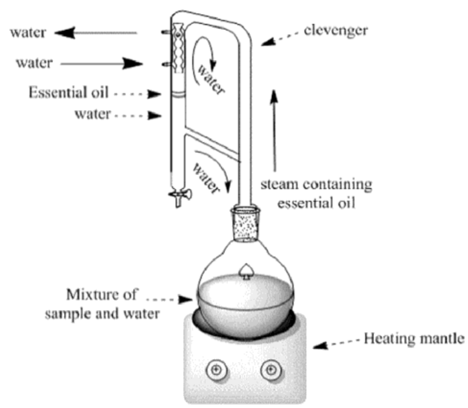


**Figure 6.** The Clevenger apparatus used for the extraction of essential oil from

To prevent any polyphenols or other essential oil components from coming into contact with the water, 1 mL of hexane was added to the condenser during the first 30 minutes after the oil began to gather in the Clevenger column. After it has cooled, exactly weigh it and compute it as follows:

$$\text{Oil \%} = \frac{\text{Weight of the oil x 100}}{\text{Weight of the material taken}}$$

The essential oil obtained was dried with sodium sulphate but was anhydrous and kept for further analysis.

For both the essential oil and the herbal tea, the ICP- MS technique was used for qualitative and quantitative heavy metal detection.

## RESULTS AND DISCUSSION

- **Salvia Rosmarinius's,** chemical profile shows that: One of the Lamiaceae plants with significant antioxidant action is rosemary.

Rosemary extracts derived using supercritical $CO_2$ extraction have been proven to be promising for inclusion into diverse foods, cosmetics, and pharmaceutical goods requiring a natural scent, colour, and antioxidant/antimicrobial component. These qualities are also required by the food sector in order to uncover possible alternatives to synthetic preservatives. [6]. The antibacterial activity of rosemary extracts was connected to their phenolic content, and carnosic acid and rosmarinic acid are most likely the major antimicrobial components of rosemary.

The GC -FID was used for the chemical composition of Salvia Rosmarinius's and the results are present in the table below:

**Table 1.** The GC – FID analysis for Salvia Rosmarinius's chemical composition.

| | Component | % |
|---|---|---|
| **1** | Thujene | 2.3 |
| **2** | alpha pinene | 10.41 |
| **3** | Camphene | 5.8 |
| **4** | sabinene | 3.1 |
| **5** | beta pinene | 7.86 |
| **6** | myrcene | 1.46 |
| **7** | alpha terpinene | 1.21 |
| **8** | p-cymene | 2.1 |
| **9** | limonene | 1.87 |
| **10** | **1,8 cineole** | **17.23** |
| **11** | terpinolene | 3.65 |
| **12** | Linalool | 0.57 |
| **13** | camphor | 7.42 |
| **14** | Borneol | 5.56 |
| **15** | terpinen-4- ol | 2.32 |
| **16** | Alpha-terpineol | 4.36 |
| **17** | trans caryophyllene | 11.23 |
| **18** | alpha Humulene | 2.03 |
| **19** | Viridiflorol | 2.25 |
| **20** | Manool | 2.14 |

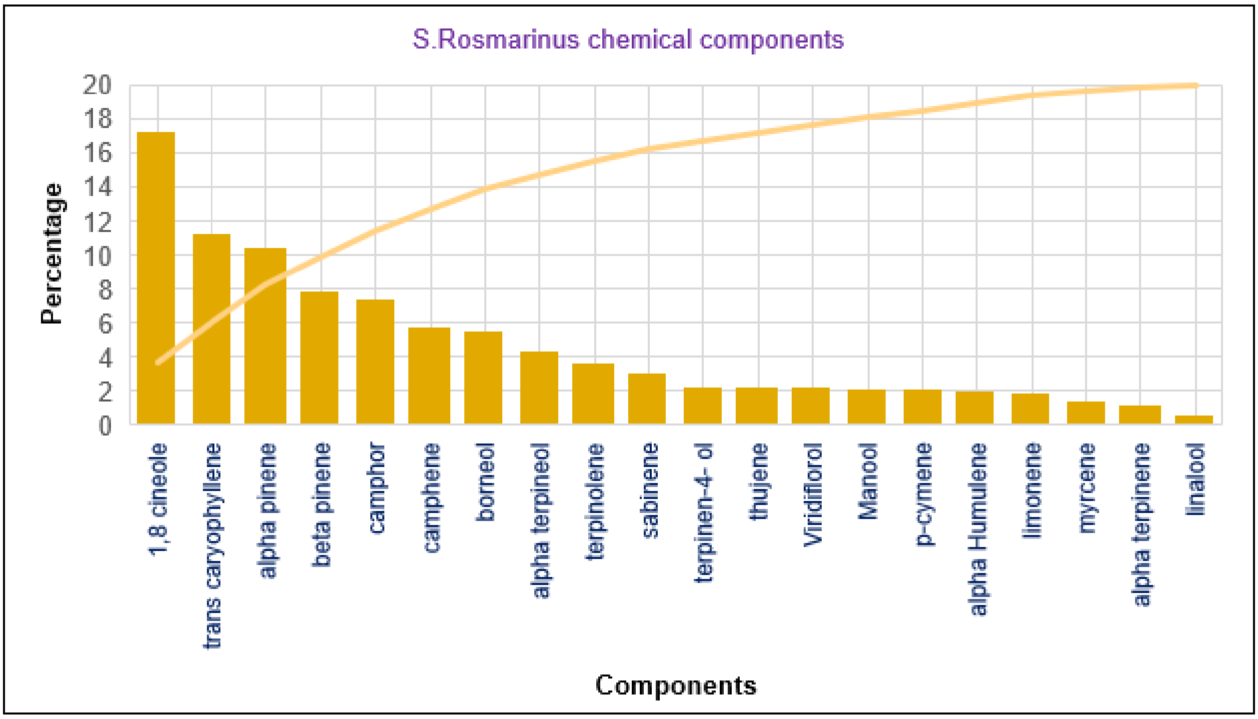


**Figure 7.** The Graphical presence of the Salvia Rosmarinus chemical components.

- **1.8- Cineole (Eucalyptol) – 17.23 % -**1,8-Cineole (also known as eucalyptol) originates mostly from plant essential oils and has many pharmacological effects, including anti-inflammatory and antioxidant properties, and has been used to treat respiratory and cardiovascular illnesses, among other things. [11].
- **Alpha pinene – 10.41%**

Antibiotic resistance modulation, anticoagulant, anticancer, antibacterial, antimalarial, antioxidant, anti-inflammatory, anti-Leishmania, and analgesic properties are among the many pharmacological activities reported, gastroprotective, anxiolytic, cytoprotective, anticonvulsant, and neuroprotective effects. [12].
The presence of Heavy metals was studied using ISO 11212 Spectrophotometry, the values recorded are presented in Table 1.

**Table 1.** The GC – FID analysis for Melissa Officinalis chemical composition.

| **Heavy Metals** | **Specifications** | **Results** | **Method** |
|---|---|---|---|
| Lead (Pb) | ≤5.0 ppm | 0.3 ppm | ISO 11212 Spectrophotometry |
| Cadmium (Cd) | ≤1.0 ppm | 0.07 ppm | |
| **Arsenic (As)** | ≤1.5 ppm | 0.12 ppm | |
| **Mercury (Hg)** | ≤0.1 ppm | <0.06ppm | |

Lead is the most abundant metal present at 0.3 ppm, still in accordance with the limits provided by WHO for plants and vegetables.

❖ **Melissa Officinalis -** Melissa officinalis includes essential oil (citroneral, citral, linalool, geraniol, and aldehydes), flavonoids, apigenin, luteolin, tannins, sesquiterpenes, caffeic acid, chlorogenic acid, rosmarinic acid, polyphenolics, and sesquiterpenes.

The GC -FID was used for the chemical composition of Melissa Officinalis and the results are presented in the table below:

**Table 2.** The chemical composition of Melissa Officinalis.

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| No. | Compound | % |
|---|---|---|
| 1 | *b- Pinene* | 0.68 |
| 2 | *Artemiseole* | 1.39 |
| 3 | *Ocinene* | 2.55 |
| 4 | ***Geraniol*** | **6.18** |
| 5 | *Linalol* | 1.35 |
| 6 | *b-Caryophyllene* | 7.76 |
| 7 | *Ocimene* | 4.67 |
| 8 | *Methyl Geranele* | 4.62 |
| 9 | *E- Citral* | 6.64 |
| 10 | *Isopulegone* | 2.45 |
| 11 | ***Citronellol*** | **14.86** |
| 12 | *Z-Citral* | 3.99 |
| 13 | ***Citronellal*** | **39.41** |
| 14 | *Humulene* | 2.24 |
| 15 | *Germacrene* | 1.2 |

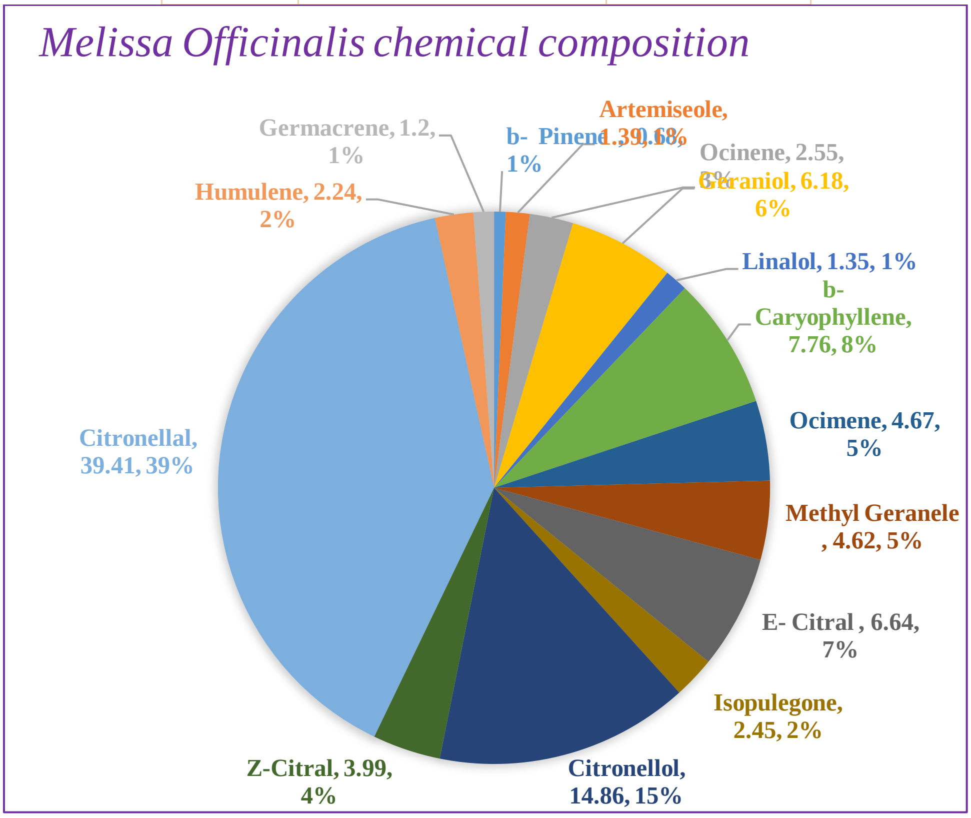



**Figure 8.** The Graphical presence of the Melissa Officinalis chemical components.

The three major components present in Melissa officinalis are:
***Citronellal – 39.41%*** It is a monoterpenoid, which gives the lemon aroma. It has a role as an antifungal metabolite.
***Citronellol – 14.86 %*** It is a monoterpene alcohol, with some beneficial properties such as antibacterial, antidepressant, antiseptic, antispasmodic, anti-inflammatory, deodorant, diaphoretic, diuretic etc.
***Genaroil 6.18%*** - It is a monoterpenoid and alcohol with certain therapeutic qualities, including antimicrobial action. (Strong inhibitory effect on many bacteria and fungi). Antioxidant properties. The

antioxidant activities of GE in several in vitro models suggest that GE may be beneficial against oxidative stress (OS), a progressive pathogenic characteristic of neurodegenerative diseases.

The presence of Heavy metals was studied using ISO 11212 Spectrophotometry, the values recorded are presented in Table 2.

**Table 2.** Spectrophotometry analysis for detection of heavy metals.

| Heavy Metals | Specifications | Results | Method |
|---|---|---|---|
| Lead (Pb) | ≤5.0 ppm | 0.27 ppm | ISO 11212 Spectrophotometry |
| Cadmium (Cd) | ≤1.0 ppm | 0.02 ppm | |
| **Arsenic (As)** | ≤1.5 ppm | 0.03 ppm | |
| **Mercury (Hg)** | ≤0.1 ppm | <0.05ppm | |

The most abundant metal is lead at 0.27 ppm followed by arsenic at 0.03 ppm. The values obtained conform to the limits provided by WHO.

- **Althaea officinalis –** Malva ( a synonym of Althea officinalis) extract has been found to contain a variety of chemicals, including phenolic derivatives, flavonoids, terpenoids, catalase enzymes, sulfite oxidase, fatty acids, specific strolls (particularly critical strolls like omega-3 and omega-6), beta carotene, and vitamins C and E, which have anti-inflammatory and antioxidant activities. **(13)**

The GC -FID analysis performed shows the chemical composition of the Albanian plant. The higher presence of Caryophyllene (E) at 11.122%, followed by alpha -Humulene at 10.48% and Ledat ol 1.356%.

***Table 3.*** The GC -FID analysis for the chemical composition of Althea Officinalis.

| | Composition | Percentage % |
|---|---|---|
| **1** | tricyclene | 0.142 |
| **2** | alpha pinene | 0.044 |
| **3** | Camphene | 0.171 |
| **4** | beta pinene | 0.132 |
| **5** | beta Myrcene | 0.112 |
| **6** | p-cymene | 0.079 |
| **7** | 1,8 cineole | 0.041 |
| **8** | E beta Ocimene | 0.1 |
| **9** | gama – terpinene | 0.062 |
| **10** | cis beta Terpineol | 0.334 |
| **11** | Camphor | 0.156 |
| **12** | neoiso 3 thujanol | 0.149 |
| **13** | Borneol | 0.12 |
| **14** | Terpinen -4-ol | 0.145 |
| **15** | Alpha-terpineol | 0.132 |
| **16** | Myrtenol | 0.133 |
| **17** | Myrtenyl acetate | 0.155 |
| **18** | Thymol | 1.374 |
| **19** | Caryophyllene ( E) | 11.122 |
| **20** | Aromanderne | 1.716 |
| **21** | alpha -Humulene | 10.48 |
| **22** | Caryophyllene oxide | 0.571 |

| | | |
|---|---|---|
| **23** | Ledol | 1.356 |

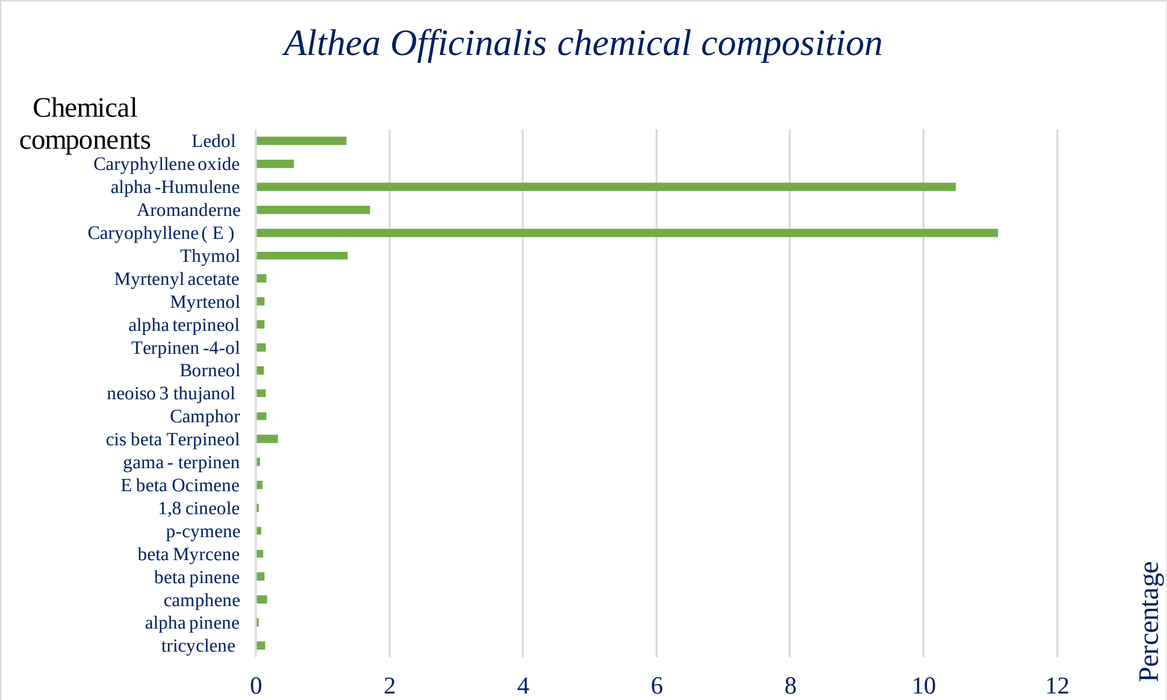



**Figure 9.** The Graphical presence of the Althea Officinalis chemical components.

The second analysis performed was the detection of heavy metals present in this plant. The ICP-MS analysis was used for detection, and the method used is Method 151, AOAC 2015.06 -19th:2019, Metals in Food.

**Table 4**. The ICP-MS analysis for detection of heavy metals.

| Heavy Metals | Results | Method |
|---|---|---|
| Lead (Pb) | 0.41 mg/kg | Method 151 AOAC 2015.06 - 19th:2019. Metals in Food |
| Cadmium (Cd) | 0.142 mg/kg | |
| Tin (Sn) | < 0.01 mg/kg | |
| Mercury (Hg) | 0.0029 mg/ kg | |

According to the analysis performed, the presence of 4 heavy metals was detected, recording lead as the most abundant at 0.41 ppm, followed by cadmium at 0.142 ppm.

- **Black tea-** Recent research has found that black tea includes potent groupings of polyphenols such as epigallocatechin gallate, theaflavins, thearubigins, an amino acid called L-theanine, as well as various additional catechins or flavonoids. (1).

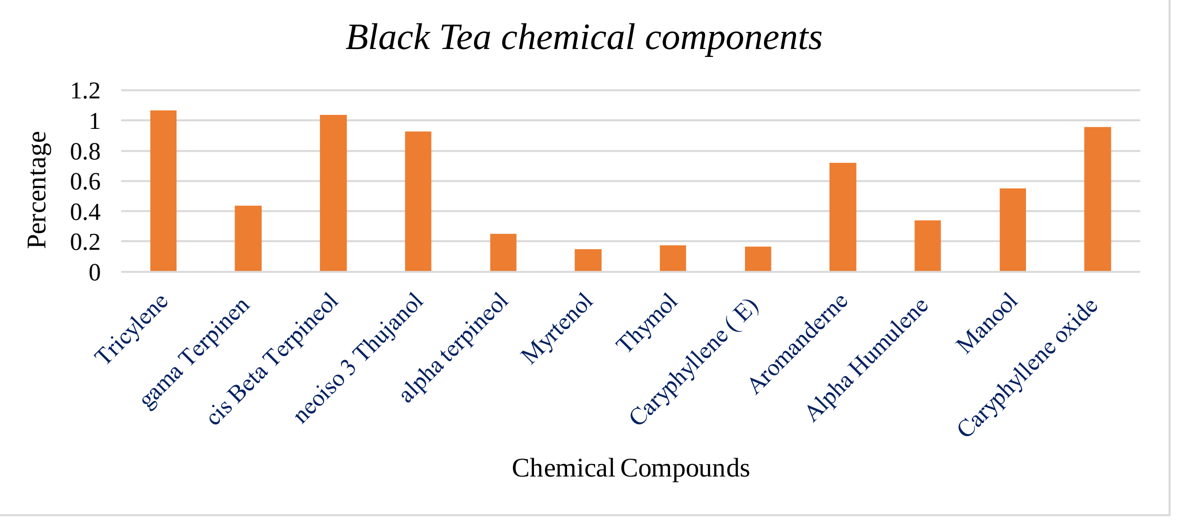
The GC- FID analysis performed shows the chemical composition of the black tea, it contains various monoterpene and terpenes as presented in Table 5.

**Table 5.** The chemical composition of black tea was obtained with GC-FID.

| | Components | % |
|---|---|---|
| **1** | Tricylene | 1.066 |
| **2** | gama Terpinen | 0.437 |
| **3** | cis Beta Terpineol | 1.035 |
| **4** | neoiso 3 Thujanol | 0.926 |
| **5** | Alpha-terpineol | 0.249 |
| **6** | Myrtenol | 0.148 |

| | | |
|---|---|---|
| **7** | Thymol | 0.176 |
| **8** | Caryphyllene (E) | 0.167 |
| **9** | Aromanderne | 0.72 |
| **10** | Alpha Humulene | 0.339 |
| **11** | Manool | 0.55 |
| **11** | Caryophyllene oxide | 0.956 |
| **12** | Other components | 93.231 |

**Figure 10.** The graphical presentation of the black tea composition.




Another analysis was performed using inductively coupled plasma mass spectrometry (ICP-MS), for the detection of heavy metals. Their results are presented in Table 6.

**Table 6.** The ICP-MS analysis for detection of heavy metals.

| Heavy Metals | Results | Method |
|---|---|---|
| Lead (Pb) | 0.19 mg/kg | Method 151 AOAC 2015.06 - 19th:2019. Metals in Food |
| Cadmium (Cd) | 0.0096mg/kg | |
| Tin (Sn) | < 0.01 mg/kg | |
| Mercury (Hg) | <0.01mg/ kg | |

The result obtained show that the most abundant metal is lead at 0.19 ppm, followed by cadmium at 0.0096 ppm.

### 2.5 Discussion

The results clearly demonstrated that the chemical profile of the plants provides an excellent insight into the benefits that these plants provide when used. It is also worth noting that different extraction methods may have an impact on the presentation of the chemical profile of the plants.

The chemical profile analysis with GC-FID was the same for the four plants, but the extraction method was different, which may be related to the presented results. The presentation of the chemical profile in the plants analyzed after the essential oil has been extracted with Clevenger appears incomplete, whereas the opposite occurs in the plants subjected to $CO_2$ extraction. The chemical profile is clearly different. The second step is to compare both methods for the same plants.

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**CONCLUSIONS**

From the results, we conclude that using $CO_2$ extraction was a more efficient method than Clevenger extraction because it was possible to capture some unstable components - highly volatile components referred to in other studies are difficult to capture with the Clevenger method.
The results obtained for the detection of heavy metals, Pb remains one of the heavy metals which is found in large amounts.

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