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EXTRACTION METHODS FOR CAFFEINE FROM *Camellia sinensis*¹

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ABSTRACT

Mints are considered to be aromatic where the leaves fresh or dried are used as culinary source. The main application of the mint is against stomach ache and chest pains. Caffeine is a substance present in tea giving the pleasant and stimulant effect. The tea samples, raw and in bags from *Mentha Spicata* were bought from local market and the fresh sample was from the garden. Extraction methods were Soxhlet with the use of methanol with three cycles and boiling tea samples with water as solvent in the period of one hour. Lead(II) acetate was used to separate tannins from caffeine followed by filtration through filter paper with black ribbon. To 5 cm³ of the filtrate was added 3 drops of 0.1% ferric chloride. A brownish green showed positive test for tannins. The determined pH values for fresh, raw and tea in bags were 5.94, 5.92, and 5.89, respectively.

KEY WORDS: caffeine, extraction, mint, tea

¹ original scientific paper

INTRODUCTION

Medicinal herbs have been interested in various scientific fields such as biotechnology, pharmaceuticals, and food science because of the phytochemical sources with the potential antioxidative activities. *Mentha* (also known as mint) is a genus of plants in the family Lamiaceae (mint family). The growing of the plant is almost during the whole year determined in the places where the environment is wet and there is moisture in the soil. Many of the plant hybrids are grown in Europe, Asia, Australia, and North America where the cultivation procedure is implemented. The length of the mints usually is from 10 to 120 cm and sometimes because of the tendency to spread unchecked, may be considered as invasive. Mints are considered to be aromatic herbs last more than two years rich with antioxidant, phenolic, and flavonoid compounds. Harvesting is performed during any time of the year where fresh leaves should be used immediately or store in the dark cool places. The ingredient in the plant shows natural source of antioxidants (Bimakr *et al.*, 2011) and the mint extracts possess antimicrobial, antiviral, insecticidal properties (Kapp *et al.*, 2013). The volatile compounds present in the plant are considered to be used in various fields such as pharmaceutical, food, confectionary and liquor industries (Bimakr *et al.*, 2011; Kapp *et al.*, 2013). It is reported that peppermint infusion may contain up to 21% of its original essential oil in the raw material, while 75% is extracted from the polyphenolic content (Kapp *et al.*, 2013).

Tea, *Camellia sinensis* is a popular inexpensive beverage and a complex mixture of caffeine and tannins (Pradhan *et al.*, 2017). Peppermint (*Mentha*) is single ingredient herbal tea, known best for the refreshing taste and strong spicy aroma and flavor using in food products, beverages, and chewing gum (Fischer *et al.*, 2020). In Eastern and Western traditions, peppermint was used in the alternative medicine in the treatment of illness and conditions such as irritable bowel syndrome, flatulence, indigestion, nausea, vomiting, cough, and bronchitis (Kapp *et al.*, 2013).

Caffeine is a colorless odorless white substance with bitter taste. It is used in the flavoring the food and it is found in tea as a stimulatory effect on central nervous system (Pradhan *et al.*, 2017). The caffeine content in various tea samples was dependent on the type of tea whether it is black, green white, oolong or it is herbal tea.

The extraction of the main compounds have been studies especially those which have increasing attention in food and pharmaceutical industries due to the effects on the central nervous system, relaxation of the smooth muscles, and myocardial stimulation such as caffeine (Bansode *et al.*, 2015; Komes *et al.*, 2009; Pradeep *et al.*, 2015). Bansode *et al.* (2015) used digestion for the extraction of caffeine from tea in hot water for 15 minutes, while 10 minutes was used in the sample preparation step using 2.5 g of the sample (Komes *et al.*, 2009).

Black Tea was generally classified to contain up to 70 mg caffeine per 30 g tea. Daily consuming of tea could be calculated in cup of tea. The caffeine in green tea falls mid-range between 35 and 45 mg in 30 g tea serving. In the comparison to the black tea caffeine is found to be less in the green tea but many factors influence to the amount such as growing, type of tea, origin of the land, and the condition for processing, drying of the tea and etc (Mumin *et al.*, 2006). The white tea is considered to be in least with amount of caffeine of all teas with only in the range of 15 to 30 mg in 30 g tea for serving. The least processed is white tea and in that way it is one of the most delicate tea varieties (Mukherjee *et al.*, 2018). Comparing the teas which are consumed in U. S. and China can be said that oolong tea is one of the most popular traditional Chinese tea with diverse flavor than any tea variants in U. A. The caffeine content is between the one of the black and green tea and the amount is between 37 and 55 mg in 30 g tea for serving (Komes *et al.*, 2009).

On the other hand there are herbal teas such as chamomile, ginger and peppermint without caffeine contain at all (Fischer *et al.*, 2020; Pradhan *et al.*, 2017). These types of teas are made from dried flowers, leaves, seeds or roots which are generally caffeine free. This is due to the absence of *Camellia sinensis* plant in most herbal teas. Herbal teas are often used as a drink in the night not being annoyed from caffeine content.

The objective of this study was to extract the caffeine more efficiency from tea samples with the use of more tea sample and more time and analyzing its content. The steam distillation method in the comparison to Soxhlet method was studied in the analysis. A simple and easy to handle thin-layer chromatography (TLC) method was used to identify caffeine in the analyzed samples.

MATERIALS AND METHODS

Samples of mint tea were bought in the local market as tea bag and raw tea, while one sample was cultivated in the garden are presented in Figure 1.

Standard caffeine (98.5%) and lead(II) acetate were purchased from Acros Organics (USA), ethyl acetate (99.8%), dichloromethane (99.8%), methanol were from Fluka (Switzerland), ethanol (70%), sodium sulfate from Alkaloid AD (Republic of North Macedonia), and glacial acetic acid and ferric chloride were bought from Merck (Germany). All reagents were with analytical purity.

Figure 1. Above-ground parts of tested mint samples: fresh mint tea sample (A), raw mint tea sample (B), bag mint tea sample (C)

DETERMINATION OF pH

The pH values of extracted samples of tea in hot water were determined using a pH-meter (Benchtop Biobase 210, pH/mV meter, PR China), given in Figure 2.

Figure 2. Picture of the pH meter

EXTRACTION METHODS

Caffeine can be dissolved in methanol well and the choice of solvent was in the accordance of the solubility (1 mg/mL). Several extraction methods such as liquid-liquid extraction followed by steam distillation and Soxhlet extraction were used in the analysis. Each tea samples (25 ± 0.0001 g) were weighted and transferred to laboratory beakers (1000 mL). In amount of 500 mL tea samples were boiled in the period of 1 h. The solid parts of the raw tea were separated with filtration through simple filter laboratory paper. The solution was 100 mL and 5 mL of lead(II) acetate 10% solution was added to the filtrates. Solution of lead(II) acetate was added in order tannins to be separated which are formed in the bottom of the backer. A black ribbon (589/1) filter paper (Germany) was used in the filtration process in order to be separated tannins from the solution successfully. In the liquid-liquid extraction dichloromethane (3×5 mL) was used in a separatory funnel. Sodium sulfate was used as a drying agent.

Thin-layer chromatography (TLC) method

TLC method was introduced in the identification of caffeine in the tea samples using caffeine as a standard. The samples of one grain were dissolved either in 1 mL methanol. A mixture of glacial acetic acid and ethyl acetate, 95:5, v/v was used as a mobile phase. The Merck pre-coated silica gel plate 5×10 cm (60F₂₅₄, 200 µm) was utilized in the TLC analysis and visualization was carried out using UV lamp from Analytikjena (Germany) at 254 nm using.

Qualitative determination of phytochemicals in *Mentha Spicata*

Determination of phytochemicals such as tannins and saponins in mint is based on the procedure given by Ezeonu and Ejikeme (2016). The test for the presence of tannins require each tea sample extract (5 mL) to be placed in a test tube where 3 drops of 0.1% ferric chloride was added to each test tube. The test of saponins is based on forming a mixture in a test tube between 5 mL of distilled water and 10 mL of the filtrate mint sample. The test tube was shaken vigorously in order to form a stable persistent froth.

RESULTS AND DISCUSSION

The results of the determination of pH for analyzed mint teas samples are given in Table I.

Table I Determination of pH for mint tea samples

Sample	Mint	pH
1	Fresh mint tea	5.94
2	Raw mint tea	5.92
3	Bag mint tea	5.89

Based on the results from Table I, it was found that the pH value was around 5.9 for all mint tea samples. The highest value of pH was determined in fresh mint tea, where the lowest value of pH was in bag mint tea sample (5.89).

The present of phytochemicals in mint tea samples were confirmed with several qualitative tests. A brownish green or a blue black colouration showed positive test for tannins in all samples (commercial and fresh tea).

The formation of emulsion on addition of three drops of olive oil showed positive result for the saponin presence in the sample.

The TLC method was included as a control method for the process. The analytical purity was indicated with TLC (one spot) where the R_f values depended on the use of mobile phase which are given in Table 2.

Table 2 Determination of R_f values for extracted and isolated caffeine in tea samples (Abazi *et al.*, 2021)

Sample*	R_f , Type of mobile phase, The first, (Glacial acetic acid and ethyl acetate, 95:5, v:v)	RSD (%)
1	0.36 ± 0.0029	0.79
2	0.36 ± 0.0023	0.64
3	0.36 ± 0.0029	0.79
4	0.36 ± 0.0028	0.25
5	0.36 ± 0.0027	0.73
6	0.36 ± 0.0026	0.26

* Standard of caffeine (1) Green tea from the grocery store for health nutrition (2) and green market (3), white tea from local market (4), black tea from the green market (5) from 2010 and (6) from 2020.

The samples were green, white tea, and two types of black tea from 2010 and from 2020.

The TLC method represents a choice to conduct analyses between the standard caffeine and caffeine obtained with several different extraction modes including tea boiling (a), Soxhlet extraction (b) and TLC analysis (c) are given in Figure 3.

Figure 3. Sample preparation (a); process of Soxhlet extraction (b), TLC analysis (c)

In Figure 4 are presented filtrated tea samples (a) and test for phytochemicals: test for tannins (b) and saponinis (c)

Figure 4. Obtained mint sample teas (a), test for tannins (b) and saponins (c)

CONCLUSION

The Soxhlet extraction procedure gave satisfactory results for extraction of caffeine from tea samples. In the *Camellia sinensis* was found to be caffeine content in the tea samples while in the herbal tea samples, caffeine was absent in the analysis. TLC method was applied for determination of caffeine content in the samples. Qualitative tests were conducted in order to determine phytochemicals in mint tea samples.

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