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Quality Control of Certain Herbal Products and Their Individual Components Used for Digestive Tract Disorders and Their Proposed Mechanism

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ANBS and GA-Gel-H designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SMK and MLA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess the quality of fifteen plants in three herbal tea products (1,2 and 3) used in the Egyptian market for the alleviation of digestive tract disorders, namely; constipation, diarrhea and flatulence, respectively and evaluate antioxidant activity of the volatile oils of some individual components of herbal teas.
Study Design: The presence of pesticides, heavy metals, microbial contaminant was documented. Moreover, some pharmacopoeial constants were calculated. In addition, the chemical compositions of their essential oils were determined and their antioxidant activities were assessed.

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Place and Duration of Study: Department of Pharmacognosy Faculty of Pharmacy, Misr University for Science and Technology, Agricultural Pesticide Committee (APC) and Micro Analytical Center, Cairo University between February 2012 and July 2013.
Methodology: Analysis of the pesticides and the essential oils were performed using GC/FID and GC/MS and determination of heavy metals was carried out using wet digestion method. Microbial contaminants were detected by serial dilution method and the pharmacopoeial constants was evaluated according to their official methods. The antioxidant activity was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging capacity assay.
Results: Out of thirteen organochlorine pesticides residues were studied p,p-DDT is present in all the samples (0.0003- 0.05 mg/kg). All the tested products were regarded as safe based on the WHO measurements. However, all the herbal teas exceeded the accepted limits of fungal (yeast) and pathogenic bacteria (*E. coli*) by the FIP. The total concentrations of aflatoxins in the three samples were 1.25, 0.52 and 3.43 µg/kg respectively. In addition, results indicated to the absence of cadmium and lead. Based on the GC analyses, altogether 112 volatile components were identified in the essential oils of nine plants belonging to Apiaceae, Asteraceae and Lamiaceae. Regarding to families Apiaceae, Asteraceae and Lamiaceae the major constituents were Anethole 93.52%, α-Bisabolol oxide B 69.55% and 1-terpinen-4-ol 33.99% respectively. The antioxidant activity confirmed that anise and dill oils showed the highest antioxidant activity as evidenced by their IC_{50} being 4.218 and 4.930 mg/ml, respectively. While, marjoram and fennel oils exhibited moderate activity displaying IC_{50} values of 12.158 and 14.413 mg/ml, respectively.
Conclusion: All the three herbal teas were considerably safe for human consumption as recommended by the different pharmacopeias. Moreover, it was proposed that their antioxidant activity might be the core of their efficacy being effective in the maintenance of the integrity and function of the gastrointestinal tract in addition to prohibition of free radicals that predispose inflammation.

Keywords: Quality control; herbal products; Egyptian market; digestive tract disorders.

1. INTRODUCTION

Herbal remedies and phytochemicals constitute the major key players of nearly all the health care systems and are still acting as the mainstay of all the available therapies, especially in the developing countries. This may be related to their better cultural acceptability and compatibility with the human body in addition to their ease of availability, low cost, efficacy, safety and less side effects [1]. Nowadays, they become of an enormous popularity and importance even among the developed countries attributing to their marvelous biological, pharmacological and chemotherapeutic activity [2].

National regulation and registration of herbal medicines varies from country to country. Where herbal medicines are regulated, they are categorized either as prescription medicines or non-prescription medicines. Within a country, a group of herbal products categorized other than as medicines may coexist. Herbal products categorized other than as medicines and foods, are becoming increasingly popular and there is potential for adverse events due to lack of regulation, weak quality control systems and loose distribution channels [3] and that is why it is mportant to begin our study.

In ensuring the quality and safety of herbal medicines, the national authorities in many Member States, as well as, other stakeholders in the provision of herbal medicines are probable to encounter numerous challenges, including the setting of standards for quality, their adoption, monitoring and enforcement. The national policy and regulations that are likely to be developed using these WHO guidelines, should also take into account all local and other special needs.

In the absence of relevant and appropriate national standards, there is a risk that these herbal medicines may be lost to traditional users and become unavailable to new users for many reasons. These reasons include: their failing to meet various trade, registration, import and export requirements; loss of confidence in these products due to the presence of real or perceived health risks; and increased reporting of adverse events involving the use of these herbal medicines [3].

Gastrointestinal tract (GIT) disorders are among the most prevalent diseases spreading worldwide between individuals of varying ages in different countries. It is worthy to mention that healthy GIT is crucial for normal digestion and absorption of nutrient that is critical for regular body growth.

Moreover, it acts as a barrier against harmful undesirable materials, also it presents the major site of biotransformation and excretion [4].

Diarrhea, constipation as well as flatulence are the most popular gastrointestinal tract complains. Constipation refers to the delay in the flow of feces through the large intestine with a consequent passage of dry, hard stool resulting in discomfort or pain. In addition of being annoying and uncomfortable, fecal impaction can be life-threatening producing pronounced circulatory, cardiac as well as respiratory problems that if not early detected, may progress resulting in death [5]. However, diarrhea is an increase in stool liquidity and frequency during the day that if persists longer than two months, it can be considered chronic diarrhea can be life-threatening causing many serious problems including dehydration [6]. While flatulence refers to the unpleasant abdominal fullness or distention with a concomitant passage of excessive amounts of intestinal gas that could be embarrassing [7].

The administration of different teas containing senna, chamomile, fennel, dill, coriander, anise as well as liquorice is common for the alleviation of constipation. Moreover, herbal preparation with achillea, verbascum, hibiscus, chicory and vine leaves was proved to be effective in the treatment of diarrhea. While, natural preparations comprising marjoram, caraway, anise, dill, chamomile, fennel and liquorice were very efficient as antispasmodic agents for the treatment of gastrointestinal tract [8-10].

With the ever-increasing consumption of herbals worldwide and their rapid expansion in the global market, safety, efficacy and quality of herbal preparations have become a major target for all health authorities. Safety assures the existence of the least acceptable limits of toxins' especially aflatoxins, toxic heavy metals, pesticides as well as microorganisms in the preparation. Efficacy ensures that the product is effective at the given dose. However, assessing the quality of the preparation deals with confirming the identity, purity, content, and biological activity of the drug [11].

In this treatise, the evaluation of three commercial herbal products that are commonly used in Egyptian market for the alleviation of digestive tract disorders was done through investigation of their microscopical properties, detection of their chemical and microbial contaminants, aflatoxins, together with the determination of certain pharmacopoeial constants, analysis of the volatile oils as well as assessing the antioxidant activity of their individual constituents.

2. MATERIALS AND METHODS

2.1 Plant Material

Herbal tea products: three commercial preparations for digestive tract disorder collected from the Egyptian market. Herbal tea-1: 100 g composed of 45 g senna leaves (*Cassia acutifolia* L. and *Cassia angustifolia* family *Fabaceae*), 10 g chamomile flower heads (*Matricaria chamomilla* L. family Asteraceae), 7 g Dill fruits (*Anethum graveolens* L. family Apiaceae), 17 g liquorice roots (*Glycyrrhiza glabra* L. family Fabaceae), 7 g fennel fruits (*Foeniculum vulgare* L. family Apiaceae), 7 g coriander fruits (*Coriandrum sativum* L. family Apiaceae),and 7 g anise fruits fruits (*Pimpinella anisum* L. family Apiaceae) and is employed as a laxative tea. Herbal tea-2: 100 g comprises of 30 g achillea leaves (*Achillea millefolium* L. family Asteraceae), 10 g verbascum flowers (*Verbascum thapus* L. family *Scrophulariaceae*), 10 g hibiscus calyx and epicalyx *(Hibiscus sabdariffa* L. family Malvaceae), 15 g chamomile flower heads (*Matricaria chamomilla* L. family Asteraceae), 5 g chicory leaves (*Chicorium intybus* L. family Astreaceae), 15 g basil leaves (*Ocimum basilicum* L. family Lamiaceae) and 15 g vine leaves (*Vitis vinifera*, family Vitaceae) and acts as anti-diarrheal agent. Herbal tea-3: Each 100 g consists of 5 g marjoram leaves (*Origanum marjorana* L. family Lamiaceae), 40 g chamomile flower heads (*Matricaria chamomilla* L. family Asteraceae), 15 g liquorice roots (*Glycyrrhiza glabra* L. family Fabaceae), and 10 g caraway fruits (*Carum carvi* L. family Apiaceae) , 10 g anise fruits (*Pimpinella anisum* L. family Apiaceae), 10 g fennel fruits fruits (*Foeniculum vulgare* L. family Apiaceae) and 10 g dill fruits (*Anethum graveolens* L. family Apiaceae), and is used to calm the gastrointestinal tract. Manufacturing date of herbal tea-1,2 and 3 is May 2011, july 2011 and November 2010 respectively. Individual plant material: fifteen crude plant materials received from the manufacturing company.

2.2 Microorganisms

Aerobic Bacteria (*Bacillus subtilis and Staphylococcus aureus*), Yeast [12] and Pathogenic Bacteria (*E. coli* and *Salmonella* species) were all supplied by Agricultural

Pesticide Committee (APC), Ministry of Agriculture, Dokki, Giza, Egypt for microbial count measurements.

2.3 Microscopical Examination of Herbal Teas

Samples for microscopical examination are prepared as follows: 2 g of the powdered drug were cleared and disintegrated through digestion with 5% potassium hydroxide in a water bath until the more resistant cells can be teased out of the more or less completely disintegrated parenchyma. Drugs containing much lignified elements are disintegrated after soaking in H_2SO_4/HNO_3 at room temperature. Microscopical examination for each herbal tea was carried out and compared to their individual components in order to detect any adulteration, substitution or absence of any component [13].

2.4 GC for Determination of Pesticide Residue

The plant extracts were prepared by immersion of 2 g of each of the dry samples in 100 ml of recently boiled distilled water for 5 minutes. Extraction and clean-up of samples were done following the official methods for analysis [14].

Analysis of the pesticides was performed using GC equipped with electron capture detector on two capillary columns, HP-5 and DB-35. Nitrogen was used as a carrier gas at a flow rate of 1 ml/min. The temperatures of injector and interface were 250°C and 300°C, respectively. The temperature program for GC was as follow; initial temperature was 100°C for 1 min, raised at rate of 25°C/min to 170°C, isothermal for 1 min, raised at a rate of 3°C to 230°C, then isothermal for 1 min, finally raised at a rate of 8°C, then isothermal for 5 min. The Codex quality assurance criteria were followed to determine the performance of the multi-residue method. Recoveries and limit of quantitation (LOQ) were determined on samples at spiking levels. The average recoveries ranged between 81% and 104% and quantitation limits between 0.003 and 0.043 mg/kg. Repeated analysis of old samples was regularly carried out to control reproducibility [15].

2.5 HPLC for Quantitative Determination of Aflatoxins

2.5.1 Sample preparation

25 g of each sample were blended with 5 g sodium chloride and 100 ml methanol: water (80:20) using a high speed blender jar for one minute, then filtered through fluted filter paper. 10 ml of the filtrate were diluted with 40 ml deionized distilled water, and then filtered through glass microfiber filter into a glass syringe barrel using marks on barrel to measure 4 ml. Pass 8 ml of filtered diluted extract (8ml = 0.4 gm sample equivalent) were completely passed through Afla Test®-P affinity column at a rate of 1-2 drops/second. 10 ml of deionized distilled water were passed through the column at a rate of 2 drops/second; the affinity column was eluted by passing one ml HPLC grade methanol through column at a rate of 1-2 drops/second then collected in a glass vial and, evaporated till dryness under stream of nitrogen (immunoaffinity chromatography).

2.5.2 HPLC for detection of aflatoxins

Sample was derevatized using 100 µl of triflouroacetic acid (TFA), which added and mixed well for 30 second, then left for 15min. 900 µl of water: acetonitrile (9:1 v/v) were added and mixed well by vortex for 30 s, then the mixture was used for HPLC analysis using reversed phase column [phenomenex C18 (250 x 4.6 mm i.d.), 5 µm from water corporation (USA)]. An isocratic system with water: methanol: acetonitrile (6:3:1). The separation was performed at ambient temperature at a flow rate of 1.0 ml/min. The injection volume was 20 µl for both standard solutions and sample extracts. The fluorescence detector was operated at wavelength of 360 nm for excision and 440 nm for emission. [16,17]

2.6 GC Analysis of Individual Components Volatile Oils in the Herbal Teas

2.6.1 Essential oil isolation

The essential oils were obtained by hydrodistillation of 100 gm of each plant using Clevenger-type apparatus for about 5 hours. The distilled oils were dried over anhydrous sodium sulphate and stored at -4°C till further analysis analyzed by GC/MS [18].

2.6.2 GC/FID analysis for volatile oils of individual components of herbal teas

The GC-FID analyses were carried out with a Varian 3400 apparatus (Varian GmbH, Darmstadt, Germany) equipped with an FID detector and a DB-5 fused-bonded capillary

column (30 m_0.25 mm i.d., film thickness 0.25 mm; Ohio Valley, Ohio, USA). The oven temperature was programmed isothermal at 45 °C for 2 min, then rising from 45 to 300 °C at 48/min, and finally held isothermal at 300 °C for 20 min; injector temperature, 250 °C; detector temp., 300 °C; carrier gas, He (2.0 ml/min); split ratio, 1 : 20. PeakSimple _ 2000 chromatography software (SRI Instruments, California, USA) was used for recording and integrating the chromatograms. Average areas under the peaks of three independent chromatographic runs were used to calculate the abundance of each component (total area = 100%).

2.6.3 GC/MS analysis for volatile oils of individual components of herbal teas

The GC/MS analyses were carried out with a Hewlett-Packard GC 5890 II gaschromatograph (Hewlett-Packard GmbH, Bad Homburg, Germany) coupled to a Thermo-Finnigan SSQ 7000 quadrupole mass spectrometer (Thermo-Finnigan, Bremen, Germany). The capillary column and the GC conditions were as described above (cf. GC-FID Analysis). The mass spectra were recorded under the following conditions: filament-emission current, 100 mA; ionization voltage, 70 eV; ion source temp., 175 °C. Diluted samples (0.5% v/v) were injected in split mode (split ratio, 1:15). The compounds were identified by comparison of their mass-spectral data and retention indices (RIs) with those of the Wiley Registry of Mass Spectral Data (8th edn.), NIST Mass Spectral Library (December 2005), and references [19-21].

2.7 Determination of heavy metals

Determination of heavy metals in samples was performed according to the official methods of analysis using wet digestion method in which 1.5 g of each powdered sample were digested in Kjeldahl flasks set at 100°C till complete digestion then diluted by deionized water and transferred quantitatively to 50 ml volumetric flask. Filtrate was analyzed using Thermo Elemental model. Solar M Atomic Absorption Spectrophotometer was used for all the measurements, the current; wavelength and slit band width of each element were adjusted automatically by the instrument software [14].

2.8 Determination of Microbial Contaminants

For determination of total viable count, dilute 2 g of herb samples were added to 40 ml portion of sterile saline solution (0.85%) and 0.1% peptone as a diluent for fungal analysis [22] in 500 ml Erlienm-eyer flask and homogenized thoroughly on an electric shaker at constant speed for 15 minutes. Tenfold serial dilutions were then prepared. 1 ml portion of each dilution was used to inoculate ten petri-dishes containing nutrient agar (for total viable aerobic bacterial count) [23] and containing yeast extract agar (for total fungal count) [24] While for detection of total coliform (pathogenic) count, MacCkoncky medium was used as a selective media for E.coli [25] and tetrathionate broths agar for salmonella species [26,27] Plates were incubated at 30-35°C for 24-72 hours.

2.9 Determination of Certain Pharmacopoeial Constants

2.9.1 Total ash

2 g of the air-dried ground material were weighed accurately in a tarred, flat-bottomed platinum or porcelain crucible and incinerated gradually at low temperature not exceeding dull redness heat, until free from carbon; then cooled in a desiccator; finally the weight of the ash is determined. If the ignition is tedious and the last traces of carbon are very difficult to burn, the crucible is left to cool and the residue is sprinkled with a small quantity of ammonium nitrate T.S., the ash is broken with a glass rod and again heated gradually to a low redness. The crucible is then cooled in a desiccator, weighed and the increase in weight of the tarred crucible gives the weight of the ash in the air-dried material. The percentage of ash (total ash) with reference to the material dried at 100ºC is calculated [18].

2.9.2 Acid insoluble ash

The total ash obtained in the above method is boiled with 25 ml dilute hydrochloric acid for 5 minutes, and then filtered through an ashless filter paper or through a tarred Gooch crucible. The residue is washed with hot water until free from chlorides. The filter paper is dried with its contents, then ignited and weighed. This weight gives the acid- insoluble ash, and the percentage of the acid insoluble ash with reference to the material dried at 100ºC is calculated [18].

2.9.3 Water soluble ash

The total ash resulting from the operation of determining the ash is boiled with 25 ml of water for 5 minutes. The mixture is filtered through an

ashless filter paper then the residue is washed with hot water. The filter paper is dried with its content, ignited and weighed. The weight of the insoluble residue is subtracted from the weight of the total ash. The difference represents the weight of the water-soluble ash, and its percentage with reference to the material dried at 100ºC is calculated [18].

2.10 Antioxidant Activity Evaluation of the Volatile oils of Some Individual Components of Herbal Teas

The radical scavenging activity of the essential oils was evaluated using diphenylpicrylhydrazyl (DPPH•) scavenging capacity assay [28]. Equal volumes of sample solutions containing 0.01–1 mg/ml of the oils and 0.2 mM methanolic solution of DPPH• were mixed and the absorbance was measured against a blank at 517 nm using a Tecan® Safire II Reader (Tecan GmbH) after incubation in the dark for 30 min at room temperature compared with DPPH• blank after background subtraction. Ascorbic acid was used as a positive control.

The percentage inhibition was calculated from three different experiments using the following equation:

$$\text{Inhibition\%} = \frac{\text{OD control - OD sample}}{\text{OD blank}}$$

Where, the inhibition % = radical scavenging activity; OD = absorption at 517 nm, and blank= negative control which contain all reagent except the volatile oil sample.

3. RESULTS AND DISCUSSION

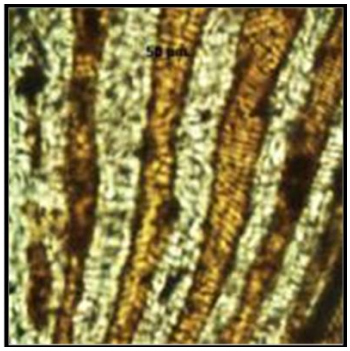
3.1 Microscopical Investigation

Microscopical investigation of the commercial herbal teas- 1, 2 and 3 revealed the presence of the main diagnostic elements of their individual components as illustrated in Fig. 1. The characteristic anise covering trichomes which are slightly curved conical unicellular hairs together with its branched vittae and underlying endocarp, the presence of the reticulate parenchyma of the mesocarp in addition to the thin-walled, polygonal cells of vittae of fennel and the abundance of large crystal sheath and orange-brown cork cells of liquorice all predominate in both the laxative and calm herbal teas. Numerous paracytic stomata, covering trichomes, crystal sheath and endocarpal fibrous layer are pronounced in the laxative preparation attributing to the presence of senna. Moreover, the appearance of the lignified parenchyma and the wavy sclerenchymatous layers from the mesocarp, together under laying endodermis showing parquetry arrangement assures the presence of coriander in the preparation. The brown fragments of vittae composing of thin-walled polygonal cells of caraway fruit together with the characteristic dill brown fragments of the vittae and the typical marjoram leaves upper epidermis, glandular, non-glandular trichomes and pollen grains confirmed their presence in the calm herbal tea. The yellowish-brown glandular trichomes in which the larger ones are sessile or with a short, unicellular stalk and a glandular head and the smaller ones are capitate with a unicellular stalk, and a rounded head composed of one or two cells in addition to the uniseriate, conical non glandular trichomes is typical to those present in the basil leaves predominates in the antidiarrheal preparation.. Moreover, tortuous fibers associated with parenchyma cells filled with calcium oxalate crystals that characterize hibiscus and candelabra hairs of verbascum flowers are observable in the antidiarrheal preparation. In addition to the presence of non-glandular trichome, small, spherical and spiny pollen grains and fibrous layer of the bract keel of achillea leaves, acicular crystals of calcium oxalate (Raphides crystals) of vine leaves, epidermal cells with anomocytic stomata and non-glandular multicellular covering trichome of chicory. However, chamomile spiny pollen grains predominate in all of the three commercial preparations.

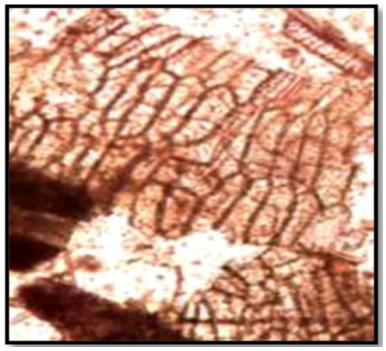
3.2 Determination of Pesticide Residue

Thirteen organochlorine pesticides residues were studied in the herbal teas including: α-HCH, β-HCH, δ-HCH, heptachlor, heptachlor-epoxide, aldrin, γ-chlordane, dieldrin, p,p-DDE, endrin, o,p-DDT, p,p-DDD and p,p-DDT. Results displayed in Table 1 showed that p,p-DDT is present in all the commercial preparations while heptachlor is present in both the laxative and antidiarrheal teas in addition to the presence of dieldrin in the former. Moreover, heptachlor-epoxide and o,p-DDT were present in calm herbal tea. However, all herbal teas are safe for use as their values do not exceed the permissible range as recommended by the different pharmacopoeias [29-31]. The detected traces of pesticides in the three products may be due to environmental contamination such as air, soil, water or human interference.

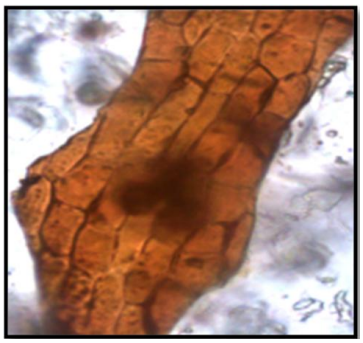
Non- glandular trichomes of Anise



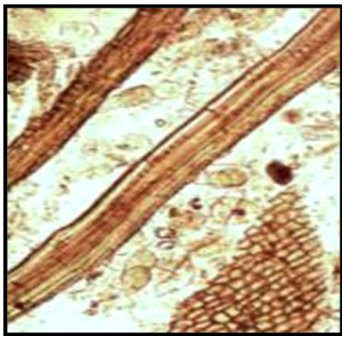
Branched vittea of Anise



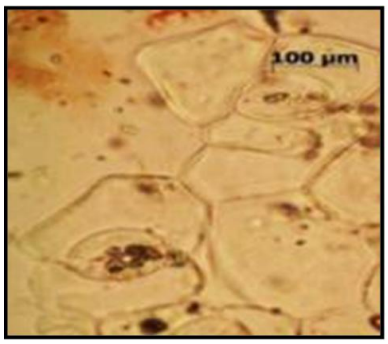
Reticulate parenchyma of Fennel



Vittea of Fennel

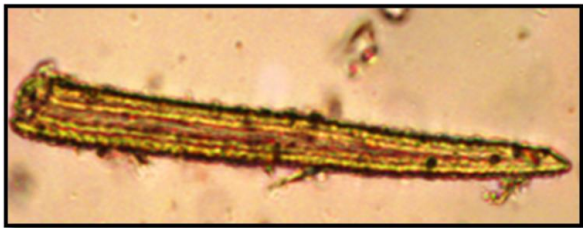


Crystal sheath and cork cells in Liquorice

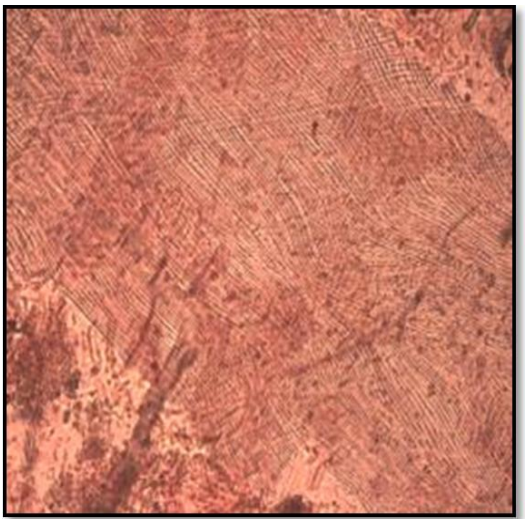


Epidermis with paracytic stomata in Senna

Non- glandular trichomes of Senna

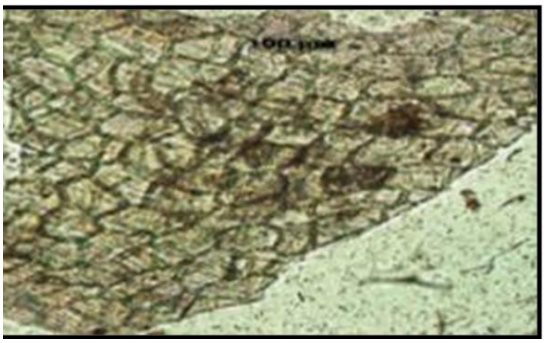


Crystal sheath in Senna

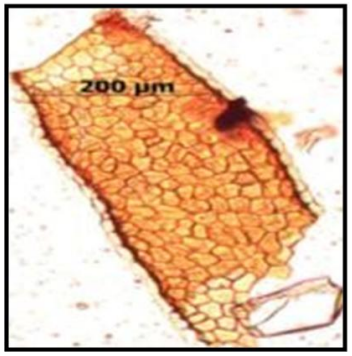


Crossed endocarpal fibers in Senna

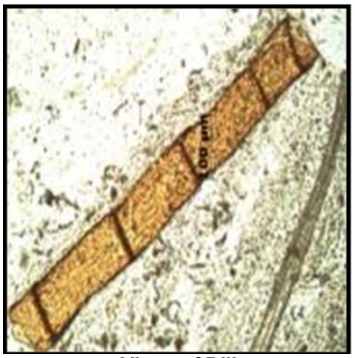
Wavy sclerenchyma from the mesocarp of Coriander



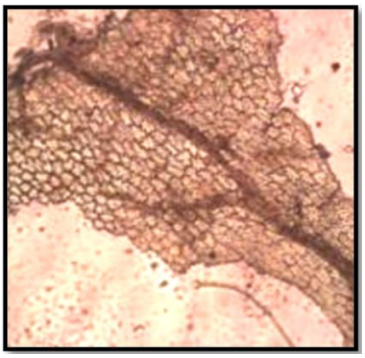
Parquetry endocarp of Coriander



Vittea of Caraway

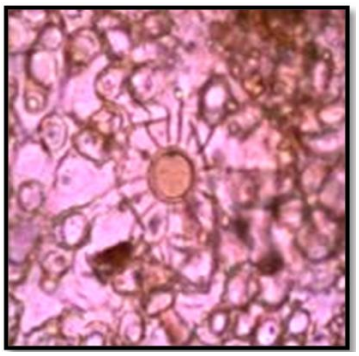


Vittea of Dill



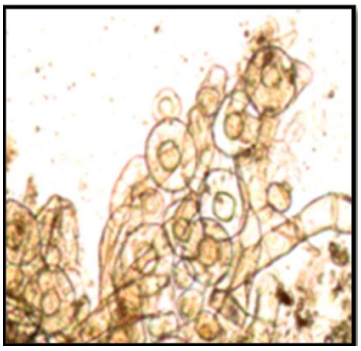
Upper epidermis of Marjoram

Non-glandular trichomes of Marjoram

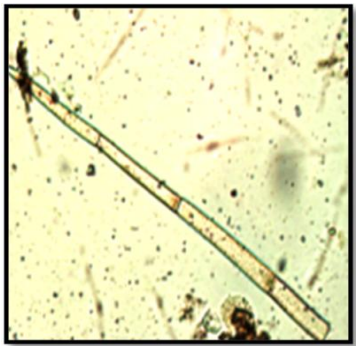


Glandular trichomes of Marjoram

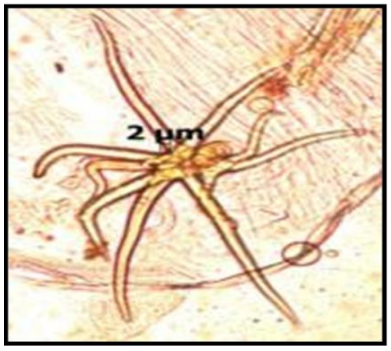
Pollen grain of Marjoram



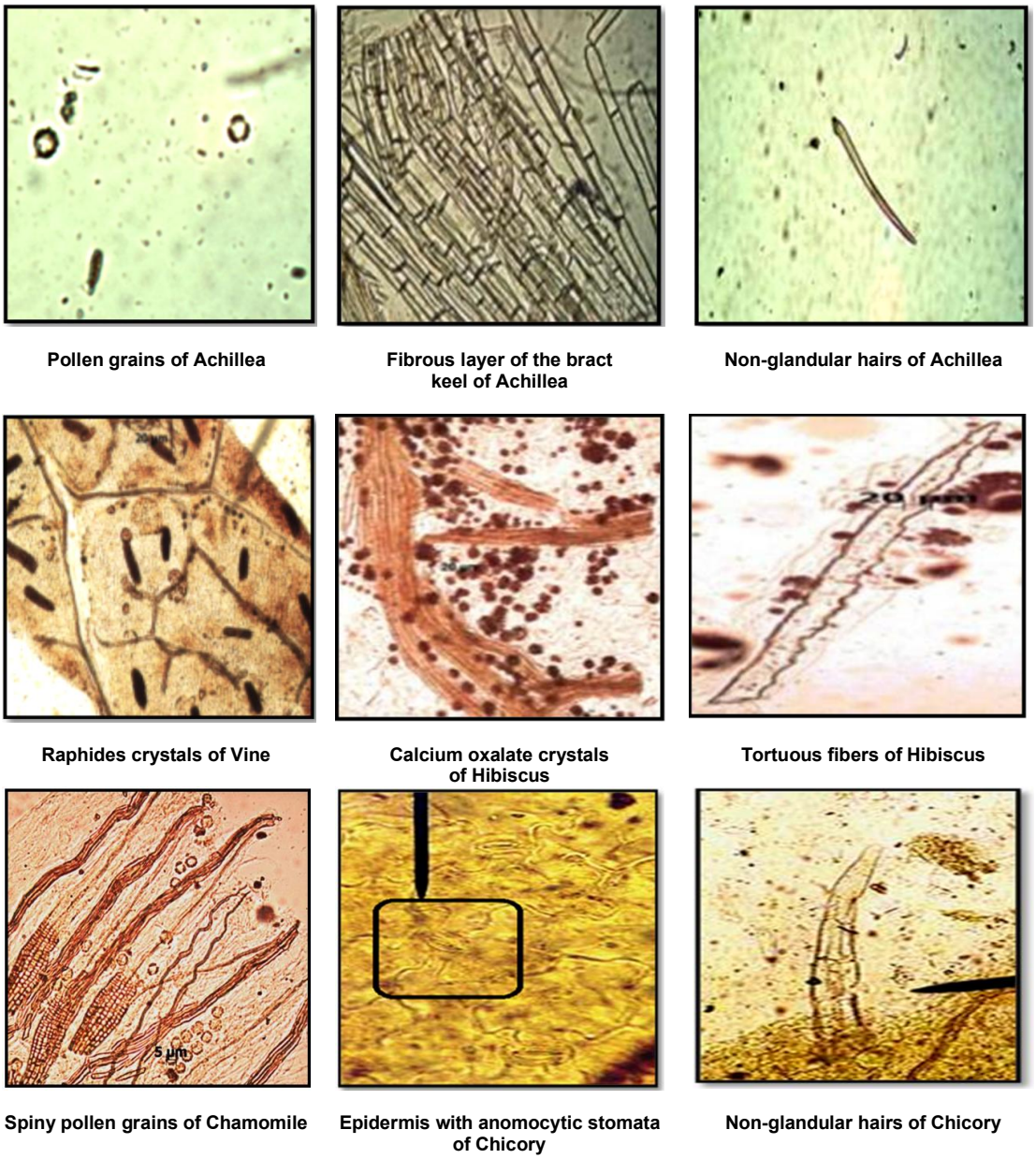
Glandular trichomes of Marjoram



Covering trichomes of Basil



Candelabra hairs of Verbascum



Pollen grains of Achillea | Fibrous layer of the bract keel of Achillea | Non-glandular hairs of Achillea

Raphides crystals of Vine | Calcium oxalate crystals of Hibiscus | Tortuous fibers of Hibiscus

Spiny pollen grains of Chamomile | Epidermis with anomocytic stomata of Chicory | Non-glandular hairs of Chicory

Fig. 1. Diagnostic elements of the different herbal teas

3.3 Determination of Microbial Contaminants

Herbs and herbal materials normally carry a large amount of microorganism, due to contamination with dust from soil representing the original habitat of bacteria and moulds or that derived from manure. In addition to the predominance of a large range of bacteria and fungi form the naturally occurring micro flora of medicinal plants and aerobic spore-forming bacteria.

Referring to the microbial contents results of the three herbal teas illustrated in Table 2, it was clear that the samples were considerably safe not exceeding the acceptable limits according to WHO measurements [32]. On the other hand, they are considered contaminated according to FIP where all the herbal teas exceeded the acceptable limits of fungal (yeast) and pathogenic bacteria (*E. coli*).

3.4 Detection of Aflatoxins

The three commercial hebal teas-1, -2 and -3 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). Results displayed in Table 3 showed that the total concentrations of aflatoxins in the three samples were 1.25, 0.52 and 3.43 µg/kg respectively which is much lower than the acceptable limits as compared with the permitted limits listed in the different pharmacopeias [32-34]. Although, aflatoxins are poorly soluble in water (10-20 µg/ml), resisting destructions by high temperature, but there is no risk of aflatoxicosis in the three commercial products due to their low concentrations.

3.5 Determination of Certain Microelements and Heavy Metals

Heavy metals pollution of food is one of the most serious problems that arise attributing to the increased consumption of fertilizers and other chemicals to meet the higher demands of food production to fulfill human needs. Lead and cadmium are the most dangerous food heavy metals contaminants where their excessive accumulation within the human bodies can cause many problems like cardiovascular, kidney, nervous and bone diseases. The reason beyond that is due to the persistence of heavy metals as they are neither biodegradable nor thermodegradable and thus accumulated and reaches to toxic levels [35].

Thus, it was felt obligatory to assess the levels of heavy metal contaminants in the three herbal adopting atomic absorption method. The results displayed in Table 4 illustrated the absence of cadmium and lead. However, comparing the daily intake dose with the acceptable daily levels, the concentrations of copper and zinc in the three commercial teas were found to fall within maximum tolerable limits, while iron was withdrawn by WHO.

3.6 Determination of Pharmacopoeial Constants

Certain important parameters and values must be followed during the quantitative and qualitative assessment of any herbal product including total ash, acid insoluble ash as well as water soluble ash. Adopting the WHO guidelines [32] for determination of ash constants for the

Table 1. Concentrations of pesticide residues (mg/kg) in commercial herbal teas

Pesticide	Herbal tea-1*	Herbal tea-2*	Herbal tea-3*
α- HCH	0.00	0.00	0.00
β- HCH	0.00	0.00	0.00
δ- HCH	0.00	0.00	0.00
Heptachlor	0.0028	0.004	0.00
Heptachlor- epoxide	0.00	0.00	0.0034
Aldrin	0.00	0.00	0.00
γ-chlordane ,	0.00	0.00	0.00
Dieldrin	0.001	0.00	0.00
p,p-DDE	0.00	0.00	0.00
o,p-DDT	0.00	0.00	0.05
Endrin	0.00	0.00	0.00
p,p-DDD	0.00	0.00	0.00
p,p-DDT	0.0003	0.003	0.05

**Average of three determinations*

Table 2. Microbial contents (total viable counts per gram) of the different commercial herbal teas

Microorganism	Herbal tea-1	Herbal tea-2	Herbal tea-3	WHO	FIP
Aerobic bacteria	4.2×10^5	6.4×10^5	5.2×10^5	10^7	$\leq 10^3 - 10^4$
Yeasts and moulds	3.1×10^5	3.0×10^5	4.7×10^5	10^4	$\leq 10^2$
Pathogenic Bacteria *E. coli*	+	+	+	10^2.	nd
Salmonella species	Nd	Nd	Nd	nd	nd

+: Found in 2 g/ 40ml (found in all ten fold serial dilution), nd: not detected

WHO: the microbial limits according to world of health organization for herbal materials that have been pretreated with boiling water or that are used as topical dosage forms [32].

FIP (Federation International Pharmaceutique) requirements for the microbial purity of non -sterile medicine not intended for topical use [33].

Table 3. Concentrations of aflatoxins G1, B1, G2, B2 and total aflatoxins (µg/kg) in herbal teas

Conc.	Herbal tea-1	Herbal tea-2	Herbal tea-3
G1	Nd	Nd	nd
B1	1.25	0.52	3.1
G2	Nd	Nd	nd
B2	Nd	Nd	0.33
Total	1.25	0.52	3.43

nd: not detected

individual official drugs constituting the herbal teas, results obtained in Table 5 proved that the values of the total ash, acid insoluble ash and water soluble ash for senna, liqourice, chamomile, fennel and coriander were around the recorded values in the published data in the Egyptian Pharmacopoeia [18] indicating that there is no adulteration or substitution in these plant constituents. However, the value of acid insoluble ash of anise and caraway are higher than the accepted limits listed in Egyptian pharmacopoeia indicating the presence of dust particles.

3.7 Volatile Oil Analysis

Volatile oil has a long history of relieving gastrointestinal tract disorders, where it was found that coriander, dill, and fennel are a marvelous trio of essential oils to an extent that these oils intake represent an obligatory need for anyone facing digestive issues on a regular basis. They act through different mechanisms, improving the entire digestive process, stimulating peristalsis, aiding digestion, diminishing gas production, showing a pronounced beneficial effect on the pancreas, gallbladder, assisting blood sugar control in addition to stimulating liver performance [36,37]. Thus, it was due to qualitatively and quantitatively estimates the volatile oil constituents present in the herbal preparations that are used in the alleviation of digestive disorders.

Based on the GC analyses, altogether 112 volatile components were identified in the essential oils of nine plants belonging to Apiaceae, Asteraceae and Lamiaceae by comparison of their mass-spectral data (MS) and retention indices (RI) with those of the Wiley Registry of Mass Spectral Data (8th edn.), NIST Mass Spectral Library (December 2005), and the literature [19-21]. Results were listed in Tables 6, 7 and 8 respectively.

3.8 Antioxidant Activity

There is no doubt that many of the serious digestive tract disorders and their fatal consequences such as inflammatory bowel disease with concomitant occurrence of abdominal pain, vomiting, diarrhea in addition to cancer that consequently produces many digestive tract disorders embracing constipation with subsequent appearance of flatulence that may be life threatening, are mainly attributed to inflammation [38,39].

However, inflammation is one of the most serious consequences of oxidative stress, and the routes that produce inflammatory mediators such as molecules adhesion and interleukins, are all resulted from oxidative damage [40]. In addition,

Table 4. Concentrations (mg/kg). of certain microelements and heavy metals in the commercial herbal teas

Heavy metals	Herbal tea-1	Herbal tea-2	Herbal tea-3	WHO
Pb	nd	nd	Nd	10
Cd	nd	nd	Nd	0.3
Cu	5.327	10.495	11.361	20
Zn	8.925	9.185	8.1667	50
Fe	18.237	22.427	17.548	*

Pb: lead, Cd: cadmium, Cu: copper, Zn: zinc, Fe: iron; WHO: maximum permissible limits recommended by world of health organization;: Withdrawn by WHO, nd: not detected.*

Table 5. Certain pharmacopeial constants of individual components of the commercial herbal teas

Sample	Total ash* %	Water soluble ash* %	Acid insoluble ash* %
Senna	6.48	5.21	1.54
Liquorice	3.145	2.59	0.87
Chamomile	7.36	4.63	2.31
Fennel	5.56	4.74	1.17
Coriander	4.59	2.60	0.95
Anise	5.47	1.12	3.33
Caraway	5.64	1.23	3.23

Average of three determinations

Table 6. Volatile constituents identified in the essential oils of anise, caraway, coriander, dill and fennel fruits (Family Apiaceae) present in the herbal teas

Compound	RI [a)] Exp.	RI [b)] Lit.	Composition %[c)]					Identification method[d)]
			Anise	*Caraway*	*Coriander*	*Dill*	*Fennel*	
α-Pinene	930	930	-	-	-	-	0.36	MS,RT
α-Phellandrene	973	1019	-	-	-	0.27	0.18	MS,RT
β-Myrecene	992	992	-	0.20	-	0.67	-	MS,RT
o-Cymene	1026	1284	-	-	-	-	0.23	MS,RT
(+)-Limonene	1029	1029	-	9.14	-	1.16	-	MS,RT
m-Mentha-1,8-diene	1031	1027	-	-	-	-	2.11	MS,RT
γ-Terpinene	1062	1062	-	-	-	-	0.15	MS,RT
Terpinolene	1062	1059	0.19	-	-	-	-	MS,RT
Fenchone	1091	1088	-	-	-	-	1.34	MS,RT
cis-Linalool oxide	1092	1088	-	-	0.06	-	-	MS,RT
Linalool	1102	1102	-	0.31	13.8	-	-	MS,RT
Camphor	1148	1147	-	-	-	53.6	0.09	MS,RT
2-Camphanone	1150	1150	-	-	0.32	-	-	MS,RT
Menthone	1158	1154	-	-	0.24	-	-	MS,RT
(-)-Terpinen-4-ol	1180	1182	-	-	1.22	-	0.30	MS,RT
α-Terpineol	1193	1193	-	-	-	-	0.11	MS,RT
Isomenthol	1196	1178	-	-	0.23	-	-	MS,RT
Methyl Chavicol	1202	1204	1.08	1.6	6.83		21.59	MS,RT
Dihydrocarvone	1207	1201	-	-	-	2.21	-	MS,RT
trans- Carveol	1224	1223	-	0.46	-	-	-	MS,RT
trans- Carveol	1238	1229	-	0.51	-	-	-	MS,RT
(R) –(+) Pulegone	1245	1235	-	-	0.21	-	-	MS,RT
Carvone	1250	1252	0.17	82.62	5.48	33.6	0.78	MS,RT
p-Anisaldehyde	1257	1252	0.41	-	-		0.57	MS,RT
Piperitone	1260	1260	-	0.31	-	2.25	-	MS,RT
p-Mentha-1,8-dien-7-al	1279	1277	-	0.26	-		-	MS,RT
Anethole	1290	1199	93.52	4.30	70.93	0.31	72.15	MS,RT
δ-Elemene	1345	1344	0.19	-	-	-	-	MS,RT
α-Elemene	1398	1398	0.12	-	-	-	-	MS,RT
α-Himachalene	1461	1451	0.39	-	-	-	-	MS,RT
α-Longipinene	1490	1352	36	-	-	-	-	MS,RT
α-Zingiberene	1503	1493	0.54	-	-	-	-	MS,RT
α-Himachalene	1517	1451	0.34	-	-	-	-	MS,RT
Myristicin	1528	1523	-	-	-	0.10	-	MS,RT
Cadina-1(10),4-diene	1533	1530	-	-	0.06	-	-	MS,RT
n-Dodecanoic acid	1569	1567	-	-	0.20	-	-	MS,RT
Apiole	1630	1682	-	0.30	-	-	-	MS,RT
Dill apiole	1631	1628	-	-	-	2.21	-	MS,RT

a) RI exp : Retention index determined experimentally on a DB-5 capillary column. b) RI lit : Published Kovats retention indices c) Contents are given as means of three analyses –, not detected. d) Identification method: compounds were identified by comparison of their mass-spectral data (MS) and retention indices (RI) with those of the Wiley Registry of Mass Spectral Data (8th edn.), NIST Mass Spectral Library (December 2005), and the literature, some compound identification was confirmed by coinjection with authentic compound (AU).

inflammation induces oxidative stress and diminishes cellular antioxidant capacity. Overproduction of free radicals interacts with cell membrane proteins and fatty acids thus, permanently affecting their role resulting in cell destruction. Moreover, free radicals can result in mutation and DNA impairment that can be an ultimate cancer predisposing factor [41].

Due to the tremendous role of antioxidants in the maintenance of the integrity and function of the gastrointestinal tract in addition to scavenging free radicals that predisposes inflammation, it was felt necessary to evaluate the antioxidant activity of the essential oils as they represent the main active constituents of the three herbal teas. Thus, the antioxidant activity of the essential oils was evaluated using DPPH assays. Results displayed in Table 9 and Fig. 2 confirmed that anise and dill oils showed the highest antioxidant activity as evidenced by their IC_{50} being 4.218 and 4.930 mg/ml, respectively. While, marjoram and fennel oils exhibited moderate activity displaying IC_{50} values of 12.158 and 14.413 mg/ml, respectively. However, caraway oil revealed the lowest antioxidant activity attaining an IC_{50} at 19.566 mg/ml as compared with the positive control Ascorbic acid that displayed an IC_{50} of 3.28 µg/ml.

Table 7. Volatile constituents identified in the essential oils of achillea and chamomile (Family Astraceae.) present in the herbal teas

Compound	RI[a)] Exp.	RI[b)] Lit.	Composition %[c)]		Identification method[d)]
			Achillea	*Chamomile*	
Linalool	1103	1103	0.65	0.06	MS,RT
Borneol	1170	1170	1.46	-	MS,RT
(-)-Terpinen-4-ol	1180	1182	2.23	0.11	MS,RT
α-Terpinol	1193	1193	l0.30	-	MS,RT
Estragole	1199	1198	1.55	0.12	MS,RT
trans-Carveol	1221	1222	-	0.03	MS,RT
cis-Carveol	1235	1230	0.35	0.02	MS,RT
Carvone	1248	1248	7.68	0.76	MS,RT
Anisaldhyde	1257	1252	-	0.06	MS,RT
Piperitone	1259	1258	1.21	-	MS,RT
MethylChavicol	1291	1204	22.29	1.60	MS,RT
2,4-Decadienal	1298	1309	-	0.06	MS,RT
Carvacrol	1306	1305	3.17	-	MS,RT
2,4-Decadienal, *(E,E)*-	1321	1321	-	0.29	MS,RT
Eugenol	1361	1360	0.48	0.07	MS,RT
n-Decanoic acid	1390	1387	-	1.21	MS,RT
α-Caryophyllene	1419	1431	9.58	-	MS,RT
(z)-B-Farnesene	1464	1458	-	4.76	MS,RT
α-Humulene	1454	1466	1.84	-	MS,RT
α-Curcumene	1480	1485	2.37	-	MS,RT
Spathulenol	1590	1588	2.30	1.90	MS,RT
Caryophyllene oxide	1597	1596	11.98	-	MS,RT
Ledol	1605	1608	4.24	-	MS,RT
Caryophyllene oxide	1623	1609	1.53	-	MS,RT
δ-Cadinol	1647	1647	1.49	-	MS,RT
τ.-Cadinol	1655	1652	-	1.02	MS,RT
α-Bisabolol oxide B	1670	1656	-	8.87	MS,RT
α-Bisabolol	1698	1685	-	8.58	MS,RT
Chamazulene	1747	1608	22.99	0.78	MS,RT
α-Bisabolol oxide B	1769	1656	-	69.55	MS,RT
Hexahydrofarnesyl acetone	1845	1846	-	0.13	MS,RT

a) RI exp: Retention index determined experimentally on a DB-5 capillary column. b) RI lit : Published Kovats retention indices c) Contents are given as means of three analyses –, not detected.

d) Identification method: compounds were identified by comparison of their mass-spectral data (MS) and retention indices (RI) with those of the Wiley Registry of Mass Spectral Data (8th edn.), NIST Mass Spectral Library (December 2005), and the literature, some compound identification was confirmed by coinjection with authentic compound (AU)

Table 8. Volatile constituents identified in the essential oils of basil and marjoram (Family Lamiaceae) present in the herbal teas

Compound	RI[a)] Exp.	RI [b)] Reported	Composition%[c)]		Identification Method [d)]
			Basil	*Marjoram*	
α-Phellandrene	1062	1053	-	0.03	MS,RT
cis-*α*-Terpineol	1104	1111	-	5.03	MS,RT
Linalool	1105	1104	16.71	-	MS,RT
Camphor	1149	1149	0.40	-	MS,RT
Isoborneol	1170	1156	0.61	-	MS,RT
1-Terpinen-4-ol	1181	1181	0.64	33.99	MS,RT
p-Menth-1-en-8-ol	1194	1195	1.66	-	MS,RT
α-Terpineol	1198	1197	-	15.01	MS,RT
Methyl Chavicol	1201	1204	6.99	-	MS,RT
trans-Piperitol	1213	1210	-	1.96	MS,RT
cis-Carveol	1224	1226	-	0.79	MS,RT
Carvone	1250	1252	0.60	4.08	MS,RT
Geraniol	1258	1259	0.84	-	MS,RT
Carvenone	1263	1253	-	1.43	MS,RT
Phellandral	1282	1272	-	0.10	MS,RT
Anethole	1295	1199	0.84	14.86	MS,RT
Carvacrol	1306	1305	-	2.36	MS,RT
Methyl cinnamate	1311	1370	3.87	-	MS,RT
α-Terpinyl acetate	1355	1354	-	0.05	MS,RT
Eugenol	1364	1362	8.39	-	MS,RT
Geranyl acetate	1385	1384	-	0.20	MS,RT
Methyl Cinnamate	1390	1381	28.90	-	MS,RT
levo-*α*-Elemene	1399	1398	0.63	-	MS,RT
Eugenol methyl ether	1407	1407	2.56	-	MS,RT
Caryophyllene	1431	1431	0.44	2.93	MS,RT
trans-*α*-Bergamotene	1445	1441	6.72	-	MS,RT
Aromadendrene, (+)-	1450	1441	-	0.24	MS,RT
α-Caryophyllene/	1465	1465	0.71	0.54	MS,RT
α-Guaiene	1473	1478	-	0.08	MS,RT
γ-Elemene	1509	1436	-	0.11	MS,RT
δ-Guaiene	1517	1505	0.87	-	MS,RT
τ-Cadinene	1526	1522	4.31	-	MS,RT
δ-Cadinene	1533	1523	-	0.21	MS,RT
(-)-Calamenene	1534	1529	1.48	-	MS,RT
Spathulenol	1590	1591	0.80	6.40	MS,RT
Caryophyllene oxide	1596	1596	0.25	2.06	MS,RT
Cubenol	1628	1628	1.56	-	MS,RT
τ- Cadinol	1655	1652	8.11	-	MS,RT
α-Cadinol	1668	1668	0.69	0.29	MS,RT
α –Bisabolol	1696	1701	0.41	-	MS,RT

[a)] RI exp : Retention index determined experimentally on a DB-5 capillary column. [b)] RI lit : Published Kovats retention indices [c)] Contents are given as means of three analyses –, not detected. [d)] Identification method: compounds were identified by comparison of their mass-spectral data (MS) and retention indices (RI) with those of the Wiley Registry of Mass Spectral Data (8th edn.), NIST Mass Spectral Library (December 2005), and the literature, some compound identification was confirmed by coinjection with authentic compound (AU).

Table 9. DPPH scavenging activity of some essential oils present in the herbal teas

Essential oil	IC_{50} (mg/ml)	Stdev
Caraway oil	19.5665444	0.95291895
Marjoram oil	12.1585261	0.56641141
Dill oil	4.93054621	0.12811971
Anise oil	4.21853919	0.23012257
Fennel oil	14.4136591	0.83255238

Data are expressed as the mean ± SD of three individual experiments.

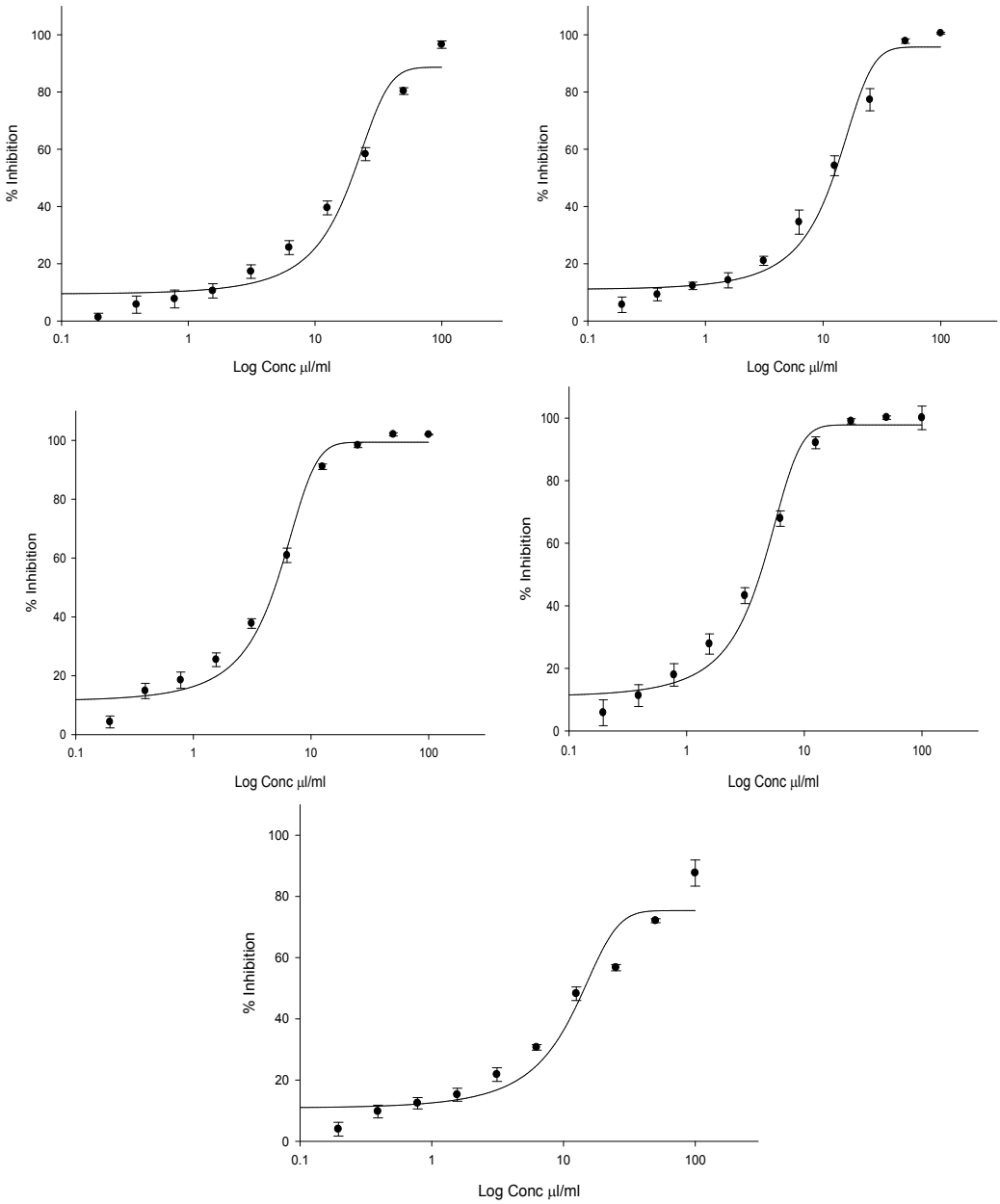


Fig. 2. DPPH scavenging activity of caraway (A), marjoram (B), dill (C), anise (D) and fennel (E)
Data are expressed as the mean ± SD of three individual experiments

4. CONCLUSION

Based on the data obtained from the various quality control assessments that were performed on the three marketed herbal teas it was clear that there was slight variation among the three products for the different evaluated parameters. However, all the products were considerably safe for human consumption not exceeding the acceptable limits as recommended by the different pharmacopeias with the exception of their microbial content in which they are considered contaminated according to FIP but safe referring to WHO measurements. Thus, the manufacturers of herbal products should try to better comply with pharmacopoeial specifications so that quality of the products could be maintained and variations could be kept within the specified limit. Moreover, it was proposed that the claimed activity of these preparations

may rely on the antioxidant activity of the essential oils as they represent the main active constituents of the three herbal teas attributing to the marvelous role of antioxidants in the maintenance of the integrity and function of the gastrointestinal tract in addition to scavenging of free radicals that predispose inflammation.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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