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Determination of Caffeine in Different Comercialy Available Green and Black Teas

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Abstract: Methyl derivatives of xanthine are a group of alkaloids commonly used for their effects as mild stimulants on various organ systems such as cardiovascular and central nervous system (CNS), respiratory system and skeletal muscles. The naturally occurring methyl xanthines are caffeine, theophylline and theobromine. The aim of this study was to determine content of caffeine in the green and black tea commercially available from Bosnian markets by simple, fast and economical methods. The simultaneous quantitative and qualitative determination was based on spectrophotometric UV/Vis method and thin layer chromatography (TLC) method. Content of caffeine in the green tea was in the range 33.90 to 110.73(mg/g), and in the black tea was in the range from 10.32 to 63.00 (mg/g). The highest content of caffeine was detected in the green tea of Slovenian manufacturer, and in the black tea of Croatian manufacture. Consuming a large amount of these types of tea could cause some health problems.

INTRODUCTION

Herbal teas play a vital role in maintaining human health and contributes to the improvement of human life. Herbs are considered as chemical laboratories capable of biosynthesizing a number of bio-molecules of different chemical classes. Tea is the most consumed plant-based beverage in the world. Caffeine is the most important naturally occurring xanthine derivate and it is found in many herbs, like tea leaves, coffee beans, kola nuts, cocoa beans etc. (Verma and Kumar, 2010). Caffeine is rapidly metabolized in human cells by demethylation: within 1–3 h of exposure to millimolar concentrations (Goth and Cleaver, 1976). Caffeine is a psychoactive stimulant known to increase alertness, elevate mood and give temporary energy boost thereby easing fatigue. It also increases the effectiveness of certain drugs, hence it is used with some over-the-counter drugs for the treatment of conditions such as migraine and cluster headaches (Ogah and Obebe, 2012). Some studies show that consuming caffeine causes increase in blood pressure, diuresis, increase in blood sugar, increase in gastric acid and pepsin secretion, increased plasma levels of fatty acids, cortisol and epinephrine, raised intraocular pressure and loss of calcium leading to bone loss (Klang, Wang and Meoni, 2002). Caffeine can make a person addicted to it and can have adverse effects because consumption of more than 1 g of caffeine can lead to death (Sapcanin, Uzunovic, Jancan et al. 2013). Intake of caffeine was considered safe if we consider the US Food and Drugs Administration classification of caffeine consumption. Under this classification, caffeine intake of 130 - 300 mg/day is low/moderate, and above 400 mg/day is high (Ogah and Obebe, 2012). Caffeine was first isolated from coffee in 1820 by a German chemist Friedlieb Ferdinand Runge (Lovett, 2005). Today, there are several chemical and physical methods for the determination of caffeine in tea leaves and other beverages. The most widely used methods for the determination of caffeine in beverages include various analytical techniques such as High Pressure Liquid Chromatography, Fourier Transform infrared spectroscopy, Near infrared reflectance spectrometry, Raman spectroscopy and capillary electrophoresis. However, such equipments and instruments are expensive and they are not available in most laboratories (Belay, Ture, Redi et al, 2008).

In this study, a method for determimnation of the content of caffeine in green and black teas commercially available from Bosnian markets is reported by using UV/Vis spectrophotometer, which is available in most laboratories. Purity and identification of isolated caffeine was determined by using thin layer chromatography (TLC). Moreover, methods for the determination of the content of caffeine in tea are easy, fast and cheap.

MATERIAL AND METHODS

Green and black tea, commercially available from Bosnian markets, produced by different manufacturers such as Croatian, Slovenian, Austrian, Turkish, Indian and Chinese companies were used in this study. Caffeine was purchased from Caelo and all other reagents and chemicals were purchased from Sigma-Aldrich Co. LLC. All chemicals were p.a. grade.

Isolation of Caffeine from Green and Black Tea Leaves

An accurately weighed amount of 5.0 g of tea was extracted with 100 ml of boiling distilled water. Solution was mixed for 10 minutes and then filtered. Caffeine was extracted from the primary filtrate into the separatory funnel. Extraction was done three times, each time with 20 mL of dichloromethane. Extract of caffeine was rinsed twice, each time with 20 mL of cold 6M NaOH. Furthermore, the extract was rinsed once with 20 mL of cold distilled water. Layer with dichloromethane was dried over anhydrous Na_2SO_4. The solution was evaporated to drieness (Vishnoi, 2003). The extracted caffeine was stored in a clean 10 mL volumetric flasks. The absorbance of the resulting solutions was then measured by using an UV/Vis spectrophotometer at 271 nm wavelength.

Preparation of caffeine standard solutions

Standard of caffeine was prepared by dissolving 1 mg of caffeine in 100 mL dichloromethane in a volumetric flask (100 mL). The working standard solutions (10, 20, 40, 60, 80 and 100 mg/L) were used in this study (Amos-Tautua Bamidele and Diepreye, 2014). The absorbance of each solution was measured at 271 nm wavelength. The absorbance values were then plotted against concentrations to generate a standard calibration curve. The results showed a good linear relationship between the absorbance and concentrations of the standard solutions.

Thin Layer Chromatography (TLC)

Purity of isolated caffeine was estimated by using TLC method and was compared with standards. This method can be applied for the determination of caffeine in different types of commercially available samples.. The test samples and standards were dissolved in ethanol-water (8:2 v/v) and applied to precoated TLC. The chromatographic separations were done on the silica gel F_{254}. TLC plates were developed with chloroform-acetone-methanol (1:1:1 v/v/v). The detection was performed under UV lamp at 254 nm and the evaluation of the chromatographic plate was based on processing of chromatographic images and Rf value was calculated (Harborne, 2005; Kumar, Niranjan, Chaluvaraju, 2010).

Quantitative caffeine determination

Quantitative analysis of caffeine was performed by using a Genesys UV-Vis Spectrometer, Model TM2. The λ_{max} was determined by scanning the standard solution from 200-400 nm and the obtained results gave an absorption spectrum, which was characterized by a single intensive absorption band located in the UV range at λ_{max} = 271 nm. Standard linear calibration curve was run to obtain the linear range of sample analysis, correlation factor was with accepted value (0.9916) and the standard calibration curve was linear over the range (10-100 µg/ml) caffeine with equation (y = 0.0344x + 0.2184). The quantitative amount of caffeine in samples (µg/ml) was then determined using the standard curve.

RESULTS AND DISCUSSION

The standard linear calibration curve obtained of spectrophotometric UV/Vis method for the determination of caffeine is presented in Figure 1. It showed a good linear relationship between the absorbance and concentrations of the standard solutions.

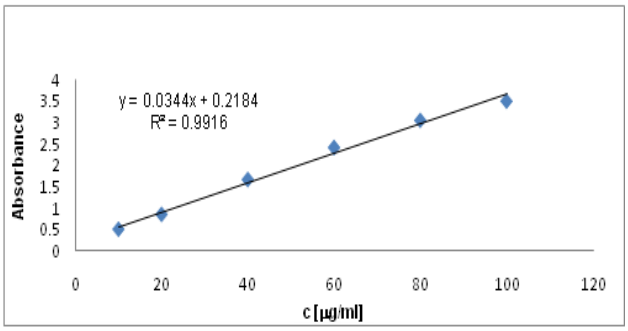


Figure 1: Calibration curves of spectrophotometric UV/Vis method for the determination of caffeine

The results obtained for caffeine in the different tea extracts are showed in Table 1.

Table 1: The content of caffeine in various tea extracts

Sample	Name	Manufacturer	µg/ml	mg/g
1	Black „*Zelenara*"	Turkey	20.74	51.80
2	White „*Zelenara*"	China	20.63	51.60
3	Green *„Podravka"*	Croatia	16.44	54.80
4	Mix black and essential oil of bergamot „*Sir Winston Tea*"	Austria	26.32	52.64
5	Green *„Pak centar"*	Bosnia	22.19	55.47
6	Green „*Teekanne*"	Austria	23.98	59.93
7	Black *„Bonito"*	India	4.13	10.32
8	Black *„Podravka"*	Croatia	25.20	63.00
9	Green „*Sir Winston Tea*"	Austria	10.17	33.90
10	Green *„1001 Cvet"*	Slovenia	33.22	110.73

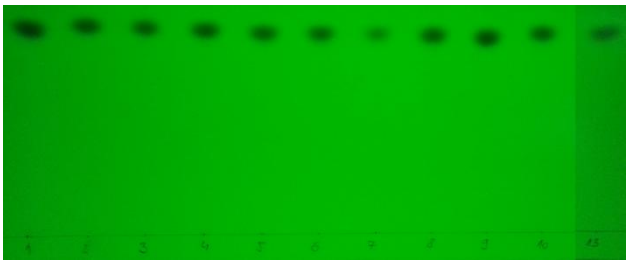
Content of caffeine in green tea was in the range 33.90 to 110.73 (mg/g), and in black tea was in the range from 10.32 to 63.00 (mg/g). The highest content of caffeine was detected in green tea from the Slovenian market, and in the black tea from Croatian market. The content of caffeine in black and green tea determined in this work is in agreement with previous works reported by Srdjenovic, Djordjevic-Milic, Grujic et al. (2008) and Bispo, Veloso, Pinheiro et al. (2002).

Identification of isolated compound by TLC and UV Spectroscopic methods.

Qualitative analysis of caffeine was performed by TLC method (Chromatogram 1.) for the determination of the caffeine in tea leaves available from Bosnian market places. UV/Vis spectroscopic study and TLC of the isolated compound were found almost similar to that of the standard caffeine Table 2. (Stahl, 2007; Harborne, 2005).

Table 2. Results of TLC and UV Spectrophotometric study of caffeine

Sample	UV-Spectra (λ_{max} in nm)	TLC Studies (Rf)
Tea leaves	271	0.79 – 0.80
Standard	271	0.80



Chromatogram 1. TLC analysis of investigated samples (1-10) and standard solution of caffeine (13)

CONCLUSION

From the results of this study it was concluded that the highest content of caffeine was detected in green tea of Slovenian manufacturer, and in the black tea of Croatian manufacturer. It was recommended that people who need caffeine restriction due to health conditions should choose products with lower caffeine contents. Since caffeine could cause some health problems, manufacturers should be required to indicate its presence and amounts on the product labels for information to consumers.

The UV/Vis spectrophotometric method employed in this study for the quantification of caffeine in tea leaves was found to be relatively fast, cheap and simple for performing. This analytical method may therefore, be recommended for the rapid quantification of caffeine in tea leaves by any educational institutions in developing countries.

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Summary/Sažetak

Derivati metil ksantina su grupa alkaloida koji se često koriste kao blagi stimulansi na različite sisteme organa, kao što su kardiovaskularni i centralni nervni sistem, respiratorni sistem i sistem skeletnih misića. Prirodni metil ksantini su kofein, teofilin i teobromin. Cilj ovog rada je određivanje kofeina u zelenim i crnim čajevima komercijalno dostupnim sa bosanskog, hrvatskog, slovenačkog, austrijskog, turskog, indijskog i kineskog tržišta, brzim i ekonomičnim metodama. Simultano kvantitativno i kvalitativno određivanje bazirano je na spektrofotometrijskoj metodi UV/Vis i tankoslojnoj hromatografiji (TLC). Sadržaj kofeina u zelenim čajevima je bio u rasponu od 33.9 do 110.73 (mg/g)., a u crnim čajevima od 10.32 do 63.00 (mg/g). Najveći sadržaj kofeina određen je u zelenom čaju sa tržišta Slovenije ,a u crnom čaju sa tržišta Hrvatske. Konzumiranje velike količine ovih čajeva može uzrokovati neke zdravstvene probleme.