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Quality Control of Herbal Products in the Egyptian Market Used for Some Gastro-Intestinal Tract Disorders

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ABSTRACT

Objectives: This study aimed to evaluate the quality of three commercial herbal tea products present in the Egyptian market; commercial herbal Calm (1), commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) in comparison with three prepared standard mixtures. **Methods:** Quality control of the three commercial herbal products and prepared standard mixtures was conducted through microscopical identification of their diagnostic elements, determination of pharmacopeial constants, and detection of contaminations. Analysis of essential oil content and estimation of the main active constituents were performed using GC/MS and HPLC respectively. Antimicrobial activity, toxicological study, anti-diabetic, anti-inflammatory and antioxidant activities were carried out on 80% methanolic extract of the three commercial herbal teas in comparison with corresponding standard mixtures. **Results:** Quality control of the three commercial herbal products revealed that some of the herbs are exhausted; some of the products are adulterated with other species rather than those of the formula; however, the three commercial herbal products are safe, free from aflatoxin, pesticides and heavy metals. They are active as antimicrobial, anti-diabetic, anti-inflammatory and antioxidant. **Conclusion:** The assessed commercial herbal products that are used in the Egyptian market for some gastro-intestinal tract disorders have proven their effectiveness as antimicrobial, anti-diabetic, anti-inflammatory and antioxidant drugs, which can be subjected for further clinical trials.

Keywords: *Chemical analysis, Gastro-intestinal disorder, Microscopical analysis, Quality control*

INTRODUCTION

Diseases of gastro-intestinal tract (GIT) are a major cause of ill-health worldwide. In developing countries infection and malnutrition are of common correlation; for example, over a billion people are infested with roundworms and hookworms, and amoebiasis that affect over 10% of the world's population. In primary care and in outpatient clinics, patients with dyspepsia and irritable bowel syndrome are very common.[1] Phytotherapy is a form of complementary and alternative therapy using plants and their extracts for healthcare. According to World Health Organization more than three fourth of the world population use herbal therapy. Herbal treatment for gastrointestinal disorders involves using herbal supplements for relief of gastrointestinal symptoms and to improve physiological function of the gastrointestinal tract. Phytotherapy is given in the form of supplements, teas, tinctures, creams and poultices.[2] 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 means 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.[3]

MATERIALS AND METHODS

Herbal tea material

Commercial herbal preparations used were collected from different batches of commercial herbal Calm (1) (HS 508/13), commercial herbal Intestinal (2) (HS 0032/12) and commercial herbal Anti diarrhea (3) (HS 0035/12) present in the Egyptian, Cairo market. Standard herbal teas were prepared from herbs obtained from Harraz, Bab Elkhalq square, Cairo, Egypt; as for prepared standard herbal tea -1 composed of 40 gm chamomile flowers, 5 gm marjoram leaves, 15 gm licorice roots, 10 gm fennel fruits, 10 gm dill fruits and 10 gm caraway fruits, as for prepared standard intestinal-2 composed of 40 gm basil leaves, 10 gm lemon grass leaves, 15 gm chamomile flowers, 15 gm fennel fruits, 10 gm anise fruits, 5 gm chicory leaves and 5 gm achillea leaves, as for prepared standard anti diarrhea-3 composed of 30 gm achillea leaves, 10 gm verbascum flowers, 10 gm hibiscus flowers, 15 gm chamomile flowers, 5 gm chicory leaves, 15 gm basil leaves and 15 gm vine leaves.

Animals

Albino mice of 25-30g body weight, adult male Albino rats of Sprange Dawely strain of 130-150 gm body weight. The animals were kept under the same hygienic conditions and on a standard laboratory diet consisting of vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%), cellulose (0.2%), casein (95%) and starch (54.3%).

Standard drugs, toxins and chemicals

Indomethacin; Epico, Egyptian Int. Pharmaceutical industries Co., A.R.E., under license of Merk & Co. INC-Rahawy, N.J., USA. Carrageenan; Sigma Co., Alloxan; Sigma Co., Metformin (Cidophage)®; chemical industries development (CID), Giza, ARE., Vitamin E (dl α-tocopheryl acetate); Pharco Pharmaceutical Co. Vitamin E is available in the form of gelatinous capsules; each contains 400mg vitamin E. Aflatoxins B1, B2, G1 and G2 were supplied from Agriculture Research Center, Central Laboratory of Residue Analysis of Pesticides and Heavy metals in Food, Dokki, Giza, Egypt. HPLC grade of acetonitrile, methanol, acetic acid and phosphoric acid were supplied through labscan limited, Stillorgan Ind. park, Co., Dublin, Irland. Analytical grade of acetone, benzene, sulphuric acid, nitric acid, hydrochloric acid, ammonia, magnesium nitrate, ammonium dihydrogen phosphate, potassium iodide, tetrahydroborate, sodium hydroxide, sodium, stanus chloride, sodium sulphate, sodium chloride, magnesium sulphate and sodium metabisulphite were supplied through Adwic, El Nasr Pharmaceutical Chemicals Co., Cairo, Egypt. Analytical grade of hexane, tetrabutyl-4-hydroxy anisole and ethyl acetate were supplied through Sigma Aldrich, St. Louis, USA.

Essential oils: Terpinene-4-ol, carvone, cis-carvone, estragole, piperitone, cadinene, spathulenol, bisabolone oxide,bisabolone oxide A, α-bisabolol, bisabolol oxide A, chamazulene,apiole, linalol, trans-α-bergamoten, α-caryophyllene, caryophyllene oxide, cis-β-farnesene, bornyl acetate, camphor, δ-elemene, anethole, α-cubebene, eugenol, methyl eugenol, isoeugenol methyl ester, methyl cinnamate, anthranilate, heptadecane, γ-muurolene, junipen, P-thymol, α-cadinol were supplied through Department of Chemistry – Faculty of Agriculture Research Park – Cairo University – Giza – Egypt.

Pesticides: Atrazine, bendiocarb, benalaxyl, bupirimate, buprofezine, carbosulfan, chlorpyrifos-ethyl, chlorpyrifos-methyl, cyanophos, diazinon, dimethoate, ethion, ethoprophos, fenitrothion, fenoxaprop-p-ethyl, fenthion, fluazifop-p-butyl, flusilazole, malaoxon, malathion, metalaxyl, paraoxon-ethyl, parathion ethyl, parathion methyl, pencycuron, pirimicarb, pirimiphos-ethyl, pirimiphos-methyl, phenthoate, phosalone, phosphamidone, profenofos, prothiofos, pyrazophos, pyriproxyfen, thiobencarb, tolcophos-methyl and triazophos were supplied through Agricultural Research Center, Central Laboratory of Residue Analysis of Pesticides and Heavy metals in Food, Dokki, Giza, Egypt.

Heavy metals: Arsenic (AS), lead (Pb), Chromium (Cr), mercury (Hg) and cadmium (Cd) were supplied through Agricultural Research Center, Central Laboratory of Residue Analysis of Pesticides and Heavy metals in Food, Dokki, Giza, Egypt.

Phenolic compounds for HPLC analysis: Gallic acid, protocatechuic acid, chlorogenic acid, catechol, vanillic acid, catechin, caffeic acid, cinnamic acid, salicylic acid, ferulic acid, rutin, rosmarinic, quercetrin, quercetin, naringinin, hespertin, kampferol and apignin were supplied through Food Technology research institute, Agricultural research center, Ministry of Agriculture and Land Reclamation, Giza, Egypt.

Esculin and inulin for HPLC analysis: Were supplied through Alfa Aesar A Johnson Matthey Company, Karlsruhe, Germany.

Saponins for HPLC analysis: Glycyrrheizic acid ammonium salt was supplied through Sigma Aldrich Chemie Co., Germany.

Biochemical kits

Biodiagnostic Kit: used for assessment of blood glucose level were supplied from Diamond Diagnostics, Egypt. Biodiagnostic Glutathione kit: used for assessment of antioxidant activity were supplied from Diamond Diagnostics, Egypt.

Microorganisms and Microbiological media

Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Shigella flexneri, Escherichia coli, Salmonella typhimurium, Candida albicans and

Aspergillus fumigatus, were supplied through The Regional Center for Mycology and Biotechnology- Faculty of Pharmacy- Al Azhar University- Nasr city- Cairo- Egypt.

Culture media for antimicrobial activity: Nutrient agar for bacteria and dextrose salt agar for fungi were supplied through The Regional Center for Mycology and Biotechnology- Faculty of Pharmacy- Al Azhar University- Nasr city- Cairo- Egypt. Culture media for microbial count: Nutrient agar, MacConkey broth, glucose azide broth, reinforced media, *Bacillus cereus* selective agar media, Vogel-Johnson agar media, chromogenic agar base, and Rose Bengal agar base were supplied through supplied through Department of Microbiology – Faculty of Agriculture Research Park – Cairo University – Giza – Egypt.

Gas chromatography for essential oil analysis

About 100 gm of each sample were subjected to hydro distillation for 3 hours using a Clevenger type apparatus. The obtained essential oil was dried over anhydrous Na_2SO_4 and stored at –10 °C in a sealed vial until use. The oil was analyzed by gas chromatographic system (Agilent 6890) system with an HP 5973 mass selective detector, and a TR-FAME (Thermo 260 M142P) (30 m, 0.25 mm ID, 0.25 µm) (70% cyanopropyl polysilphenylene siloxane) capillary column. The temperature of the injector and the transfer line were 200 °C and 250 °C respectively. The oven temperature was programmed as follows: Initial temperature: 80 °C for 2 min, increase 3 °C/min up to 230 °C, and then hold at 230 °C for 5 min. The carrier gas was Helium, and the flow rate was (1.5 ml/min). The amount of sample injected was about 1µl (5µl/1 ml solvent) and the ionization energy was 70 eV. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds. Also, probability merge search software and NIST MS spectra search program were used.[4]

Gas chromatography for pesticides detection

Addition of 10 ml acetonitrile and the internal standard on 10 gm of sample, agitate intensively; then add NaCl, $MgSO_4$ and buffering salts for phase separation and PH adjustment, agitate intensively and centrifuge (Raw extract). Take an aliquot of the upper organic phase and subject it to dispersive SPE cleanup (d-SPE) by mixing it with $MgSO_4$ and a sorbent (e.g. PSA) to remove water and undesired co-extractives, agitate shortly and centrifuge (Final extract). The final extract will be analysed by gas chromatography system equipped with two electron capture detectors (ECD) for organochlorine pesticides. Gas Chromatography (Agilent 6890 series) equipped with two nitrogen phosphorus detectors (NPD) for organophosphorus and organonitrogen pesticides. For results confirmation, samples were injected into two capillary columns with different polarities (HP-PAS-5: 0.32 mm×0.52 um×25 m and DB-1701P: 0.32 mm×0.25 um×5 m). Conditions: N_2 constant flow, 1.3 ml/min; inlet temperature, 225°C; injection volume, 1 µl (split less); initial oven temperature, 90°C, held for 2 min, then a 20°C/min ramp to 150°C followed by a 6°C/min ramp to 270°C and held for 18 min.[5]

HPLC detection for aflatoxin

Stock solution of aflatoxins 1000 µg/ml was prepared by dissolving 1 mg of aflatoxin standards (3.6) in 1 ml benzene/acetonitrile (98:2). The stock solution was stored at – 20 °C. Intermediate solution: Individual standards of 10 µg/ml of B1, B2, G1 and G2 were prepared by diluting appropriate volume of the stock standard solution in benzene acetonitrile (98:2). The exact concentration of the intermediate solution was determined by spectrophotometry according to Association of Analytical Communities (AOAC). Mixture solution of aflatoxins B1, B2, G1 and G2 of 1 µg/ml was prepared by diluting appropriate volume of individual solutions in benzene acetonitrile (98:2) and stored in small portions (1 ml each portion) in small vials and kept in freezer at –20°C.

Working solutions: Intermediate aflatoxin mixture was evaporated to dryness and derivatized using TFA in a pre-column. The weakly fluorescent B1 and G1 were transformed into their highly fluorescent hemiacetals B2a and G2a. The highly fluorescent G2 and B2 were not affected by this conversion due to their saturated structure.[6]

Extraction procedure: Test portions of 25 g (hot chili,) dry the samples by blending with 200 ml chloroform and 25 ml of sodium chloride solution (10%) in a high speed blender for 2 minutes. The extracts were filtered and an aliquot of 40 ml was evaporated on rotary evaporator at 40°C. The residue was dissolved in 4 ml hexane ethyl acetate (1:1) and 0.5 ml was subjected to Gel permeation chromatography (GPC) clean-up. Purification and derivatization of aflatoxins: A 0.5 ml was passed through GPC system at a flow rate of 2.5 ml/min. 32 ml (13 min) was dumped and 37 ml (15 min) was collected in a 100 ml pear-shaped flask. The solution was evaporated on rotary evaporator at 40°C. Aflatoxins were derivatized by addition of 100 µl of TFA and 200 µl hexane to the residue and shaken occasionally for 0.5 min. The flask was allowed to stand for 5 min at room temperature. A 900 µl of acetonitrile-water (1:9) was added and the flask was shaken occasionally for 0.5 min and let to stand for 5 minutes. A 100 µl of the aqueous layer was subjected to HPLC analysis. Aflatoxins were separated and quantified by reversed phase-HPLC using C18 analytical column. The mobile phase was deionized water methanol acetonitrile (60:25:15), at a flow rate of 0.7 ml/min. Aflatoxins were detected by fluorescence detector at the excitation and emission wavelengths of

360 and 440 nm respectively. The injection volume was 100 µl. [6]

HPLC analysis of polyphenolic compounds

Hewllet Packared Cairo, Egypt (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet detector set at 280nm for polyphenolic compounds and quaternary HP pump (series 1100). About 30 grams of each sample was homogenized in 100 ml of 70% acetone containing 0.5% sodium metabisulphite for 1 min using an Ultra-Turrax blender. Three successive extractions with 70% aqueous acetone were carried out at 4ºC for 30 min. After removing acetone under vacuum at 35ºC, carotenoids were separated by three successive extractions with petroleum ether, the three organic phases were combined, filtered on Whatman filter paper to eliminate residue of aqueous phase, and evaporated to dryness under vacuum at 35ºC. The residue was dissolved in MeOH (3 ml), then filtered through an aerodisc filter prior to HPLC analysis and assigned as petroleum ether extract (PE). Ammonium and metaphosphoric acid were added to the aqueous phase. Phenolic compounds were then extracted with ethylacetate (1:1, v/v) three times. The three organic phases were combined and treated as for the PE phase. Methanolic extract was filtered through an Aerodisc filter (0.45 µm) before HPLC analysis and assigned as ethyl acetate extract (EA).[7]

HPLC analysis of flavonoid compounds

Hewllet Packared (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet detector set at 330nm for flavonoids and quaternary HP pump (series 1100).

About 0.5 gm of each sample was weighed into a 100 ml Erlenmeyer flask then dispersed in 40 ml of 62.5% aqueous methanol containing 2gm/L of 2,(3)-tert-butyl-4-hydroxyanisole (BHA). The mixture was then ultrasonicated for 5 min. To this extract 10 ml of 6 M HCl was added. The sample was bubbled with nitrogen for 40-60 seconds after which the flask was sealed tightly. Hydrolysis was carried out in a shaking water bath at 90 ºC for 2 hrs. After hydrolysis the sample was allowed to cool, and then it was filtered, made up to 100 ml with methanol, and ultrasonicated for 5 min. Before quantification by HPLC the sample was filtered through a 0.2 µm membrane filter. Separation and determination of flavonoids were performed by reversed phase HPLC (RP-HPLC) diode array detection (DAD), wave lengths used for identification and qualification of flavonoids, with the diode array were 330 nm, using a Inertsil C18 column (GL Sciences, Inc., Japan), ODS-3(150mm X 4.0 mm, 3µm) with a guard column Inertsil C18, 3µm (GL Sciences, Inc., Japan). Temperature of the column oven was set at 35 ºC. Gradient elution was employed for flavonoids with a mobile phase consisting of 50 mM H_3PO_4, PH 2.5 (solution A) and acetonitrile (Solution B) as follows. The flow rate of the mobile phase was 0.7 ml/min, and the injection volumes were 10 µl of the standards and sample extracts. All flavonoids were quantified using the external standard method. Quantification was achieved using diode array detection (DAD), based on peak area.[8]

Atomic absorption spectrophotometric detection of heavy metals

Blank solution was prepared by mixing 6 ml of heavy metal free nitric acid and 4 ml of heavy metal free hydrochloric acid in a digestion flask. Carry out the digestion in the same manner as for the test solution. Measure the content of cadmium, chromium and lead by standard addition method, using reference solutions of each heavy metal.[9]

***Arsenic and mercury**:* Measure the content of arsenic and mercury in comparison with the reference solutions of arsenic or mercury at a known concentration by direct calibration using an automated continuous flow hydride vapor generation system. The absorbance value of the blank solution is automatically subtracted from the value obtained with the test solution.[9]

Arsenic: To 19 ml of the test solution or of the blank solution, add 1 ml of a 200 gm/l solution of potassium iodide. Allow the test solution to stand at room temperature for about 50 min or at 70 °C for about 4 min.[9]

Acid reagent: heavy metal free hydrochloric acid; reducing reagent: 6 gm/l solution of sodium; tetrahydroborate in a 5 gm/l solution of sodium hydroxide.[9]

Mercury: To 19 ml of the test solution or of the blank solution, add 1 ml of a 200 gm/l solution of potassium iodide. Allow the test solution to stand at room temperature for about 50 min or at 70 °C for about 4 min.[9]

Insoluble ash and acid insoluble ash

Total ash: The CEM Corporation (Matthews, NC) has developed a series of instruments for dry and wet ashing as well as other microwave digestion series known as “microwave assisted chemistry”. Programmed microwave wet digesters and muffle furnaces decrease time closed vessels are used for ashing. The systems allow for programming temperatures that can dehydrate, then ash and exhaust the system, twenty minutes were shown to be adequate for the plant materials.[3]

Soluble and insoluble ash in water: These measurements are an index of the fruit content of preserves and jellies. Weigh the total ash, add 10 ml distilled water, cover the crucible and heat nearly to boiling, filter on ashless filter paper and rinse with hot distilled water several times, dry and re-ash filter paper at least 30 min, weigh and calculate as percent water

insoluble ash, calculate soluble ash by subtracting insoluble ash.[3]

Soluble and insoluble ash in acid: Weigh the ash, add 25 ml 10 %HCL to total ash or water insoluble ash, cover the crucible and boil for 5 min, filter on ashless filter and rinse with hot distilled water several times, dry and re-ash filter paper at least 30 min, weigh and calculate as percent acid insoluble as, calculate soluble ash by subtracting insoluble ash.[3]

Antimicrobial activity

Antimicrobial activity was determined using the agar well diffusion. The assay method as described by Holder and Boyce.[10] DMSO was used as a negative control and Ampicillin (10 mg/disc) for gram positive bacterial strains and Gentamicin (10 mg/disc) were used as positive control for gram negative bacterial strains, Amphotericin B were used as a positive control for fungi. The plates were done in triplicate. Bacterial cultures were incubated in nutrient agar at 37°C for 24 hrs while the other fungal cultures were incubated in saboroud dextrose agar at (30-32°C) for 3-7 days. Solutions of 10 mg /ml of Ampicillin, Gentamicin and Amphotericin B were used as standard for comparison. Antimicrobial activity was determined by measuring the inhibition zone.[10]

Pharmacological activity

The dried commercial herbal teas and their prepared standard mixtures (1), (2) and (3) (500gm) were separately subjected to exhaustive extraction with 80% aqueous methanol under reflux (50 ºC) and were dried under vacuum (50 ºC) to give dry total extract of 17g, 20g, 23g, 19g, 18.5g and 21g respectively.

Toxicological study

Determination of the LD_{50} of the aqueous methanolic extract of commercial herbal teas and their prepared standard mixtures was estimated according to Karber[11]; Paget and Barnes [12]**.** Preliminary experiments were done to determine the minimal dose that kills all animals (LD_{100}) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses, each dose was injected in a group of 6 animals by subcutaneous injection. The mice were then observed for 24 hours and symptoms of toxicity and mortality rates in each group were recorded and the LD_{50} was calculated.[11,12]

Hypoglycemic activity

Male albino rats of Sprague Dawely Strain (130-140 g) were injected intraperitoneal with alloxan (150mg/kg b.wt.) to induce diabetes mellitus.[13] Hyperglycaemia was assessed after 72 hours by measuring blood glucose level after 1 and 2 months intervals[14]. Diabetic rats received 100mg/kg b.wt. of commercial herbal teas and their prepared standard mixtures and 100 mg/kg b.wt of metformin drug as reference drug.

At the end of each study period blood samples were collected from the retro orbital venous plexus through the eye canthus of anaesthetized rats after an overnight fast. Serum was isolated by centrifugation and the blood glucose level was measured.[14]

Acute anti-inflammatory activity

This effect was determined according to the method described by Winter *et al.*, 1962. The rats received 100 mg/kg b.wt of the extract of commercial herbal teas and their prepared standard and received 20 mg/kg of the reference drug Indomethacin.[15]

Antioxidant activity

Glutathione in blood was determined according to the method of Beutler.[16] The absorbance was measured at 405 nm[16]. Calculation of Glutathione conc.: In blood= A_{sample} X 66.66 mg/dl.

RESULTS AND DISCUSSION

Microscopical examination

Commercial herbal teas compared to their corresponding prepared standard herbal teas and compared to reported data [17] confirmed the presence of diagnostic elements of their constituents among which are; very abundant pollen grains which are spherical with three pores and a spiny and warty exine and Outer epidermis of the corolla showing glandular trichomes of chamomile flowers, Non-glandular, multicellular and uniseriate hair, labiacious hair and capitate hair of **Majorana** leaves, endocarp forming parquetry arrangement of Fennel fruits, endocarp composed of a layer of thin walled, lignified cells of **Dill** fruits, branched brown fragments of vittae and covering trichomes, which are conical, slightly curved of anise fruits, endocarp composed of a layer of fairly large cells with thin, slightly lignified walls and sclereids of the mesocarp which occur in large groups of caraway fruits, the very abundant fibers which occur in groups surrounded by calcium oxalate prism sheath and the fairly abundant fragments of orange-brown cork composed of thin-walled cells of **Licorice**, Covering trichomes , they are uniseriate, conical, composed of two or three and occasionally up to six cells, with slightly thickened and warty walls and pollen grains are fairly large, spherical with six pores and furrows; rounded of basil leaves, simple unicellular, non-glandular hairs scattered in the adaxial and abaxial surfaces of lemon grass leaves, branched, anastomosing laticeferous vessels with dark brown content and Non-glandular and uni-cellular trichomes of **Chicory** leaves, tricolporate pollen grains with spiny exine, unicellular covering trichome from the leaf, disk floret, sclerenchyma and epidermal cells at the margine of a phyllary, papillae of the stigma of both **Achillea** flowers and leaves, stellate

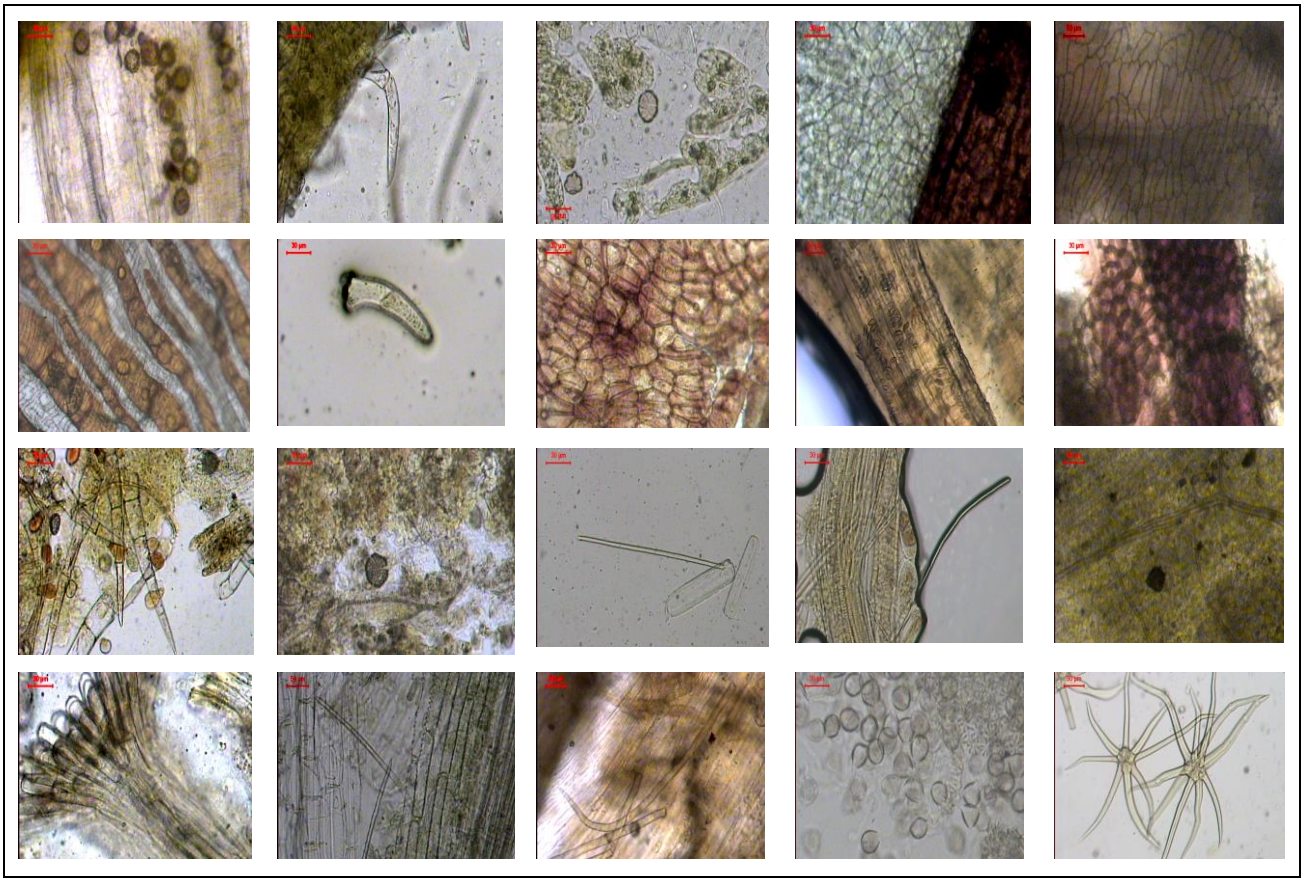

Figure 1. Diagnostic elements of the different herbal teas

hair, which is the hair that is split into several strands at the free end and the 3- colporoidate prolate pollen grains with reticulate exine of **Verbascum** flowers, stellate hair and cluster crystals of Ca-oxalate of hibiscus flower and calcium oxalate raphides of the leaf of **Vine** leaves (**Figure 1**).

Determination of certain pharmacopoeial constants

Adopting the methods of WHO guide lines total ash, water insoluble ash and acid insoluble ash were determined for commercial and standard herbal teas (1), (2) and (3). Results obtained were tabulated in Table 1.

The values of the total ash, water insoluble ash, and acid insoluble ash obtained from commercial herbal Calm (1) are different from those obtained from the prepared standard herbal calm (1) which indicates presence of adulteration.

The values of the total ash obtained from commercial herbal Intestinal (2), and commercial herbal Anti diarrhea (3) are near to the values obtained from the prepared standard herbal intestinal (2), and standard herbal anti-diarrhea (3), while the values of water insoluble ash, and acid insoluble ash obtained from commercial herbal Intestinal (2), and commercial herbal Anti diarrhea (3) are completely different from those obtained from the prepared standard mixtures, which indicates presence of adulteration.

Detection of pesticide residues

Medicinal plant materials may contain pesticide residues, which accumulate as a result of agricultural practices, such as spraying, treatment of soils during cultivation and administration of fumigants during storage. It is therefore recommended that every country producing medicinal plant materials should have at least one control laboratory capable of performing the determination of pesticides using a suitable method[3]. Chlorpyrifos is detected in commercial herbal Calm (1), commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) below LOQ (Table 2). On the other hand, malathion is not detected in commercial herbal Calm (1). The other pesticides like atrazine, bendiocarb and benalaxyl are not detected in commercial herbal teas (1), (2) and (3).

The European Pesticides database established maximum residual limits (MRLs)[18] are given in Table 3. From the results which were given in Table 3, Commercial herbal Calm (1), commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3)

Table 1. Pharmacopoeial constants of commercial and prepared standard teas

Commercial Material	Total ash %	Water insoluble ash %	Acid insoluble ash %	Standard Material	Total ash %	Water insoluble ash %	Acid insoluble ash %
Commercial herbal Calm (1)	8.09	2.46	0.35	Standard herbal calm (1)	10.03	1.17	0.53
Commercial herbal Intestinal (2)	11.12	2.34	0.52	Standard herbal Intestinal (2)	11.36	1.55	0.86
Commercial herbal Anti-diarrhea (3)	10.4	3.34	0.72	Standard herbal Anti diarrhea (3)	10.70	2.73	0.38

Table 2. The limit of quantitation (LOQ) and the concentration of pesticides detected in commercial herbal Calm (1), commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3)

Compound	LOQ (mg/kg)	Concentration (mg/kg)		
		Commercial herbal Calm (1)	Commercial herbal Intestinal (2)	Commercial herbal Anti-diarrhea (3)
Chlorpyrifos	0.15	Detected below LOQ	Detected below LOQ	Detected below LOQ
Malathion	0.15	Not detected	Detected below LOQ	Detected below LOQ

contain residues of chlorpyrifos which is below LOQ (<0.15 mg/kg) and below MRLs (<0.1 mg/kg). Commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) contain residues of malathion which is below LOQ (<0.15 mg/kg) and below MRLs (0.5 mg/kg). So all pesticidal residues are within limits, and they may be coming from environmental contamination such as air, water or soil. From the results which were given in table (3), Commercial herbal Calm (1), commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) contain residues of chlorpyrifos which is below LOQ (< 0.15 mg/kg) and below MRLs (< 0.1 mg/kg). Commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) contain residues of malathion which is below LOQ (< 0.15 mg/kg) and below MRLs (0.5 mg/kg). So all pesticidal residues are within limits, and they may be coming from environmental contamination such as air, water or soil.

Table 3. MRLS of certain pesticides

Compound	MRLs mg/kg
Malathion	0.5
Chlorpyrifos	0.1

Determination of certain heavy metals

Contamination of herbal materials with heavy metals can be attributed to many causes. These include environmental pollution (i.e. contaminated emissions from factories and leaded petrol and contaminated water including runoff water which finds its way into rivers, lakes and the sea, and some pesticides), soil composition and fertilizers.[3]

Certain heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr) and arsenic (As) were determined in the commercial herbal products using atomic absorption spectrometry after high pressure microwave digestion.

From Table 4, we can conclude that the concentration of heavy metals in commercial herbal Calm (1), commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) were found to be within maximum tolerable limits.

Detection of microbial contaminants

Detection of different groups of bacteria and fungi

All the parts of plants (root, leaf, and flower) naturally have a high level of microorganisms, bacteria and fungi, especially molds. Microbial contamination could be a result of inappropriate harvesting, cleaning of the raw plant material, and unhygienic processing of the plants, unsuitable transport and storage.[19]

It could be concluded that all the samples were contaminated with bacteria, fungi and yeasts contaminants. Concerning the three commercial herbal samples under investigation; the total bacterial count, *Fecal Streptococci* and *coliforms* lie within WHO limits for bacterial contamination, while the three samples are heavily contaminated with *Escherichia coli*.

Regarding fungal contamination of the three samples, we have found that commercial herbal Calm (1)

Table 4. The concentration (mg/kg) of certain heavy metals present in commercial herbal Calm (1), commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) and WHO limits

Herbal product	Cr	Cd	Hg	Pb	As
Commercial herbal Calm (1)	------	0.032	------	0.178	------
Commercial herbal Intestinal (2)	------	0.019	------	0.24	------
Commercial herbal Anti diarrhea (3)	------	0.028	------	0.454	------
WHO limits	2 ppm	0.3 mg/kg	0.5 mg/kg	10 mg/kg	5 mg/kg

Cr, Chromium; Cd, Cadmium; Hg, Mercury; As, Arsenic

Table 5. Microbial contents of samples of commercial herbal Calm (1), commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3)

Microbial Group	Commercial herbal Calm (1)	Commercial herbal Intestinal (2)	Commercial herbal Anti- diarrhea (3)
Total plate count (cfu/g)	5×10^4	5.3×10^4	2.6×10^4
Total *coliforms* (MPN/g)	1.1×10^4	4.5×10^3	1.1×10^4
Total *Staphylococci* (cfu/g)	Nil	Nil	Nil
Escherichia coli (MPN/g)	1.5×10^3	4.5×10^2	2.5×10^3
Fecal Streptococci (MPN/g)	4	Nil	Nil
Clostridium perfringens (MPN/g)	Nil	Nil	Nil
Bacillus cereus (cfu/g)	Nil	Nil	Nil
Staphylococcus aureus (cfu/g)	Nil	Nil	Nil
Detection of *Salmonella spp.*	Nil	Nil	Nil
Total fungi (cfu/g)	4.5×10^4	2.9×10^4	2.5×10^3
Total yeast (cfu/g)	4.9×10^3	1.2×10^2	8.5×10^2
Aspergillus niger (cfu/g)	30	85	Nil
Aspergillus flavus (cfu/g)	75	75	20
Total *Fusarium* (cfu/g)	Nil	Nil	Nil
Total *Penicillium* (cfu/g)	2.4×10^4	1.7×10^3	30

and commercial herbal Intestinal (2) are heavily contaminated with fungi while commercial herbal Anti diarrhea (3) contaminated with fungi, but still lies within WHO limits (Table 5). So, the results obtained lies within WHO guide lines [3] for the three samples under investigation except for the presence of *Escherichia coli* which is outside the WHO limits and fungal contamination of commercial herbal Calm (1) and commercial herbal Intestinal (2).

Detection of aflatoxin

Aflatoxin are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus *Aspergillus flavus* and *Aspergillus parasiticus* on variety of food products. Among 18 different types of aflatoxin identified, major members are aflatoxin B1, B2, G1 and G2.[20] No aflatoxins were detected in all the three samples commercial herbal Calm (1), commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3).

Investigation of essential oil

Analysis of essential oil content in commercial herbal Calm (1)

GC/MS analysis of essential oil of Commercial herbal Calm (1) (**Figure 2**) revealed the presence of: Terpinene-4-ol (1.13%) as a finger print for *Majorana hortensis* Moench leaves, carvone (26.42%) as a finger print for *Carum carvi* L. fruits& *Anethum graveolens* L. fruits, estragole (52.58%) as a finger print for *Pimpinella anisum* L. fruits& *Foeniculum vulgare* Mill fruits, cadinene (0.67%), spathulenol (0.25%), bisabolone oxide (0.4%) and bisabolone oxide A(2.96%) as a finger print for *Matricaria chamomilla* L. flowers. The absence of chamazulene and α-bisabolol which are the main

active consituents of *Matricaria chamomilla*, absence of fenchone the main acive consituent of *Foeniculum vulgare* Mill, absence of limonene main active constituent of *Foeniculum vulgare* Mill, *Carum carvi* L and *Anethum graveolens* L and absence of anethole the main active constituent of *Pimpinella anisum* L. It also revealed the presence of essential oil apiole (10.03%) characteristic for *Petroselinum crispum* and piperitone (0.6%) characteristic for *Mentha piperita* which are not characteristic oil constituents for the plants in the herbal tea under investigation.

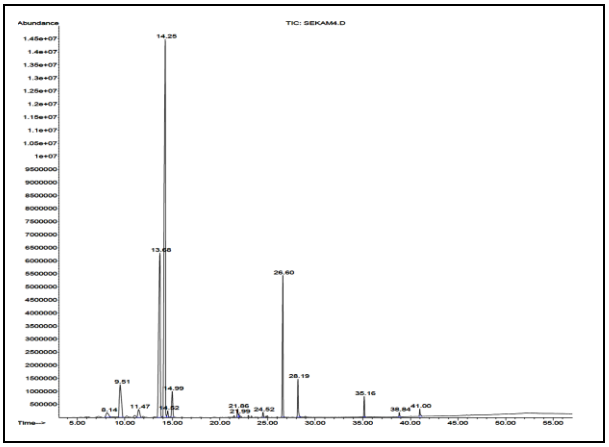

Figure 2. GC/MS analysis of the essential oil of commercial herbal Calm (1).

Analysis of essential oil content in commercial herbal Intestinal (2)

GC/MS analysis of essential oil of Commercial herbal Intestinal (2) (**Figure 3**) revealed the presence of: Linalol (10.75%), trans-α-bergamoten (0.68%), camphor (0.29%), α-cubebene (0.4%), methyleugenol (1.95%), methyl cinnamate (4.83%) and eugenol (4.8%) as a finger print for *Ocimum basilicum*L. leaves, α-caryophyllene (0.45%), Cis-β-farnesene (0.35%), cadinene (0.48%), spathulenol (0.64%), α-bisabolol (0.16%), bisabolone oxide (0.33%), bisabolol oxide A (2.45%) and chamazulene (0.14%) are finger print for *Matricaria chamomilla* l. flowers, δ-elemene (1.55%) as finger print for both *Ocimum basilicum*L. leaves and *Matricaria chamomilla* L. flowers, estragole (24.16%) as a finger print for both *Pimpinella anisum* L. fruits and *Foeniculum vulgare* Mill fruits, anethole (40.33%) as a finger print for *Pimpinella anisum* L. fruits, iso eugenol methyl ester (0.16%) as a finger print for *Pimpinella anisum* L. fruits. The absence of fenchone which is the main active constituent of *Foeniculum vulgare* Mill, absence of all the characteristic active constituent of *Cymbopogon citratus* (citral, nerol, geranicol, citronellal, terpinolene and geranyl acetate)[21]. It also revealed the presence of volatile oil which is apiole (0.13%) characteristic for *Petroselinum crispum* L.

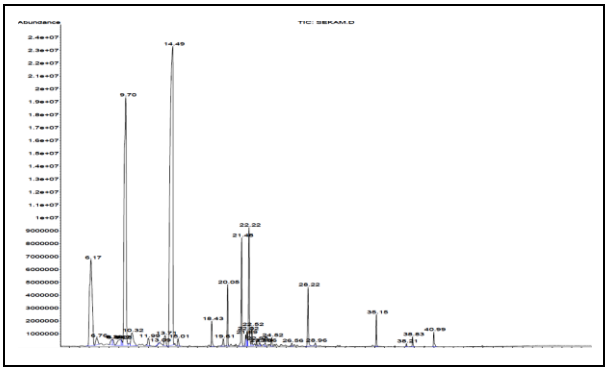

Figure 3. GC/MS analysis of the essential oil of commercial herbal Intestinal (2)

Analysis of essential oil content in commercial herbal Anti diarrhea (3)

GC/MS analysis of essential oil of Commercial herbal Anti diarrhea (3) (**Figure 4**) revealed the presence of: Linalol (0.56%), trans-α-bergamotene (1.46%), γ-muurolene (3.42%), α-cubebene (1.43%), methyl eugenol (3.15%), and methyl cinnamate (4.6%) as finger print for *Ocimum basilicum* L. leaves, β-farnesene (2.88%), bisabolone oxide (2.26%), bisabolol oxide A (11.07%), α- bisabolol (1.19%) , cadinene (2.81%) , spathulenol (2.34%) and chamazulene (3.88%) as finger print for *Matricaria chamomilla* L.flowers. The presence of bornylacetate (1.61%) finger print for *Achillea millefolium* L. flowers, cis carvone (2.45%) finger print for *Achillea ligustica* flowers, thymol (0.62%) finger print for *Achillea santolina* flowers, α-cadinol (0.92%) finger print for *Achillea tenuifolia* flowers and *Achillea millefolium* L. flowers, γ- cadinene (9.84%) as finger print for both *Ocimum basilicum* L. leaves and *Matricaria chamomilla* L. flowers, caryophyllene oxide (1.06%) as finger print for *Achillea millefolium* L. flowers.

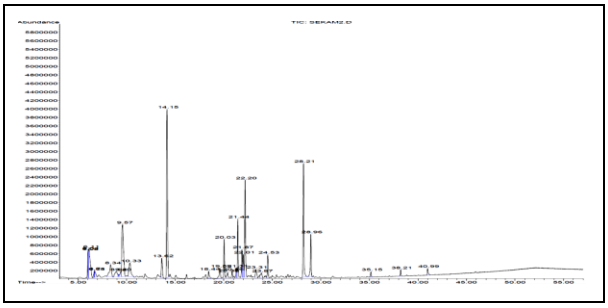

Figure 4. GC/MS analysis of the essential oil of commercial herbal Anti diarrhea (3)

From the previous results we conclude that commercial herbal Calm (1), commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) contain exhausted herbs, also commercial herbal Calm (1) may be adulterated by *Parsely* (apiole) and *Mentha*

Table 6. HPLC results of phenolic compounds present in commercial herbal Intestinal (2) (µg/100g)

Test items	Retention time	Commercial herbal Intestinal (2) (µg/100g)	Plant and part used
Gallic acid	6.959	74.84	
Catechin	8.495	2245.81	
Chlorogenic acid	8.725	9385.14	- *Achillia millefolium* L. leaves
Caffeic acid	10.229	1561.96	
Vanillic acid	11.203	1717.77	
Coumarin	13.955	239.12	- *Matricaria chamomilla* flowers - *Cichorium intybus* L. leaves
Cinnamic acid	15.528	2719.89	

Table 7. HPLC results of phenolic compounds present in commercial herbal Anti diarrhea (3) (µg/100g)

Test items	Retention time	Commercial herbal Anti- diarrhea (3) (µg/100g)	Plant and part used
Gallic acid	6.959	66.63	- *Vitis vinifera* leaves
Protocatechuic acid	8.322	1924.61	- *Hibiscus sabdariffsa* flowers
Catechin	8.495	1364.53	- *Vitis vinifera* leaves
Chlorogenic acid	8.725	11470.40	- *Achillia millefolium* L. leaves
Catechol	9.238	8290.01	- *Vitis vinifera* leaves
Caffeic acid	10.229	1116.29	- *Vitis vinifera* leaves
Vanillic acid	11.203	2100.05	- *Vitis vinifera* leaves
Ferulic acid	11.965	2425.16	
Cinnamic acid	15.528	4222.86	

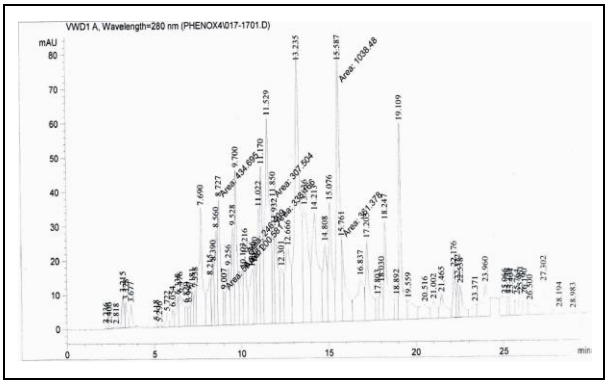

Figure 5. HPLC chromatogram of phenolic compounds in commercial herbal Intestinal (2)

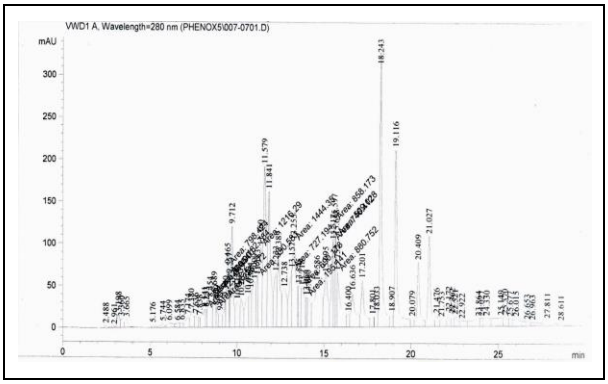

Figure 6. HPLC chromatogram of compounds in commercial herbal Anti diarrhea (3)

(piperitone), moreover commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) contain different species of *Achillea*.

HPLC analysis

HPLC analysis of polyphenolic compounds

It has been found that commercial herbal Intestinal (2) (**Figure 5**) contains chlorogenic acid in concentration (9385.14 µg/100g) which confirms the presence of *Achillia millefolium* L. leaves, and coumarins in concentration (239.12 µg/100g) which are present in both plants *Matricaria chamomilla* flowers and *Cichorium intybus* L. leaves. The presence of gallic acid, catechin, caffeic acid, vanillic acid and cinnamic acid which are not charactristic constituents for any of the herbs under investigation.

HPLC analysis of commercial herbal Anti diarrhea (3) (**Figure 6**) revealed the presence of gallic acid in concentration (66.63 µg/100g), catechin in concentration (1364.54 µg/100g), catechol in concentration (8290.01 µg/100g) and caffeic acid in concentration (1116.29 µg/100g) which are the main active constituents of *Vitis vinifera* leaves. Presence of ferulic acid (2425.16 µg/100g), and cinnamic acid (4222.86 µg/100g) confirms the presence of *Verbascum phlomoides* flowers, presence of vanillic acid (2100.05µg/100g) which is present in both plants *Vitis vinifera* leaves and *Verbascum phlomoides* flowers. Finally, presence of protocatechuic acid (1924.61 µg/100g) which confirms the presence of *Hibiscus sabdariffa* flowers, and chlorogenic acid (11470.40µg/100g) confirms the presence of *Achillia millefolium* L. leaves. The presence of ferulic acid and cinnamic acid which are active constituents of *Verbascum phlomoides.*

HPLC analysis of flavonoids

HPLC analysis of commercial herbal Intestinal (2) (Table 8) revealed the presence of rosmarinic in concentration (39.03mg/100g), rutin in concentration (32.75 mg/100g), quercitrin in concentration (2.76 mg/100g), kampferol (6.14 mg/100g) and apigenin (4.45 mg/100g). Presence of rutin confirms the presence of *Achillea millefolium* L. flowers, quercitrin confirms the presence of *Matricaria chamomilla* L. flowers and presence of apigenin confirms the presence of *Matricaria chamomilla* L. flowers and *Achillea millefolium* L. flowers.

Table 8. HPLC results of flavonoids present in commercial herbal Intestinal (2) (mg/100g)

Test items	Retention time	Commercial herbal Intestinal (2) (mg/100g)	Plant and part used
Rosmarinic	9.286	39.03	
Rutin	10.088	32.75	- *Achillea millefolium* L. flowers
Quercitrin	10.764	2.76	- *Matricaria chamomilla* L. flowers
Kampferol	12.481	6.14	
Apigenin	13.285	4.45	- *Matricaria chamomilla* L. flowers - *Achillea millefolium* L. flowers

Table 9. HPLC results of flavonoids present in commercial herbal Anti diarrhea (3) (mg/100g)

Test items	Retention time	Commercial herbal Anti-diarrhea (3) (mg/100g)	Plant and part used
Rosmarinic	9.286	1.55	
Rutin	10.088	2.66	- *Vitis vinifera* leaves - *Verbascum thapsus* flowers - *Achillea millefolium* L. flowers
Quercitrin	10.764	5.33	- *Hibiscus sabdariffa* flowers
Narenginin	11.596	2.65	- *Cichorium intybus* L. leaves
Hesperidin	11.936	4.97	- *Verbascum thapsus* flowers
Quercitin	11.986	3.56	- *Vitis vinifera* Leaves - *Verbascum thapsus* flowers - *Hibiscus sabdariffa* flowers
Kampferol	12.481	6.36	- *Verbascum thapsus* flowers - *Hibiscus sabdariffa* flowers - *Vitis vinifera* leaves
Apigenin	13.285	4.31	- *Vitis vinifera* leaves - *Verbascum thapsus* flowers - *Achillea millefolium* L. flower

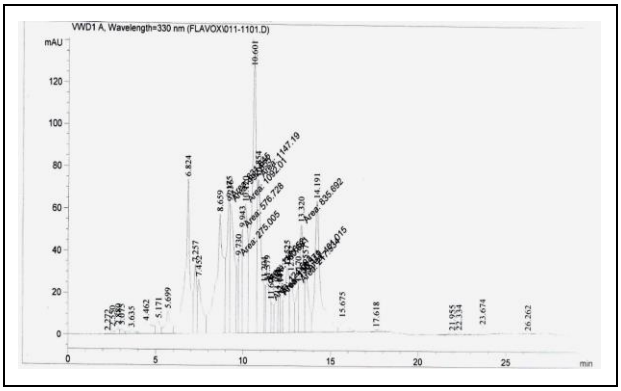

Figure 7. HPLC chromatogram of flavonoids in commercial herbal Intestinal (2)

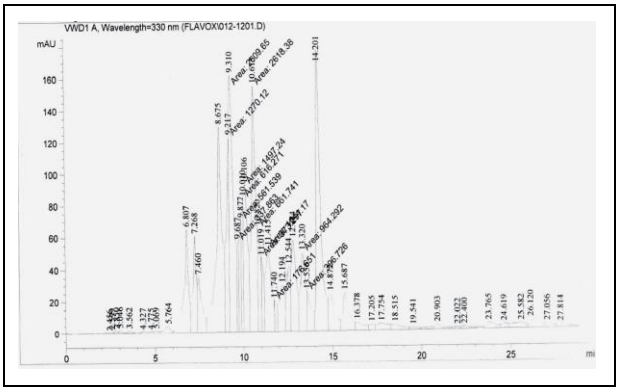

Figure 8. HPLC chromatogram of flavonoids in commercial herbal Anti- diarrhea (3)

HPLC analysis of flavonoid content in Commercial herbal Intestinal (2) (**Figure 7**) revealed presence of rosmarinic acid and Kampferol which are not characteristic constituents to any of the herbs under investigation

HPLC analysis of flavonoid content in Commercial herbal Anti diarrhea (3) (Table 9) revealed presence of rosmarinic acid which is not a characteristic constituents to any of the herbs under investigation. So it is concluded that commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) are adulterated.

Biological study

Antimicrobial study

Commercial herbal Anti diarrhea (3) and standard anti diarrhea did not show any antibacterial activity against *Shigella flexneri*. Commercial herbal Anti-diarrhea (3) proved to be more reactive as antibacterial than standard anti diarrhea against *Escherichia coli* with inhibition zone (14.6 ± 0.25, % of inhibition 64%) versus standard intestinal inhibition zone (11.3 ± 0.25, % of inhibition 49.6%), and *Salmonella typhimurium* with inhibition zone (16.2 ±

Table 10. Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (20mg/ml) concentration extracts

	***Aspergillus fumigatus* Fungi**	***Candida albicans* Fungi**	***Staphylococcus aureus* Gm +ve**	***Bacillus cereus* Gm +ve**	***Clostridium perfringens* Gm +ve**	***Shigella flexneri* Gm -ve**	***Escherichia coli* Gm -ve**	***Salmonella typhimurium* Gm -ve**
Commercial herbal Calm (1)	16.8±0.44 (73.7%)	-ve	17.3±0.2 (59.9%)	17.6±0.25 (59.1%)	15.6±0.63 (85.2%)	14.2±0.25 (73.2%)	14.6±0.44 (64%)	18.3±0.58 (63.5%)
Commercial herbal Intestinal (2)	13.4±0.58 (58.8%)	-ve	15.2±0.58 (52.6%)	16.4±0.44 (55%)	13.6±0.63 (74.3%)	11.6±0.63 (59.8%)	13.6±0.58 (59.6%)	16.7±0.44 (57.9%)
Commercial herbal Anti-diarrhea (3)	-ve	-ve	-ve	-ve	-ve	-ve	14.6±0.25 (64%)	16.2±0.37 (56.3%)
Standard Calm (1)	11.1±0.44 (48.7%)	-ve	13.2±0.37 (45.7%)	16.3±0.58 (54.7%)	11.3±0.63 (61.7%)	12.3±0.44 (63.4%)	14.6±0.25 (64%)	17.2±0.37 (59.7%)
Standard Intestinal (2)	18.3±0.19 (80.3%)	-ve	19.3±0.58 (66.8%)	20.3±0.44 (68.1%)	22.6±0.63 (123.5%)	15.2±0.58 (78.4%)	13.9±0.44 (60.9%)	20.3±0.63 (70.5%)
Standard Anti-diarrhea (3)	-ve	-ve	-ve	-ve	-ve	-ve	11.3±0.25 (49.6%)	13.2±0.37 (45.8%)
Amphotericin B	22.8±0.11 (100%)	20.7±0.22 (100%)	-	-	-	-	-	-
Ampicillin	-	-	28.9±0.14 (100%)	29.8±0.15 (100%)	18.3±0.44 (100%)	-	-	-
Gentamicin	-	-	-	-	-	19.4±0.16 (100%)	22.8±0.22 (100%)	28.8±0.24 (100%)

0.37, % of inhibition 56.3%) versus standard intestinal inhibition zone (13.2 ± 0.37, % of inhibition 45.8%).

The MICS (µg/ml) were performed for the tested samples showed anti-bacterial and antifungal activity against tested organisms using sabouraud agar as culture media for fungi, and Amphotericin B as positive control for fungi, and nutrient agar as culture media for bacteria using Ampicillin as positive control for gram positive bacteria, and Gentamicin as positive control for gram negative bacteria.

The results (Table 10) obtained showed that the MICS of both standard calm (15.63µg/ml) and standard intestinal (3.9µg/ml) against *Aspergillus fumigatus* were less than the MIC obtained in case of Commercial herbal Calm (1) (500µg/ml), and Commercial herbal Intestinal (2) (250µg/ml). The MICS also of both standard calm and standard intestinal against the three gram positive bacterial species, and the three gram negative bacterial species were less than that obtained from Commercial herbal Calm (1) and Commercial herbal Intestinal (2).

From these results we conclude that both standard calm and standard intestinal are more effective in low concentrations than Commercial herbal Calm (1) and Commercial herbal Intestinal (2).

Toxicological studies

Determination of the LD_{50} of the aqueous methanol extract of commercial herbal Calm (1), commercial herbal Intestinal (2), commercial herbal Anti diarrhea (3) and the prepared standard mixtures was estimated according to Karber[11]; Paget and Barnes.[12] LD_{50} for commercial herbal Calm (1), commercial herbal Intestinal (2), commercial herbal Anti diarrhea (3) and the prepared standard mixtures up to 5 gm/kg b.wt. is safe.

Anti-diabetic activity

The anti-diabetic activity of commercial herbal Calm (1), commercial herbal Intestinal (2), commercial herbal Anti diarrhea (3) and the prepared standard mixtures was assessed and the result (Tables 11, 12) revealed that, the most potent one is commercial herbal Calm (1) with percent of change (25.5%, 45.4%) followed by Standard calm with percent of change (25.9%, 41.6%), commercial herbal Anti diarrhea (3) with percent of change (26.4%, 31.5%), Standard anti diarrhea with percent of change (18.6%, 27.1%), Standard intestinal with percent of change (26.1%, 33.2%) and the least potent one is commercial herbal intestinal (2) with percent of change (19.0%, 33.2%).

Commercial herbal Calm (1) showed anti diabetic activity higher than its prepared standard mixture, while the standard intestinal showed anti diabetic activity higher than commercial herbal Intestinal (2) and on the other hand commercial herbal Anti diarrhea (3) showed anti diabetic activity higher than standard anti diarrhea.

Acute anti-inflammatory activity

The anti-inflammatory activity of commercial herbal Calm (1), commercial herbal Intestinal (2), commercial herbal Anti diarrhea (3) and the prepared standard mixtures were done (Table 13) and we have found that the most potent one is standard Calm (1) with

Table 11. Effect of commercial herbal Calm (1), commercial herbal Intestinal (2), commercial herbal Anti diarrhea (3) extracts and metformin drug on blood glucose level in alloxan induced diabetic rats (n=6)

Group Time	Diabetic Non treated	Diabetic Treated with commercial herbal calm (1) (100 mg/kg)		Diabetic Treated with commercial herbal intestinal (2) (100 mg/kg)		Diabetic Treated with commercial herbal anti diarrhea (3) (100 mg/kg)		Diabetic. Treated with Metformin (100 mg/kg)	
	M±S.E	M±S.E	% of change	M±S.E	% of change	M±S.E	% of change	M±S.E	% of change
Zero	259.2±9.4	263.4±8.9	--------	256.4±9.8	------	251.3±8.9	-------	264.5±9.3	---------
2Weeks	261.3±9.7	196.2±7.9*	25.5	207.6±9.2*	19.0	210.2±9.7*	16.4	183.1±6.8*	30.8
4Weeks	266.5±9.8	143.8±6.4*	45.4	173.7±6.9*	32.3	172.1±7.3*	31.5	87.6±4.2*	66.9

** Statistically significant from zero time at P < 0.01*[22]

Table 12. Effect of standard Calm, standard Intestinal, standard Anti-diarrhea extracts and metformin drug on blood glucose level in alloxan induced diabetic rats (n=6)

Group Time	Diabetic Treated with St. calm. (100mg/kg)		Diabetic Treated with St. intestinal (100 mg/kg)		Diabetic Treated with St. anti-diarrhea (100 mg/kg)	
	M±S.E	% of change	M±S.E	% of change	M ± S.E.	% of change
Zero	255.7±8.2	---------	261.2±8.5	---------	248.6±8.8	----------
2Weeks	189.4±7.1	25.9	193.1±7.8	26.1	202.3±8.2*	18.6
4Weeks	149.3±5.8	41.6	174.5±7.2*	33.2	181.2±8.1	27.1

percent of change 60.7%) followed by commercial herbal Calm (1) with percent of change (57.1%), commercial herbal Intestinal (2) with percent of change (53.3%), commercial herbal Anti diarrhea (3) with percent of change (48.3%), St intestinal with percent of change (46.5%) and the least potent one is standard anti diarrhea with percent of change (45.0%).

Standard Calm has the highest anti-inflammatory activity than Commercial herbal Calm (1), while commercial herbal Intestinal (2) and commercial herbal Anti diarrhea (3) showed high anti-inflammatory activity than their corresponding prepared standard mixtures.

Antioxidant activity

Antioxidant activity of commercial herbal (1), (2), (3), prepared standard mixtures extracts and vitamin E drug in male albino rats were done (Table 14). The most potent herbal tea is commercial herbal Calm (1) with percent of change (2.8%) followed by standard calm with percent of change (3.3%), commercial herbal Intestinal (2) with percent of change (11.3%), standard intestinal with percent of change (12.9%), commercial herbal Anti diarrhea (3) with percent of change (13.5%) and the least potent one is standard anti diarrhea with percent of change (16.3%).

Table 13. Acute anti-inflammatory activity of commercial herbal Calm (1), commercial herbal Intestinal (2), commercial herbal Anti diarrhea (3) and the three prepared standard mixtures extract of plant in male albino rats (n=6)

Group	Dose in b.wt. mg/kg	% edema	
		Mean ± S.E.	% of change
Control	1m saline	61.3±1.8	
Commercial herbal Calm (1)	100	26.3±0.4*	57.1
Standard calm	100	24.1±0.6*	60.7
Commercial herbal Intestinal (2)	100	28.6±0.5*	53.3
Standard Intestinal	100	32.8±0.9*	46.5
Commercial herbal Anti diarrhea (3)	100	31.7±1.2*	48.3
Standard Anti diarrhea	100	33.7±1.3*	45.0
Indomethacin	20	21.6±0.4*	64.8

** Statistically significant different form control group at P < 0.01.*

Table 14. Antioxidant activity of commercial herbal Calm (1), commercial herbal Intestinal (2), commercial herbal Anti diarrhea (3) and prepared standard mixtures extracts and vitamin E drug in male albino rats (n=6)

Group	Blood glutathione (mg%)	% change from control
Control (1 ml saline)	36.3 ± 1.4	-
Daibetic	21.4 ± 0.3*	41.1
Diabetic + Vitamin E (7.5 mg/kg)	35.9 ± 1.1	1.1
Diabetic + Commercial herbal Calm (1) (100 mg/kg)	35.3 + 0.9	2.8
Diabetic + Commercial herbal Intestinal (2). (100 mg/kg)	32.2 ± 0.8*	11.3
Diabetic + Commercial herbal Anti diarrhea (3). (100 mg/kg)	31.4 ± 0.6*	13.5
Diabetic + St. calm (100 mg/kg)	35.1 ± 1.2	3.3
Diabetic + St. intestinal (100 mg/kg)	31.6 ± 0.9*	12.9
Diabetic + St. anti-diarrhea (100 mg/kg)	30.4 ± 0.7*	16.3

** Statistically significant different form control group at P< 0.01.*

Commercial herbal Calm (1) showed the highest antioxidant activity in comparison to standard Calm, while Commercial herbal Intestinal (2) and Commercial herbal Anti diarrhea (3) showed also high anti-oxidant activity than their corresponding prepared

standard mixtures, as all compared to standard antioxidant drug.

CONCLUSION

Quality control of the three commercial herbal products revealed that some of the herbs are exhausted; some of the products are adulterated with other species rather than those of the formula; however, the three commercial herbal products are safe, free from aflatoxin, pesticides and heavy metals. They are active as antimicrobial, anti-diabetic, anti-inflammatory and antioxidant.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

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