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Evaluation of tea and coffee products commercialized in Egypt using HPTLC

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ABSTRACT

Tea and coffee have been consumed for hundreds of years ago for their flavour and stimulating properties. Nowadays, caffeine received an increasing interest in food and pharmaceutical industries due to its CNS stimulant properties, peripheral vasoconstriction, relaxation of the smooth muscle and myocardial stimulation. Caffeine constitutes an average of up to 4% of tea leaf and 1.5% of coffee bean. The aim of this study is to assess the quality of tea and coffee products present in the Egyptian market. Quality assessment was performed based on caffeine levels by using high performance thin layer chromatography (HPTLC). The results showed the quality variation among the products examined of both tea and coffee. With Ahmed tea London English breakfast showing the highest caffeine content (535 mg/ cup) and El Gawhara Green tea showing the lowest (3 mg/ cup). The used method is simple, rapid, reliable and suitable for evaluation of quality of caffeine containing preparations.

Key words: *Camellia sinensis*, *Coffea Arabica*, Caffeine, High Performance Thin Layer Chromatography, Quality variation, Adulteration.

INTRODUCTION

Tea (*Camellia sinensis* family Theaceae) and coffee (*Coffea Arabica* family Rubiaceae) are the most consumed drinks in the world. Consumption of tea is part of people's daily routine, as an everyday drink and as a therapeutic aid in many illnesses (Bhatt *et al*., 2010)

According to the Food and Agriculture Organization of the United Nations statistics (FAO STAT), the average yearly consumption of tea and coffee per capita in Egypt is 0.93 kg and 0.3 kg, respectively (FAO stat, 2015). However, examining the Egyptian population one finds that the percentage of children (under 14 years and most probably not consuming any tea or coffee) is 33% (The World Bank official site, 2015), resulting in a dramatic increase in the average consumption rate of tea and coffee to 1.38 kg and 0.44 kg, respectively. Thus, the estimated

average consumption of tea and coffee extracts is 1.0 and 0.16 L/person/day, respectively, in Egypt.

In this concern, teas could be classified into three major types: non-fermented green tea (produced by drying and steaming the fresh leaves); semifermented oolong tea (produced by partial fermentation of leaves before drying); and fermented black and red (pu-erh) tea (undergo full fermentation before drying and steaming) (Zuo *et al*., 2002).

Obviously worldwide, black tea is the most widely consumed (80%), whereas green tea is drunk throughout Asia; oolong tea is popular in China and Taiwan (Wu and Wei, 2011). Chemically, the main secondary metabolite composition of tea and coffee is polyphenols, alkaloids (caffeine, theophylline, and theobromine), volatile compounds and tannins (Ushir *et al*., 2011). Biologically,tea possesses a ray of medicinal uses such as being antioxidant, lower risk of several types of gastrointestinal and lung cancers (Amantana *et al*, 2002; Kondo *et al*., 2002; Xie *et al*., 1998), antimutagenic, antidiabetic, antibacterial, anti-inflammatory, and hypocholesterolemic qualities (Amantana *et al*, 2002; Kondo *et al*., 2002; Fenf *et al*., 2001; Xie *et al*., 1998). Furthermore, caffeine possesses stimulatory effects (Zuo *et al*., 2002). However, large doses of caffeine, more than 250 mg per day, can lead to a condition known as caffeinism. This phenomenon usually combines caffeine dependency causing unpleasant physical and mental conditions including nervousness, irritability, restlessness, insomnia, headaches, and heart palpitations after caffeine use (Iancu *et al*., 2007).

The increase in the dose up to 500 mg or more results in a state of CNS over-stimulation called caffeine intoxication (American Psychiatric Association, 2013). The symptoms of caffeine intoxication may include restlessness, fidgeting, anxiety, excitement, insomnia, flushing of the face, as well as increased urination, gastrointestinal disturbance, muscle twitching, a rambling flow of thought and speech, irritability. Also, intoxication may include irregular or rapid heartbeat, and psychomotor agitation. In much larger overdoses, mania, depression, lapses in judgment, disorientation, disinhibition, delusions, hallucinations, or psychosis may occur, and rhabdomyolysis (Winston *et al*., 2005; Verkhratsky, 2005).

Extreme overdose can result in death (Holmgren *et al*., 2004; Alstott *et al*., 1973). The LD_{50} of caffeine in humans is dependent on individual sensitivity; but is estimated to be about 150 to 200 milligrams per

kilogram of body mass or roughly 80 to 100 cups of coffee for an average adult (Peters, 1967) .

The aim of the present study is to determine the concentration of caffeine using HPTLC inorder to compare teas and instant types of coffee of different sorts, grades, and producing companies. A total of 16 tea and 13 coffee samples commercialized in Egypt have been analyzed.

MATERIALS and METHODS

Standard solutions:

A solution of caffeine standard was prepared by dissolving 30.0 mg accurately weighed, in 10.0 mL methanol (Merck, Germany) in a volumetric flask. This is the stock solution. It was further diluted for preparing three point calibration curve.

Samples:

Instant coffee, Leaf tea and Teabags, commercially available from three major supermarkets (Fathala, Carrefour and Metro) in Alexandria, Egypt were randomly sampled and used for this study.

Preparation of the coffee and tea solutions:

The coffee solution was prepared by addition of 200.0 ml boiling water to 2.0 g of the sachets in a 250.0 ml conical flask and stirred by a glass rod as directed on the packaging. However, in case of tea it was prepared by 2 methods: the first method (infusion) was prepared by addition of 200.0 ml boiling water to 2.0 g of the leaf tea and teabags and stirred by a glass rod, the second method (decoction) was prepared by addition of 200.0 ml boiling water to 2.0 g of the leaf tea and teabags and stirred by magnetic bar on a hot plate at 100 ^{0}C for 3 min. The solution was then filtered through a cotton wool and the residue was washed with distilled water (3 X 10.0 ml). The tea solution was combined, then cooled to room temperature and finally diluted to 250.0 ml with distilled water.

For extraction of caffeine, coffee and tea solutions were transferred to a separating funnel and rendered alkaline. These were extracted with three successive quantities of 30.0, 20.0 and 10.0 ml of chloroform. The combined chloroformic extracts were washed with 10.0 ml water. The washed chloroformic extracts were transferred to a flask, the chloroform distilled off, dried over anhydrous Na_2SO_4 and the remaining residue dissolved in 10.0 ml methanol in a volumetric flask.

High performance thin layer chromatography:

A Camag HPTLC system equipped with an automatic TLC sampler, TLC scanner 3 and integrated software WINCATS version 1.4.1 was used for the analysis. Chromatography was performed on 20 cm × 10 cm HPTLC plates coated with silica

gel $60F_{254}$ (E. Merck) of 200 µm layer thickness for the quantification of caffeine. Standard and samples were applied to the plates as 5 mm long bands, 5 mm apart by the use of a Camag Linomat (V) sample applicator equipped with a 100 µl microsyringe and an automatic TLC sampler under a flow of Nitrogen gas.

Detection and estimation of caffeine:

The linear ascending development was carried out in a Camag glass twin through chamber (20 cm × 10 cm) previously saturated with 20.0 mL mobile phase [Chloroform: Methanol (9:1 v/v)] at room temperature (25^{o}C). Plates were developed to a distance of 80 mm; the TLC plate was then air dried. Quantitative evaluation of the plate was performed in an absorbance-reflectance mode at λmax = 254 nm, using a slit width 6 × 0.4 mm, data resolution 100 mm Step^{-1}, scanning speed 20 mm s^{-1} and baseline correction was used.

Validation of the method:

Linearity: Standard solutions were prepared. Chromatogram was developed after application of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/mL working standard solution. A three-point calibration was obtained by fitting peak areas to the amount (mg) of the compound by least squares regressions (Figure 1, Table 1). Thus, it is recommended to be used in the linear part only. The correlation coefficient was found to be 0.99985 (Figure 2).

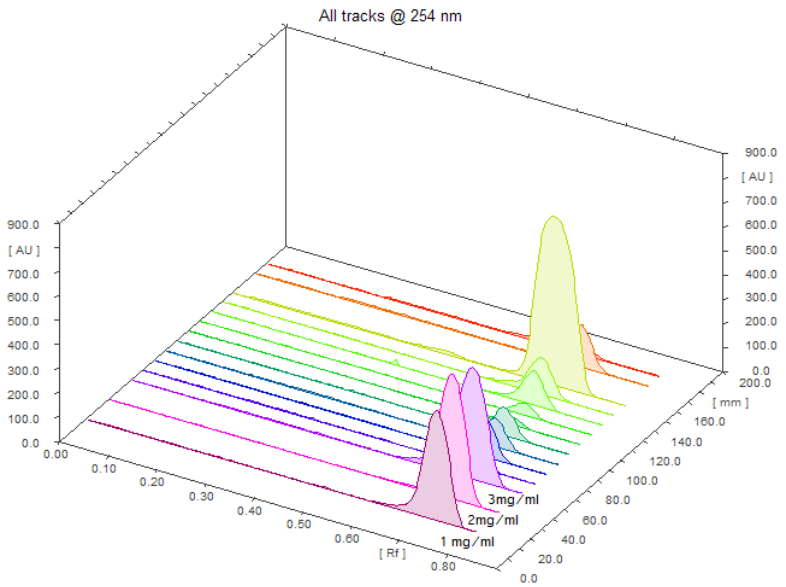

Figure 1: HPTLC chromatogram of caffeine standard solution in different concentrations (20, 40 and 60 mm) followed by samples 1-12.

Table 1: Validation data of caffeine standards obtained from HPTLC chromatogram.

Vial	Rf Value	Peak Height	Peak Area	Caffeine Standard concentration (mg/ml)
1	0.72	450.45	12549.86	1
2	0.71	511.78	14313.59	2
3	0.70	455.87	15974.91	3

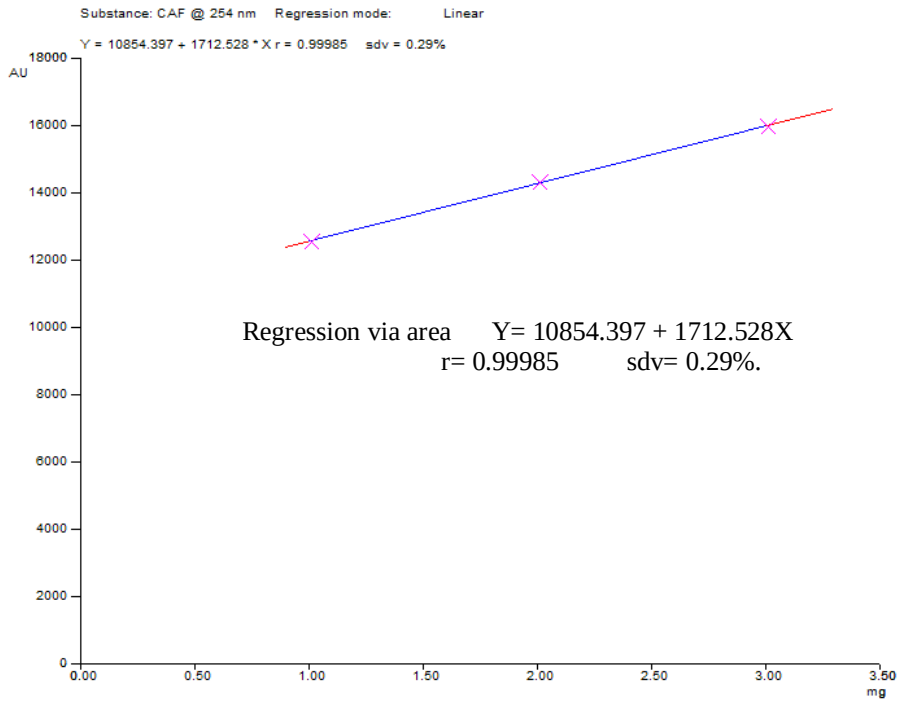

Figure 2: Validation Data for Calibration curves of caffeine peak area obtained from HPTLC.

RESULTS AND DISCUSSION

Results of total caffeine calculated per a cup of tea or coffee in various tea and coffee brands are given in Tables 2 and 3, respectively. The tables show a wide variation in the concentration between the different brands.

Caffeine concentration is higher in case of black teas, showing values between 117 to 535 mg/cup of tea decoction, and 100 to 334 mg/cup of tea infusion whereas green teas ranged from 3 to 158 mg/cup of tea decoction and 2 to 117 mg/cup of tea infusion.

Table 2: Caffeine content per serving of tea

Tea Brands	Caffeine (mg/cup)	Weight (gm)/ serving	Manuf. Date	Expiry date	Notes
Ahmed tea London English breakfast TB (dec)	535	2.2	2/2011	2/2015	-States that it is Ceylon tea and others -Packed in Sri Lanka
Ahmed tea London English breakfast TB (inf)	334	2.2			
Ahmed tea London Earl Grey TB (dec)	182	2	6/2011	6/2014	-States that it is Ceylon tea and others -Packed in Sri Lanka
Ahmed tea London Earl Grey TB (inf)	148	2			
British tea company England TB (dec)	202	2.3	8/2010	8/2013	-States that it is Ceylon tea. -Packed in Egypt.
British tea company England TB (inf)	152	2.3			
El Arosa dust tea (dec)	213	2	1/2011	1/2014	-AEQ 559/2005
El Arosa dust tea (inf)	169	2			
El Arosa dust tea (dec)	213	2	1/2011	1/2014	-AEQ 559/2005
El Arosa dust tea (inf)	169	2			
El Gawhara dust (dec)	117	2			
El Gawhara dust (inf)	100	2			
El Gawhara Green tea (dec)	3	2	3/2011	2/2014	-AEQ 559/2005
El Gawhara Green tea (inf)	2	2			
El Gawhara Golden TB (dec)	259	2	6/2009	6/2012	-AEQ 559/2005
El Gawhara Golden TB (inf)	199	2			
Lipton yellow label granulated tea (dec)	109	2	7/2010	7/2012	-AEQ 559/2005
Lipton yellow label granulated tea (inf)	96	2			
Lipton yellow label dust tea (dec)	403	2	12/2011	12/2013	-AEQ 559/2005
Lipton yellow label dust tea (inf)	252	2			
Lipton Clear Green TB (dec)	28	1.5	7/2011	6/2012	-Packed in the Arab united Emiratis
Lipton Clear Green TB (inf)	23	1.5			
Lipton yellow label TB (dec)	123	2.2			
Lipton yellow label TB (inf)	66	2.2			
Rose tea dust (dec)	440	2	5/2011	5/2014	-States that it is Kenyan tea
Rose tea dust (inf)	279	2			
Sprouting China Green tea (dec)	158	2	6/2011	5/2014	-AEQ 559/2005
Sprouting China Green tea (inf)	87	2			
Temple tea China Green TB (dec)	124	1.8	6/2011	5/2014	-AEQ 559/2005
Temple tea China Green TB (inf)	102	1.8			

Abbreviations: dec= decoction, inf= infusion, TB= teabag, AEQ= according Egyptian quality standards, BN= batch number.

Table 3: Caffeine content per serving of coffee

Coffee Brands	Caffeine (mg/cup)	Weight (gm)/ serving	Manuf. date	Expiry date	Notes
Alicafé Classic	503.8	20	12/2011	12/2013	-Produced in Malaysia -BN: LE04
Alicafé cappuccino with Ginseng	95.9	20	11/2011	11/2013	-Contains ginseng extract -Produced in Malaysia -BN:XB49
Alicafé with essence of Tongkat Ali and Ginseng	280.2	20	11/2011	11/2013	-Contains ginseng and Tongkat extract -Produced in Malaysia -BN: WB01
City café cappuccino	233.3	17	11/2011	5/2013	-Produced in Syria -BN: B27228
Coffee Break cappuccino	216.8	25	11/2011	6/2013	-Produced in Egypt -AEQ 4465/2004
Kenton cappuccino	194.5	12.5	5/2011	11/2012	-Produced in Turkey -BN: 003
Kochane Za Smak Mokate cappuccino	155.9	12.5	6/2011	12/2012	-Produced in Poland
Lafesta cappuccino vanilla	188.2	12.5		7/2012	-Produced in Romania -BN: 40291AD
Misr café	314.1	2.2	10/2011	10/2013	-AEQ 517-1474/2005
Nescafé cappuccino	232.2	12.5	10/2011	10/2012	-Produced in South Korea -BN: 43318296/100550948DW
Nescafé classic	130.9	1.8	11/2011	11/2013	-AEQ 517-1474/2005 -BN: 20350891B
Per'l cappuccino with collagen	128.4	20	9/2011	9/2013	-Produced in Malaysia -BN:WB11
Shaheen cappuccino Hazelnut	306.5	12.5	11/2011	5/2013	

Abbreviations: dec= decoction, inf= infusion, TB= teabag, AEQ= according Egyptian quality standards, BN= batch number.

These results raise a question about El Gawhara Green tea brand, where the very low concentration indicates complete adulteration of the tea. Additionally, the concentration of caffeine in Lipton Clear Green TB brand which indicates a low grade of the tea leaf used.

Examination of red tea results for caffeine shows that Ahmed tea London English breakfast TB is the brand with highest caffeine content, thus one serving of this tea will result in getting the maximum recommended daily intake. Furthermore, the great difference in caffeine concentration between Lipton yellow label dust tea and Lipton yellow label TB (3.3 and 3.8 times more for the dust tea

decoction and infusion, respectively) suggests a partial extraction of caffeine before preparation of the teabags.

For coffee, the results of caffeine concentration show that Alicafé Classic and Alicafé Cappuccino with Ginseng brands possess the highest and lowest concentrations, respectively.

CONCLUSION

Although caffeine is a GRAS drug, overdoses results in CNS over stimulation ranging from restlessness to hallucination or psychosis (Winston *et al*., 2005). Furthermore, extreme overdose could be lethal depending on individual sensitivity and body weight. It is estimated that the LD50 of caffeine in human 150-200 mg/ kg body weight (American Psychiatric Association, 2013; Kerrigan and Lindsey, 2005). Accordingly, for a 70 kg adult 10 gm caffeine could be lethal and this is easily reached by 18 cups of Alicafé Classic coffee or Ahmed tea London English breakfast TB (dec). Consequently, brands that serve very high concentration of caffeine is not an advantage because one could easily reach overdose or even lethal dose. Thus, there should be a governmental regulation in Egypt to force manufacturers of caffeine containing products to state the caffeine content on the packaging.

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تقييم لمنتجات الشاي و القهوة التي تسوق في مصر باستخدام HPTLC

حاتم محمد محمد مكى

يستخدم الشاي والقهوة منذ مئات السنين لمذاقهما و خواصهما المنبهة. و حاليا يتلقى الكافيين اهتماما في الصناعات الغذائية و الدوائية نتيجة خواصه المنبهة للجهاز العصبي و تضيق الأوعية المحيطة و الرخاء العضلات الملساء و تحفيزة لعضلة القلب. يكون الكافيين متوسط 4% من اوراق الشاي و 1.5% من حبوب القهوة. الهدف من هذه الدراسة هو تقيم جودة منتجات الشاي و القهوة في السوق المصري معتمدا على معدلات الكافيين باستخدام HPTLC. اظهرت النتائج ان هناك فروقات في الجودة للانواع الخاضعة للدراسة. فوجد ان شاي أحمد لندن بريكفاست يحتوي على اعلي كم من الكافيين (535 مجم / كوب) و أقَل كمية في شاي الجوهرة (3 مجم / كوب). الطريقة المستخدمة بسيطة و سريعة و يعتمد عليها لتقيم التحضيرات التي تحتوي على كافيين.