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Qualitative and quantitative analysis of caffeine in some commercial brands of tea consumed in India

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ABSTRACT

Caffeine is a common organic molecule found in many beverages such as coffee, tea, energy drinks and cola, which make the drinks addictive. Caffeine has drawn more attention due to its physiological effects beyond its stimulatory effect on central nervous system, hence it is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs. Caffeine content in various energy drinks and beverage varies from 10 to 50 mg of caffeine per serving; however the US Food and Drug Administration (FDA, 2006) limits the maximum amount in carbonated beverages to 6 mg/oz. Large amount of caffeine consumption can cause physiological and psychiatrically dependence. The aim of this study is to determine the concentration of caffeine in tea brands available in India to ensure whether the caffeine concentration in the follow tea as per FDA recommendation or not. There are few reputed brands like Taj, Red Label, Agni and other local brands like Mohini, and Krishna Gopal were studied, by using simple and fast standard UV-Visible spectrophotometric method. The minimum caffeine level was observed in the Mohini brand tea, while Taj tea brand sample showed the highest caffeine content.

Keywords: Caffeine, Tea, UV-Visible spectrophotometric method.

INTRODUCTION

"A cup of tea would restore my normality." - Douglas Adams [1]

Tea is one of the most ancient and popular beverages consumed around the world. It is a complex mixture containing a variety of substances such as tannins, and caffeine [2]. The tannins behave differently when the tea is brewed some of the tannins get hydrolyzed, forming acidic compounds in the tea. The brown color of tea is due to the pigments such as flavonoids and chlorophylls which are dissolved in the hot water when the tea is brewed [3]. Caffeine ($C_8H_{10}N_4O_2$) was first discovered in the tea in 1827 by the German chemist Friedrich Ferdinand Runge in 1819 and was named "Theine". It was later found in mate and various other plants, eventually it was found that theine of tea was identical with the Caffeine of coffee and the term Theine then was dropped [4]. Caffeine is known as 1, 3, 7-trimethyl xanthine or 3, 7 dihydro-1, 3, 7-trimethyl-1-H purine-2, 6-dione [5]. The actual source of caffeine is coffee beans, kola nuts, cocao pods and dried leaves of *Camellia sinensis*. Other botanicals that contain caffeine are dry herb *Yerba mata* and *Guarana* [6]. Caffeine is used in wide range of pharmaceuticals formulations and beverages, ayurvedic medicines and has the potential of being used in Pan masala, chocolates, etc. Caffeine occurs as a colorless odorless white powder, silky glistening needles with bitter taste. When ingested, it stimulates the central nervous system and can temporarily increase blood pressure and heart rate [7, 8]. A normal dose of caffeine is generally considered to be 100 mg, which is roughly the amount found in a cup of coffee. Caffeine is quickly and completely removed from the brain. Its effect is short-lived and it tends not to negatively affect concentration or higher brain functions [9]. However, continued exposure to caffeine leads to developing a tolerance to it. Too much caffeine can result in caffeine intoxication, which is characterized by nervousness, irritability, anxiety, tremulousness, muscle twitching (hyperreflexia), insomnia, headaches, respiratory alkalosis, and heart palpitations [10]. The Food and Drug Administration (FDA) defines caffeine as a generally recognized as safe (GRAS) substance. However, FDA specifies that the maximum amount in carbonated beverages is limited to 0.02% (FDA 2006). Therefore, the highest legal amount of caffeine allowed in a 355 mL (12oz) can of soft drink is about 71mg [11]. Pregnant women who consume high amounts of caffeine have increased risk of miscarriage, difficult birth and delivery of low-weight babies [17]. Research has shown links between heavy caffeine consumption and osteoporosis, high blood pressure, heart disease, heart burn, ulcers, severe insomnia and infertility [18]. Caffeine has diuretic properties when administered in sufficient doses to subjects who do not have a tolerance for it. The diuretic effect of coffee causes excretion of fluid through the kidney, which may lead to dehydration [19]. Caffeine was classified as a drug of abuse by the International Olympic Committee (IOC) when present in urine at concentration levels of more than 12µg/ml [20]. According to Directive 2000/13/EC, foodstuffs

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which contain caffeine must mention in the label or in the ingredient list. Caffeine used as a flavoring in the production or preparation of a foodstuff must be mentioned by name in the immediately after the term "flavoring". Besides to above Directive 2002/67/EC of 18 July 2002, directed any drinks contain caffeine in excess of 150 mg/L must also provide a warning message on the label followed by an indication of the caffeine content such that: "High caffeine content (X mg/100 ml)" [21]. Therefore, determination of caffeine in soft and energy drink for assurance of food safety and quality control is mandatory. Tea is one of the common beverages which contain caffeine and the concentration of caffeine in tea powder depends upon the various parameters while processing/manufactured. At present, there is no such standard followed for caffeine content in tea brands. So in this context this article will highlight the concentration of caffeine in some of the tea brands available in India.

Table 1: Occurrence of caffeine in some common foods and drugs [12-16]

A. Food stuffs	
Coffee	80-125 mg per cup
Coffee, decaffeinated	2-4 mg per cup
Tea	30-75 mg per cup
Cocoa	5-40 mg per cup
Milk chocolate	6 mg per oz
Baking Chocolate	35 mg per oz
Coca cola	46 mg per oz
B. Drugs	
Anacin, bromo-seltzer, midol	32 mg per tablet
Excedin extra strength	65 mg per tablet
Dexatrin,Dietac, Vivacin	200 mg per tablet
Dritan	16 mg per tablet
No doz	100 mg per tablet

MATERIALS AND METHODS

Materials

The dried tea leaves were brought form the local market and identified by Botanist cum Taxonomist Prof. J. Soodhi. Different sample of brands of tea such as Taj, Red Label, Agni, Tata, along with local brands like Mohini, and Krishna gopal were collected from the local market of Yamunanagar. The major solvents like methanol, acetone, dichloromethane, chloroform, ethyl acetate and petroleum ether are issued from the college chemical store. All the chemicals and solvents used in this research were laboratory grade.

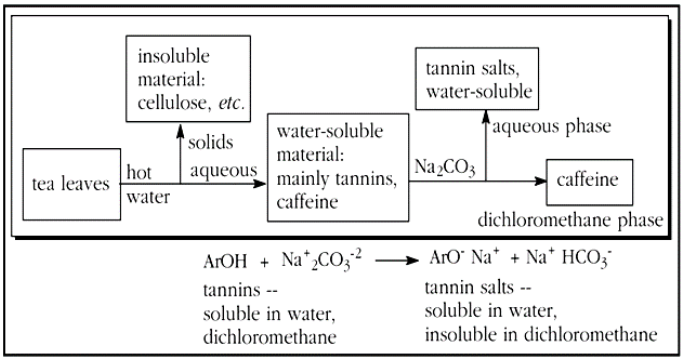


Figure 1: Extraction protocol for tea for isolation of caffeine

Methods

Isolation of caffeine: 100gms dried tea leaves were powdered with help of a motor and pestle placed in a beaker and 1 liter distilled water was added to it. Mixture was digested on a water bath for 20-30 minutes. It was then filtered through a muslin cloth. To the hot filtrate, 10ml 0.1M sodium carbonate was added to solubilize tannin. This is because the aromatic alcoholic compounds (i.e., tannin) react with sodium carbonate to form sodium salt of respective compound. The salt produce gets easily soluble in water. The resulted solution was transformed in to the 1 lit pear shaped separating funnel as shown in Fig. 2(a). The aqueous phase was partitioned with non polar solvent like dichloromethane. The dichloromethane layer was collected and solvent was distilled off. Residue obtained was then re-crystallized with ethanol as shown in Fig. 2(e)

Characterization of Caffeine: The isolated caffeine was characterized by following procedures.

Solubility study: The isolated compound was taken in a test tube and the solubility study was carried out by considered the different solvents from non-polar to polar. The solubility of caffeine in water, ethyl acetate, ethanol, carbon tetrachloride, methanol, chloroform, dichloromethane and acetone were measured by the gravimetric method which reported by early investigator. For each measurement, an excess of known mass of caffeine was added to a known mass of solvent. Then, the equilibrium cell was heated to the required temperature with continuous stirring. To ensure equilibrium, un-dissolved solid and solution were allowed to settle for 48hrs before sampling. For each measurement, an excess amount of caffeine was added to a known mass of solvent. Then, the equilibrium cell was heated to the required temperature with continuous stirring. After 48hrs, the stirring was stopped and the solution was kept still for 48hr. Then, the excess solid could be observed in the lower part of the equilibrium cell. The sample of the upper part of the solution was withdrawn with a suitable warmed pipette to another weighed vial. The vial was closed tightly and weighed to determine the mass of the sample. Then, the vial was placed in an oven to evaporate the solvent. After the evaporation of the solvent, the vial was dried for another 5hrs and reweighed to determine the mass of the solid. Thus, the solid concentration of the sample could be determined. All the solubility experiments were conducted six times to check the reproducibility

Melting point: Small amount of isolated compound was taken in a melting point capillary tube and tapped in such that the sample settled in the bottom of the tube. The tube then placed in the sample hole of a digital melting point and obverted the temperature of melting point.

pH of 1% w/v solution

1%w/v solution of isolated compound was prepared and the pH was measured in a calibrated pH meter.

Moisture content

An accurate and precise moisture determination is often essential in resolving the issues where water is involved. The moisture content of the isolated compound was carried out by Karl Fischer titrimetric method.

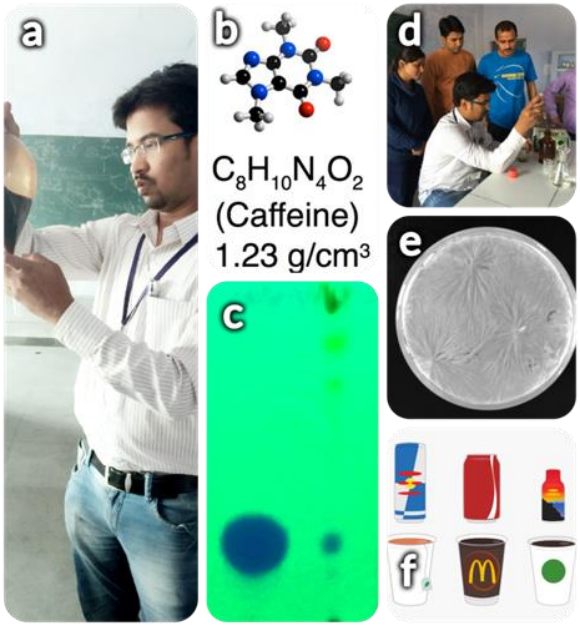


Figure 2: (a) & (d) Isolation process of Caffeine, (b) Molecular Structure of caffeine, c) TLC of Isolated caffeine, (e) Crystal of Caffeine, (f) Sources of Caffeine

Determination of ash

Loss on ignition is a test used in analytical chemistry, particularly in the analysis of minerals. It consists of strongly heating ("igniting") a sample of the material at a specified temperature, allowing volatile substances to escape, until its mass ceases to change. Accurately 3 grams of the powder is transferred to porcelain crucibles which were previously calcined, cooled and weighed. The samples are charred in a muffle furnace at 450 ºC for 2 hours. After cooling in a desiccator, they are weighed on an analytical balance. This procedure is repeated until obtaining constant weight. The ash percentage obtained in triplicate, is calculated in relation to the dried drug

Heavy metals

1gm of the isolated compound was taken in a transparent flat-bottomed glass tube, with a capacity of approximately 70 mL and the external mark corresponding to a volume of 45-50 mL with an internal diameter of 23 mm. The tubes used should be equal in both the inner diameter and in other aspects, since the comparison is direct. The tubes should be observed from above, against a white background. The standard volume used varies according to the specified in the monograph under analysis.

Thin layer chromatography

Thin Layer Chromatography (TLC) Purity of isolated compound (caffeine) was checked using TLC method and compared with standard. The test sample and standard was dissolved in chloroform and applied to pre-coated TLC plates with Silica gel (Merck, 60F) using Chloroform: acetone: methanol (1:1:1) solvent system and detection was done by putting TLC plates in iodine chamber. Then Rf value was calculated. The TLC plate was shown in fig. 2 (c)

Assay of caffeine content in different sample of tea with UV-VIS Spectrophometry

Ultraviolet Spectra were recorded using Shimadzu UV-Vis Spectrometer, Model 1700S, and distilled water was used as solvent for the dilution of sample as well as blank. The λmax value of test sample and standard was recorded [14-15]. A 100 ml stock standard of caffeine was prepared by dissolving 10 mg of caffeine in 100 ml of purified water. The solubility was further enhanced with ultrasonic bath with aid of heat. The working standards were prepared by pipetting 1, 1.5, 2, 2.5, 3, 3.5, and 4ml of the stock standard solution into separate volumetric flask and finally the volume make up with distilled water to get the desire concentration. The absorbance of different concentrations of 10, 15, 20, 25, 30, 35 and 40 ppm were analyzed using UV spectrophotometer at 272 nm wavelength and a standard calibration was plotted by considering absorbance in y-axis and concentration in x-axis.

Sample preparation and Analysis: 5gm of tea powder was taken in a 250ml beaker and it was allowed to boil over a bunsen burner for 30 min. The solution was filtered and the water was evaporated by rotavapour to get a solid residue. The sample was kept in dessicator till further analysis. 10mg of the sample was weighed accurately and dissolve in 100ml methanol and subsequent dilution was carried to obtain a 20ppm solution. Similar procedure was adopted the rest of the different brands of tea samples.

Preliminary Phyto-chemical analysis [22]

The different aqueous extracts were prepared by taking 5gm of sample in a clean round bottom flask and 250ml of distilled water was added. it was allowed to boil over a bunsen burner for 30 min. The solution was filtered and the water was evaporated by rotavapour to get a solid residue. The sample was kept in dessicator till further analysis. The preliminary Phytochemical analysis was performed through standard official procedure for the identification of different class of secondary metabolites present in different aqueous extract of tea sample.

RESULTS

Characterization of Caffeine

Solubility studies

Solubility of caffeine decreases in the order of chloroform, dichloromethane, acetone, ethyl acetate, water, methanol, ethanol, and carbon tetrachloride. The solubility of caffeine in chloroform showed a higher value than those in the other solvents. Thus, chloroform is a better solvent to separate and purify caffeine from solutions. The other characteristic parameter are highlighted in table no. 2.

Table 2: Characteristics of caffeine

Particular		Range/ Standard value
Appearance	White crystal [As shown in fig 2. (e)]	White crystalline powder
Molecular formula	$C_8H_{10}N_4O_2$	$C_8H_{10}N_4O_2$
Molecular weight	194.19	194.19
Density at 20^{0}C	1.23	1.23
Melting point	237. 7^{0}C	238^{0}C
pH of 1% solution	5.91	6.9
Water at 80^{0}C	0.41%	Not more than 0.5%
Residue on ignition	0. 0861%	Not more than 0.1%
Heavy metals	*0.00089%*	Not more than 0.001%

Thin layer chromatography (TLC)

The rf value of the isolated compound is found to be 0.53 with the mobile phase chloroform: acetone: methanol (1:1:1).

Preliminary phyto-chemical analysis

Preliminary phyto-chemical analysis of aqueous extract of different sample was listed in table no. 3.

Table 3: Preliminary phyto-chemical analysis of aqueous extract of different sample

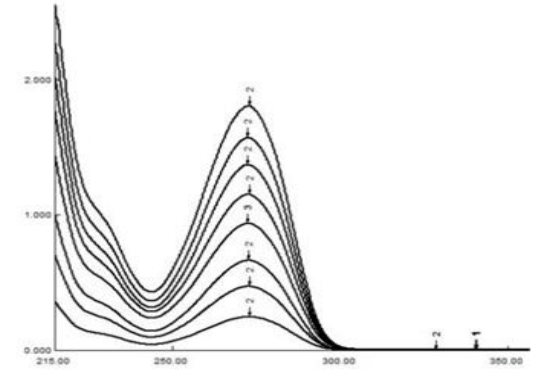
Sl. No.	Tests	Aq. extract of Mohini	Aq. Extract of Taj	Aq. Extract Agni	Aq. Extract Krishna Gopal	Aq. Extract Red Label
1.	Alkaloids	- *	- *	- *	- *	- *
2.	Carbohydrates	-	+	-	+	+
3.	Gums/Mucilage	-	-	-	-	-
4.	Tannins	+	+	+	+	+
5.	Flavonoids	+	+	-	-	+
6.	Saponins	-	-	-	-	-
7.	Sterols	-	-	-	-	-

(+): Present, (-): absent

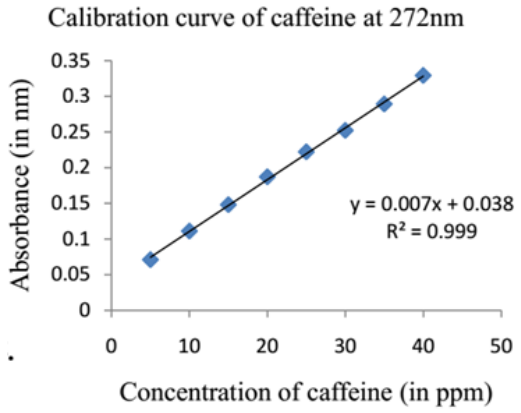
* All sample gives negative test for the general alkaloids test like Dragendroff's test, Mayer's test, Hager's test, Wagner's test, but it gives positive test to Murexide test, which signify the presence of purine alkaloids like caffeine.

Assay of caffeine content in different sample of tea with UV-VIS Spectrophometry

There was difference of caffeine contents in each sample of different brands of tea. The quantity of caffeine was quantified with help of calibration curve it found that Mohini brand contains 274.27 to 276.63 mg per 100gm (Avg. value 275.45mg per 100gm), Krishna gopal ranged from 289.56 to 290.82 mg per 100gm (Avg. value 290.24mg per 100gm), Agni ranged from 386.32 to 388.85 mg per 100gm (Avg. value 387.58mg per 100gm), Red label ranged from 412.42 to 413.98 mg per 100gm (Avg. value 413.2 mg per 100gm), Taj ranged from 488.14 to 489.92 mg per 100gm (Avg. value 489.03mg per 100gm).



(a)



(b)

Figure 3: (a) Overlay spectra of caffeine at 272 nm, (b) Calibration curve of caffeine at 272 nm

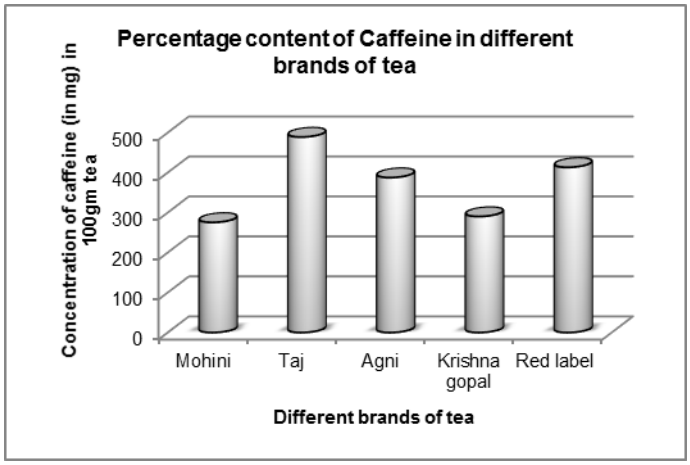


Figure 4

CONCLUSION

The caffeine content found in the five different brands were determined by UV-Visible spectrophotometry. The spectral analysis concluded that the highest amount of caffeine was found in Taj and lowest in local brand, Mohine followed by Krishna gopal. The others doesn't show the remarkable difference in the caffeine content. A similar type results has shown in preliminary phytochemical analysis report for all sample of tea. Although all sample don't give positive test to general alkaloid but in contrast it give the positive test for murexide test which signify the presence of purine alkaloids. This analytical measurement was undertaken primarily to assess the compliance of content levels of the caffeine and their daily intake doses with the permissible levels.

Source of Support: Nil.

Conflict of Interest: None Declared.

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