ABSTRACT

Plant-based health products play, nowadays, an important role in the global drug market. Despite this constant rise in commercial importance, the processing of herbal drugs lacks reliable scientific assessment and is often performed without strict regulations. To guarantee the quality of the final product, well-defined specification criteria should be established for raw materials before manufacture. The quality and correct identity of crude powdered plants or plant organs can be checked via inspection of sensory, microscopical and physicochemical characteristics. Liver disorders represent a threatening health problem in Egypt. Herbs are frequently used as supportive complementary remedies to alleviate these ailments. The objective of this study was to establish reliable criteria for proper identification of three hepatoprotective herbal drugs viz., seeds of *Linum usitatissimum* L. (flax seeds) and *Trigonella foenum-graecum* L. (fenugreek seeds), and leaves of *Rosmarinus officinalis* L. (rosemary leaves). The powdered samples were microscopically examined and subjected to proximate, physicochemical and nutritional analysis. The present work will, thus, provide helpful information on the quality of these herbal materials to ensure genuineness, safety and efficacy prior incorporation in pharmaceutical formulations.

**Key words:** Quality control, flax, fenugreek, rosemary, hepatoprotective.
INTRODUCTION

The global commercial importance of herbal drugs is continuously increasing. Nowadays, plant materials are employed throughout both developed and developing countries as home remedies, over-the-counter drugs, and raw materials in pharmaceutical industries. The processing of plant-based health products is still lacking adequate scientific assessment, and marketing is often performed without mandatory regulations. The validity of phytopharmaceuticals as rational drugs is thus hindered and necessitates the establishment of strict standardization criteria to evaluate their performances, limitations, optimal dosages, contraindications and applications. Since reproducible efficacy and safety of the final product depend primarily on adequate selection of the raw material, this should be performed on the basis of well-defined specific characteristics which guarantee the quality of the crude drug. Examination of sensory, macro-and microscopic characters constitute the first step for checking the identity and purity of powdered drugs. Besides, recent physicochemical and molecular technologies are frequently employed for herbal drugs profiling and standardization prior further processing [1-4].

The liver is the largest gland in the human body; it plays a vital role in glycogen storage, protein synthesis and detoxification, as well as in metabolism of proteins, carbohydrates and fats. Exposure to a wide variety of insults such as alcohol, drugs, chemicals, viruses and bacteria makes the liver one of the most frequently injured organs. Liver disorders are thus common clinical problems which necessitate a safe and effective drug therapy to prevent further cirrhosis and carcinoma [5, 6]. The traditional approach to management of chronic liver diseases is to regulate and support liver functions, in addition to gastrointestinal and immune systems through antioxidant, anti-inflammatory, antiviral and immunomodulatory mechanisms [6].

Focusing our interest on hepatoprotective herbs is of prime importance, particularly that the prevalence of liver disorders constitute a threatening health problem in Egypt [7]. In this respect, three local herbal products viz., seeds of Linum usitatissimum L. (flaxseeds) and Trigonella foenumgraecum L. (fenugreek seeds) and leaves of Rosmarinus officinalis L. (rosemary leaves) were selected and subjected to standardization. Selection was on the basis of their protective action either directly on the liver or through controlling metabolic alterations in the liver associated with other illnesses. Special emphasis was made on
establishment of reliable microscopical and physicochemical criteria which could permit the safe and efficient incorporation of these raw materials in herbal formulations.

Linum usitatissimum L. (flax or linseed, Linaceae), is a widespread food and fiber crop native to the region extending from eastern Mediterranean, through Western Asia and Middle East to India. Flax is reputed as an excellent source of: insoluble and soluble dietary fibres owing to its seed hull polysaccharide content and highly polyunsaturated edible oil rich in linolenic acid; in addition it is the richest dietary source of phytoestrogen and antioxidant lignans [8-14]. The integration of flaxseed or its derived lignans in diet is reported to potentiate hepatoprotection [11, 15]. Trigonella foenumgraecum L. (fenugreek, Fabaceae) is one of the oldest medicinal plants, originating in India and Northern Africa. The seeds mature in long pods and are medicinally consumed as extracts or powders. Fenugreek seeds are commonly consumed as a condiment and lactagogue [16]; moreover, they are a good source of many essential elements such as iron, phosphorus and sulfur [17]. The hepatoprotective and antioxidant effects of fenugreek seeds were reported [18-20] and its extract exhibited immunomodulatory activity in mice [21]. Rosmarinus officinalis L. (rosemary, Lamiaceae) is native to the Mediterranean region. The leaves are commonly used as a flavouring food additive and its extract was proven to exert potent hepatoprotective activity against a variety of hepatotoxic agents [22-26].

This study aimed to provide helpful information on the quality of these herbal materials to ensure genuineness, safety and efficacy prior incorporation in pharmaceutical formulations. In this respect, the powdered samples were microscopically examined and subjected to proximate, physicochemical and nutritional analysis.

MATERIALS AND METHODS

Plant material

Identified and certified samples were utilized in this study. Mature seeds of Linum usitatissimum L. (cultivar Sakha 1, flaxseeds) and of Trigonella foenumgraecum L. (cultivar Giza 30, fenugreek seeds) were purchased, in June and October 2011, respectively, from the Agricultural Research Center (Giza, Egypt). Rosmarinus officinalis L. (rosemary) leaves were collected from herbs growing at the Horticulture Research Institute (Giza, Egypt), during the flowering season, in April 2012. The identity of the plant materials was confirmed and voucher specimens (numbered 19.9.2013.1, 19.9.2013.2 and 19.9.2013.3, respectively)
deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University (Cairo, Egypt).

The individual samples were air-dried, powdered, packed in dark-coloured tightly closed containers and saved for further investigation.

**Sensory and microscopical examination**

Sensory characters (appearance, colour, odour and taste) of the three powdered drugs were recorded followed by microscopical and histochemical examination. A light microscope image analyzer Leica DMLB, connected to Leica JVC digital ½ inch CCD camera and Leica QWin software, Wetzlar, Germany, was used for microscopical inspection.

**Determination of moisture and ash contents**

Proximate analysis of the seeds and leaves was performed by adopting the procedures of the Association of Official Analytical Chemists (A.O.A.C., 2000) [27]. The analytical standards determined included the moisture, total ash, acid- and water-insoluble ashes contents. All values were estimated gravimetrically and expressed as % w/w with respect to the air-dried material.

**Moisture content:** the loss in weight recorded upon heating a known amount of the air-dried powdered sample, at 100 or 105°C till constant weight was taken as a measure of the moisture content.

**Total ash content:** accurately weighed amount of the air-dried powdered sample was introduced in a previously ignited and tarred empty silica crucible. The sample was uniformly spread in the crucible, and then ignited by gradual heating from 500 up to 600°C. The crucible containing the total ash was allowed to cool in a desiccator till constant weight, weighed and the total ash calculated by difference.

**Acid-insoluble ash content:** a weighed amount of the total ash was boiled with dilute HCl, followed by filtration through an ashless filter paper and washing the insoluble residue with dilute HCl. The filter paper containing the residue was then ignited in a tarred silica crucible, processed as for total ash determination and the acid-insoluble ash content deduced.

**Water-insoluble ash content:** gravimetric determination of the water-insoluble ash was performed similarly as for the acid-insoluble ash except that distilled water was used instead
of HCl and that ignition was performed at 450 up to 500°C.

**Determination of crude fibres content**

The crude fibres content was analyzed by means of a Gerhardt Fibretherm Laboratory Instrument FT12, Gerhardt UK Ltd. The analytical procedure relies on boiling an accurately weighed amount of the air-dried powdered sample with sulphuric acid, washing with water till free from acidity, followed by boiling the residue with potassium hydroxide and rinsing with water till free from alkalinity. The non-soluble residue was then dried (at 105°C) weighed and incinerated (at 600°C) until ash is formed. The difference between the ash content and the non-soluble residue in relation to the weight of the initial sample represents the crude fibres content.

**Determination of total carbohydrate content**

The total carbohydrate content was estimated by adopting the phenol-sulfuric acid colourimetric method as described by Dubois et al. 1956 [28]. The powdered plant material was subjected to complete acid hydrolysis (1M H₂SO₄) followed by neutralization with barium carbonate and filtration. An aliquot of the clear neutral hydrolysate was treated with phenol (5%) and H₂SO₄ (96%). The optical density of the yellow-orange colour produced was measured at 490 nm, against a blank, using a Perkin Elmer Lambda 11 UV/VIS Spectrophotometer (Perkin Elmer, Massachusetts, USA).

**Determination of total protein content and amino acid composition**

**Total protein content:** The powdered specimens were digested with H₂SO₄, for nitrogen determination according to Kjeldahl in a Kjeldatherm Gerhardt, laboratory instrument, (Gerhardt Lab. supplies Co., UK). The digested samples were then distilled in presence of NaOH in a Gerhardt Vapodest 50s laboratory instrument (Gerhardt Lab. supplies Co., UK) and the released ammonia measured [27]. The total nitrogen content (% w/w) was determined and total protein deduced.

**Total and free amino acids analysis:** Total amino acids were determined by adopting the method of Doi et al. 1981 [29]. Acid hydrolysis was carried out according to the procedure of Block et al. 1958 [30]. For the amino acid profiling, the air-dried ground samples (100 mg) were hydrolyzed in sealed tubes with 6 N HCl (10 ml, 110°C, 24 h). A definite volume (1 ml) of each of the acid hydrolysates was treated with successive amounts of distilled water and subjected to vacuum distillation at 80°C to remove any residual HCl and water evaporated to
dryness. The residue was dissolved in the loading buffer (2 ml, 6.2M, pH 2.2) followed by filtration (0.45µ membrane). Amounts of free amino acids were determined by using an Automatic Amino Acid Analyzer AAA 400 (Ingos Ltd., Czech Republic) [31]. The analytical conditions adopted were: flow rate, 0.2 ml/min; buffer pressure, 0-50 bar; reagent pressure, 0-150 bar; reactor temperature, 121°C.

**Determination of total lipid content**

Total lipids (free and conjugated with proteins) were determined using a rapid soxhlet extraction system, Gerhardt Soxtherm System (Gerhardt Lab. supplies Co., UK) [27]. Tested samples were first digested with boiling HCl to liberate the conjugated lipids. The digestion solution was filtered and the fat remaining in the filter left to dry, extracted with petroleum ether and the solvent evaporated. The dried residue was weighed and total lipid content (% w/w) calculated.

**Determination of total phenolics**

The percentage of total antioxidant phenolics in the air-dried powdered samples was determined spectrophotometrically by adopting the Folin-Ciocalteu method [36]. Phenolics were extracted with 80% methanol and the absorbance of the blue color produced on treatment of an aliquot amount of the extract with Folin-Ciocalteu reagent and NaHCO₃ (7.5%) was measured at 760 nm (after 15 minutes, at 45°C), against a blank sample. Gallic acid (Sigma-Aldrich Chemicals, Germany) was used to compute the standard curve. Determinations were carried out in triplicates; results were the mean values ± standard deviations and expressed as mg gallic acid equivalents per gram dry extract (mg GAE/g). The total phenolics content (% g/100g dry wt.) was then calculated.

**Determination of macro- and micro-minerals**

Trace elements were estimated by inductively coupled plasma atomic emission spectroscopy. The powdered samples were introduced into pressure vessels and digested in an Advanced Microwave Digestion System (Milestone Inc. Shelton, CT, US) under controlled temperature and pressure. The concentrations of Calcium (Ca), Magnesium (Mg), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn) and Selenium (Se) were determined by means of a Thermo Scientific iCAP 6000 Series Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (Thermo Electron Ltd., Cambridge, UK). Argon gas was used for excitation of the element atoms [32].
Determination of vitamin content

The dietary antioxidant vitamins A, C and E were spectrophotometrically determined in the powdered herbal materials according to published procedures [33-35].

**Vitamin A:** the content of β-carotene (a pro-vitamin A) was estimated according to Nagata and Yamashita 1992 [33]. The dried ethanol extract (100 mg) was vigorously shaken with acetone–hexane mixture (4:6, 10 ml) and filtered. The absorbance of the filtrate was measured at four different wave lengths 453, 505, 645 and 663 nm and the β-carotene content and corresponding Vitamin A calculated as follows:

\[
\text{β-carotene (mg/100 ml)} = 0.216 A_{663} - 1.22 A_{645} - 0.304 A_{505} + 0.452 A_{453}.
\]

Vitamin A (IU/100 g) = (β-carotene concentration)/0.6

**Vitamin C:** extraction of Vitamin C from each powdered sample was carried out with oxalic acid (0.05 M solution, 24 hours, away from light). The extract was filtered and aliquot amount of the filtrate (2.5 ml) transferred to an amber-coloured volumetric flask and treated with oxalic acid (0.05 M solution, 2.5 ml), metaphosphoric acid / acetic acid solution (0.5 ml), sulphuric acid (5% solution, 1 ml) and ammonium molybdate (5% solution, 2 ml). The absorption of the blue colour produced was measured at 760 nm and Vitamin C content in the different samples deduced from a pre-established standard curve [34].

**Vitamin E (α-tocopherol):** the spectrophotometric estimation of Vitamin E was performed by adopting the method of Nasar et al. 2009 [35], based on Emmorie-Engel reaction: tocopherols reduce ferric to ferrous ions, which on addition of α,α'-dipyridyl a red coloured complex is produced. Tocopherols and carotenes were extracted from the powdered herbal materials by means of petroleum ether and the absorbance of the extract measured at 450 nm. A correction was made for carotenoids after addition of FeCl₃. The absorbance of the solution was then read after 90s at 520 nm. The concentration of vitamin E was calculated as follows:

\[
\text{Vitamin E (mg / 100 g)} = \frac{AT - AC}{AS}; \text{ where AT, AC and AS represent the absorbance of test, carotene and standard samples, respectively. The IU of Vitamin E is the biological equivalent of 2/3 mg dl-α-Tocopherol, or of 1 mg of dl-α-tocopherol acetate; thus the concentration of Vitamin E in IU/100 g was deduced from the following equation:}
\]

\[
\text{Vitamin E (IU/100g) = Vitamin E (mg/100g) X 0.67}
\]
RESULTS AND DISCUSSION
The microscopical elements of powdered flaxseeds, fenugreek seeds and rosemary leaves as represented in Figures 1, 2 and 3, respectively, were found in agreement with reported description \cite{2, 37-39}. Results of physicochemical analysis of the three samples including moisture, total ash, acid-insoluble ash, water-insoluble ash and crude fibres contents, as well as total carbohydrates, nitrogen, proteins, amino acids, lipids and phenolics contents are gathered in Table (1); recorded data were the average of three determinations and calculated on dry weight basis. Total amino acid profiles of the different samples are displayed in Table (2), macro- and micro-mineral contents are listed in Table (3) and vitamins A, C and E contents in Table (4).

Sensory and microscopic characteristics
**Powdered flaxseed** is yellowish brown in colour with readily visible dark reddish brown fragments of the testa. It has a characteristic odour and a mucilaginous oily taste. It is characterized microscopically (Figure 1) by the presence of the following:

![Microscopic images of flaxseed](image)

Figure (1): Powdered seeds of *Linum usitatissimum* L. A. mucilaginous epidermis, B. sclerenchymatous cells, C. mat-like structure, D. thickened parenchyma, E. pigment cells, F. oil globules, G. endosperm and cotyledon cells, H. cotyledon cells (zoomed in).
1. Fragments of tabular epidermal cells containing stratified mucilage stained red with ruthenium red (1 A).

2. Fragments showing yellowish-brown longitudinally elongated sclerenchymatous cells (1 B) with pitted, thick and lignified walls, generally appearing crossed by thin walled elongated collapsed cells (hyaline layer) on one side and by rounded somewhat thickened parenchyma on the other (mat-like structure) (1 C).

3. Fragments of rounded somewhat thickened parenchyma from the hypodermis (1 D).

4. Dark Brown fragments showing pigment cells formed of polygonal flattened cells with pitted walls and reddish-brown contents (1 E).

5. Numerous oil globules, stained red with sudan III (1 F).

6. Fragments of the endosperm and cotyledons showing polyhedral cellulosic parenchymatous cells with slightly thick walls which contain oil globules and aleurone grains stained yellow with picric acid (1 G & H).

**Powdered fenugreek seed** is yellowish in colour, with strong characteristic odour and a mucilaginous slightly bitter taste. It is characterized microscopically (Figure 2) by the presence of the following:

1. Fragments of the testa showing palisade-like epidermal cells (2 A) that are radially elongated with thick cellulosic lamellated walls, and a conical-shaped lumen, narrow at the upper extremity and rounded at the base, in surface view, composed of small polygonal cells with thickened pitted walls and lumina narrower at the top (2 B) than the base (2 C).

2. Fragments of the testa showing basket-like cells of the sub-epidermal layer (hypodermis) (2 D). Cells being large, narrow at the upper end, wide at the base and constricted in the middle, with radial walls having bar-like thickenings (side view), and appearing polygonal with bar-like thickenings extending to the upper and lower walls (surface view).

3. Parenchyma of the aleurone layer (2 E) formed of elongated, rectangular cells with slightly thickened walls containing aleurone grains consisting of globoids only.

4. Fragments of endosperm cells which are polygonal to elongated, unevenly thickened and filled with stratified mucilage (2 F) stained blue with methylene blue.

5. Fragments of cotyledons with parenchymatous thin walled elongated cells containing fixed oil and aleurone grains stained yellow with picric acid (2 G).
Figure (2): Powdered seeds of *Trigonella foenum graecum* L. A. palisade like epidermis (side view), B. palisade like epidermis (top view), C. palisade like epidermis (basal view), D. hypodermis (side and surface views), E. aleurone layer, F. endosperm, G. cotyledon cells

**Powdered rosemary leaf** is greyish-green to yellowish-green in colour with an aromatic fragrant odour and spicy, bitter and aromatic taste. It is characterized microscopically (Figure 3) by the presence of:

1. Fragments of lower epidermal cells with straight to sinuous thin walls.
2. Fragments of the upper epidermis (**3 B**) with straight, slightly thickened and pitted walls.
3. Fragments of hypodermis underlying the upper epidermis composed of uneven cells showing thick beaded anticlinal walls (**3 C**).
4. Numerous non-glandular multicellular branched trichomes of the lower epidermis (**3 D**) and unicellular covering trichomes of the upper epidermis and multicellular branched trichome with two branches only (**3 E**).
5. Glandular trichomes with short, unicellular stalks and composed of 8 cells radiating heads (**3 G**) and few others, with unicellular stalks and spherical, unicellular heads (capitate hair) (**3 A**).
6. Fragments of lignified wood fibres with straight or undulating walls having narrow lumina and acute tapering apices (3 H).

7. Fragments showing xylem vessels with spiral, annular, scalariform and reticulate thickenings (3 I).

Figure (3): powdered leaves of *Rosmarinus officinalis* L. A. lower epidermis, B. upper epidermis, C. underlying hypodermis of upper epidermis with glandular labiaceous trichome, Cap. Capitate hair, D. non-glandular multicellular extensively branched trichomes of the lower epidermis, E. multicellular branched trichome with two branches only, F. unicellular nonglandular trichome, G. labiaceous glandular trichomes, H. fibers, I. xylem vessels.

**Proximate composition**

Results recorded in Table (1) revealed that fenugreek seeds have the highest moisture and total carbohydrates contents 7.91 and 5.83 %, respectively. Moreover, they also showed the highest percentage of total nitrogen, total protein, and total and free amino acids contents 6.4, 23.5, 20.54 and 8.63 %, respectively. On the other hand, flaxseeds have the highest crude
fibre and total lipid contents 40.52 and 15.93 %, respectively. In contrast, rosemary leaves were characterized by the highest amounts of phenolics 2.73 % and all types of ashes (9.01, 5.77 and 2.08 % for total, water-insoluble and acid-insoluble ash contents, respectively).

Table (1): Proximate composition, amino acids and phenolic content in flaxseeds, fenugreek seeds and rosemary leaves

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% g/100g in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flaxseeds</td>
</tr>
<tr>
<td>Moisture</td>
<td>5.32</td>
</tr>
<tr>
<td>Total ash</td>
<td>4.44</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.51</td>
</tr>
<tr>
<td>Water insoluble ash</td>
<td>3.66</td>
</tr>
<tr>
<td>Crude fibres</td>
<td>40.52</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>10.12</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>5.8</td>
</tr>
<tr>
<td>Total protein</td>
<td>21.8</td>
</tr>
<tr>
<td>Total amino acids</td>
<td>15.12</td>
</tr>
<tr>
<td>Free amino acids</td>
<td>7.74</td>
</tr>
<tr>
<td>Total lipids</td>
<td>15.93</td>
</tr>
<tr>
<td>Total phenolics</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Amino acids composition

Results documented in Table (2) showed that leucine was the main determined essential amino acid (2.972, 2.290 and 1.2265 g/100 g) in fenugreek seeds, flaxseeds and rosemary leaves, respectively while methionine was the least identified essential amino acid in flax and fenugreek seeds (0.184 and 0.049 g/100 g, respectively) and was totally absent in rosemary leaves. Glutamic acid was the main detected non-essential amino acid (5.587 and 5.476 g/100 g) in flax and fenugreek seeds, respectively, while aspartic acid was the most abundant of this group (2.204 g/100 g) in rosemary leaves. Meanwhile, proline was the non-essential amino acid detected in the lowest amount in both flax seeds (0.047 g/100 g) and rosemary leaves (0.042 g/100 g) being absent in fenugreek seeds.

The importance of the major detected amino acids leucine and glutamic acid is that: leucine acts as a muscle shelter and as fuel; it helps curing of bones, skin, and muscle tissue, and is suggested for recovery from surgery; it lowers raised blood sugar levels. Glutamic acid is vital in the metabolism of sugars and fats and is utilized in healing of ulcers \[39\].
Table (2): The amino acid profile of flaxseeds, fenugreek seeds and rosemary leaves

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Amount g/100 g in</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flaxse</td>
<td>Fenugreek seeds</td>
<td>Rosemary leaves</td>
</tr>
<tr>
<td>Essential amino acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>0.781</td>
<td>0.954</td>
<td>0.281</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.361</td>
<td>1.733</td>
<td>0.619</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.290</td>
<td>2.972</td>
<td>1.265</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.498</td>
<td>2.671</td>
<td>0.825</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.184</td>
<td>0.049</td>
<td>0.000</td>
</tr>
<tr>
<td>Phenyl alanine</td>
<td>0.814</td>
<td>0.802</td>
<td>0.395</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.580</td>
<td>0.532</td>
<td>0.378</td>
</tr>
<tr>
<td>Valine</td>
<td>2.200</td>
<td>1.729</td>
<td>1.011</td>
</tr>
<tr>
<td>Non-essential amino acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine*</td>
<td>2.740</td>
<td>2.751</td>
<td>1.786</td>
</tr>
<tr>
<td>Arginine*</td>
<td>2.146</td>
<td>0.049</td>
<td>0.334</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>3.629</td>
<td>4.788</td>
<td>2.204</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>5.587</td>
<td>5.476</td>
<td>1.499</td>
</tr>
<tr>
<td>Glycine*</td>
<td>4.730</td>
<td>4.077</td>
<td>2.005</td>
</tr>
<tr>
<td>Proline*</td>
<td>0.047</td>
<td>0.000</td>
<td>0.042</td>
</tr>
<tr>
<td>Serine</td>
<td>1.267</td>
<td>1.820</td>
<td>0.466</td>
</tr>
<tr>
<td>Tyrosine*</td>
<td>0.630</td>
<td>0.726</td>
<td>0.475</td>
</tr>
<tr>
<td>Total essential amino acids</td>
<td>9.708</td>
<td>11.442</td>
<td>4.774</td>
</tr>
<tr>
<td>Total non-essential amino acids</td>
<td>20.776</td>
<td>19.687</td>
<td>8.811</td>
</tr>
<tr>
<td>Total determined amino acids</td>
<td>30.484</td>
<td>31.129</td>
<td>13.585</td>
</tr>
</tbody>
</table>

* Amino acids also classified as semi-essential (or conditionally essential) amino acids.

Macro- and micro-minerals content

The concentrations of Calcium (Ca), Magnesium (Mg), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn) and Selenium (Se) were measured are displayed in Table 2. Fenugreek seeds revealed the highest amounts of Ca, Fe, Mn and Cr (1286.6, 83.2, 3.16 and 0.55 mg/100 g, respectively). On the other hand, the highest content of Mg, Zn, Cd and Cu (295.4, 5.87, 1.896 and 1.896 mg/100g, respectively) was observed in flaxseeds. Equivalent amounts of Se (0.9mg/100g) were detected in flaxseeds and rosemary leaves being slightly lower (0.82 mg/100g) in fenugreek seeds.
Table (3): Macro and micro elements in flaxseeds, fenugreek seeds and rosemary leaves

<table>
<thead>
<tr>
<th>Element</th>
<th>% mg /100g in</th>
<th>Flaxseeds</th>
<th>Fenugreek seeds</th>
<th>Rosemary leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td></td>
<td>284.2</td>
<td>1286.6</td>
<td>276.0</td>
</tr>
<tr>
<td>Mg</td>
<td></td>
<td>295.4</td>
<td>197.1</td>
<td>142.3</td>
</tr>
<tr>
<td>Cd</td>
<td></td>
<td>0.052</td>
<td>0.016</td>
<td>0.008</td>
</tr>
<tr>
<td>Cr</td>
<td></td>
<td>0.346</td>
<td>0.55</td>
<td>0.19</td>
</tr>
<tr>
<td>Cu</td>
<td></td>
<td>1.896</td>
<td>1.426</td>
<td>1.442</td>
</tr>
<tr>
<td>Fe</td>
<td></td>
<td>61</td>
<td>83.2</td>
<td>10</td>
</tr>
<tr>
<td>Mn</td>
<td></td>
<td>1.74</td>
<td>3.16</td>
<td>2.05</td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td>5.87</td>
<td>4.79</td>
<td>3.26</td>
</tr>
<tr>
<td>Se</td>
<td></td>
<td>0.9</td>
<td>0.82</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Vitamins A, C and E contents
Rosemary leaves appeared the richest material in all tested vitamins (Vitamins A, C and E; 2863.68 IU/100 g, 42.37 mg/100 g and 2142.65 IU/100 g, respectively). The lowest amounts of vitamins C and E were found in flaxseeds (20.56 mg/100 g and 1122.08 IU/100 g, respectively) and the least vitamin A content was detected in fenugreek seeds (971.7 IU/100 g).

Table (4): The vitamin content of flaxseeds, fenugreek seeds and rosemary leaves

<table>
<thead>
<tr>
<th>Sample Vitamin</th>
<th>Vitamin A (IU/100 g)</th>
<th>Vitamin C (mg/100 g)</th>
<th>Vitamin E (IU/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flaxseeds</td>
<td>Fenugreek seeds</td>
<td>Rosemary leaves</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>1015.97</td>
<td>971.7</td>
<td>2863.68</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>20.56</td>
<td>26.55</td>
<td>42.37</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1122.08</td>
<td>1653.26</td>
<td>2142.65</td>
</tr>
</tbody>
</table>

CONCLUSION
Consumers of natural remedies are continuously increasing worldwide. This necessitates strict regulatory measures during processing of herbal raw materials prior manufacture and launching of final products in the market. The sensory, microscopic, physicochemical and nutritive characteristics, established in this study for flax and fenugreek seeds and rosemary
leaves could be considered as reliable scientific criteria for identity and purity. These data supported by comprehensive chemical and biological profiling will allow implementation of these hepatoprotective botanicals in pharmaceutical industries.

REFERENCES