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Authentication of various commercially available crude drugs using different quality control testing parameters

Kaneez Fatima[1]*, Shaukat Mahmud[1], Hina Yasin[2], Rana Asif[1], Kiran Qadeer[3] and Iqbal Ahmad[3]

[1]Department. of Pharmacognosy, Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan
[2]Department. of Pharmacognosy, Faculty of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[3]Department. of Pharmaceutical Chemistry, Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan

Abstract: The object of this study is to investigate the quality of various plant materials used in the preparation of herbal formulations using different methods of standardization to confirm their purity, safety and efficacy. However, it is uncertain whether these raw materials comply with the standards prescribed in the pharmacopeias. In the present study six raw materials' i.e. *Foeniculum vulgarae, Curcuma longa, Aloe barbadensis, Plantago ovata, Zingiber officinale* and *Glycyrrhiza glabra* have been obtained from the market and various quality control tests including microscopic evaluation, physico-chemical characteristics, thin layer chromatography (TLC), spectrophotometric assay (British Pharmacopoeia) and Fourier transform infrared spectroscopy (FTIR) have been performed to determine their compliance with the standards. The TLC has been used for the identification of the active ingredients on comparison of their R_f values with the reference standard. FTIR Spectra of these materials have been obtained to assign the functional groups present in the components of a particular material. Although these findings provide a significant data to herbal drug manufacturers for authentication of commercially available plant materials used in various herbal formulation.

Keywords: *Plant materials, Powder microscopy,* TLC, FTIR, Quality control tests.

INTRODUCTION

The herbal system of medicine is widespread in India, Pakistan and Bangladesh and the Hakeems practicing this system are qualified from recognized Institutions of Eastern Medicine. It is also gaining importance in other developing countries as well as in Europe and North America. This system of medicine can play an important role in our national health care program (Bisset, 1994; Roberts and Tyler, 1997; Barnes *et al*., 2007; Andreescu, 2008; Der Mardersian and Riedlinger 2006) In view of the absence or relatively low side effects of herbal formulations, people are more inclined to use these formulations to get relief from various (Anubhuti *et al*., 2011; Bhutani, 2003). It is very important to ensure the quality of herbal medicines and to carry out quality control tests on the plant material used in herbal formulations. The efficacy, safety, purity, and quality of herbal formulations must be ensured before their use to achieve optimum benefits in the treatment of diseases. A very important element in the standardization of herbal formulations is the assessment of the quality of plant materials used in their manufacturing (Burke *et al*., 2011). If the material meets certain standards, the formulation would be of the desired quality and thus beneficial to the patient (British Pharmacopeia 2016, WHO, 1998; EMEA, 2005; Ahmad and Usmanghani, 2003; Capassu *et al*., 2000; Kunle, 2012).

Quality control tests and specifications for the assessment of identity, purity and potency of several plant materials are available in the herbal and allopathic drug pharmacopoeias (e.g. British Pharmacopoeia, Indian Herbal Pharmacopoeia, Chinese Herbal Pharmacopoeia and other guidelines WHO, USP, EMEA, 2005). It is therefore, necessary to confirm their compliance with the prescribed standards. The quality of the Plant raw material is reflected in the quality of the herbal formulations incorporating them as essential ingredients.

The standardization and quality control of herbal medicines involves several steps though the resources of the raw material play a key role in assuring the quality and stability of herbal preparations (Shrikumar *et al*., 2006). Thus, the plant raw materials have been standardized by using commercially available standard in various techniques like thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), ultraviolet/visible spectrometry (UV/Vis), infrared-spectrometry (IR), nuclear magnetic resonance (NMR) spectrometry, mass spectrometry (MS) etc. These techniques alone or in combination, can be successfully used to conduct the tests required for the standardization and control of the quality of both the raw materials and the finished herbal products (Zang, 2012; Bele, 2011; Gaedeke, 2003; Mukherjee, 2002).

Standardization has been defined by American Herbal Product Association as "The body of information and control necessary to produce material of reasonable consistency". This can be achieved through minimizing

**Corresponding author:* e-mail: kaneezfatima@baqai.edu.pk

the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes (Waldesch *et al.*, 2003; Srivastava *et al.*, 2009).

Methods of standardization should encompass all aspects to confirm the quality of the herbal drugs including the exact identification of the sample, macroscopic and microscopic test, ash values (for determining the inorganic content) and extractive values (water soluble extractives, ethanol-soluble extractives, ethyl acetate-soluble extractives, ether-soluble extractives for the estimation of active constituents of their relevant nature) phytochemical evaluation (test for sugar, protein, alkaloids, tannins etc) volatility, tests for xenobiotics, microbial evaluation, toxicity index and biological evaluation (Houghton, 1998; Panchawat and Rathor, 2003). In these tests, the phytochemical profile is of special significance provide a data for primary constituents (sugar, protein) and secondary constituents (alkaloid, tannins, resins) of a plant raw material which directly affect the activity of the herbal drugs. The IR fingerprint profiles serve as a reference to the phytochemical profile of the drug in ensuring the quality, while quantification of the marker compounds would serve as an additional parameter in assessing the quality of the sample (Calixto, 2000; Patil and Rajani, 2010). Phytochemical standardization encompasses all possible information generated with regard to the chemical constituents such as sugar, protein, volatile oil, alkaloids, resins, tannins etc present in a herbal drug. The phytochemical evaluation for standardization purpose (Baquar, 1998) includes the following:

1. Preliminary testing for the presence of different chemical group such as carbohydrate, amino acids, volatile oil, alkaloids, resins, tannins, phenols, terpenes.
2. Quantification of chemical groups of interest (e.g., total alkaloids, total phenolics, total triterpenic acids, total tannins).
3. Establishment of fingerprint profiles.
4. Multiple marker-based fingerprint profiles.
5. Quantification of important chemical constituents.

In addition, the study of various parameters such as pharmacodynamics, pharmacokinetics, dosage, stability, shelf-life, toxicity evaluation and chemical profiling of the herbal formulations is considered essential (Patel and Saluja, 2002; Mosihuzzaman and Choudhary, 2008). The evaluation of heavy metals contamination, according to Good Agricultural Practices (GAP), in herbal drug standardization is equally important (Bauer, 1998).

MATERIALS AND METHODS

Collection and identification of crude drugs

Six commercially available crude drugs were collected from the local market of Karachi that included *Foeniculum vulgarae, Curcuma longa, Aloe barbadensis, Plantago ovata, Zingiber officinale and Glycyrrhiza glabra*. These Plant materials were sampled in the range of 3-5 according to their availability. The Samples of the Plant material were coded as: *Foeniculum vulgarae* (F1-F5), *Curcuma longa* (T1-T5), *Aloe barbadensis* (A1-A3), *Plantago ovata* (I1-I5), *Zingiber officinale* (G-1-G-3) *and Glycyrrhiza glabra* (L1-L5). All these samples were authenticated by the expert.

The authentic reference standards of the constituents of the crude drugs were obtained from Sigma Aldrich Co.

Evaluation of crude drugs

The Plant materials were subjected to various quality control tests mentioned in the British Pharmacopoeia (BP2016) to conform their authenticity. The techniques used for the evaluation of crude drugs included microscopic evaluation, physicochemical parameters (foreign matter, Moisture content, ash value, solubility, fluorescent test, and swelling index), thin layer chromatography (TLC), Ultravoilet and visible spectrophotometric analysis (Double beam spectrophotometer) and Fourier transformer infrared spectroscopy (FTIR).

Ultravoilet and visible spectrophotometery

1. 0.1g of the powdered drug was dissolved in 60ml of glacial acetic acid.
2. The solution was placed in a water-bath at 90°C for 60 min. 2.0g of boric acid and 2.0g of oxalic acid were added and then again placed in a water-bath at 90°C for 10 min.
3. It was allowed to cool, and then diluted to 100.0ml with glacial acetic acid and mixed it well.
4. 5.0ml of the clear supernatant was taken and diluted to 50.0ml with glacial acetic acid.
5. The absorbance of the solution was determined at 530 nm using glacial acetic acid as the compensation liquid (British pharmacopeia 2013)

Fourier transform infrared spectroscopy (FTIR)

Ten milligram of the dried extract powder wasused to prepare a 100mg KBr disc. The disc was placed in the FTIR spectrometer (Shimadzu, IR Affinity 1, Japan),and th spectrum obtained in the range of 400 to 4000cm^{-1} with a resolution of 4cm^{-1} (Ashokkumar and Ramaswamy 2014)

RESULTS

Various quality control tests performed to examine the authenticity of different commercially available crude drugs according to BP (2016) specifications are:

Microscopic evaluation

Foeniculum vulgarae

The images of microscopy of fennel powder were recorded with the help of a camera mounted on the

microscope. The following features were observed as shown in fig. 1. The characteristic feature of the microscopic examination of different drug are given in table 1.

Table 1: Characteristics feature of powder microscopy *Foeniculum vulgarae*

A = Mesocarpal cells
B = Tannins
C = Endocarp
D = fragments of the endocarp, in surface view
E = Fibers
F = endosperm fragments (Fa) small calcium oxalate cluster crystals (Fb) aleurone grains
G = fiber bundles from the carpophores consistency is paramount
H = numerous fiber bundles Ha from the ridges, (Hb) often accompanied by narrow spiral vessels
I = Pitted Parenchyma
J = Oil Droplets

Curcuma longa

The microscopic features of turmeric powder are shown in fig. 2 and given in table 2.

Table 2: Characteristics feature of powder microscopy *Curcuma longa*

A = parenchyma with orange- yellow or yellowish-brown secretory cells
B = starch granules
C = xylem vessels
D = reticulate vessels
E = parenchyma
F = cork cells
G = fibre
H = trichome
I = Single xylem vessel

Plantago ovata

The images of microscopy of husk powder are shown in fig. 3. The characteristic feature of a microscopic examination of husk powder are given in table 3.

Table 3: Characteristics feature of powder microscopy *Plantago ovata*

A = Fragments of the episperm with polygonal cells filled with mucilage
B = Epidermal cells of the testa in surface view
C = Epidermis of the testa in surface view
D = Part of a cotyledon in sectional view
E = Layers of the cotyledons in surface view
F = pitted vessels
G = Part of the radicle of the embryo.

Zingiber officinale

The microscopic images of the sample of *Zingiber officinale* are shown in fig. 4 and the characteristic features of ginger powder are given in table 4.

Table 4: Characteristics feature of powder microscopy *Zingiber officinale*

A = abundant starch granules, simple, flattened, oblong or oval or irregular with a small point hilum situated at the narrower end.
B,C = abundant reticulate vessels, fairly large, isolated
D = fragments of brown cork, usually seen in surface view
E =fragments of brown cork, in transverse section
F =thin-walled parenchyma of the ground tissue
G = fragments containing (Ga) vessels with reticulate thickening (Gb) often accompanied by narrow, thin-walled cells containing brown pigment and (Gc) amyliferous parenchyma.
H = agglomerated starch granules
I = groups of large, thin-walled, septate fibres, with one wall frequently dentate

Glycyrrhiza glabra

The microscopic images of the sample *Glycyrrhiza glabra* are shown in fig. 5. The characteristic microscopic features of liquorice powder are given in table 5.

Table 5: Characteristics feature of powder microscopy *Glycyrrhiza glabra*

A = Part of a group of smaller vessels with bordered
B = Fragment of a large vessel with elongated pits
C = part of medulary ray in radial longitudinal pits
D = Starch granules
E = Part of a group of fibers with incomplete calcium oxalate prism sheath
F = Cork in surface view
G = Layers of cotyledons in surface view H = part of cork and cortex in sectional view I = Reticulate vessels J = fragments of parenchymatous tissue along with vessels

Physicochemical tests

All the crude drugs were subjected to different physicochemical tests and the results obtained are presented in table 6.

Foreign matter

The percent foreign matter of *Foeniculum vulgarae* samples are between 0.123-0.771 and *Plantago ovate* samples are between 0.26-1.07.

Moisture content

The moisture content of fennel sample and ginger samples (table 6) was found to be in the range of 5.38 - 6.23%. and 6.0-8.6%. The level of moisture for fennel and ginger is not reported in BP (2016). However, moisture content of turmeric, aloevera, liquorice and husk samples was found to be in the range of 6.91- 8.72%, 3.54-4.83%, 7.42-8.95% and 6.34 - 9.06% respectively (table 6). Thus, the moisture content of all samples was found to be in the range of maximum limit of 12.0% reported in BP (2016). The low content of moisture is good for the quality of material.

Table 6: Physicochemical Parameters of the selected samples of *Foeniculum vulgarae, Curcuma longa, Aloe barbadensis, Plantago ovata, Zingiber officinale and Glycyrrhiza glabra*

<table>
<tr><td>Samples</td><td colspan="5">Physicochemical Parameters</td></tr>
<tr><td>Foeniculum vulgarae</td><td colspan="2">Foreign matter</td><td colspan="2">MC (Moisture Content) $^{+}$ 1</td><td>Total ash</td></tr>
<tr><td>F1</td><td colspan="2">0.165</td><td colspan="2">5.57</td><td>7.40</td></tr>
<tr><td>F2</td><td colspan="2">0.771</td><td colspan="2">5.38</td><td>6.89</td></tr>
<tr><td>F3</td><td colspan="2">0.123</td><td colspan="2">5.30</td><td>6.93</td></tr>
<tr><td>F4</td><td colspan="2">0.331</td><td colspan="2">6.23</td><td>7.49</td></tr>
<tr><td>F5</td><td colspan="2">0.221</td><td colspan="2">5.46</td><td>7.91</td></tr>
<tr><td>Curcuma longa</td><td colspan="3">MC^{+} 0.5</td><td colspan="2">Ash value</td></tr>
<tr><td>T1</td><td colspan="3">7.39</td><td colspan="2">6.33</td></tr>
<tr><td>T2</td><td colspan="3">7.72</td><td colspan="2">6.47</td></tr>
<tr><td>T3</td><td colspan="3">8.72</td><td colspan="2">6.09</td></tr>
<tr><td>T4</td><td colspan="3">6.91</td><td colspan="2">7.77</td></tr>
<tr><td>T5</td><td colspan="3">7.56</td><td colspan="2">5.40</td></tr>
<tr><td rowspan="2">Aloe barbadensis</td><td colspan="2">Solubility</td><td rowspan="2">Fluorescent test</td><td rowspan="2">Moisture Content</td><td rowspan="2">Ash value</td></tr>
<tr><td>Water</td><td>Ethanol</td></tr>
<tr><td>A-1</td><td>Partly soluble</td><td>Soluble</td><td>++</td><td>3.54</td><td>11.0</td></tr>
<tr><td>A-2</td><td>Slightly soluble</td><td>Soluble</td><td>++</td><td>4.75</td><td>9.50</td></tr>
<tr><td>A-3</td><td>Slightly soluble</td><td>Soluble</td><td>+++</td><td>4.83</td><td>25.1</td></tr>
<tr><td>Plantago ovata</td><td>Foreign matter</td><td>MC</td><td colspan="2">Ash value</td><td>Swelling index</td></tr>
<tr><td>I-1</td><td>1.06</td><td>6.34</td><td colspan="2">5.11</td><td>10.1</td></tr>
<tr><td>I-2</td><td>0.26</td><td>8.11</td><td colspan="2">2.55</td><td>50</td></tr>
<tr><td>I-3</td><td>0.56</td><td>9.06</td><td colspan="2">1.66</td><td>60</td></tr>
<tr><td>I-4</td><td>1.07</td><td>6.45</td><td colspan="2">2.70</td><td>10</td></tr>
<tr><td>I-5</td><td>0.54</td><td>7.85</td><td colspan="2">1.12</td><td>10</td></tr>
<tr><td>Zingiber officinale</td><td colspan="2">MC</td><td colspan="3">Ash value</td></tr>
<tr><td>G1</td><td colspan="2">6.0</td><td colspan="3">7.6</td></tr>
<tr><td>G2</td><td colspan="2">6.5</td><td colspan="3">5.7</td></tr>
<tr><td>G3</td><td colspan="2">8.6</td><td colspan="3">3.0</td></tr>
<tr><td>G4</td><td colspan="2">6.2</td><td colspan="3">5.9</td></tr>
<tr><td>G5</td><td colspan="2">6.5</td><td colspan="3">4.5</td></tr>
<tr><td>Glycyrrhiza glabra</td><td colspan="2">M C</td><td colspan="3">Ash value</td></tr>
<tr><td>L1</td><td colspan="2">7.42</td><td colspan="3">5.3</td></tr>
<tr><td>L2</td><td colspan="2">7.99</td><td colspan="3">5.3</td></tr>
<tr><td>L3</td><td colspan="2">8.95</td><td colspan="3">4.7</td></tr>
<tr><td>L4</td><td colspan="2">7.68</td><td colspan="3">13</td></tr>
<tr><td>L5</td><td colspan="2">6.99</td><td colspan="3">2.6</td></tr>
</table>

(+) = intensity of fluorescent behavior, (-) = absence of fluorescent behavior.

Ash content

The Ash content is one of the important physicochemical parameter to assess the quality of drugs such as fennel ginger, turmeric, has been found in the range of 6.89-7.91, 3.0-7.6, 5.40-7.77% respectively (table 6). All the samples comply with the BP limit of 10.0. 6.0, 8.0 percent respectively. It indicates that all the samples comply with the BP standard and have ash values within the specified limit of inorganic matter. However, the ash content in aloevera sample was found to be in the range of 9.5-25.1%. These values do not comply with the BP limit of 2.0%.

Fluorescent test

All the samples of alovera are soluble in ethanol and partly soluble in water as mentioned in BP (2016). The fluorescent behavior of aloevera samples is reported in table 6. All the samples of aloevera (A-1 to A-3) shows a yellowish green fluorescence under UV light at 365nm with varying intensity represented by (+) such as sample A-3 is more fluorescent as compared to A-1 and A-2.

Swelling index

The swelling index of husk sample is given in table 6. According to BP limit (Minimum 40) three of the samples, i.e. I-1, I-4 and I-5 that is 10.1, 10, 10 respectively not comply with the standard and this may be due to some adulteration in the sample. While sample I-2 and I-3 were 50 and 60 are up to the pharmacopoeial standard.

***Thin layer chromatography* (TLC)**

TLC studies revealed the presence of anethole in the samples of fennel (F-1 to F-5) with a R_f value 0.79 that corresponds to the standard spot of anethole (fig. 6). The samples of turmeric (T-1, to T-5) were chromatographed

on TLC plates along with the standard curcumin, demethoxy curcumin (DMC) and bisdemethoxy curcumin (BDMC). The R_f values of these compounds are given in table 7. All the samples showed the spots of Curcumin, DMC, and BDMC that corresponded to the standard spots as mentioned in BP (2016). The R_f value of standard curcumin is 0.71 while those of the test samples are between 0.70-0.72 which is within the range of the standard. The slight deviation in R_f may be due to high water content and any variation in the solvent system. Similarly the standard DMC and BDMC have R_f value 0.53 and 0.42 whereas the R_f value of test samples for DMC is 0.59-0.63 and BDMC is 0.48-0.50 (fig. 7)

All the samples of alovera showed the spot of barbaloin corresponding to the standard spot as shown in fig. 8. The R_f value is found to be 0.80. Detection of barbaloin was performed at 340nm. Plate was scanned with Camag TLC scanner -III. The three dimensional pattern of the standard and test sample used revealed the presence of super imposable spots with identical R_f values of 0.80 for both the standard and the test samples.

Table 7: Qualitative determination of the selected samples of *Foeniculum vulgarae, Curcuma longa, Aloe barbadensis, Plantago ovata, Zingiber officinale and Glycyrrhiza glabra by TLC technique.*

Samples	R_f value		
Foeniculum vulgarae	Anethole		
Standard	0.79		
F1	0.79		
F2	0.79		
F3	0.79		
F4	0.79		
F5	0.79		
Curcuma longa	(Curcumin)	(DMC)	(BDMC)
Standard	0.71	0.53	0.42
T1	0.72	0.63	0.50
T2	0.73	0.62	0.50
T3	0.70	0.59	0.48
T4	0.71	0.61	0.50
T5	0.71	0.61	0.50
Aloe barbadensis	Barbaloin		
Standard	0.80		
A1	0.80		
A2	0.80		
A3	0.80		
Plantago ovata	Xylose	Galactose	
Standard-1(Xylose)	0.27	---	
Standard-2 (Galactose)	---	0.48	
I-1	0.27	0.50	
I-2	0.32	0.53	
I-3	0.31	0.51	
I-4	no spot	0.52	
I-5	no spot	0.52	
Zingiber officinale	Standard (Resorcinol)	Gingerol-1	Gingerol-2
---	0.56		
G1		0.55	0.54
G2		0.55	0.54
G3		0.55	0.54
G4		0.57	0.56
G5		0.56	0.55
Glycyrrhiza glabra	Standard (Glycerrihitic acid)	R_f value	
---	0.84		
L1		0.83	
L2		0.83	
L3		0.84	
L4		0.84	
L5		0.84	

Table 8: Spectrophotometric assay of *Curcuma longa* and *Aloe barbadensis*

Curcuma longa	Absorbance At 530 nm	Percent content(curcumin)
T1	0.420	1.78
T2	0.381	1.62
T3	0.467	1.98
T4	0.410	1.74
T5	0.439	1.87
Aloe barbadensis	Absorbance At 512 nm	Percent content (barbaloin)
A1	0.463	30.26
A2	0.451	29.51
A3	0.472	30.88

Table 9: FTIR spectral assignments of *Foeniculum vulgarae, Curcuma longa, Aloe barbadensis, Plantago ovata, Zingiber officinale* and *Glycyrrhiza glabra.*

Name of Plant	Absorption Frequency (cm^{-1})	Types of Absorption	Interference
Foeniculum vulgarae	2900	Stretching	Alkanes(CH_3, CH_2, CH)
	2380	Mid, sharp	Phosphorous present
	1740	Stretching	Aldehyde present
	1550	Stretching	Nitro compound present
	1270	Stretching	Aromatic amine may be present
	1050	Stretching	Aliphatic amine may be present
Curcuma longa	2400	Stretching Mid, sharp	Phosphorous present
	1680	Stretching	Alkenes present
	1550	Stretching	Nitro compound present
	1300	Stretching	Alkyl halides present
	1000	Stretching	Carboxylic, alcohol, ester may be present
Aloe barbadensis	3300	Stretching	Amine (N-H) group present (characteristic of amino acid)
	2850	Stretching	Alkane present (CH)
	1655	Stretching	Carbonyl group present C=O
	1610	C=C Stretching	Indicating the presence of Vinyl ether and Aloin compound
	1320	symmetric stretching	aromatic nitro compounds NO2
	1240	stretching vibrations	C-O groups of esters and phenols
	1100	Stretching vibrations	*C = S* present
	780	Stretching	*C-H out of plane deformation*
Plantago ovata	2400	Stretching	Phosphorous may be present
	1710	Stretching	aldehydes present
	1550	Stretching	Nitro compound present
	1000	Stretching	alkenes may be present
Zingiber officinale	2900	Stretching	Alkanes(CH_3, CH_2, CH)
	2380	Mid, sharp	Phosphorous present
	1500	Stretching	Aromatic ring may be present
	1180	Bending	Alkene may be present
	1000	Stretching	Carboxylic group may be present
Glycyrrhiza glabra	1650	Stretching	Primary amine present
	1550	Stretching	Nitro compound present
	14500	Stretching	Aromatic ring may be present
	1240	Stretching	Aromatic amine present
	1050	Stretching	Carboxylic group may be present

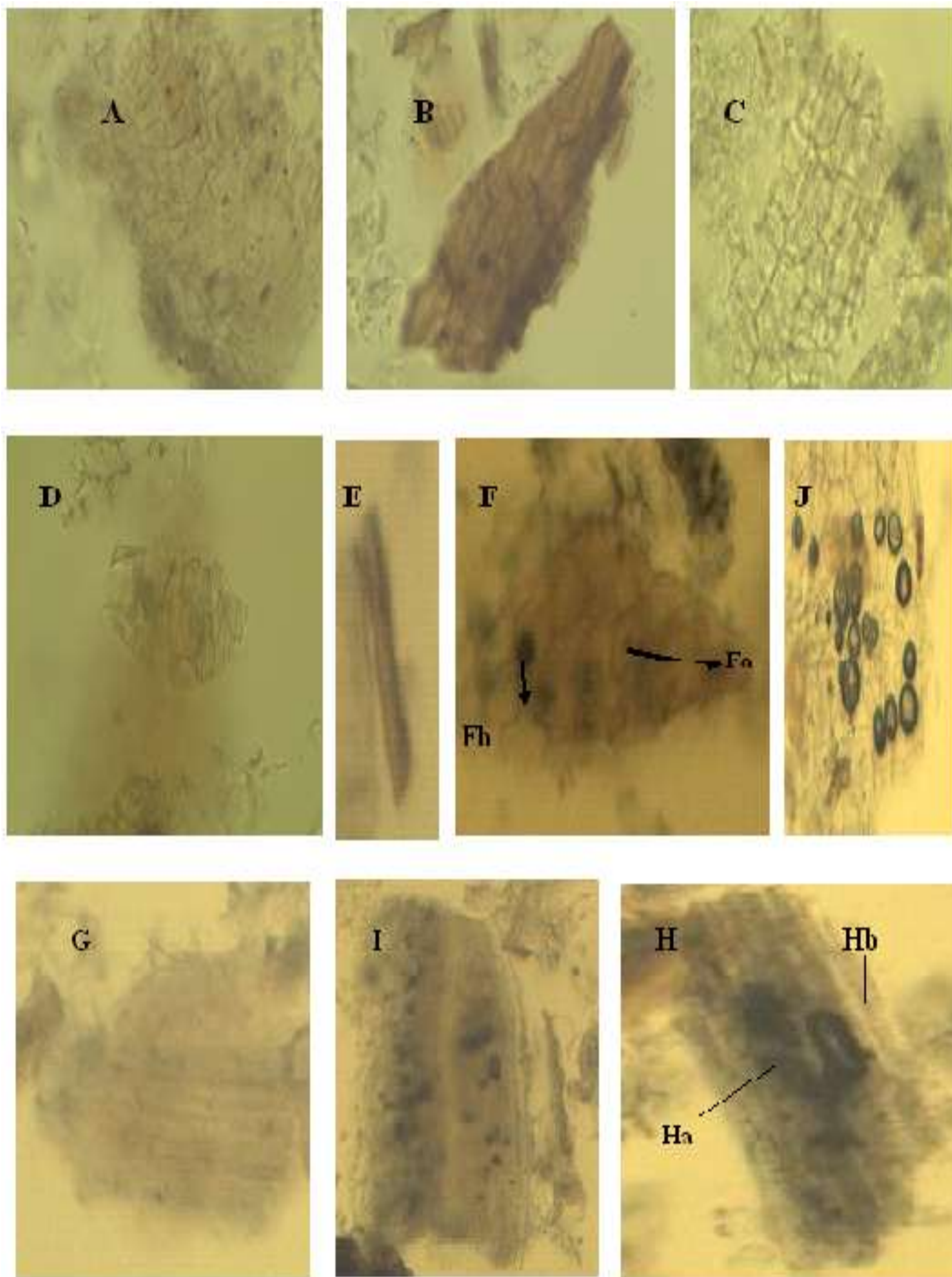

Fig. 1: Powder Microscopy of *Foeniculim vulgarae*

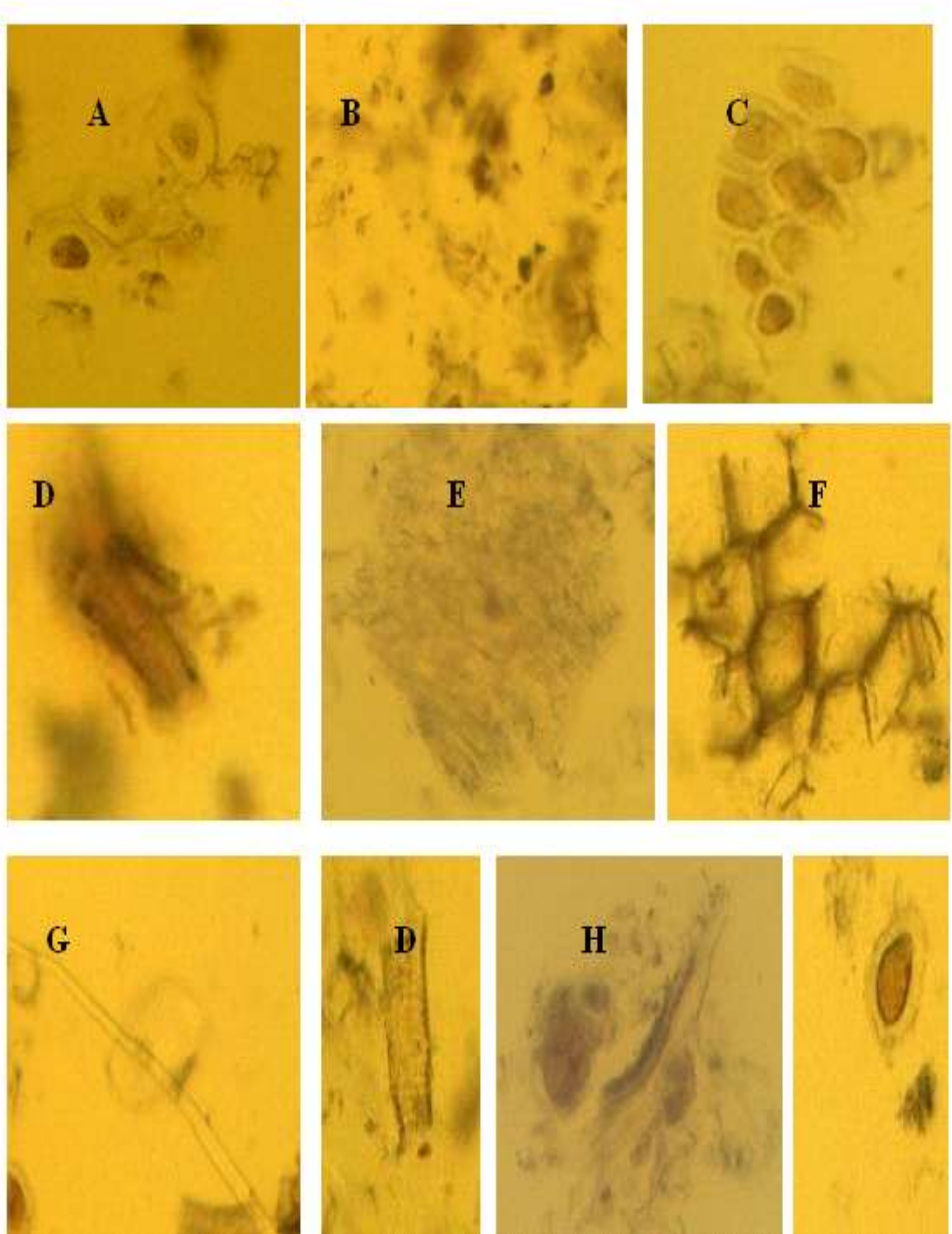

Fig. 2: Powder microscopy of *Curcuma longa*

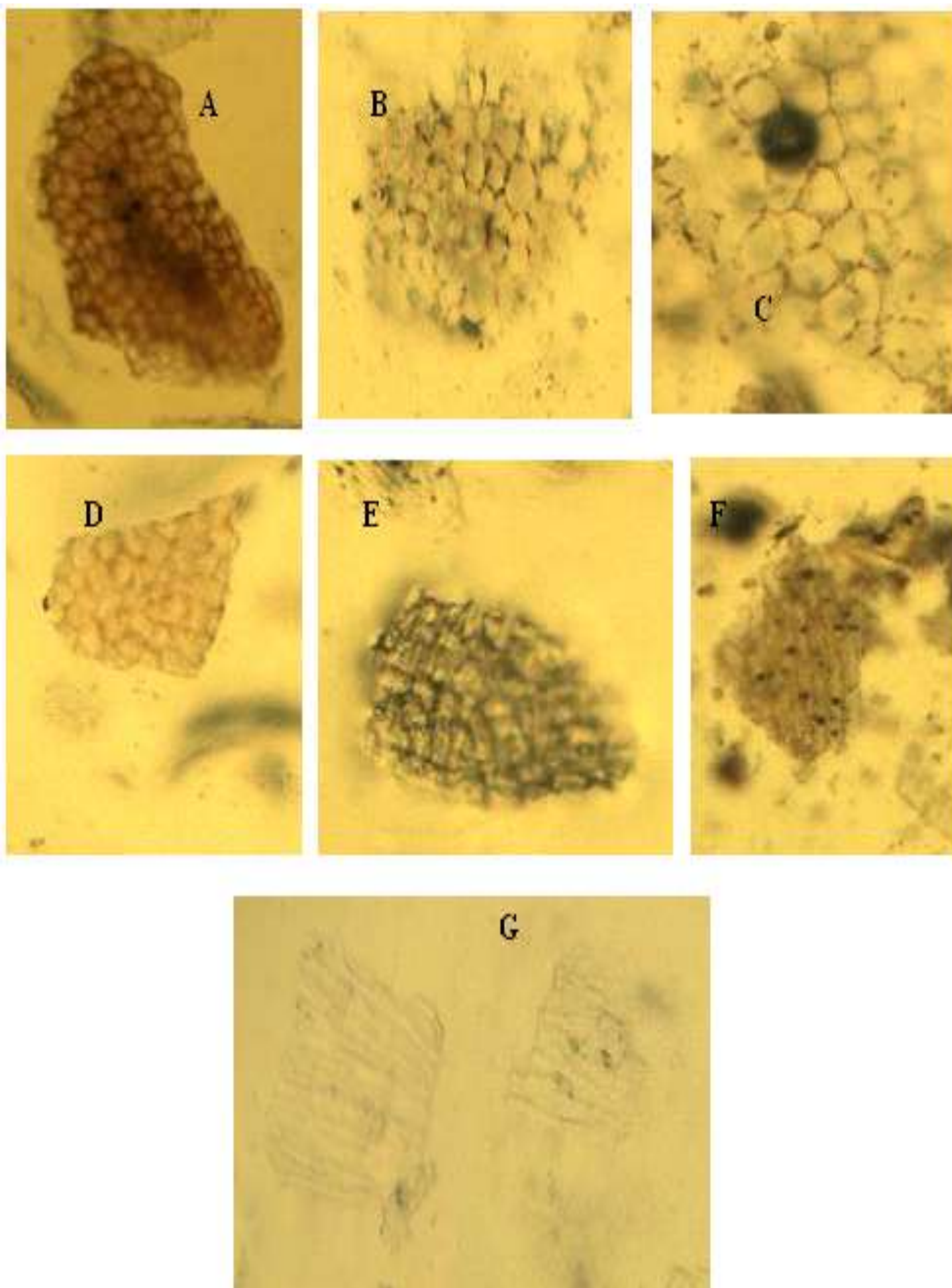

Fig. 3: Powder microscopy of *Plantago ovata*

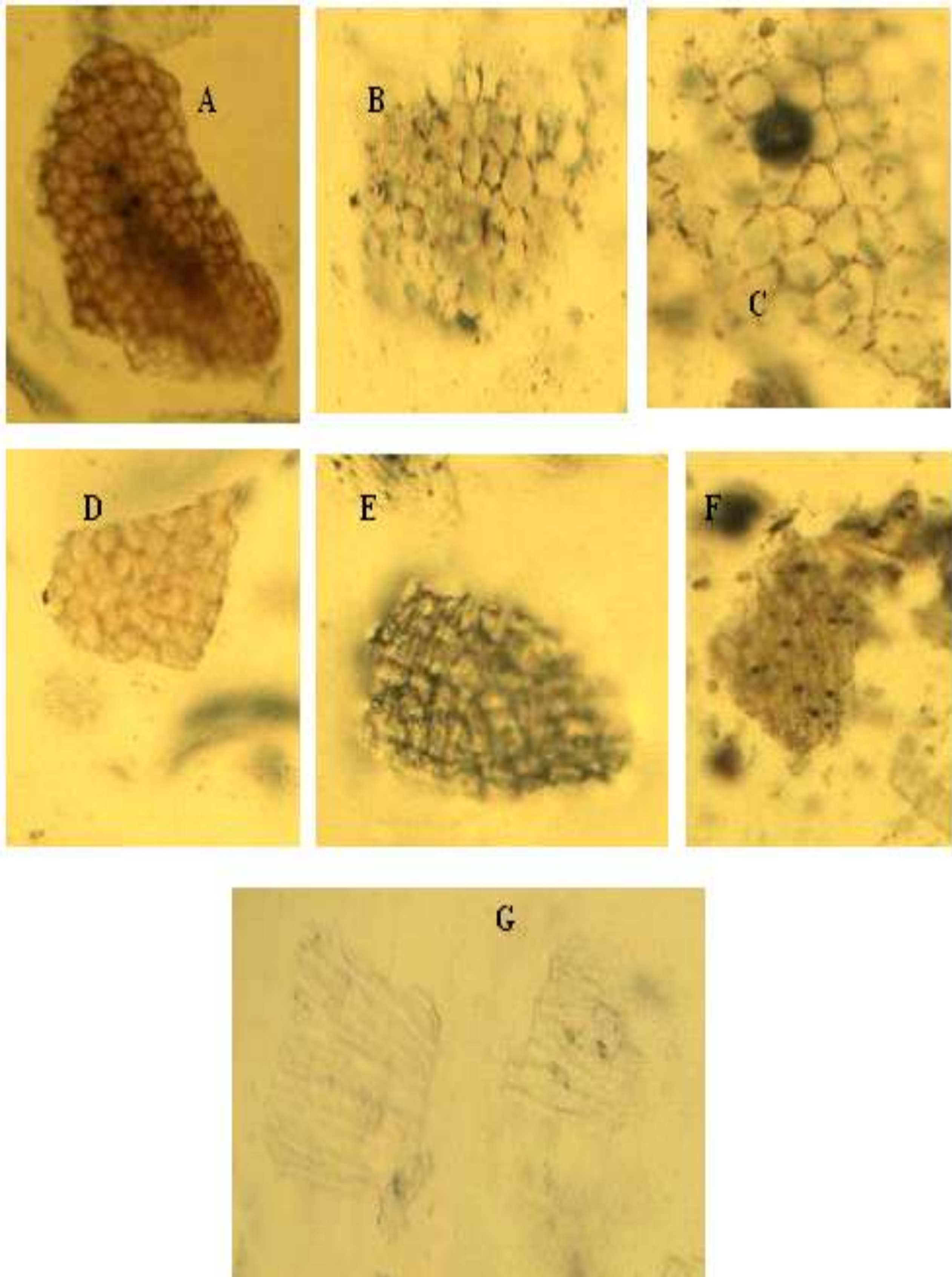

Fig. 3: Powder microscopy of *Plantago ovata*

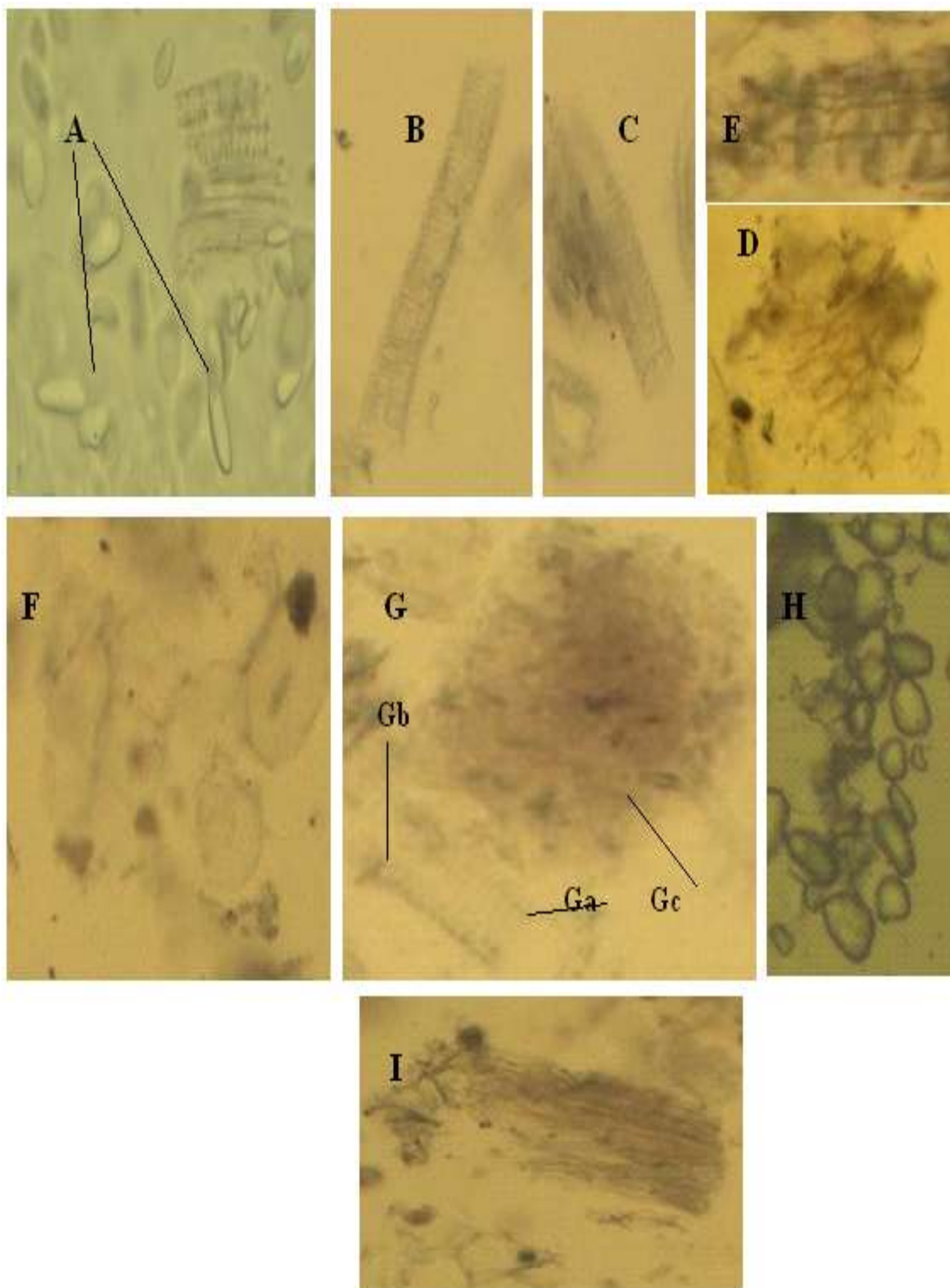

Fig. 4: Powder Microscopy of *Zingiber officinale*

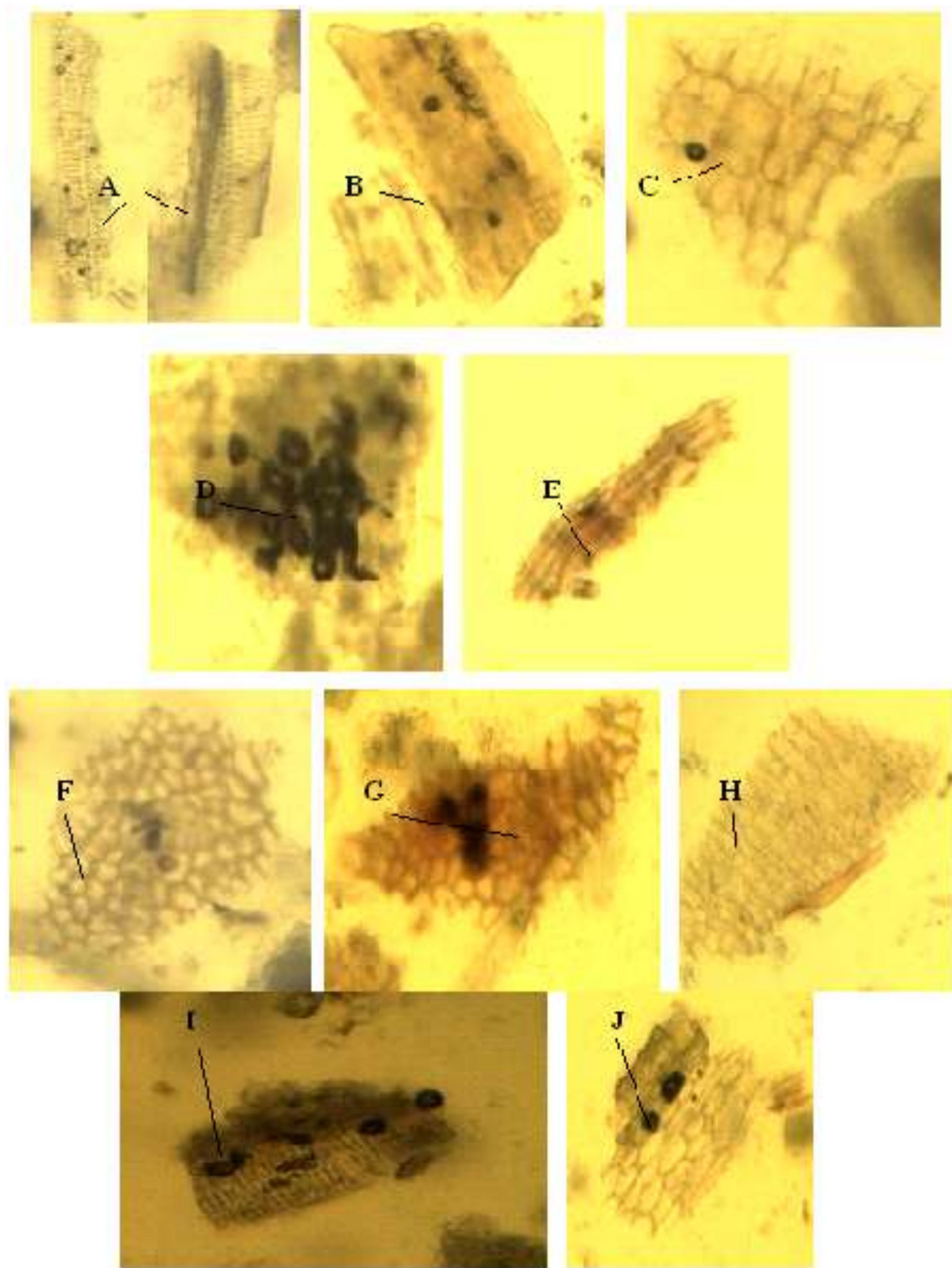

Fig. 5: Powder microscopy of *Glycyrrhiza glabra*

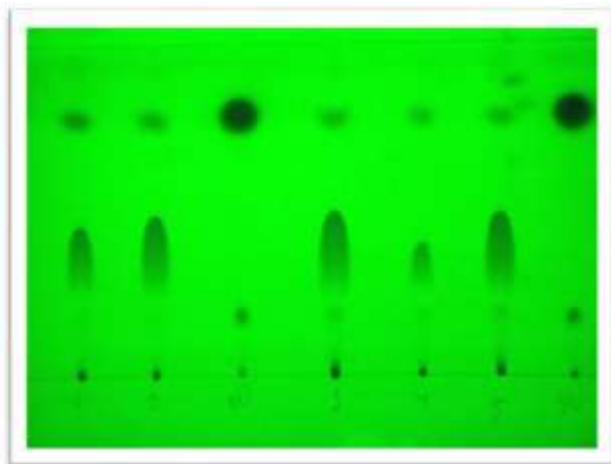

Fig. 6: TLC of *Foeniculum vulgarae*

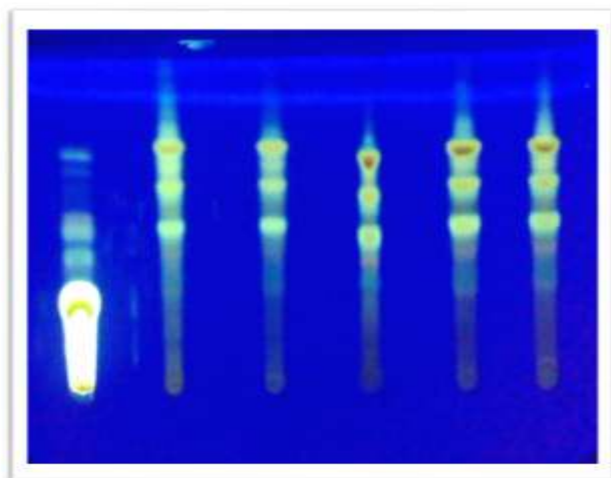

Fig. 7: TLC of *Curcumma longa*

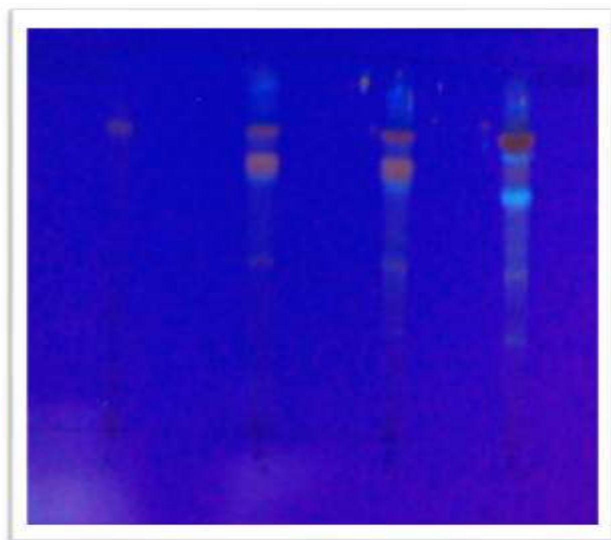

Fig. 8: TLC of *Aloe barbadensis*

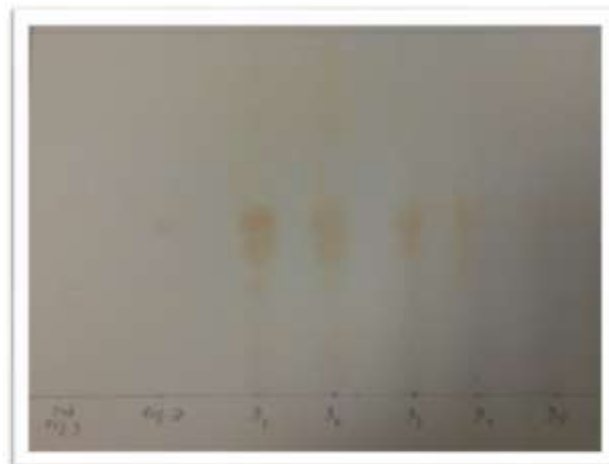

Fig. 9: TLC of *Plantago ovata*

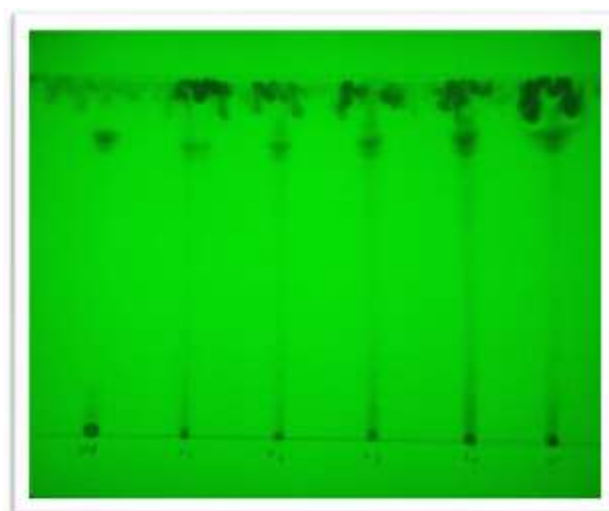

Fig. 10: TLC of *Glyccyrhiza glabra*

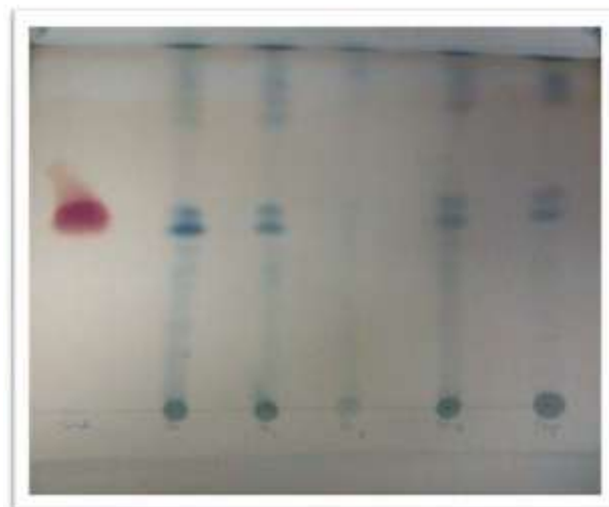

Fig. 11: TLC of *Zingiber officinale*

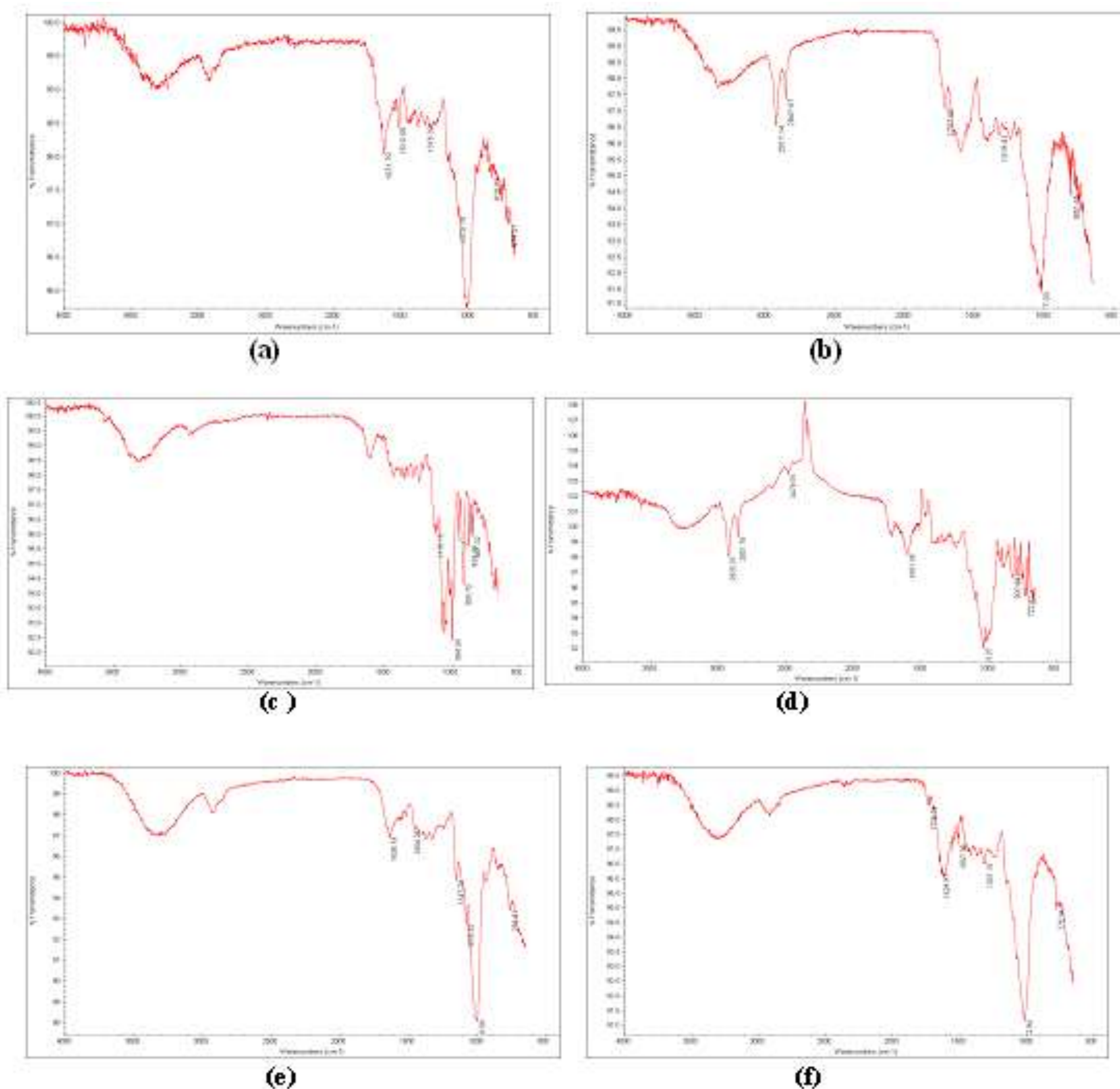

Fig. 12: FTIR spectra of herbal material: (a) *Foeniculum vulgarae (b) Curcuma longa (c) Aloe barbadensis (d) Plantago ovata (e) Zingiber officinale (f) Glycyrrhiza glabra.*

All the samples of ispaghoula were compared with the standard xylose and galactose spots by TLC and showed spot, except samples I-4 and I-5 which do not show the spot for xylose. The spots corresponding to the standard spot with different R_f values are given in table 7.

The R_f values of ginger sample G-1 to G-5 are given in table 7. TLC studies revealed the presence of gingerol-1 and gingerol-2 in the samples of ginger as shown in fig. 11 The R_f value of gingerol-1 varies from 0.55-0.57 while that of Gingerol-2 from 0.54-0.56 and corresponded to the standard spot of Resorcinol, R_f 0.56. (fig. 11) The resorcinol is used as a standard to compare the spots of gingerol in the sample because it has a similar basic structure as that of gingerols. Thus, all the samples show intense violet zones (gingerol) as mentioned in the B.P. (2016) and comply with the standard spot.

TLC shows the presence of glycerrihitic acid in the samples of liquorice. The R_f value of sample L-1 to L-5 varies from 0.83-0.84 and corresponds to the standard spot of glycerrihitic acid with R_f of 0.84 (fig.10).

Spectrophotometric assay

Curcuma longa

The assay of curcumin in turmeric samples T-1-T-5 has been carried out by ultravoilet and visible spectrophotometer by absorbance measurement at 530 nm and the percent content is given in table 8. BP (2016) specifies the limit of dicinnamoyl methane derivatives,

expressed as curcumin, a minimum of 1.0 per cent. All the samples of turmeric T-1-T-5 have percent curcumin between 1.62-1.98%. Thus, all show compliance with the prescribed limit.

Aloe barbadensis

The absorbance of test samples (A1 to A-3) at 512nm is given in table 8. The percent Barbaloin was found to be between 29.5-30.9 that complies with the BP limit (minimum 28.0%).

Fourier transforms infrared spectroscopy (FTIR)

The FTIR spectra of all the crude drug samples (*Foeniculum vulgarae, Curcuma longa, Aloe barbadensis, Plantago ovata, Zingiber officinale and Glycyrrhiza glabra)* are shown in fig. 12 and the details of the spectral assignments are given in table 9.

DISCUSSION

The specified quality control test given in BP 2016 were pefromed in order to conform it authenticity and purity. Firstly, microscopic features of each sample was examined that confirm the originality of samples at cellular level as shown in table 1-5. Moreover the physicochemical stability of plant raw materials were checked by various physicochemical test such as, foreign matter, moisture content, ash content, fluorescent test, swelling index mentioned in the monograph. Afterward various analytical techniques have been applied likewise TLC, spectrophotometric assay and FTIR to identify and determine the percent active constituents and functional group present in selected samples.

All the samples meet the limit of pharmacopoeial standard (maximum 1.5%) and have a low content of foreign matter indicating good quality of the material (British pharmacopeia 2016).

Thus, All the samples of have show lesser value of moisture content which supports a good quality material (British pharmacopeia 2013). The excessive moisture in herbal raw material may lead to promotion of mould and bacterial growth, and subsequently to deterioration and spoilage of the drug. Therefore, low content of moisture is good for maintaining the quality of the material (Anubhuti *et al*. 2011). The Ash content is one of the important physicochemical parameter to assess the quality of drugs such as ginger. It has been found in the range of 3.0-7.6 for sugar samples (table 6). All the samples comply with the BP limit of 6.0%. The inorganic matter present in crude drug may catalyse degradation reaction and thus affect the stability of the active ingredients. Since, The moisture content of the liquorice samples was found to be in the range of 7.42-8.95%. The level of moisture for liquorice is a maximum 10.0% as mentioned in BP (2016). Thus, all the present samples of liquorice comply with the prescribed limit. The low content of moisture is good for the quality of material.

Ash content

Ash content is another physicochemical parameter to assess the quality of a drug. It has been found that the ash content of fennel sample lies in the range of 6.89-7.91 (table 6) All the samples comply with the BP limit of 10.0. Determination of ash content indicates the presence of inorganic matter in crude drugs to trace the amount of minerals such as as Na^+ (Sodium), K^+ (Potassium), and Ca^{2+} (Calcium). Ash value is one of the important quality control parameter to check and approve any raw material for the preparation of finished products. Though, the ash values of test samples of turmeric are within a range of 5.40-7.77%. The maximum ash value specified in BP (2016) is 8.0 percent. It indicates that all the samples comply with the BP standard and have ash values within the specified limit of inorganic matter. However, the ash content in aloevera sample was found to be in the range of 9.5-25.1%. These values do not comply with the BP limit of 2.0%. It indicates that aloevera samples have high content of inorganic matter that may affect the quality of finished herbal product. On this basis the raw material can be rejected.

The Ash content indicates a high amount of inorganic matter and may be rejected on this basis for use in the preparation of drug products. However all the samples were complied with the BP limit except aloevera which would be rejected upon this basis and does not qualify to be use in formulation.

The fluorescent behavior of aloevera samples was analysed which indicates varied fluorescent behavior among samples.

The swelling index is one of the important physicochemical parameter which contributes to the pharmacological activity of raw Ispaghula husk. It is used as a bulk forming laxative and the main pharmacological action is due to its mucilage content. The swelling properties of the mucilage make it absorb water in gastrointestinal canal thereby increasing the volume of feces and promoting peristalsis so it is used in treatment of chronic constipation (Auzi *et al*., 2006). Though ispaghula husk qualify QC (quality control) test upon basis of its swelling index.

***Thin layer chromatography* (TLC)**

TLC identify and determine the purity of sample by corresponding with standard spot under specified solvent system Anubhuti *et al*. (2011). The slight deviation in R_f value may be due to high water content and any variation in the solvent system. All the spots of fennel sample were corresponded to standard spot anethole and confirm its purity. Pandey *et al*. (2012) has performed HPTLC to check barbaloin in alovera using a mobile phase of

acetate-methanol-water (10:21:1). Similarly selected samples of aloevera also showed the spots of barbaloin in similar manner. However, The sugar composition of ispaghula husks (g/100g dry matter) includes rhamnose 2.1g, mannose 1.1g, arabinose 17.6, galactose 3.7, xylose 47.4, glucose traces (Marteau *et al*., 1994). All the samples were compared with the standard xylose and galactose spots by TLC and showed spot, except samples I-4 and I-5 which do not show the spot for xylose. Thus, all the samples of plantago ovata do not comply with the pharmacopoeial standard (fig. 9.) and throw some doubts on its quality and purity due to difference in R_f values and absence of the spots of some sugars component. TLC of ginger showed the presence of gingerol-1 and gingerol-2 in the samples of ginger. Rai *et al*. (2006) have proposed a sensitive and accurate HPTLC method to determine the quantity of 6-gingerol in rhizomes of Zingiber officinale. Methanol extracts of rhizomes from three different sources were chromatographed and n-hexane and diethyl ether (40:60v/v) was used as the mobile phase. The R_f of 6-gingerol was found to be 0.40. The proposed HPTLC method for monitoring 6-gingerol in ginger can be used for routine quality testing of ginger extracts.

The presence of glycerrihitic acid in the samples of liquorice was determined. Thus all the samples show violet zone corresponding to the zone of glycyrrhetic acid as observed in the chromatogram obtained with the reference solution according to BP (2016) and thus comply with the standard.

Spectrophotometric assay

The percent content of curcumin in turmeric samples was determined by spectrophotometer at 530 nm expressed as a minimum of 1.0 per cent which significantly determines the percentage of active constituent in given turmeric samples that would ultimately affect the potential pharmacological effects of crude drug. Simultaneously the percent Barbaloin was determined in the selected samples of aloevera which eventually affect the activity may be due to barbaloin.

The FTIR assignments of all the crude drug samples (*Foeniculum vulgarae, Curcuma longa, Aloe barbadensis, Plantago ovata, Zingiber officinale and Glycyrrhiza glabra)* showed the presence of various functional groups which would be helpful in determination and structure elucidation of various constituent in selected samples.

CONCLUSION

The main predicament in Pakistan is the assessment or standardization of herbal product or the raw material used in commercially available formulation. Regrettably, there is no perception in herbal drug producer to ensure and certify the quality of their products or the raw material used in the formulation. Therefore, the study was subjected to different methods and protocols for evaluation of raw herbs. The commonly used herbs in management of various ailments have been selected i.e. *Foeniculum vulgarae* (fennel), *Curcuma longa (*turmeric), *Aloe barbadensis* (alovera), *Plantago ovate* (husk), *Zingiber officinale* (ginger) *and Glycyrrhiza glabra* (liqourice). Some of the herbal samples were found to confirm the quality standards while others did not comply probably due to adulteration or being spurious or substandard raw material. In case of the standardization of herbal raw material proper botanical identification has prime importance to utilize them as a raw material for herbal as well as cosmaceutical industries. Sometimes spoiled or adulterated material, improper cultivation, collection, harvesting or adverse effects during processing may cause severe health problems. In this situation the key step is to confirm the authenticity of the required raw material by physicochemical methods (quantitative and qualitative) of determination. Essentially, this study provide significant data to create awareness about quality testing of herbal raw material among herbal, cosmaceutical and pharmaceutical industries of the country that would be beneficial for the therapeutic outcomes of commercially available herbal products.

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