Quality Control of Certain Slimming Herbal Products Present in the Egyptian Market

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Abstract: Two commercial slimming herbal tea products present in the Egyptian market *viz*; Sekem Herbal Tea (commercial herbal tea-**1**) and Royal Regime Tea (commercial herbal tea-**2**) were quality-evaluated compared to two prepared standard mixtures; prepared standard herbal tea-**1**, composed of mixture of herbs of Sekem Herbal Tea (chicory, marjoram, nettle and senna leaves, liquorices roots, celery fruits and calendula flowers) and prepared standard herbal Tea-**2** composed of mixture of herbs of Royal Regime Tea (fennel, senna and chicory). Quality control of both commercial and prepared herbal teas was conducted through microscopical identification of their diagnostic elements, determination of certain heavy metals and pharmacopeial constants and detection of aflatoxins content and total microbial count. Quality control was also conducted through HPLC quantitative estimation of main active constituents of the commercial and prepared standard herbal teas, where results revealed that as for the percentages of sennoside A in commercial herbal tea-**1** and its standard tea were 58.87 and 56.70, respectively, while its percentage in commercial herbal tea-**2** and its standard tea were 59.30 and 55.17, respectively, as for esculetin percentages in commercial herbal tea-**1** and its standard tea were 0.41 and 0.73, respectively, while its percentages in commercial herbal tea-**2** and its standard tea was the same 0.17 and as for scopoletin percentage in commercial herbal tea-**1** and its standard tea were 0.19 and 0.18, respectively, which all within the reported standard limits. Quality control was also conducted through GC/MS of the volatile oil constituents, the percentage yields of volatile oils, which were obtained by hydrodistillation of both commercial tea-**1** and its corresponding standards tea were 1.8 and 2.0 V/W, respectively, while that of commercial tea-**2** and its corresponding standard tea were 2.0 and 2.2 V/W, respectively. GC-MS analysis revealed that the major oil components of both commercial teas and their corresponding prepared standard teas were nearly the same with slight significant different percentages. Lipid profile tests (cholesterol, triglycerides and total lipids) were carried out in induced hypercholesteremic rats and after eight weeks of oral treatment with aqueous extracts of commercial tea**s-1** and **-2** and their standard teas, showing significant reduction in cholesterol, triglycerides and total lipids plasma levels. Sekem herbal tea decreased the glucose levels by 10.7% in normoglycemic rats after 30 minutes of glucose oral administration and by 8.3% in STZ-induced diabetic rats after 30 days treatment; while it's prepared standard tea caused 5.7 and 4.5% reduction, respectively. Royal Regime Tea decreased the glucose levels in normoglycemic and hyperglycemic rats by 3.0 and 6.0% reduction, respectively; while it's prepared standard tea decreased the blood glucose level by 9.6 and 8.3% in normoglycemic and hyperglycemic rats after 30 minutes and 30 days of treatment, respectively.
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1. Introduction:

The use of medicinal plants for treating diseases is the oldest existing method that humanity has tried to cope with illness, in high-income countries, the widespread use of phytotherapy declined at the end of the first era of the twentieth century, due to the development and production of synthetic medicine, however during the past few decades, the use of phytotherapy started to increase even in industrial countries, while in low- and –middle -income countries, phytotherapy never stopped being important as the only therapeutic system to which certain people could refer (Crellin *et al*., 1989). It is important that the conditions for the correct and appropriate use of phytotherapeutic methods to follow the criteria of safety, efficacy and quality; safety in the meaning of assuring the presence of the least acceptable limits of aflatoxins, pesticides, toxic heavy metals and micro organisms in the drug, efficacy means that the drug must be efficient in the given dose, while quality means evaluating the identity, purity, content, and other chemical, physical and biological properties of the drug (WHO, 2007). A healthy weight is crucial for a long and healthy life, being obese or overweight

increases the risk of heart attack, high blood cholesterol, high blood pressure and diabetes (Grundy, 2004; Haslam and James, 2005; Caballero, 2007; Shoelson *et al.*, 2007). Drinking certain slimming herbal teas prevents fat absorption, expelling it out of the body with wastes and getting rid of water accumulated in the body, thus leading to weight loss (Dewick, 1999). According to the most recent statistics conducted by the World Health Organization, nearly 70 percent of Egyptian adults are overweight (WHO, 2010). This encouraged the authors to evaluate two commercial slimming herbal tea products that are widely used in Egyptian market *viz*; Sekem Herbal Tea and Royal Regime. Though these commercial herbal teas are used as slimming drugs, some of their individual constituents reported to have other pharmacological activities (Evans, 2002; Gulcin *et al.*, 2004; Mimica-Dukic and Popovic, 2007; Meena *et al.*, 2010), thus it deemed interested to the authors to quality evaluate these herbal tea products comparing them with prepared standard teas as well as to investigate their hypocholestermic and hypoglycemic activity.

2. Materials and Methods

Plant material:

Herbal tea material: commercial preparations used were collected from different batches present in the Egyptian market; samples of batch numbers (HS0305/09, HS0334/09 and HS0335/09) of Sekem herbal tea (slimming) with production dates of (2/09, 6/09 and 7/09, respectively) and samples of batch numbers (06020, 06504 and 06681) with production dates (5/09, 6/09 and 7/09 respectively) of royal regime tea. Standard herbal mixture teas were prepared from herbs collected from the Experimental Farm, Faculty of Pharmacy, Cairo University; as for prepared standard herbal tea-**1** composed of 20 gm *Chicorium intybus* L., 15 gm *Urtica dioica* L., 15 gm *Majorana hortensis* L. and, 10 gm *of Cassis angustifolia* L. leaves, 15 gm *Glycyrhizza glabra* L. roots, 15 gm *Apium graveolens* L. fruits and 10 gm *of Calendula officinalis* L. flowers, as for prepared standard herbal tea-**2** composed of 50 gm *Foeniculum vulgare* L., 30 gm *Cassia angustifolia* L. and 20 gm of *Chicorium intybus* L.

Animals

Adult male albino rats (*Rattus norvegicus*) weighing from 22 – 28 g obtained from the Animal Houses, Faculty of Veterinary Medicine, Alexandria University, Egypt, were used for antihyperlipedemic, hypoglycemic and antidiabetic activity.

Microorganisms:

The bacterial strains*; Bacillus cereus, B. polymexa B. sphaericus, B. subtilus, Micrococcus* spp and *Staphylococcus epidermidis* and the fungi; *Aspergillus candidus; A. niger, A. versicolor, Fusarum equiseti, F. oxysporum; Mucor pusillus* and *Penicillum* spp., were all supplied through Department of Microbiology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, were used for microbial count measurement.

Solvents and chemicals:

Acetonitrile, methanol, phosphoric acid, acetic acid formic acid (analytical and HPLC grade), anhydrous CH_3COONa, $MgSO_4$, and Na_2SO_4 were all supplied through E. Merck, Darmstadt, Germany. Aflatoxins B_1, B_2, G_1 and G_2 were supplied from, the Central Laboratory of Residue Analysis of Pesticides and Heavy Metals at Food, Agriculture Research Center Dokki, Giza, Egypt. Kits for lipid profile test; commercial kits for serum total cholesterol, triglycerides and total lipids were supplied from Diamond Diagnostics Co., Cairo, Egypt.

Authentic reference Materials:

Sennoside A, esculetin and scopoletin, were purchased from Sigma Chemical Co. St. Louis, Mo, USA. The authentic samples for pesticide analysis were supplied through Central Laboratory of Residues Analysis of Pesticides and Heavy Metals from Food – Agriculture Research Center, Giza, Egypt.

LC/MS/MS for determination of pesticide residue:

Agilent Technologies 7890 A, Triplet Quadrupole MS Agilent Technologies 7000 B; column Phenomenex 50 mm column Aqua C-18 with internal diameter: 0.32 mm and film thickness 5 µm, Flow rate; 0.2 ml/min, Mobile phase; Solvent A: H_2O / 0.1 formic acid, Solvent B: acetonitrile / 0.1% formic acid with Mass spectrometer Elecrospray ionization, Multiple Reaction and monitoring (MRM) mode was used according to scheme of pesticide analysis of Chen *et al*. (2011) as follows; fifty grams of each tea sample under investigation was comminuted in a disintegrator for 1 min., 5 g was transferred into 50 ml centrifugal tube having 10 ml H_2O + 10 ml acetonitrile (containing 1% acetic acid), vortexed for 3 minutes, set for 1 h, 5 g anhydrous CH_3COONa + 4 g anhydrous $MgSO_4$ were added, vortexed for 1 min., the tubs were immediately cooled in ice bath for 5 min., centrifuged for 5 minutes at 5000 rpm, samples were then subjected to SPE clean – up [(SPE column was conditioned with 10 ml acetonitrile/ toluene 3:1 (containing 1% acetic acid)], 1 ml of the extract was subjected to CC eluted with 20 ml acetonitrile/toluene 3:1 (containing 1% acetic acid). The effluent was concentrated to 1 ml by evaporating with weak N_2 stream at 40°C the residue was reconstituted in 1ml acetonitrile (1%acetic acid), filtered with 0.2 acetonitrile (1%acetic acid) and with 0.2 µm organic filter and then injected into LC/MS/MS for analysis.

Preparation of the samples for HPLC analysis:

One hundred and fifty grams from each of commercial herbal tea-**1** and **-2**, and their

corresponding prepared standard teas were extracted using Soxhlet apparatus with ethanol till exhaustion, evaporated to dryness under reduced pressure (40°). Seven mg of each residue was dissolved separately in 1 ml of methanol and 10 µl of each prepared solution was subjected to HPLC.

HPLC for quantitative determination of active constituents:

HPLC Agilent 1100 series, Quaternary pump, equipped with a Hypersil 100 RP-18 column (5µm, 250 × 4 mm), flow rate 1.5 ml/min, samples injector (20 ml) and UV photodiode array detector was used for quantitative determination esculetin, scopoletin and sennoside A. Isocratic elution at room temperature using methanol: water 35: 65 V/V (adjusted to pH 3.5 with phosphoric acid) as mobile phase, and UV detection at 320 nm for esculetin. Isocratic elution at room temperature using methanol: water 15: 85 V/V (phosphoric acid was added till pH 2) as a mobile phase and UV detection at 330 nm for scopoletin. Isocratic elution at room temperature using methanol: 2% acetic acid 70:30 V/V as mobile phase and detection at 254 nm for sennoside A. Standard calibration curves were established using different concentrations of authentic esculetin, scopoletin and sennoside A (Hayashi *et al.,* 1980).

GPC/HPLC for quantitative detection of aflatoxins:

Aflatoxins were detected after extraction of the herbal samples with dichloromethane:water (10:1) by clean up gel-permeation chromatography (GPC) using a column packed with Bio-beads S-X3 and dichloromethane : hexane (3:1) as eluent for clean-up of extracts prior to separation and quantification of aflatoxins by HPLC. The eluent fraction containing the aflatoxins is concentrated by evaporation under reduced pressure and the aflatoxins separated by Agilent 1100 HPLC on an ODS reverse phase column (C-18, 4.6 mm × 150 mm, 3.5µm) with fluorescent UV detector (365 nm), isocratic elution at room temperature using deionized water: methanol: acetonitrile (50:40:10) as mobile phase with a flow rate of 0.8 ml/min and injection volume 10 µl (Hetmanski and Scudaamore, 1989).

Preparation of volatile oils:

Volatile oils of herbal teas were prepared by hydrodistillation using Clevenger apparatus (Egyptian Pharmacopeia, 2005). The obtained oils were dried over anhydrous sodium sulfate and stored at -4°C till analyzed by GC/MS.

GC/MS for volatile oils analysis:

Analysis was performed on a Shimadzu GC/MS QP2010 USA, samples were injected into an XTI5 MS column (0.25 µm, 20 m × 0.25 mm), oven temperature: 60-260°C, program rate; 2°C/min., injector port temperature: 250°C, detector; FID/250°C, carrier gas: Helium, flow rate; 6 ml/min., linear velocity: 44.7 cm/sec.

Determination of microbial contaminants:

A total of eighteen samples were tested; nine samples of commercial herbal tea-**1** (three samples of each of the three product batches) and nine samples of commercial herbal tea-**2** (three samples of each of the three product batches). One g of each sample was mixed with 9 ml sterile peptone water and then 10 fold serial dilutions were made. Five level-spacing 1 logarithmic unit were investigated by pipetting 1 ml from each level in a plate, 15 ml of nutrient (agar for bacterial count, Sabouraud dextrose agar for fungal count and Mackonky agar for pathogenic coliform count), were added. The contents were allowed to solidified and inverted plates were incubated at 37°C, examined after 2 days for both bacterial and coliform count and after 7 days for fungal count and suitable dilution were counted (Baker and Breach, 1980).

Hypocholesterermic activity:

Thirty male rats were fed with a diet supplied with 1% cholesterol and beef fat for 4 weeks to become hypercholesteremic. The hypercholesteremic rats were then randomly allocated into 5 equal groups; control hypercholesteremic group, second and third groups receiving aqueous extracts of commercial herbal tea-**1** and its prepared standard tea, respectively, fourth and fifth groups receiving aqueous extracts of commercial herbal tea-**2** and its prepared standard tea, respectively. Basal blood samples were taken before treatment by puncturing the inner canthus of the eye under light ether anesthesia and after overnight fasting. Blood from each rat was left to clot, then centrifuged for about 10 minutes at 300 rpm to obtain a clear serum, and stored frozen at -20°C until assayed for total cholesterol, triglycerides and total lipids using specific kits (Saravanan *et al*., 2007).

Hypoglycemic and antidiabetic activity:

Oral glucose tolerance test (OGTT) in hypoglycemic rats

The oral glucose tolerance test was performed on normoglycemic five groups of rats fasted for 15 hrs. The normoglycemic rats were then randomly allocated into 5 equal groups; control group receiving saline, second and third groups receiving aqueous extracts of commercial herbal tea-**1** and its prepared standard tea, respectively, fourth and fifth groups receiving aqueous extracts of commercial herbal tea-**2** and its prepared standard tea, respectively. Thirty minutes later, glucose (1.25 g/kg body weight) was orally administered to each rat. Blood samples were obtained from each rat by sinocular puncture under light ether anesthesia at -30 (half hour before drug administration), 0 time (just before glucose administration, 30, 60, 90, 120 and 150 min. for

determination of glucose according to commercial kits (Waynforth and Flecknell, 1992).

Induction of diabetes

Rats were fasted overnight before inducing diabetes with Strerptozotocin (STZ). The rats were given an intraperitoneal injection of STZ, freshly dissolved in saline at a dose of 65 mg/kg body weight, so as to induce diabetic state with blood glucose levels > 200 mg/ml. At this dose of STZ (120 mg/kg body weight) given, diabetic induction was 90% with a mortality rate of 20%. Blood glucose was monitored after STZ treatment to confirm the diabetic state and the diabetic rats were included in the experiment ten days after STZ treatment. Diabetic rats were divided into five groups; control group diabetic group, second and third groups receiving aqueous extracts of commercial herbal tea-**1** and its prepared standard tea, respectively, fourth and fifth groups receiving aqueous extracts of commercial herbal tea-**2** and its prepared standard tea, respectively. Animals were continued with the administration for 60 days and were used to assess the effect of different treatments on the change of blood glucose at 0, 15, 30 and 60 days (Waynforth and Flecknell, 1992).

Statistical analysis

All the data were presented as mean ± standard deviation. Differences between treated groups were analyzed for statistical significance by Student's test. Differences were regarded significant at $p<0.05$ level of significance. The statistical analyses were performed with the software Graph Pad Prism, version 3 for windows, Graph Pad Software (San Diego, CA, USA).

3. Results and Discussion

The microscopical examination of commercial herbal teas-**1** and -**2** compared to their corresponding prepared standard herbal teas (Herbal tea-**1** and -**2**) and compared to reported data (Jackson and Snowdon, 1990), confirmed the presence of diagnostic elements of their constituents among which are; the branched anastomosing laticeferous vessels with dark contents and the non-glandular multicellular hair of chicory; the unicellular stellate hair, fragments of crystal layers of CaOx clusters, large cystoliths hair containing dense granular masses of $CaCO_3$ and glandular hair with uniseriate stalk and biseriate head of nettle; the non-glandular unicellular warty cuticle hair, crystal sheath (lignified fibers with thick wall, wide lumen and tapering ends surrounded by parenchyma cells containing prisms of Ca Ox crystals) and clusters of CaOx crystals of senna; the bordered pitted large lignified xylem vessels, cork cells of polygonal shape and thick straight anticlinal wall, starch granules (simple, oval and rounded with no striations) and crystal sheath of liquorice; the bulbous papillae with an adherent pollen grain, glandular hair with biseriate ovoid head and biseriate stalk, non-glandular hair conical biseriate multicellular hair, fragments of fibrous layer of anthers of slightly thickened walls associated with small elongated sclerenchyma with slightly thickened walls of large pits and spiny pollen grains of calendula; the nonglandular multicellular uniseriate hair with warty cuticle, glandular capitate hair (one-celled ovoid head and one-celled short stalk), glandular labiaceous hair (eight radiating celled-head and one celled-stalk) of marjoram; the cylindrical long vittae with brown content; lignified fibro–vascular tissue of small thin-walled fibers and vessels with spiral and annular thickening, epicarp cells with narrow tangentially elongated thin walled-cells arranged in groups parallel to one another oriented in one direction (non-parquetry structure) of endocarp of celery; the brown simple (nonbranched) vitti and reticulate parenchyma of fennel. The microscopical examination confirmed the absence of any substitutions or adulteration (Figure 1).

Determination of certain heavy metals and certain pharmacopeial constants

Because metal contaminants cause chronic toxicity problems as; lead causes anorexia, constipation, severe abdominal cramps, peripheral neuritis, encephalopathy, renal dysfunction, anemia, mild jaundice, psychological disorder and olegospermia; cadmium causes renal injury, liver damage, anemia yellow stained teeth and distributes calcium metabolism (osteomalacia; decrease bone density), hypercalcinuria and renal stones (De Smet et al., 1992), thus the level of certain heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr) and arsenic (As) were determined adopting atomic absorption method (Ghaedi *et al.*, 2008). The results in Table 1 showed that the concentrations of heavy metals in both commercial teas were found to be within maximum tolerable limits (WHO guidelines, 2007).

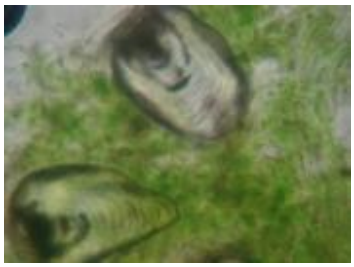
Certain specific parameters and values must be followed during the quantitative and qualitative estimations of any herbal drug either in single or in a mixture form, among which are total ash, acid insoluble ash and water soluble ash. Adopting the guidelines of WHO (2007), for determination of ash constants for both commercial teas and their corresponding prepared standard herbal teas, as well as for each herb constituting the herbal teas separately, results obtained (Table 2) showed that the values of the total ash, acid insoluble ash and water soluble ash were around the recorded values in the published data (British Pharmacopoeia, 1999; Egyptian Pharmacopoeia, 2005), indicating that there is no adulteration or substitution in these plant constituents.

Laticeferous vessels of Chichorium X = 280

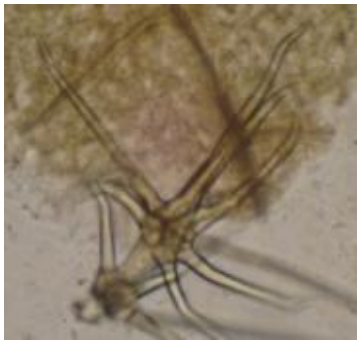
nonglandular hair of Chichorium X = 400

Glandular hair of Nettle X = 340

Fragment of crystal layer of Ca.Ox clusters of Nettle X = 140

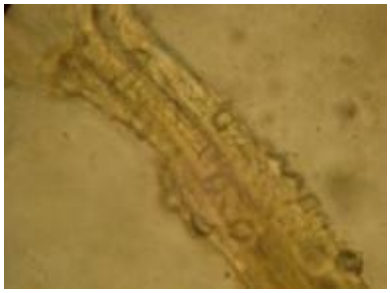


Cystolysith hair of Nettle X = 280



Stellate hair of Nettle X = 140

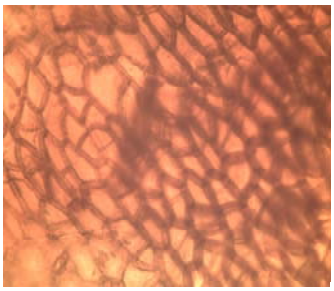
Warty cuticle hair of Senna X = 140



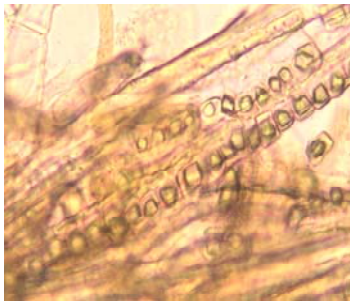
Crystal sheath of Senna X = 250

Clusters of Ca.Ox crystals of Senna X = 280

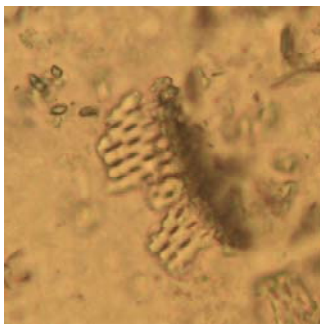
Fig. (1): Dignostic elements of the different herbal teas



Cork cells of Liqurice X = 250

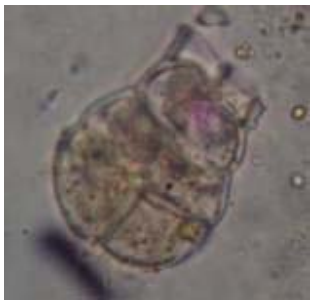


Crystal sheath of Liqurice X = 250



Pitted xylem vessels of Liquorice X = 100

Spiny pollen grains of Calendula X = 420

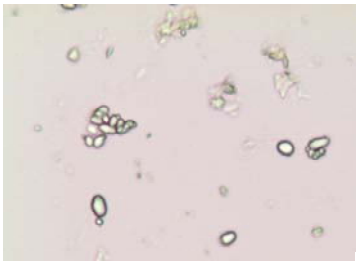


Glandular hair of Calendula X = 340

Nonglandular hair of Calendula X = 340

Fibrous layer of anther of Calendula X = 420

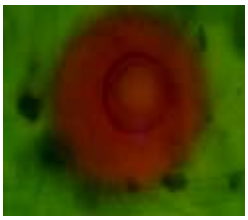
Bibrous papillae of Calendula X = 250



Starch granules of Liqurice X = 100

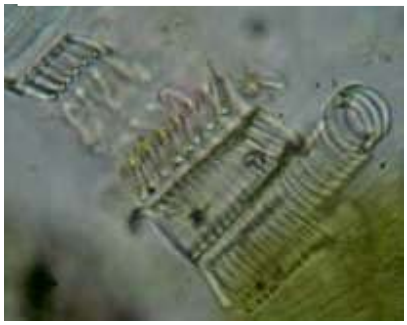
(cont.) **Fig. (1):** Dignostic elements of the different herbal teas

Non-parquetory endocarp of Cellery X = 280

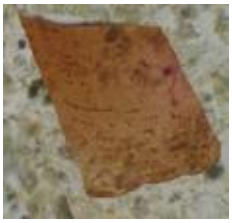


Labiaceous hair of Marjoram X = 280

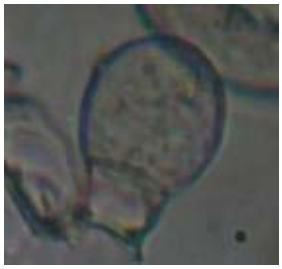
Nonglandular hair with warty cuticle of Majoram X = 140



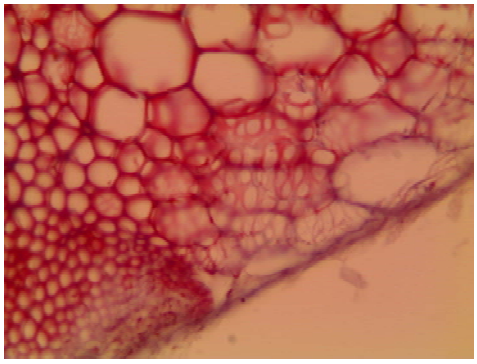
Spiral and annularxylem vessel of Cellery X = 340



Vittea of Cellery X = 140



Capitate hair of Majoram X = 340



Reticulate parenchyma of Fennel X = 280

Vittea of Fennel X = 140

(cont.) **Fig. (1):** Dignostic elements of the different herbal teas

However in case of celery, total and acid insoluble ash pointed to 13.91% and 3.07% respectively, which are higher than that reported in B.P. (should be not more than 10% and 2%, respectively), this difference may be attributed to cultivation of the plant in different habitat. Statistically there is no significant difference between the parameters of commercial and their corresponding prepared standard teas confirming that the commercial teas are not adulterated.

Determination of pesticide residue

Pesticides residues detected in samples of commercial herbal tea-1 were propamocarb, metalaxyl and ortho- phenyl phenol (OPP), while malathion, chlorpyrifos profenofos and OPP were detected in commercial herbal tea-2 in concentrations within limits (MRLs database, 2011) (Table 3). Pesticide residues detected are may be found as a result of environmental contamination such as air, soil and water according to guidelines of pesticide residue contamination, (IFOAM EU Group, 2012).

Table (1): Concentration (mg/kg) of heavy metals in commercial herbal teas-**1** and **-2**

Sample	Cr	Cd	Hg	Pb	As
Commercial herbal tea-**1** (Sekem herbal tea)	1.10	0.064	0.08	0.17	0.15
Commercial herbal tea-**2** (Royal regime tea)	-	0.032	0.05	0.12	0.04
WHO standard limits	2.00	0.300	0.50	10.00	4.00

Cr, Chromium; Cd, Cadmium; Hg, Mercury; Pb, lead; Ar, Arsenic

Table (2): Pharmacopeial constants of commercial and prepared standard teas together with the individual powdered herbs

Herbal ingredient or Herbal tea	Total ash		Water-soluble ash		Acid-insoluble ash	
	Weight (g)	% W/W	Weight (g)	% W/W	Weight (g)	% W/W
Calendula flower	0.2311	9.75	0.1106	4.67	0.0256	1.12
Celery fruit	0.4613	13.91	0.1105	3.33	0.0862	3.07
Chicory leaf	0.2424	8.46	0.1194	4.17	0.0230	0.92
Fennel fruit	0.1893	8.41	0.0539	2.40	0.0082	0.28
Liquorice root	0.1610	6.4	0.0562	2.23	0.0160	0.61
Marjoram leaf	0.3199	12.4	0.0271	1.05	0.0782	3.17
Nettle leaf	0.7574	27.1	0.1569	5.62	0.0454	1.88
Senna leaf	0.2880	9.36	0.0562	1.83	0.0274	0.99
Commercial herbal tea-1	0.4447	16.47	0.1568	5.81	0.0214	0.85
Standard herbal tea-1	0.4533	16.60	0.1625	6	0.0232	0.93
Commercial herbal tea-2	0.3455	12.08	0.906	3.17	0.0180	0.71
Standard herbal tea-2	0.3426	11.8	0.0859	2.96	0.0180	0.6

Each figure represents mean of three determinations

Table (3): Concentrations of pesticide residues in commercial herbal teas compared to database

Pesticide	**Concentration (mg/kg)**		
	Herbal tea-1	**Herbal tea-2**	**MRLs**
Propamocarb (carbamate fungicide)	0.19	0.00	0.20
Metalaxyl (phenylamide fungicide)	0.03	0.00	0.10
O-Phenyl Phenol (phenolic biocide & fungicide)	0.02	0.02	0.10
Malathion (organophosphate)	0.00	0.06	0.50
Chlorpyrifos (chlorinated organo thiophosphate)	0.00	0.01	0.10
Profenofos (organothiophosphate)	0.00	0.02	0.02

Detection of aflatoxins

Among the large variety of mycotoxins, were aflatoxins, which are considered as the most dangerous. Aflatoxins are mutagenic and they produce hepatocarcinoma even when given in very low doses for laboratory animals. Expired and non-expired samples from both commercial hebal tea-1 and -2 were investigated for detection of B-series aflatoxins (coumarin nucleus fused to a bifuran unit in addition to a pentenone ring) and of G series (six-membered lactone ring) compared to standards B_1, B_2, G_1 and G_2, which achieved separation peaks in less than 5.5 min (Barbas and Dams, 2005), while none of the herbal samples showed any corresponding peaks. Thus no aflatoxins were detected in any of the samples under investigation confirming the safety of these drugs even if had been taken by mistake after their expiry date.

Microbial contaminants:

Results of microbial contents of both commercial herbal tea-1 and -2 as shown in Table 4, revealed that the samples were considerably different, according to WHO (2012) the aerobic bacteria in dried and instant products should not exceed 10^3

cfu/g, thus the examined samples could be roughly categorized according to their initial bacterial counts into samples of low microbial content showing bacterial counts from 0 - 10^2 cfu/g, which was found in 10 samples representing 55.6 % of the total 18 samples, moderate microbial content showing bacterial counts from 1^2 - 1^3 cfu/g, which was found in 8 samples representing 44.4 % of the total samples and high microbial content of bacterial counts more than 1^3 cfu/g, which was not found in any of the tested samples. The obtained results were within the limits of reported data (Alexander, 1978) stating that the microbial loads in medicinal plants are usually due to contamination with dust from soil which is considered the main habitat of bacteria and fungi. Yeast was detected in two samples only representing 11.1% of the total samples. The high incidence of Bacillus spp. is in agreement with that reported data (Baxter and Holzapfel, 1982), the domination of Aspergillus spp. in all examined samples is in harmony with reported data (El-Zawahry et al., 1991). Coliform, which is a Gram-ve Bacilli was not detected in any of the investigated samples. In conclusion both commercial teas were slightly contaminated with fungi and bacteria that are non pathogenic organisms (Roy and Chourasia, 1990).

Table (4): Microbial contents of different batches of commercial herbal tea-**1** and -**2**

Herbal tea/ batch No.	Sample No.	Count (cfu/gm)	Bacterial species	Count (cfu/gm)	Mould species
Herbal tea-1 HS0305/09	1	1.0 × 10	*B. polymexa*	0	-
	2	1.0 × 10	*Micrococcus* spp.	1.0 ×10	*M. pusillus*
	3	1.0 × 10	*Micrococcus* spp.	0	-
Herbal tea-1 HS0334/09	4	2.0 × 10	*B. subtilus*	0	-
	5	0	-	1.0×10^2	*F. oxysporum*
	6	0	-	0	-
Herbal tea-1 HS0335/09	7	1.0×10^3	*S. epidermidis*	0	-
	8	0	-	2.0×10^2	*A. candidus*
	9	3.0 × 10	*B. subtilus*	0	-
Herbal tea-2 06020	10	2.0 × 10	*B. subtilus*	0	-
	11	2.0 × 10	*B. subtilus*	1.0 ×10	*A. niger*
	12	4.0 × 10	*S. epidermidis*	0	-
Herbal tea-2 06504	13	1.0 × 10	*B. sphaericus*	0	-
	14	1.0 × 10	*S. epidermidis*	0	-
	15	1.0 × 10	*B. subtilus*	0	-
Herbal tea-2 06681	16	1.0×10^2	*B. subtilus*	1.0 ×10	*A. versicolor*
	17	1.0 × 10	*S. epidermidis*	0	-
	18	3.0 × 10	*B. cereus*	1.0×10^3	*F. equiseti*

A., Aspergillus; B., Bacillus; F, Fusarium; S., Staphylococcus

Analysis of volatile oils

The percentage yields of each oil components of both commercial herbal teas and their corresponding prepared standard herbal teas were presented in Table 5, the percentage yields were determined according to the Egyptian pharmacopoeia (2005) and oil components were identified by comparing their mass spectra with the published data (Adams, 2004). The percentage yield of volatile oil of commercial herbal tea-1 and its prepared standard were 1.8 and 2 % V/W respectively. Comparing oil constituents of commercial herbal tea-1 and 2 with their corresponding prepared standard herbal tea-1 and -2, it was found that the major oil components were nearly the same but with different percentages. Difference in the volatile oil composition to some extent is governed by climatic and drying conditions as well as the production process (Halim et al., 1990; Okoh et al., 2008; Verma et al., 2010; Bishr et al., 2012).

HPLC analysis of active constituents:

HPLC was carried out for quantitative estimation of the main active constituents of the plant components which do not contain volatile oil in commercial herbal teas-1 and -2 and their corresponding prepared standards. Sennoside A, esculetin, and scopoletin were used for standardization of senna, chicory and nettle, respectively. HPLC chromatograms of commercial herbal tea-1 and -2 as well as their corresponding prepared standard teas were compared to HPLC chromatograms of the authentic standards sennoside A and esculetin for their equivalent retention time peaks, while scopoletin was only determined for commercial herbal tea -1 and its corresponding prepared herbal tea -1 (Table 6). Results showed that the concentrations of the measured active constituents calculated from the standard curves of sennoside A of senna, esculetin of chicory and scopoletin of nettle (Figs 2, 3 and 4, respectively) were within the reported limits (British

Pharmacopeia, 1990), showing no significant differences, confirming that the herbs used in the commercial teas are not exhausted.

Table (5): The major oil components present in both commercial herbal teas and their prepared standard herbal teas identified by GC/MS

Retention time/min	Base peak m/z	Oil component	Percentage in Herbal tea-1	Percentage in prepared standard Herbal tea-1	Percentage in Herbal tea-2	Percentage in prepared standard Herbal tea-2
7.22	93.00	α - Terpinene	1.19	3.29	-	-
7.70	68.00	Limonene	48.23	2.17	2.72	1.92
8.81	93.00	γ - Terpinene	1.80	5.64	-	-
10.07	93.00	Terpinolene	0.33	1.70	-	-
10.20	81.00	Fenchone	-	-	2.68	1.84
14.64	71.00	Terpin -4-ol	6.85	23.87	-	-
15.44	59.00	α - Terpineol	0.88	7.75	-	-
15.79	148.00	Methyl chavicol	0.56	9.05	65.06	59.04
20.34	135.00	Carvacrol	0.82	25.04	-	-
21.10	148.00	E- anethole	-	-	28.29	31.89
27.99	41.00	Z- Caryophyllene	0.89	1.13	-	-
31.92	41.00	Aromadendrene	2.71	0.79	-	-

Table (6): HPLC analysis of both commercial herbal teas and prepared standard herbal teas

Retention time/min	Active constituent	Percentage in Herbal tea-1	Percentage in prepared standard Herbal tea-1	Percentage in Herbal tea-2	Percentage in prepared standard Herbal tea-2
1.15	Sennoside A	58.87	56.70	59.30	55.17
5.28	Esculetin	0.41	0.73	0.17	0.17
27. 10	Scopoletin	0.19	0.18	-	-

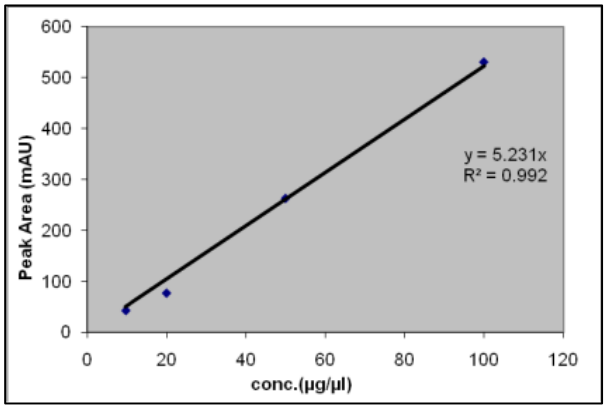


Fig. (2): Standard curve of sennoside A

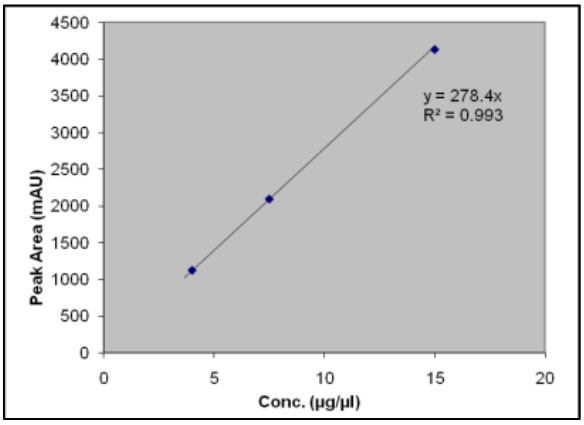


Fig. (3): Standard curve of Esculetin

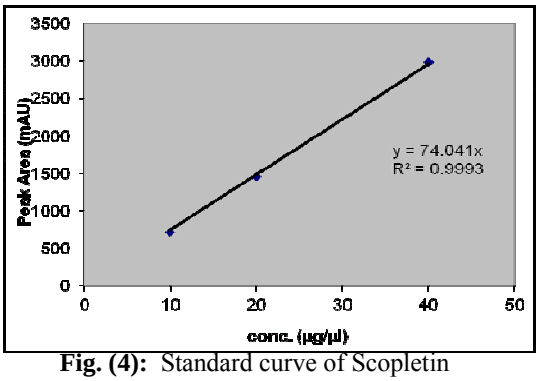


Fig. (4): Standard curve of Scopletin

Biological activity

Hypocholesterermic activity

Results of hypolipidemic activity of both commercial and standard herbal teas-1 and -2 in experimentally induced hypercholesteremic rats (Saravanan *et al.*, 2006) showed that administration of both commercial and standard herbal tea-1 (400 mg/kg) and commercial and standard herbal tea-2 (360 mg/kg) for 8 weeks had significantly reduced total cholesterol, triglycerides and total lipids (Tables 7, 8 and 9). The prepared standard teas showed slightly more effect on lipid profile tests in

hypercholesteremic rats than commercial teas. Standard herbal tea-1 showed more decrease in cholesterol and triglyceride levels 16.36% and 5.91%, respectively than the commercial tea after 8 weeks. While the commercial herbal tea-1 showed more decrease in total lipids by 8.14% than the standard tea after 8 weeks. Standard herbal tea-2 showed more decrease in cholesterol, triglycerides and total lipids by 4.24%, 3.97% and 6.29%, respectively than the commercial tea after 8 weeks.

Hypoglycemic and antidiabetic activity:

The effect of commercial herbal teas-1 and -2 their corresponding prepared standard teas on fasting glucose levels in normoglycemic rats (Table 10) showed no statistical difference in the initial basal glycemic levels between the studied groups. The plasma glucose levels gradually decreased after one hour of oral administration of the aqueous extracts of the herbal formulations. The commercial herbal tea-1 decreased the blood glucose by 10.7% level from 30 to 60 minutes after glucose administration, while the prepared standard herbal tea-1 decreased the level by 5.74%. The commercial herbal tea-2 decreased the blood glucose level by 3.01% while the prepared standard herbal tea-2 decreased the blood glucose level by 9.63% from 30 to 60 minutes after glucose administration in normoglycemic rats. The minimum value of plasma glucose was reached 2.5 hours after treatment. The hypoglycemic effect of the teas under study was statistically significant from 1 to 2.5 hours.

In STZ-induced diabetic rats, plasma glucose levels were 3.1 times higher than those in normal rats. Oral administration of the tea decoctions by diabetic rats showed significant reductions in the plasma glucose levels after 30 days of treatment. The maximum effect was observed after 60 days of treatment by all the tested tea preparations (Table 11). Chicory leaves contain inulin which regulates lipid/glucose metabolism and reported to have antihyperglycemic and antihyperlipidemic effects (Kaur *et al.*, 1989). Celery leaves is reported to have antihperlipidemic and diuretic effect, regulating body fluids and helps getting rid of excess fluid out of the body (Tsi *et al.*, 1996). Liquorice roots are reported to decrease the blood glucose levels in diabetic rats and decreases total body fat, by preventing the accumulation of excessive total body fat and visceral fat (Tominaga *et al.*, 2009). Nettle leaves possess antihyperglycemic effect and is reported to decrease body weight (Wagner *et al.*, 1989).

Table (7): Effect of the herbal teas on the total cholesterol level in hypercholesteremic rats (n=6)

Treatment	Duration of treatment (weeks)		
	0	4th week	8th week
	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)
Control	132.40 ± 2.71	129.20±4.43	127.80±4.95
Commercial herbal tea-**1** (400 mg/kg)	142.40 ± 7.14	126.40 ± 7.28	122.80 ± 5.12
Standard herbal tea-**1** (400 mg/kg)	138.80 ± 4.33	110.00 ± 6.97	97.00 ± 2.98
Commercial Herbal tea-**2** (360 mg/kg)	135.00 ± 7.29	132.20 ± 2.78	103.80 ± 5.47
Standard herbal tea-**2** (360 mg/kg)	133.80 ± 4.77	114.80 ± 3.51	97.20 ± 3.47

Table (8): Effect of the herbal teas on the triglycerides (mg/dl) level in hypercholesteremic rats (n=6)

Treatment	Duration of treatment (weeks)		
	0	4th week	8th week
	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)
Control	87.80±1.98	86.60±2.73	90.60±2.87
Commercial herbal tea-**1** (400 mg/kg)	88.20 ± 2.22	86.00 ± 3.05	81.40 ± 3.98
Standard herbal tea-**1** (400 mg/kg)	86.60 ± 3.53	81.80 ± 2.08	74.80 ± 1.66
Commercial Herbal tea-**2** (360 mg/kg)	89.40 ± 2.23	84.20 ± 1.59	81.40 ± 1.81
Standard herbal tea-**2** (360 mg/kg)	89.80 ± 2.29	83.60 ± 2.04	78.20 ± 2.22

Table (9): Effect of the herbal teas on the triglycerides (mg/dl) level in hypercholesteremic rats (n=6)

Treatment	Duration of treatment (weeks)		
	0	4th week	8th week
	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)
Control	492.60 ± 10.88	502.00 ± 7.94	528.40±26.80
Commercial herbal tea-**1** (400 mg/kg)	497.80 ± 13.47	443.60 ± 20.04	389.00 ± 9.58
Standard herbal tea-**1** (400 mg/kg)	466.20 ± 20.68	429.80 ± 17.54	402.20 ± 8.75
Commercial Herbal tea-**2** (360 mg/kg)	491.60 ± 17.26	432.60 ± 18.15	390.60 ± 7.30
Standard herbal tea-**2** (360 mg/kg)	488.80 ± 15.43	382.60 ± 16.19	357.60 ± 15.84

Table (10): Effect of the herbal teas on fasting blood glucose levels (mg/dl) in normoglycemic rats (n=6)

Treatment	Time (min.)						
	-30	0	30	60	90	120	150
	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)
Control	63.50±1.73	66.17±2.09	148.00±2.31	150.33±2.09	148.00±3.57	144.67±3.70	137.67±3.76
% Change		4.2	123.7	1.6	-1.5	-2.3	-4.8
Commercial herbal tea-**1** (400 mg/kg)	65.17±2.88	66.67±2.03	138.33±2.08	123.50±3.73	110.67±4.42	107.50±6.06	105.50±3.81
% Change		2.3	107.5	-10.7	-10.4	-2.9	-1.9
Standard herbal tea-**1** (400 mg/kg)	67.17±1.40	68.50±1.48	145.00±1.98	136.67±3.76	126.33±3.77	119.00±5.77	116.00±6.32
% Change		2.0	111.7	-5.7	-7.6	-5.8	-2.5
Commercial herbal tea-**2** (360 mg/kg)	66.50±3.97	68.33±2.99	144.17±2.66	139.83±2.80	132.33±3.61	130.33±3.42	121.67±5.51
% Change		2.8	111.0	-3.0	-5.4	-1.8	-6.4
Standard herbal tea-**2** (360 mg/kg)	66.17±2.06	67.00±1.55	135.00±2.41	122.00±2.98	111.00±4.07	98.67±3.27	96.33±2.40
% Change		1.3	101.5	-9.6	-9.0	-11.1	-2.4

Table (11): Effect of the herbal teas on blood glucose levels (mg/dl) in diabetic rats (n=6)

Treatment	Days of treatment		
	0	30	60
	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)	Mean ± SE (mg/dl)
Control	208.33±13.46	226.83±5.11	221.67±4.66
% Change		8.9	-2.3
Commercial herbal tea-**1** (400 mg/kg)	225.83±4.13	207.17±3.24	191.67±3.29
% Change		-8.3	-7.5
Standard herbal tea-**1** (400 mg/kg)	228.67±4.99	218.33±4.40	202.50±2.19
% Change		-4.5	-7.3
Commercial herbal tea-**2** (360 mg/kg)	223.33±4.74	210.00±7.18	200.33±12.59
% Change		-6.0	-4.6
Standard herbal tea-**2** (360 mg/kg)	224.83±4.35	206.17±4.56	189.83±3.81
% Change		-8.3	-7.9

Conclusion

Both commercial teas have nearly the same biological activities, although they contain different constituents and in different percentages as commercial herbal tea-2 contains the same amount of chicory and three times the amount of senna present in commercial herbal tea-1, on the other hand commercial herbal tea-1 contains nettle, majoram, liquorice celery and calendula, while commercial herbal tea-2 contains fennel. Thus though the investigated commercial products are indicated for reducing body weight, this study revealed their value as hypocholestermic and hypoglycemic drugs.

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