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Antioxidant Activity of Aqueous Extracts of Different Caffeine Products

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Abstract

The antioxidant activity of water extracts (cold and hot) of six caffeine products were carried out. The extracts were screened for total polyphenol contents and antioxidant activity using DPPH, ABTS methods and reducing power method at 50 and 100 µg/ml after 15 min and 30 min using DPPH, ABTS BHA and Caffeine as standard compounds. The results indicated that, the hot water extracts for different caffeine products showed higher antioxidant activity than those of cold extracts and this activity was time and concentration dependent. In addition, the activity was higher against ABTS radical more than DPPH and reducing power methods. Also, there is a positive correlation between the antioxidant and reducing compounds presented in water extracts of different caffeine products. The results of HPLC showed that fresh tea leaves are rich in flavanol monomers known as catechins. The most abundant catechin derivatives in green tea are EGC, EGCG and GC. On the other hand EGCG and GC are major catechin derivative in different caffeine product except El-Fakher tea and Cacao. Generally, these beverages had high antioxidant capacities and total phenolic contents, and could be important dietary sources of antioxidant phenolic for prevention of diseases caused by oxidative stress.

Keywords: caffeine product, antioxidant activity, phenolic contents

Abbreviations: DPPH: 2, 2 diphenyl-1-picrylhydrazyl; ABTS: 2,2'- azino-bis (ethylbenzthiazoline-6-sulfonic acid); BHA: butylated hydroxyl anisole

Introduction

Tea is derived from terminal three leaves of shoots of tea plant *Camellia sinensis* (L.) O. Kuntze (syn. *Thea sinensis* L.) family Theaceae. It is the most popular non-alcoholic beverage in the world (Longo *et al.,* 2008). Recent experimental studies have recognized that tea exhibits a significant health protecting activity due to its high polyphenol content (Manzocco *et al.,* 1998). Tea polyphenols are the most significant group of tea components and have a wide range of pharmaceutical properties including antioxidative, anticarcinogenic and antiarteriosclerotic (Atoui *et al.,* 2005; Dufresne and Farnworth, 2001). A number of dissimilar chemical reactions initiated by an enzyme, polyphenol oxidase during fermentation, are of practical value in commercial manufacture of black tea. The primary polyphenols are oxidized during the fermentation process and are transformed to compounds with tanning properties. The oxidized polyphenols in black tea are responsible for briskness, strength, color, taste and pungency of the black tea infusion. These polyphenols remain intact in green tea as steaming of leaves following plucking in green tea manufacture inactivates the enzyme polyphenol oxidase. The natural polyphenols in green tea include (-)-epigallocatechin-3- gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3- gallate (ECG), and epicatechin (EC). The highest concentration is of EGCG followed by EGC, ECG and EC in decreasing order (Bradfield *et al.,* 1948; Nakabayashi, 1991). The pharmacological importance of these catechins decreases in the order of EGCG, EGC, ECG and EC. Other minor catechins, (+)-gallocatechin (GC), (-)-gallocatechin gallate (GCG), (-)-catechin gallate (CG) and (+)-catechin (C) are also present in tea (Yamamoto *et al.*, 1997). The green tea contains 30 to 42% polyphenols on the dry weight basis and a cup of green tea contains about 300 to 400 mg of polyphenols (Balentine *et al.,* 1997). In fact, evidence obtained from archeological investigation has found that tea has been consumed by humans for over 500,000 years (McKenna *et al.,* 2000). Tea is particularly rich in polyphenols, including catechins, theaflavins and thearubigins, which are thought to contribute to the health benefits of tea. Tea polyphenols act as antioxidants in vitro by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. It is well known that free radical induced oxidative stress is an important factor in the etiology of many pathological processes. Much attention has been focused on the antioxidants in food that may have beneficial physiological effects. Polyphenols constitute an important source of dietary antioxidants, being distributed widely in fruits, vegetables, cereals and beverages including red wine, tea, coffee and cocoa (Richelle *et al.,* 2000). Roasting markedly affects the composition of the coffee polyphenols through the Maillard reaction and confers to coffee its pleasant taste and aroma (Richelle *et al.,* 2000). In addition, carbohydrate caramelization and pyrolysis of organic compounds occur (Belitz and Grosch, 1999). However, although natural antioxidants are lost during heating,

the overall antioxidant properties of coffee brews can be maintained or enhanced by the formation of new antioxidants such as the Maillard reaction products (Nicoli *et al.,* 1997a). Tea is found in many different varieties however, the most commonly consumed are black, green and oolong tea. The differences between them are related to the way that the leaves are processed during manufacture. Green tea is unfermented, with the leaves being heated or steamed soon after being picked to prevent the fermentation that takes place with black tea (Segal, 1996). Black tea is simply fermented green tea which is prepared through a method of rolling that releases juices and enzymes from the leaves which begins the fermentation process. Oolong tea is partially fermented green tea, providing a mid-point between green and black tea (Segal, 1996).

Green tea is rich in catechins which are a class of polyphenols with a flavonoid structure (McKenna *et al.,* 2000). Much of the attributed therapeutic benefits of green tea are due to the presence of four major catechins - epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). It is the phenolic structure of the catechins that contribute to their potent antioxidant activity because they are able to bind to metal ions and prevent their participation in peroxidase reactions (Abdel-Rahim and El-Beltagi, 2010; Afify and Shalaby, 2009; Afify *et al.,* 2011; Cooper *et al.,* 2005; Shallan *et al.,* 2010a, 2010b). In addition, they act as free radical scavengers, regenerate α-tocopherol and terminate lipid peroxidation chain reactions (Abdel-Rahim and El-Beltagi, 2011; El-Beltagi and Mohamed, 2010; El-Beltagi *et al.,* 2011; Mohamed *et al*., 2010; Stewart *et al*., 2005). Green tea solids are comprised of approximately 30% - 42% catechins with a typical cup of green tea containing 10-30 mg EGCG (McKenna *et al.,* 2000).

The aim of the current study was to investigate the relationship between antioxidant capacity (Using three different mechanisms) and total phenolic content, reducing compounds and catechin derivative of aqueous extracts of different caffeine products.

Materials and methods

Chemical reagents

All chemicals and reagents were of analytical grade and obtained from either Sigma-Aldrich or Merck Chemical Co. (Darmstadt, Germany).

Sample collection

Gawhara green tea, Ahmed tea, El-Arosa tea, El-Fakher tea, Abd El-Maboed Coffee and Koruna Cacao were collected from traditional market, Giza, Egypt.

Preparation of extract

A known weight of the dried and grinded samples (Caffeine products) was extracted three successive times with hot water (100°C) and cold water (4°C) in dark place (Flask covered with aluminum foil) at room temperature (25°C). The collected extracts were filtered using filter paper (Whatman number 102, 18 cm), Filtrate was evaporated to remove the water solvents using rotary evaporator, and the obtained residues (crude extracts) were kept in refrigerator at (4°C) until use (Rossenthaler, 1930).

Determination of total phenolic content

Phenolic compounds were determined based on a method described by Singleton *et al*. (1999). Briefly, a known weight was extracted with distilled water. 1 ml of distilled water extract was mixed with 1 ml of Folin Ciocalteu reagent. After 3 min, 1 ml of saturated sodium carbonate solution (20%) was added to the mixture and adjusted to 10 ml with distilled H_2O. The reaction mixture was kept in the dark for 1 h with intermittent shaking. The absorbance was measured at 725 nm using a spectrophotometer (Jenway, UV/Visible 1613). Phenolic contents were calculated on the basis of the standard curve for Gallic acid as stander.

Determination of total reducing compounds

Total reducing compounds were determine by titration of tea extracts using iodine as oxidizing agent and starch as indicator and expressed ascorbic acid as recorded by Davies *et al.* (1991).

Assay of antioxidant activities

Antioxidant activity of different extracts was determined using the following methods:

DPPH radical assay

Scavenging effect of 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical was measured by the method of Chou *et al.* (2009). Where, 0.1 ml of 1 mM methanol solution of DPPH was incubated with various concentrations of dry sample crude extract (50 and 100 µg/ml). After 30 and 60 min incubation periods at room temperature, absorbance of the resulting solution was recorded at 517 nm. DPPH radical scavenging activity was expressed as the inhibition percentage and was calculated as (absorbance of control - absorbance of sample/absorbance of control) × 100. Standard butylated hydroxyl anisole (BHA) was used for comparison and the dose response curve of promising extract compared with stander compounds at different concentrations were carried out.

ABTS radical cation scavenging assay

This assay was based on the ability of different substances to scavenge 2,2'- azino-bis (ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\square+}$) radical cation in comparison to a standard (BHA, at 50 and 100 µg/ml). The radical cation was prepared by mixing a 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 4-16 hrs until the reaction was completed and the absorbance was stable. The $ABTS^{\square+}$ solution was diluted with ethanol to an absorbance of 0.700±0.05 at 734 nm for measurements. The photometric Assay was conducted

on 0.9 ml of ABTS$^{\square+}$ solution and 0.1 ml of tested samples (50 and 100 µg/ml in MeOH solution) and mixed for 45 s, measurements were taken at 734 nm after 1 min. The antioxidative activity of the tested samples was calculated by determined the decrease in absorbance at different concentrations by using the following equation:

E= ((Ac-At)/Ac) x 100

where: At and Ac are the respective absorbance of tested samples and ABTS$^{\square+}$ (Re *et al.,* 1999).

Reducing power

The reducing power was determined by the method of Chou *et al*. (2009). The distilled water extracts (0.25, 50 and 100 µg/ml) was mixed with 0.25 ml of sodium phosphate buffer (200 mM, pH 6.6) and 0.25 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. then 0.25 ml of 10% trichloroacetic acid was added to the mixture to stop the reaction, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (0.5 ml) was mixed with 0.4 ml of deionized water and 0.1 ml of 0.1% ferric chloride solution to allow for standing for 10 min. The absorbance was measured at 700 nm. A higher absorbance indicated a higher reducing power, and standard BHA was used for comparison.

HPLC analysis of catechin derivatives

The analytical determinations of phenolic compounds for different caffeine products were carried out using reverse phase-high performance liquid chromatography in isocratic mode. The Waters HPLC system equipped with automated gradient controller, 510 pumps, U6K injector, 481 detector, 746 data module and Waters µ-bondapak C18 column (3.9 x 300 mm), was used for the analysis. Elution was carried out at ambient temperature between 24 to 28°C using water: methanol: acetic acid (70: 30: 0.5) as a mobile phase at a flow rate 1.0 ml/min. All extracts were prepared in triplicate and each extract was analyzed in triplicate. The UV detection was carried out at 280 nm.

Statistical analysis

All experimental results were expressed as means ± S.D. Analysis of variance was performed by ANOVA procedures. The results with P<0.05 were regarded to be statistically significant. Data were statistically analyzed using Co state Statistical Package (Anonymous, 1989).

Results and discussion

Total phenolic content

Six kinds of commercial caffeine products (El-Gawhara green tea, Ahmed tea, El-Arosa tea, El-Fakher tea, Abd El-Maboed Coffee and Koruna Cacao) infusions were obtained from traditional markets in Giza-Cairo, which represent main categories of the infusions made in Egypt. The total phenolic contents of 6 infusions were estimated using the Folin-Ciocalteu method, which relies on the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline medium, and it is a simple and rapid method (Singleton and Rossi, 1965). As shown in Tab. 1, the total phenolic contents varied from 1.68 ± 0.06 in El-Fakher tea to 2.28±0.06 in El-Gawhara green tea, gallic acid equivalent (g GAE)/100g. El-Gawhara green tea and Abd El-Maboed Coffee had the highest total phenolic content (2.28±0.06 and 2.28±0.07 g GAE/ 100 g respectively), El-Fakher tea showed the lowest total phenolic content (1.68±0.06 g GAE/100 g) in cold water tested samples. While in the hot water tested samples, the highest total phenolic content was the green tea and Ahmed tea (2.44±0.03 and 2.38±0.05 g GAE/ 100 g), El-Arosa tea recorded the lowest total phenolic content (1.39±0.04 g GAE/100 g).

Reducing compounds (including all water soluble reducing compounds) percentage in cold water extracts recorded the highest values in Koruna Cacao (28.3%±0.22) and El-Gawhara green tea (25.8%±0.22), while Ahmed tea showed the lowest value (5.28%±0.07). Therefore, the reducing compound percentage in hot water extracts showed the highest values in El-Fakher tea (34.3±0.51) and Koruna cacao (26.4±0.25), while Ahmed tea also showed the lowest value (6.6±0.12).

Antioxidant activities

The antioxidant activity of caffeine products for cold and hot water extracts were measured using *in vitro* assays including the reducing power assay and the assay evaluating the free radical scavenging capacity such as the DPPH and ABTS methods with two concentrations 50 and 100 (µg/ml) (Tab. 2, 3, 4). The results revealed that the antioxidant activity of water extracts were time and concentration dependent. In addition to, the antioxidant

Tab. 1. Total phenolic contents and Percentage of reducing compounds of caffeine products

Solvent	Samples	Total Phenolic contents (g GAE/100g d.w.)	Reducing compounds %
Cold water	El-Gawhara green tea	2.28 ± 0.06	25.8 ± 0.22
	Ahmed tea*	1.75 ± 0.03	5.28 ± 0.07
	El-Arosa tea*	2.14 ± 0.05	19.8 ± 0.15
	El-Fakher tea*	1.68 ± 0.06	23.76 ± 0.14
	Abd El-Maboed coffee	2.28 ± 0.07	16.3 ± 0.12
	Koruna cacao	1.76 ± 0.04	28.3 ± 0.22
Hot water	El-Gawhara green tea	2.44 ± 0.03	11.88 ± 0.17
	Ahmed tea*	2.38 ± 0.05	6.6 ± 0.12
	El-Arosa tea*	1.39 ± 0.04	14.52 ± 0.11
	El-Fakher tea*	1.87 ± 0.05	34.3 ± 0.51
	Abd El-Maboed coffee	1.87 ± 0.07	15.28 ± 0.17
	Koruna cacao	2.12 ± 0.04	26.4 ± 0.25
Standard (BHA)		2.28 ± 0.07	25.8 ± 0.16
Standard (Caffeine)		1.75 ± 0.03	5.28 ± 0.09
LSD 5%		0.086	0.40

* Black tea; Ahmed tea (import tea from England), El-Arosa tea (export tea), El-fakher tea (popular tea)

activities determined by the three methods proved that hot extracts showed high antioxidant activities than cold extracts with different samples (green tea, Ahmed tea El-Arosa tea, El-Fakher tea, Coffee, Cacao) compared with BHA and Caffeine as standard. The antioxidant activities of green tea cold water measured by the two radical scavenging methods (DPPH and ABTS) are much closed to each other (71.2%±0.38, 74.5%±0.48, at 50 (µg/ml) after 15 min) (Tab. 1, 2, 3). On the same time as a general trend hot extracts showed more antioxidant activities than cold extracts measured at concentration of 50 (µg/ml) such as cold green tea (71.2±0.38) against hot green tea (81.3±0.39), cold Ahmed tea (70.5±0.32) against hot Ahmed tea (88.2±0.42). This high level of antioxidant activities in tea is consistent with several other reports of hot water extract of black and green tea (Atoui *et al.,* 2005; Dreosti, 1996).

Ahmed tea and Coffee showed the highest antioxidant activities at 100 µg/ml (81.9%±0.58, 81.8%±0.66) after 30 min in DPPH method with cold water extracts. While with hot extracts; Ahmed tea and Coffee showed the highest antioxidant activates at (100 µg/ml) concentration, (89.7±0.32, 90.5±0.24) after 30 min respectively. The study reveals that the consumption of these spices would exert several beneficial effects by virtue of their antioxidant activity (Nicoli *et al.,* 1997a; b).

Tab. 2. Antioxidant activity (%) of cold and hot extracts of caffeine sources against DPPH method

Solvent	Samples	Antioxidant activity (%) At 50 µg/ml		Antioxidant activity (%) At 100 µg/ml	
		15 min	30 min	15 min	30 min
Cold water	El-Gawhara green tea	71.2 ± 0.38	72.9 ± 0.41	76.1 ± 0.48	79.3 ± 0.42
	Ahmed tea*	70.5 ± 0.32	74.6 ± 0.45	70.6 ± 0.47	81.9 ± 0.58
	El-Arosa tea*	45.1 ± 0.28	53.7 ± 0.37	51.7 ± 0.49	73.2 ± 0.41
	El-Fakher tea*	61 ± 0.23	61.9 ± 0.33	61.9 ± 0.39	75.6 ± 0.39
	Abd El-Maboed coffee	69 ± 0.45	74.5 ± 0.38	68.9 ± 0.34	81.8 ± 0.66
	Koruna cacao	33.9 ± 0.31	45.1 ± 0.50	39.4 ± 0.45	66.6 ± 0.52
Hot water	El-Gawhara green tea	81.3 ± 0.39	87.3 ± 0.62	79.5 ± 0.15	87.3 ± 0.42
	Ahmed tea*	88.2 ± 0.42	88.6 ± 0.58	88.3 ± 0.20	89.7 ± 0.32
	El-Arosa tea*	75.5 ± 0.37	77 ± 0.56	75.8 ± 0.22	84.6 ± 0.29
	El-Fakher tea*	88.3 ± 0.36	89.7 ± 0.64	88.6 ± 0.17	89.5 ± 0.18
	Abd El-Maboed coffee	88.6 ± 0.21	88.6 ± 0.51	88.8 ± 0.26	90.5 ± 0.24
	Koruna cacao	78 ± 0.20	79.16 ± 0.43	77.1 ± 0.22	88.3 ± 0.21
Standard (BHA)		77.4 ± 0.20	80.5 ± 0.58	80.4 ± 0.24	86.9 ± 0.35
Standard (Caffeine)		33.2 ± 0.12	40.7 ± 0.36	36.3 ± 0.15	47.7 ± 0.17
LSD 5%		0.496	0.94	0.35	0.49

* Black tea; Ahmed tea (Imported tea from England), El-Arosa tea (exported tea), El-fakher tea (popular tea)

Tab. 3. Antioxidant activity (%) of cold and hot extracts of caffeine sources against ABTS method

Solvent	Samples	Antioxidant activity (%) At 50 µg/ml		Antioxidant activity (%) At 100 µg/ml	
		15 min	30 min	15 min	30 min
Cold water	El-Gawhara green tea	74.5 ± 0.48	76.5 ± 0.31	80.8 ± 0.41	84.2 ± 0.45
	Ahmed tea*	65.3 ± 0.46	65.6 ± 0.41	77.4 ± 0.34	78.5 ± 0.38
	El-Arosa tea*	50.3 ± 0.31	52.4 ± 0.32	55.0 ± 0.20	72.2 ± 0.39
	El-Fakher tea*	60.5 ± 0.39	59.3 ± 0.32	68.9 ± 0.28	67.8 ± 0.35
	Abd El-Maboed coffee	68.1 ± 0.42	68.0 ± 0.36	78.6 ± 0.38	82.5 ± 0.39
	Koruna cacao	42.1 ± 0.35	45.1 ± 0. 48	50.6 ± 0.39	67.8 ± 0.30
Hot water	El-Gawhara green tea	82.5 ± 0.32	83.6 ± 0.34	88.3 ± 0.34	89.6 ± 0.29
	Ahmed tea*	86.5 ± 0.42	87.5 ± 0.21	85.9 ± 0.40	89.1 ± 0.28
	El-Arosa tea*	80.0 ± 0.49	82.5 ± 0.31	85.6 ± 0.37	84.8 ± 0.38
	El-Fakher tea*	85.3 ± 0.29	91.8 ± 0.53	88.3 ± 0.32	91.3 ± 0.40
	Abd El-Maboed coffee	84.7 ± 0.30	85.6 ± 0.32	87.3 ± 0.35	88.5 ± 0.37
	Koruna cacao	80.2 ± 0.38	82.4 ± 0.30	85.6 ± 0.25	88.9 ± 0.23
Standard (BHA)		80.2 ± 0.39	81.6 ± 0.51	85.9 ± 0.23	88.7 ± 0.19
Standard (Caffeine)		50.1 ± 0.16	50.9 ± 0.21	54.3 ± 0.18	58.6 ± 0.14
LSD 5%		0.59	0.62	0.54	0.52

* Black tea; Ahmed tea (imported tea from England), El-Arosa tea (exported tea), El-fakher tea (popular tea)

The highest antioxidant activities of tea product due to the high content of total phenolic compounds as well as the reducing compounds such as cold green tea, (2.28±0.06; 25.8±0.22); and cold coffee (2.28±0.07, 16.3±0.12).

Reducing power of a compound served as a significant indicator of its potential antioxidant activity. In concentration 50 and 100 (µg/ml), the antioxidant activity of reducing powers of Abd El-Maboed coffee and El-Fakher tea in cold water extract showed the maximum values (62.10±0.19) and (55.80±0.09), while in hot water extracts El-Gawhara green tea and Koruna cacao were the higher values (70.65±0.15) and (62.38±0.15) in the concentration 50 (µg/ml) (Tab. 4). Also in concentration 100 (µg/ml), the antioxidant activity of reducing powers of El-Gawhara green tea and El-Fakher tea recorded the higher antioxidant activities (91.64±0.15) and (90.84±0.14). Specifically, reducing powers of hot water extracts showed good antioxidant activities than those of cold water extracts and contained a lot of phenolic compounds. Hence, it is supposed that those antioxidant activities may be due to high level of total phenolic compounds (Hwang *et al.,* 2010).

Tab. 4. Relative percentage of antioxidant activity of cold and hot water extracts determined by reducing power

Solvent	Samples	Antioxidant activity (%)	
		50 (µg/ml)	100 (µg/ml)
Cold water	El-Gawhara green tea	42.6 ± 0.11	70.0 ± 0.13
	Ahmed tea*	50.89 ± 0.12	72.51 ± 0.17
	El-Arosa tea*	41.2 ± 0.10	71.65 ± 0.16
	El-Fakher tea*	55.80 ± 0.09	82.30 ± 0.37
	Abd El-Maboed coffee	62.10 ± 0.19	88.40 ± 0.34
	Koruna cacao	42.70± 0.02	74.90 ± 0.16
Hot water	El-Gawhara green tea	70.65 ± 0.15	91.64 ± 0.15
	Ahmed tea*	61.45 ± 0.21	89.61 ± 0.11
	El-Arosa tea*	40.0 ± 0.12	70.62 ± 0.09
	El-Fakher tea*	58.6 ± 0.20	90.84 ± 0.14
	Abd El-Maboed coffee	60.84 ± 0.16	89.67 ± 0.10
	Koruna cacao	62.38 ± 0.15	88.54 ± 0.12
Standard (BHA)		40.78 ± 0.09	87.94± 0.13
Standard (Caffeine)		30.87 ± 0.08	40.62 ± 0.07
LSD 5%		0.26	0.20

* Brown tea; Ahmed tea (imported tea from England), El-Arosa tea (exported tea), El-fakher tea (popular tea)

Tab. 5. Correlation coefficient between different antioxidant methods and reducing compounds as well as phenolic compounds

	ABTS	DPPH	R. power	Phenolic	Red. com
ABTS		0.887857	0.662047	0.79073	0.307982
DPPH			0.86345	0.543765	0.371244
R. power				0.386846	0.499476
Phenolic					0.351879
Red. com					

These results proved by studying correlation coefficient between antioxidants methods DPPH, ABTS and reducing power method, phenolic compound and reducing compounds as shown in Tab. 5. The results in Tab. 5 showed that, there are positive correlations between different antioxidant methods and reducing compounds in caffeine extracts. The correlation coefficients among different antioxidant methods and phenolic and reducing compounds are between 0.30 and 0.88. There are good correlation (r= more than 0.5) achieved between ABTS and DPPH methods (r= 0.88); ABTS and phenolic compounds (r= 0.79); DPPH, ABTS and reducing power methods (r= 0.66 and 0.86 respectively) but another trend (weak correlations; r= less than 0.5) were observed in other relations; between ABTS and Reducing compounds (r= 0.30); DPPH and reducing compounds (r= 0.37); Reducing power method and reducing compounds (r= 0.49) and Phenolic compounds and reducing compounds (r= 0.35).

The antioxidant properties of green coffee, which are mainly due to the polyphenols, such as chlorogenic acid or caffeic acid available to act as an antioxidant can be increased (light and medium coffees) or maintained (dark coffee) by formation of new antioxidants, i.e. Millard reaction products during the roasting process. Melanoidins are brown polymers formed by the Maillard reaction during the roasting of coffee beans and account for up to 25% of the dry matter. It has recently been shown by the ABTS method that coffee melanoidins have significant antioxidant activity *in vitro* (Borrelli *et al.,* 2002; Brudzynski and Miotto, 2011). Polyphenols in green tea as well as in different caffeine product possess much more potent antioxidant activity than well-known antioxidants such as vitamin C and vitamin E. The main antioxidant found in green tea called epigallocatechin gallate, or EGCG is at least 100 times more effective than vitamin C and 5 times better than vitamin E at protecting cells (Ahmed *et al.,* 2004) and proposed as a cancer chemopreventative (Saffari and Sadrzadeh, 2004). Because of their high antioxidant activities, it could be speculated that these extracts will be beneficial for the diseases caused by oxidative stress. Although, EGCG has been shown to inhibit tumor growth/invasion by inhibiting the gelatinase activity (Cheng *et al.,* 2003; Garbisa *et al.,* 2001). There are a number of studies documenting the beneficial health effects of green tea consumption. Most of these studies place emphasis on the anti-cancer properties of green tea, which have now been attributed, at least in part, to the ability of green tea polyphenols to inhibit the gelatinases (Cheng *et al.,* 2003; Garbisa *et al.,* 2001). Therefore, it is tempting to suggest that green tea polyphenol EGCG or compounds derived from it may serve as lead agents in the design of more potent and effective inhibitors of collagenases for use therapeutically to block the pathogenesis of arthritis (Ahmed *et al.,* 2004).

The dose response curves of promising sample (Green tea) compared with stander compounds were analyzed

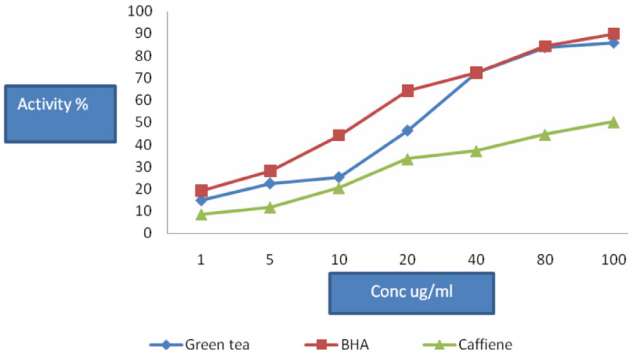

Fig. 1. The dose response curve of promising sample (Green tea) compared with stander compounds

and the results showed in Fig. 1. Revealed that, there is positive correlation between the concentration of sample and antioxidant activity against DPPH radical.

HPLC analysis

HPLC analysis of catechin and catechin derivative of tea and tea product as well as coffee and cacao showed that the major components of catechin derivative are different according to the source (Tab. 6.). The major catechin derivative in green tea are (EGC, EGCG, GC) which represents (28.11, 32.60, 20.42%), while in Ahmed tea, Coffee and El-Arosa tea (EGCG, GC) are represents (30.65, 40.86%), (38.87, 30.23%) and (23.19, 55.4, El-Fakher tea, Cacao (GC) represents (3.8, 2.8, 1.8), respectively. The antioxidant activity of the tea and tea product related the major component of catechin derivative which differ from product to another, on the other hand antioxidant activity in coffee and cacao may be related to the addition browning reaction during processing (Friedman *et al.,* 2005; Kang *et al.,* 2010; Rusak *et al.,* 2008).

Tab. 6. Relative percentage of phenolic compounds separated by HPLC

Sample no.	EGC	EGCG	GC	ECG	+C	EC
green tea	28.11	32.6	20.42	2.31	2.17	14.3
Ahmed tea*	14.0	30.65	40.86	3.83	5.1	5.5
El-Arosa tea*	11.5	23.19	55.4	0.25	2.96	6.60
El-Fakher tea*	15.3	0.0	69.8	1.83	5.69	7.35
Coffee	14.0	38.87	30.23	7.88	5.50	3.45
Cacao	4.25	0.0	95.70	0.0	0.0	0.0

EGC: Epigallocatechine, EGCG: Epigallocatechingallate, GC: Gallocatechin, ECG: Epicatechin gallate, +C: Catechin, EC: Epicatechin. * Black tea; Ahmed tea (imported tea from England), El-Arosa tea (exported tea), El-fakher tea (popular tea)

Conclusions

It can be conclude that, the water extracts of different caffeine products had high antioxidant activity especially green tea when compared with the synthetic and natural stander and this activity was time, compounds and concentration dependent.

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