ABSTRACT

Tea is among the most consumed drinks worldwide with broad health benefits, such as cardiovascular protection and anticancer effect. The chemical compositions of the tea change greatly. The evaluation of the composition of nutrients in tea plants cultivated in different countries is of great importance from the perspective of quality, standards, nutrition, and health. The objective of this study was to use different methods for analysis to determine if the tea extracts contain phenols, flavonoids, saponins, terpenoids and tannins. The determination of phytochemicals is very important for many reasons. From the phytochemicals analysis it was conducted that the presence of tannins was found only in mint leaves and flowers. The analysis showed that flavonoids, saponins, terpenoids and phenols were present in all samples. The pH value was determined. Salvia officinialis had the highest pH value (7.35), while for the mint leaves and flowers the pH value was 6.45 respectively 7.02. TLC analysis of tea extracts was done for confirmation and visualization of bioactive compounds. ATR-FTIR was used to identify the functional groups as well as the possible structures of the compounds found in these plants. It can be concluded that these plants contain different bioactive compounds and can be recommended as plants of phytopharmaceutical importance.

Keywords: FTIR, mint, pH values, tea, TLC.

I. INTRODUCTION

Tea (Camellia sinensis L.) is one of the most consumed non-alcohol beverages in the world after water, due to its pleasant taste, rich flavours, aroma, and broad health benefits to human beings [1]. Tea plant is made from tender shoot tips and leaves and comprises several categories of beverages resulted from distinct processing treatment of the tea. Green and black tea are the most known tea in the world, while white tea is a rare tea type, yellow and oolong tea are known as well [2], [3]. The chemical compositions of the tea changes greatly, thus affecting the potential effect on health. Phytochemical investigations from tea plants have shown the presence of different chemical compounds such as flavonoids, phenolic compounds, catechins, alkaloids, tannins, volatile constituents, saponins, caffeine and amino acids as main active ingredients having strong antioxidant activities, anti-carcinogenesis and hepatoprotective activities [4–6].

Different investigations have shown that polyphenols and caffeine are the major components responsible for the different bioactivities and characteristic sensory properties of teas. The strong antioxidant activities of tea infusions are attributed to polyphenols and phenolic compounds, which are rich natural source of antioxidant. They are related with a range of chronic disease such as arteriosclerosis, diabetes mellitus, cancer and liver injury [1], [3], [7–9].

In recent years, saponins and polysaccharides as natural products from tea were also identified for their beneficial properties to health. Saponins are one of the major bioactive secondary metabolites in tea plants, with different activities. Saponins are a group of compounds that have specific characteristics such as strong foaming, emulsifying, dispersing and wetting properties as well as active pharmacological activities, including anti-cancer, anti-inflammatory, insecticidal, antibacterial, hemolysis, antioxidant, anti-hypertensive, weight loss, nerve stimulation or neuroprotection and fish toxicity, and insecticide activities [2], [10–12].

Flavonoids are the main group of phenolic compounds found in most plant foods and in particularly high concentrations in tea. Flavonoids consist of different subgroups such as anthocyanidins, flavanones, flavonols, flavones, flavonoids and isoflavones. Flavonoids are the most abundant and diverse of all bioactive non-nutritive components of foods. Phenolic compounds are used for application of biological activities. Phenolic acids are divided in hydroxybenzoic acids and hydroxycinnamic acids [13].

The flavanol composition in the phenolic content of tea is significant and leads to quantification. Different components
are found in flavabols like galloatechin, catechin gallate, galloatechin gallate, epicatechin, epigalloatechin, epicatechin gallate and epigalloatecin gallate. In literature it is reported that the presence of different phenolic acids such as gallic acid, p-coumaric acid, quinic acid derivatives, caffeoylquinic acid isomers, and caffeoyl glucose, contribute for understanding the phenolic composition of tea [13].

Tannins are another group of compounds that can be found in tea extracts and have various beneficial effects on health. Tannins have antioxidant and antimicrobial activities. Due to antimicrobial properties, tannins act as plant defensive agents and protect trees from fungi, pathogens, insects, viruses, and herbivorous animals [14]. In addition to the beneficial effects, this group of compounds also has undesirable attributes, such as precipitation of proteins, inhibition of digestive enzymes and prevention of the use of vitamins and minerals. The foods rich in tannins are considered to have low nutritional value and are harmful to health [7]. The main groups of tannins are condensed tannins (flavonoids without a sugar core) and hydrolyzable tannins (ellagic and gallic acids with a sugar core mainly glucose) [14]. Preparation of herbal products from different plants is done with different extraction techniques. Tea also can be extracted with different methods. Natural products extracted with different methods may yield various chemical components. Among the extraction's methods, extraction with water as a solvent is definitely the simplest, easiest, and greenest method. The extraction process can be affected from many factors [3], [12].

In this study, determination of pH in the tea extracts, the qualitative analysis of the tea extracts, TLC analysis and ATR-FTIR analysis were examined. The determination of phytochemicals such as flavonoids, saponins, terpenoids and phenols content of tea is very important in evaluation of the standard and quality of tea as well as any possible implications to health. Since tea is a drink that is part of our daily consumption, the evaluation of the composition of nutrients especially those mentioned above and their antioxidant capacity in tea plants cultivated in different countries is of great importance from the perspective of quality, standards, nutrition, and health.

II. MATERIALS AND METHODS
A. Sample Data
1) Sample collection
   Samples of mint (Mentha spp.) tea (flowers and leaves) and Salvia officinalis L. were cultivated in the garden in the western part of North Macedonia. The samples were collected and were placed in a shady place to dry.
2) Chemicals
   Ethanol was purchased from Alkaloid AD (Republic of North Macedonia), ferric chloride and glacial acetic acid were bought from Merck (Germany), sodium hydroxide, hydrochloric acid, chloroform, sulphuric acid, propan-2-ol, toluene, preparative TLC Silica gel plate. All reagents were with analytical purity grade.
3) Preparation of tea extracts
   Each sample was weighed (5 g) and placed in a laboratory glass where it was added 100 ml of distilled water and heated in magnetic stirrer for 10 minutes. The decoction was cooled and filtered through simple filter paper, and it was proceeded with other analysis.
   4) Determination of pH tea samples
      Using a pH-meter (Benchtop Biobox 210, pH/mV meter, PR China) were determined the pH values of extracted tea samples.
   5) Phytochemical analysis
      The prepared extracts were used for phytochemical analysis.
B. Qualitative Analysis of the Tea Extracts
1) Test for tannins
   Analyzes were conducted using the method by [15]. In a specific volume of the filtrate were added 2-3 drops of 1% ferric chloride solution. The filtrate which had a green color changed to dark-green or blue-black, indicating the presence of tannins.
2) Test for saponins
   In the obtained filtrate was added 5 ml of distilled water and heated until the mixture formed stable white foam. The formation of the white emulsion with the addition of distilled water showed a positive result.
3) Test for flavonoids
   To the aqueous filtrate of each water extract, 2 ml of diluted NaOH solution 1.0 M was added, followed by the addition of 2 ml of concentrated hydrochloric acid.
4) Test for terpenoids
   To the obtained filtrate from the tea was added 2 ml of chloroform and 3 ml of concentrated sulfuric acid to form a layer where the color changes. The presence of a reddish-brown color at the interface indicates positive results for the presence of terpenoids.
5) Test for phenols
   For the determination of phenols in the tea extracts, 5 ml of distilled water was added and then a few drops of the 5% iron chloride solution. The formation of a dark green color indicates the presence of phenolic compounds.
C. Preparative Thin Layer Chromatography (TLC)
   Silica gel plate was used for the study. The mobile phase solvent system consisting of Benzene: Acetic acid: Water (125:72:3) was used for the separation of alkaloids. The extracts of Mint leaves and flowers and the extract from Salvia officinalis plant were deposited at the concentrated band 1.5 cm from the edge of its respective TLC plate with the help of capillary tubes and allowed to dry.
1) Development of chromatogram
   After the application of the extracts on the TLC plate, the plate was kept in TLC chamber and then mobile phase was allowed to move through adsorbent phase up to ¾th of the plate. TLC was performed for alkaloids in the solvent system: Benzene: Acetic acid: Water (125:72:3).
   The Merck pre-coated silica gel plate 5×10 cm (60F254, 200 μm) was used in the TLC analysis and visualization was carried out using UV lamp from Analytikjena (Germany) at 254 nm using.
D. ATR-FTIR Spectroscopic Analysis

ATR-Fourier transform infrared (ATR-FTIR) was used to identify the characteristic functional groups in the samples. With those analysis can be obtained information about the structure of the molecule as well as the frequency absorbed in the spectrum. Small quantities of the samples were placed into the infrared spectrometer for collecting the spectrum data. The IR spectrum was obtained using Shimadzu, IRRSpirit Fourier Transform Infrared Spectrophotometer. QATR-S Single reflection ATR Accessory. The samples were scanned in the range from 4000 to 400 cm\(^{-1}\) with 20 scans at a resolution of 4 cm\(^{-1}\). The air spectrum was used as background. The peak values of the FTIR were recorded and analyzed. Each FTIR spectra was analyzed with LabSolutions IR and used in comparison with the literature.

III. RESULTS AND DISCUSSION

The determined pH values of each analyzed tea sample are given in Table 1. From the results it can be seen that the pH value for Salvia officinalis was 7.35, while for the mint leaves was 6.45 and for the mint flowers was 7.02. Salvia officinalis had the highest pH value, where the lowest pH value was determined in mint leaves. According to [16] the pH values for green tea from the grocery store for health nutrition and green market were 5.80 respectively 5.19, while in our results the pH value for the mint leaves and flowers were 6.45 respectively 7.02. According to the [17], the pH value of the green tea is 6.663, this pH value is closer to our results. Those differences in the pH values can be from the treatment and processing of the tea, the soil where the tea is grown, the way of collection, the place of storage and the drying temperature of the plant.

<table>
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<tr>
<th>TABLE I: DETERMINATION OF pH VALUES FOR TEA SAMPLES</th>
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<td>Tea sample</td>
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<td>Mint leaves</td>
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<td>Mint flowers</td>
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<td>Salvia officinalis</td>
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The results obtained for qualitative analysis are presented in Table II. The presence of phytochemicals in mint tea samples (leaves and flowers) and Salvia officinalis were confirmed with qualitative analyzing, while the presence of bioactive components was confirmed using TLC. From the table the presence of tannins was found only in mint leaves and flowers while in Salvia officinalis the test for tannins showed that tannins were not present in this plant. In nutrition science tannins are considered as bioactive components [14], [18]. Generally, tannins can be referred to as tannic acids which are natural sources. Tannin are active ingredient found in plant-based medicines, it can be found in foodstuffs, i.e., grapes, wine, fruit juice, blackberries, strawberries, walnuts, cashew nuts, hazelnuts, mangoes, tea and beer in food industries [15], [14]. The analysis showed that flavonoids, saponins, terpenoids and phenols were present in all the samples. From the results it we can conclude that phytochemicals are soluble in aqueous solution, which increases the possibility of consuming tea and in this way the body is supplied with bioactive ingredients.

<table>
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<th>TABLE II: QUALITATIVE DETERMINATION OF THE PHYTOCHEMICAL COMPONENTS OF DIFFERENT TEA EXTRACTS</th>
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By using thin layer chromatography for the mint (flowers and leaves) and Salvia officinalis extracts were performed qualitative analysis. The solvent system confirmed the presence of alkaloids, which are bioactive components in the parts of the mint and Salvia officinalis plant. Purple spot was observed under short UV light of 254 nm confirming the presence of alkaloids. The analysis by thin-layer chromatography is performed for visualization and confirmation of the presence of chemical components. The results are shown in Fig. 1.

![Fig. 1. TLC profile for alkaloid compounds.](Image)

Lane 1: Mint leaves; Lane 2: Mint flowers; Lane 3: Salvia officinalis.

The FTIR spectrum that was obtained during the analysis of the different types of tea was used to identify the functional groups of the active ingredients based on the peak value in the infrared radiation region. The bands obtained in the FTIR spectrum of the stretching, bending vibrations and the
functional groups of each analyzed tea sample were identified by comparing with others researches. The FTIR-ATR spectrum of the mint leaves is shown in Fig. 2, while in the Table III are presented the different FTIR absorption bands for tea samples. The absorption at the region 3300-3600 cm\(^{-1}\) is due to the stretching of hydroxyl groups. The region from 2800-2950 cm\(^{-1}\) is due to the symmetric stretching of saturated (sp3) carbon that corresponds to C-H stretching vibrations and CH\(_2\). The region from 1500-1600 cm\(^{-1}\) is assigned to the bending mode of N-H and to aromatic domain. The vibrational absorption band at 1370 cm\(^{-1}\) was assigned to rocking of methyl group. A notable band at 1250 corresponds to stretching vibrations of carbonyl C=O. The region from 1000-1140 cm\(^{-1}\) is due to the absorptions of stretching vibrations of C-O. The regions lower than 1000 cm\(^{-1}\) correspond out of plane to C-H bending vibrations. In the FTIR spectrum of *Salvia officinalis* (Fig. 3) few more bands are shown in comparison with the spectrum of mint leaves. In the region from 1600-1750 are shown bands that correspond to bending vibrations N-H, and C=O of ester, while the region from 1300-1450 cm\(^{-1}\) is due to stretching vibrations CO (amide) and C-.

![FTIR-ATR spectra of mint leaves.](image1)

![FTIR-ATR spectra of Salvia officinalis.](image2)

C stretching’s from phenyl groups [19], [20]. The presence of functional groups for phenolics and flavonoids in the presented spectra are widely reported for their antioxidant potential. They have antibacterial, antifungal, antiviral, hepatoprotective, immunomodulating and anti-inflammatory properties [21].

IV. CONCLUSIONS

This study summarizes the modern approach for analysis of extractable tannins, flavonoids, saponins, terpenoids, alkaloids, and saponins in the mint and *Salvia officinalis* plant. The alkaloids were isolated and purified with preparative thin layer chromatography. The pH value was determined in the extract of mint and *Salvia officinalis*, the pH value was different for the three extracts. FTIR spectroscopy is considered as a fast technique for investigation of the fingerprint region and to find the functional groups and the composition of the plants. It can be concluded that these plants contain different bioactive compounds and can be recommended as plants of phytopharmaceutical importance. However, it is recommended that other studies to be done to fully ascertain their bioactivity, toxicity profile, effect on the ecosystem and agricultural products.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

REFERENCES


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