Gravimetric Estimation of Caffeine in Different Commercial Kinds of Tea Found in the Iraqi Market

Maha N. Hamad*,[1] and Dhuha A. Abdul-Hussain*

***Department of Pharmacognosy, College of Pharmacy, University of Baghdad, Baghdad,Iraq .**

Abstract

Caffeine (1,3,7-trimethylxanthine), which is the most widely consumed stimulant in the world, had been isolated and estimated gravimetrically in fifteen different commercial kinds of tea found in the Iraqi market.The kinds of tea were chosen according to their differences in the degree of fermentation and the method of processing i.e. black , gray and green . The isolated caffeine was identified by melting point, sublimation, TLC, chemical tests, UV , IR , HPLC and CHNO analysis.

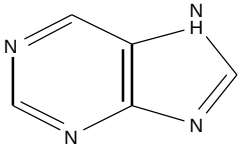
Key words: Caffeine, Purine, tea.

الخلاصة

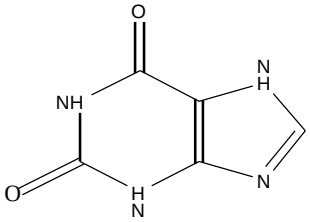
كافايين (1, 3 , 7 –ثلاثي مثيل زانثين) الذي يعتبر من أكثر المواد المنبهة إستعمالاً في العالم, تم فصله وتعيين كميته بطريقة وزنية في خمسة عشر نوعاً مختلفاً من الشاي الموجود في الأسواق المحلية. تم أختيار نماذج من الشاي على درجات مختلفة من التخمير و طرق مختلفة للتحضير اي الاسود والرصاصي والاخضر. تم تشخيص الكافايين المعزول بطرق مختلفة منها قياس درجة الذوبان , التسامي , وكروماتوغرافيا الطبقة الرقيقة , وفحوصات كيمياوية , وطيف الأشعة فوق البنفسجية والأشعة تحت الحمراء والكروماتوغرافيا السائلة ذات الكفاءة العالية وحساب نسب عناصر الكربون ,الهيدروجين,النيتروجين والاوكسجين.

Introduction

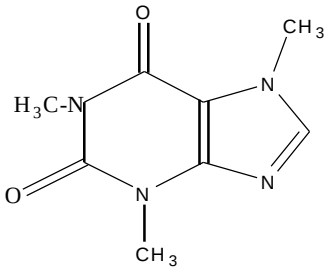
Thea or tea consists of the prepared leaves and leaf buds of *camellia sinensis* (formerly known as *Thea sinensis*) of the Theaceae family. There are three main commercial types of tea: green , oolong (gray) and black, depending on the method of processing. The leaves may be fermented or left unfermented. Fermented teas are referred to black tea, unfermented teas as green tea and partially fermented teas as oolong tea.Black tea is prepared by heaping the fresh leaves until fermentation has begun, then they are rapidly dried artificially with heat, while green tea is prepared by rapidly drying the freshly picked leaves in copper pans over a mild artificial heat, or the leaves are often rolled in the palm of the hand as they dry. Gray tea is partially fermented by heaping then they are dried on artificial heat. Tea contains caffeine (theine) and small amounts of adenine, theobromine, theophylline, gallotannic acid and volatile oil.[1,2]Caffeine (1,3,7-trimethylxanthine), the molecular formula of which is $C_8H_{10}N_4O_2$, is the most widely consumed stimulant in the world can be considered to be constructed from the purine ring system, which is important biologically, being found in nucleic acids and nucleotide and in few organisms as alkaloids.[3]Caffeine was first discovered in tea in 1827, and was named theine , and later it was found in mate , coffee and various other plants and the term theine was then dropped.[4]Purines are considered pseudo alkaloids since they are not derived from an amino acid precursor.[5]



Purine



Xanthine



Caffeine

1Corresponding author E- mail : manoha_1957@yahoo.com
Received : 28/4/2010
Accepted : 8/8/2010

Caffeine acts as a CNS stimulant , mild diuretic, it increases the heart rate and blood pressure and stimulate gastric secretions. It also acts as a natural pesticide that paralyze and kills certain insects feeding on the plants. [6,7] Caffeine with UV can kill some kinds of algae and there are evidences that it enhances mutations in bacteria and viruses and also induce chromosome damage. [8,9,10,11]Caffeine is an ingredient of several dozen proprietary products, for the most part, these combination with acetyl salicylic acid, ascorbic acid, codeine, paracetamol, and other analgesics and antipyretics.Caffeine is found in a number of beverages ingested by people in addition to tea as coffee, and to some extent cocoa. Other , less commonly used sources of caffeine include the plants guarana, and yerba mate' which are sometimes used in the preparation of teas, and recently ,energy drinks. Tea leaves contain 1-4% caffeine, while coffee 1-2% yet a cup of brewed coffee contains about 100-150 mg caffeine while a cup of tea contains 60-75 mg. caffeine is also a common ingredient of soft drinks such as cola. Soft drinks typically contain about 10-50 mg of caffeine per serving . The range of caffeine contents in various beverages is shown table 1

Table 1 : range of caffeine in various beverages

Approximate caffeine content of various beverages	Range of mgs of caffeine
Coffee (5oz. cup)	40-170
Soft drinks (12 oz. can)	10-50
Black tea (one tea bag)	25-110
Oolong tea (one tea bag)	12-55
Green tea (one tea bag)	8-30
Decaffeinized tea(one tea bag)	1-4
Energy drinks (12 0z. can)	75-90

In this paper we have estimated caffeine gravimetrically in fifteen different kind of tea found in the market black, gray and green tea .

Materials and method

- Samples of tea were chosen randomly to represent black, gray and green tea in the form of tea bags or unpacked form.
- All reagents are anhydrous solvents were of analar type and generally used as received from the commercial suppliers.
- Silica gel used in the form or ready made aluminum plates of silica gel GF_{254} , Merck Co.
- UV was run in methanol , IR was run in KBr disk.
- HPLC was done using Knauer/ Germany HPLC.
- Standard caffeine is from Evans Medical Ltd , Liverpool.

Isolation of caffeine

25 gm of tea were boiled with 200ml of water for fifteen minutes in a covered beaker . The extract was filtered through muslin and the mark was re boiled with 120ml of water for five minutes, filtered and the mark over the muslin was washed with 70ml of boiling water, the muslin was then squeezed till exhaustion. The combined extracts were cooled and mixed with 4gm of sodium carbonate with stirring, then transferred to a separatery funnel and partitioned with three successive portions of methylene chloride each of 50ml (i.e. 3X50ml), each time the funnel was inverted back and forth ten times. The methylene chloride layers were combined together and dried over anhydrous sodium sulfate , filtered and evaporated to dryness under vacuum. The obtained caffeine was re crystallized from boiling ethanol, filtered and weighed. The percentages of caffeine was calculated as w/w. The extraction procedure is shown in diagram(1).

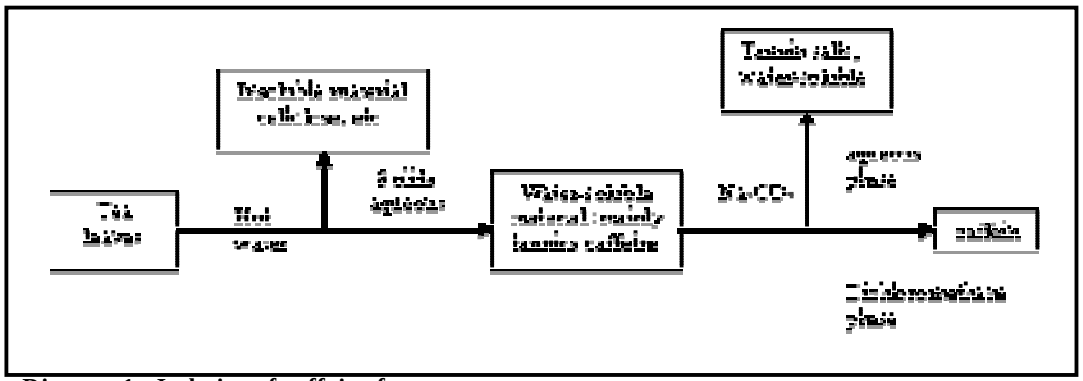


Diagram 1 : Isolation of caffeine from tea

Two samples of each kind were used and the average weights of the isolated caffeine were taken for calculation of the percentages and comparison.

Identification of Caffeine

The isolated caffeine was identified by several methods :

- It was identified by measuring its melting point and compare it with standard caffeine and also using a mixed sample from isolated caffeine and the standard .[12]The results are shown in table (2).

Table 2 : Melting points of isolated and standard caffeine

Sample	Melting point
Isolated caffeine	236.7°C
Standard caffeine	237.3°C
Mixed isolated and Standard caffeine	237°C

Then caffeine was identified by two chemical tests :

1- Murexide test : Isolated caffeine gave a purple color.
2- Isolated caffeine was treated with hydrogen peroxide and 2% HCL. After evaporation to dryness , a bright red color was obtained. The color turn purple upon addition of drops of 5% ammonia. [13]

Also caffeine was identified by sublimation and this process was achieved by introducing a small quantity of caffeine in a porsalen dishand covered with a watch glass , the porsalen dish was subjected to heat while the watch glass was covered with a plastic sack containing ice .Upon heating caffeine started to sublime and condense on the lower surface of the watch glass. Caffeine was also identified by TLC using silica gel GF_{254} plates developed in three different mobile phases and comparing the R_f values of isolated caffeine with standard using single and mixed spots , and detection was done under UV_{254}nm. [14,15,16,17]

Mobile phases used are:

Mobile phase I : Ethyl acetate : acetic acid 95:5 .

Mobile phase II : Chloroform : ethyl acetate : formic acid 5: 4 : 1

Mobile phase III : petroleum ether : methylene chloride : ethyl acetate 1: 1 : 2.

The result of TLC are shown in table (3)

Table 3 : R_f values of isolated and standard caffeine

Mobile phase	Isolated caffeine Rf value	Standard Caffeine Rf values
I	0.226	0.257
II	0.490	0.516
III	0.110	0.110

- The UV absorption spectrum exhibits a pair of absorption bands peaking at (209) and (272)nm with a shoulder between them [18] . The UV spectrum of the isolated caffeine is shown in fig.(1)

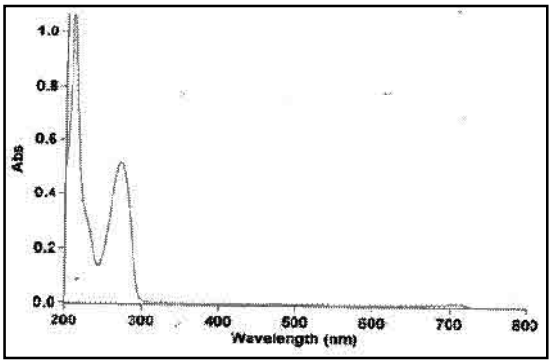


Figure 1 UV spectrum of the isolated caffeine

Also caffeine was identified by IR [19] and the spectrum is shown in fig. (2)

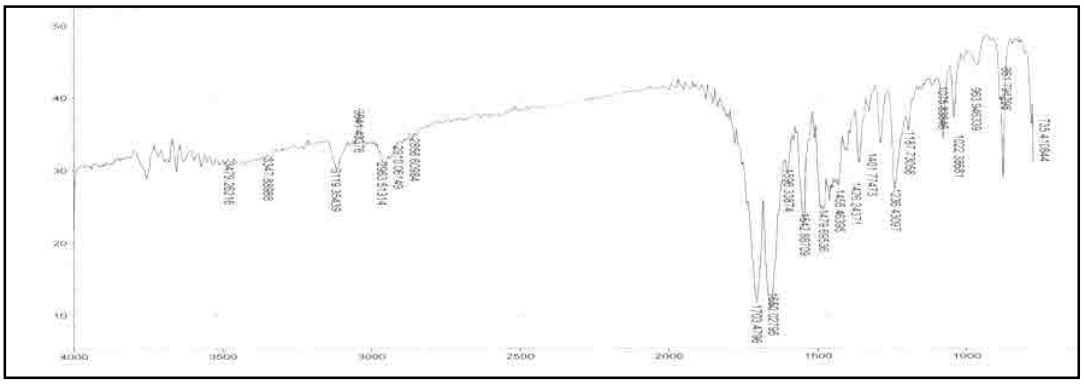


Figure 2 : IR spectrum of the isolated caffeine

Also caffeine was identified by CHNO analysis ,whereby the percentage of each element measured and compared with the calculated one.The results are shown in table 4.

Table 4 : CHNO analysis of caffeine

Element	Calculated percentages	Found percentages
Carbon	49.484	49.877
Hydrogen	5.155	5.199
Nitrogen	28.866	29.109
Oxygen	16.495	16.709

Caffeine was finally identified by HPLC [18] using C_{18} 5x150mm column and a mobile phase composed of methanol/water 90:10 with flow rate of 0.8ml/minute and detection with UV detector at 275nm. The retention time of the isolated caffeine was compared with that of the standard. The results are shown in figures 3 and 4.

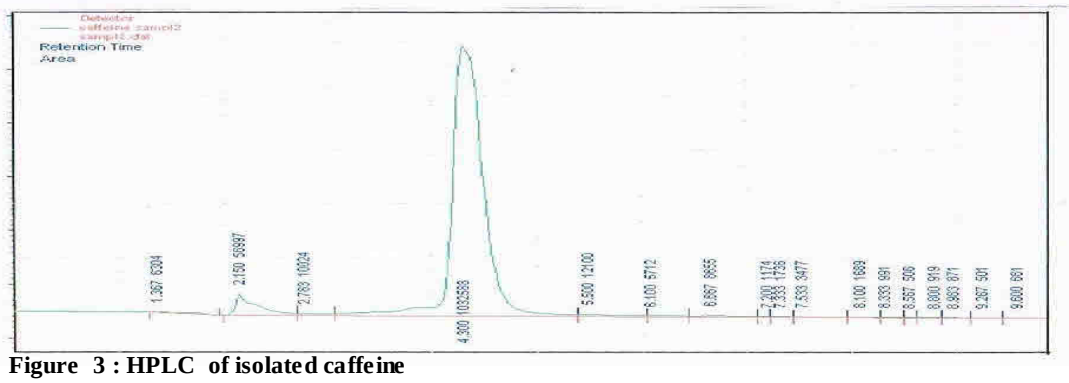


Figure 3 : HPLC of isolated caffeine

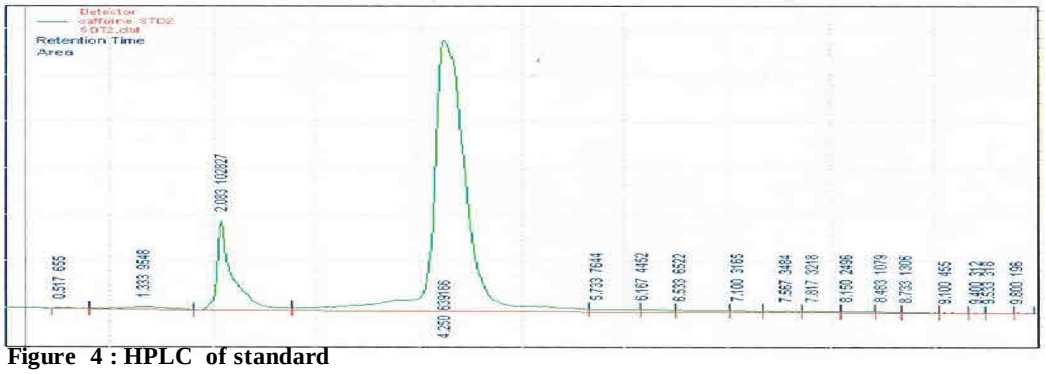


Figure 4 : HPLC of standard

Results and Discussion

The average weights and percentages of the caffeine isolated from each kind of tea are shown in table (5) and fig. (5). The idea of this method of isolation of caffeine is to extract the water soluble materials in the tea leaves in a hot water . (the solubility of caffeine in water is 22 mg/ml at 25°c , 180 mg/ml at 80°c and 760 mg/ml at 100°c) . The caffeine is extracted from the water after cooling with dichloromethane (140 mg/ml) than in water (22 mg/ml), it readily dissolves in the dichloromethane.However, the tannins are slightly soluble in dichloromethane but upon addition of sodium carbonate to the extract the tannins will be converted to phenolic anions (since phenols are acidic enough to be converted to phenolic salts i.e. deprotenation of OH group) upon addition of sodium carbonate). The phenolic salts thus formed are not soluble in dichloromethane , soluble in water, as shown below:

$$\underset{\substack{\text{tannins soluble}\\ \text{in water, dichloromethane}}}{ArOH} + Na^{+}{}_2CO_3^{-2} \longrightarrow \underset{\substack{\text{tannins salts}\\ \text{soluble in water}\\ \text{insoluble in dichloromethane}}}{ArO^{-}Na^{+}} + Na^{+}HCO_3^{-}$$

Table 5 :The percentage of caffeine in different kinds of tea

no	Tea brand	Weight of caffeine In 25 gm of tea	% of caffeine
1	Al-Otoor (black)	0.481	1.924
2	Al-Rabeea (black)	0.351	1.40
3	Mahmood (black)	0.322	1.288
4	Ahmad (black)	0.310	1.240
5	Al-Wazza (black)	0.294	1.176
6	Al-Tuffaha (black)	0.238	0.952
7	Ahmad (black , Tea bags)	0.2	0.800
8	Ration tea (black)	0.180	0.720
9	Lipton (black , tea bags)	0.090	0.360
10	Al-Okozay (gray , tea bags)	0.110	0.440
11	Al-attar (green , tea bags)	0.341	1. 364
12	Lipton (green , tea bags)	0.327	1.308
13	Ahmad (green , tea bags)	0.221	0.884
14	Green tea (un packed)	0.130	0.520
15	Alokozay (green , tea bags)	0.120	0.480

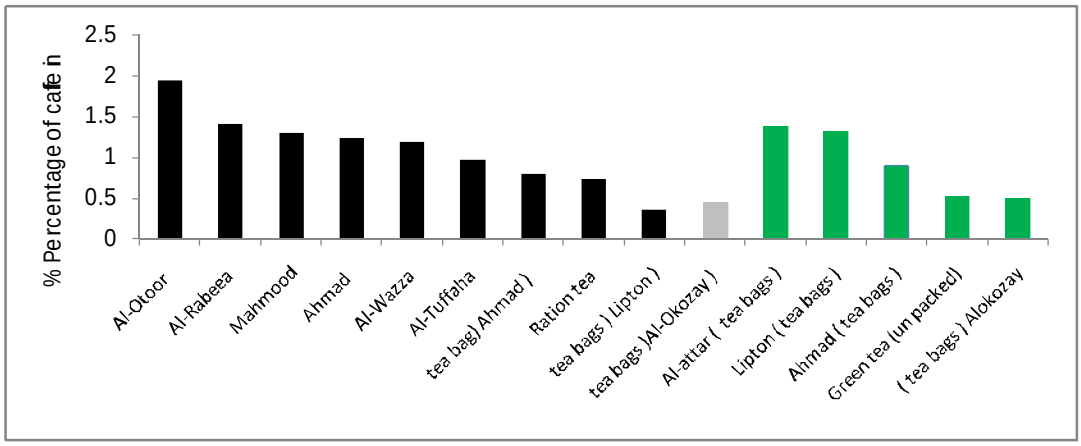


Figure 5 : percentages of caffeine in different kinds of tea

Caffeine being a xanthine derivative was first identified by the murexide test. The core of the reaction is apparently that caffeine through a number of steps , is oxidized into the intermediate that ultimately condenses to murexoin. The ammonium salt of murexoin is the principal contributor to the purple color of the final solution. [20]The quantitative differences obtained in different kind of tea is probably due to the method of processing of each kind since caffeine sublimes with out decomposition upon exposure to heat, therefore it should be expected that caffeine could be lost during fermentation and processing. Also the differences of caffeine quantity and consequently the percentage may be due to different time of harvesting of leaves of the plant.

Acknowledgement

We are deeply greatfull to Mr. Dhulfiqar A. Abid (MSc.) and Mrs. Sahar M. Shakir (MSc.) and Mr. Zaid M. Abdul-Khalik for running the UV , IR , and HPLC spectrum.

References

1. Tyler V.E. , Brady L.E., "Pharmacognosy" 9th edition, Lea and Febiger Philadelphia 1988.
2. Trease G.E. and Evans W.C., "Pharmacognosy" 15th edition , WB Saunders Co. Ltd London 2002.
3. Dey P.M. , Harborne J.B. , "Plant Biochemistry" Academic press 1997.
4. Weinberg BA., Bealer BK., "The world of caffeine" Routledge, Newyork and London 2001.

5. Cordell G.A., "Introduction to alkaloids" John Wiley and Sons , Inc Canada 1981, 6.
6. Jinno, D. , "Comperhensive Medical chemistry" Pergamon Press 1996.
7. Eaton K., "Caffeine could be healpful" Las Vegas Review Journal , 2010, 23.
8. Srivastava B.S., Kumar H.D., Singh H.N. , "The effect of caffeine and light on killing of the blue-green algae *Anabaena doliolum* by UV radiation" Archives of Microbiology 1971, 78(2), 139-144.
9. Siderpoulos A.S., Shankel D.M., "Mechanism of caffeine enhancement of mutations induced by sub lethal UV dosages" J. Bacteriology 1968, 96(1), 198-204.
10. Lytle C.D., "The effect of caffeine on the survival of UV-irradiated *Herpes simplex* Type 1 virus" J. General virology 1974, 24, 381-383.
11. Cremer C., Cremer T., and Gray J. W., "Introduction of chromosome damage by UV light and caffeine" Cytometry 1982, 2(5), 287-290.
12. The Merck index , 8th edition Merck and Co. Inc. Rahway, NJ, USA 1996.
13. Cave' A., "Pharmacognosy , Phytochemistry, medicinal plants" 3rd edition , Intercept Ltd, England 1995, 882-883.
14. Fenske M., "caffeine determination in human saliva and urine by TLC andUV absorption densitometry" Chromatographia 2007, 65(3-4) , 233-238.
15. Pavlik J.W., "The detection of caffeine in commercial products" J. of Chemical education 1973, 50(2), 134.
16. Franciszek B., Sabina A., Urszula H., "Densitometric determination of caffeine in tea beverages after TLC e-separation" Chemia analityczna, 2006, 51(4), 603-611.
17. Stahl, E., "Thin layer chromatography" Springer-Verlag Berlin. Heidlberg N.Y. 1969.
18. Pavlova V., Petrovsk S., "Simultaneous determination of amphetamine, methamphetamine, and caffeine in seized tablets and HPLC" Acta Chromatographica, 2007, 18, 157-164.
19. Cook D., Regnier Z.R., "The infrared spectra of caffeine salts" Canadian Journal of Chemistry, 1967, 45, 2895-2897.
20. Pedersen O., "Pharmaceutical chemical analysis : Methods for identification and limit tests" 2006, 86-88.