Pharmacognostical and Phytochemical evaluation of

Atasi (Linum usitatissimum L.)

Ila Tanna*, Preeti Pandya, Harisha C R, V J Shukla & Chandola H M

*Department of Roga Nidana & Vikriti Vijnana, 1,2Pharmacognosy Laboratory, 3Pharmaceutical Chemistry Laboratory & 4Kayachikitsa Department, Institute for Post Graduate Training & Research in Ayurveda, Gujarat Ayurved University, Jamnagar- 361 008, Gujarat
E-mail: dr.ashka@yahoo.com

Received 23.01.12, revised 04.03.13

Atasi commonly called Flax seed, is a richest plant source of omega 3 fatty acids. It promotes transmission of the chemical messengers that facilitate communication between nerve cells and are associated with emotional stability (e.g. serotonin) and positive emotions (e.g. dopamine). Omega-3s have been linked to depressive conditions such as bipolar disorder, unipolar depression, borderline personality disorder, premenstrual syndrome and perinatal depression. A detailed investigation of flax seed was carried out. The microscopic characters of transverse section of seed show epidermis, pigment layer, cotyledon consist of protein mass with aleuron grains and abundant globule of fixed oil. Powder microscopic characters include lignified sclerides, aleurone grains & fatty oil globules, etc. Physicochemical parameters of flax seed revealed fixed oil content (42.05%), whereas the physicochemical parameters of flax seed oil revealed loss on drying (0.19 %w/w), refractive index (1.48), specific gravity (0.92), acid value (0.33), iodine value (170.90), saponification value (192.8). Thin layer chromatographic study of the unsaponified matter of flax seed oil showed the presence of seven and four spots in short UV, long UV, respectively. The information generated by this particular study provides relevant pharmacognostical and physicochemical data needed for proper identification and authentication of flax seed and flax seed oil.

Keywords: Atasi, Flax seed, Pharmacognosy, Physicochemical, TLC

IPC Int. Cl.®: A61K 36/00, D01C, G01N 30/00, A01D 22/23, A01D 8/00

Atasi (Linum usitatissimum L.) (common flax, linseed) possess sweet (madhura), bitter (tikta) taste, unctuous (snigdha), hot potency (ushna virya) and pungent end metabolism (katu vipaka). It alleviates vata and also pacifies pitta and kapha. Flax seed oil has sweet taste (madhura rasa), promoting digestion and metabolism (Agneya guna), heavy (guru), unctuous (snigdha), hot (ushna) properties and promotes vitality (bala). It pacifies vata dosha and increases pitta & kapha. It is useful in skin diseases (kushtha), worm infestation (krimi), dyslipidemia (medo dosha), inflammatory condition (vrana shotha), etc.¹

Nerve tissue possesses one of the highest concentrations of fatty acids in the body, with approximately 20% of the dry weight of the brain comprised of long chain fatty acids. These long chain fatty acids provided in food are essential for both the structure and function of nerve cells. Omega-3s promote transmission of the chemical messengers that facilitate communication between nerve cells and are associated with emotional stability (e.g. serotonin) and positive emotions (e.g. dopamine). Omega-3s have been linked to depressive conditions such as bipolar disorder, unipolar depression, borderline personality disorder, premenstrual syndrome and perinatal depression. Omega-3 fatty acids affect brain derived neurotrophic factor, which encourages synaptic plasticity, provides neuroprotection, enhances neurotransmission and has antidepressant effects². Analyses of blood fatty acids show that depressed people have lower levels of omega-3s and higher levels of omega-6s compared with people who are not depressed. For example, several studies reported lower EPA and DHA levels in depressed compared with non-depressed people, whereas total omega-3s [including Alpha-linolenic acid (ALA)] were reduced in all depressed patients except in one study. Overall, the reports showed that the severity of depression was greater as the concentrations of eicosapentanoic acid (EPA), docosahexanoic acid (DHA), ALA and total omega-3s fell. Although some of these findings were not of statistical significance, there was a consistent shift away from omega-3s toward omega-6s in depressed people³. Flax seed is

¹Corresponding author
Table 1—Amount of Omega 3 FA in flax seed & flax seed oil

<table>
<thead>
<tr>
<th>Source</th>
<th>Total essential fatty acids (gm)</th>
<th>Omega 6 FA (gm)</th>
<th>Omega 3 FA (gm)</th>
<th>Ratio (Omega 6:omega3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flax seed oil</td>
<td>8.9</td>
<td>1.7</td>
<td>7.2</td>
<td>1:4</td>
</tr>
<tr>
<td>(1 tbsp ~14 gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground flax seed</td>
<td>2</td>
<td>0.4</td>
<td>1.6</td>
<td>1:4</td>
</tr>
<tr>
<td>(1 TBSP-7 gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

the best plant source of omega 3 fatty acids. Atasi is a richest source of ALA which can be endogenously converted to longer chain Omega 3 fatty acids; EPA & DHA. Flax seed oil deliver greater amount of Omega 3s compared to ground flax seed & it is the easiest form to take (Table 1).

Aim and objectives
To carry out the pharmacognostical and phytochemical analysis of Flax seed and Flax seed oil.

Materials and methods
Flax seeds were purchased from Lallubhai Vrajlal Gandhi Ltd., Ahmedabad in the month of December 2010. Pharmacognostical evaluation of flax seed including organoleptic, transverse sections, powder microscopy and histo-chemical tests were carried out. Transverse sections were made by free hand sections, powder microscopy flax seed was carried out with and without staining. Slides were first studied under distilled water (without staining), then stained with Phloroglucinol and Concentrated HCl to identify lignified elements. Photomicrographs were taken using Corlzeiss Binocular Microscope (10×40) attached with camera. Oil was obtained (25%) by grinding & expression pressing method.

Thin layer chromatography
Thin Layer Chromatographic study of the Unsapofiable matter of Flax seed oil was carried out on Silica gel G plate using Hexane: Diethyle ether: Gl.Acetic acid – 5:4.5:0.5 (V/V) as solvent system. The developed plate, after drying was sprayed with LB reagent (Libberman buchard) spray reagent followed by heating at 110°C for 10 minutes. From the chromatogram the number of spots and their Rf values were calculated.

Sample preparation
Unsaponified matter of Flax seed oil was diluted with diethyl ether and used for spotting.

Mobile phase Hexane: Diethyle ether: Gl.Acetic acid – 5:4.5:0.5 (V/V)
Stationary phase Silica Gel G (Merck)
Spray reagent LB reagent (Libberman buchard)
Visualization At Short UV - 254 nm and Long UV - 366 nm

Results and discussion

Macroscopic
Atasi consists of dried, ripe seeds of Linum usitatissimum L. (Family: Linaceae), an erect annual herb, 0.6-1.2 m high, extensively cultivated throughout the plains of India upto an altitude of 800 m, capsule ripen by end of June, dried seeds separated from capsule by thrashing. Seed small, brown, glossy with minutely pitted surface, elongated-ovoid, flattened, rounded at one end and obliquely pointed at the other, near which on one edge, a light depression enclosing hilum and micropyle, embryo consisting of two yellowish-white, flattened planoconvex cotyledons and a radical, nearly fills the seed and completely surrounded by a thin, whitish endosperm, both endosperm and embryo oily, tests mucilaginous when soaked in water, odour, characteristic, taste, oily when chewed (Fig. 1).

Organoleptic characters
Organoleptic characters like mucilaginous sweetish in taste, whitish yellow in colour and bland odour.

Transverse section
Transverse section of seed showed testa consists of isodiametric cells with mucilaginous outer walls, collenchymatous cells of middle layer of seed coat
cylindrical, single layered, yellowish brown, longitudinally elongated, thick, and lignified and with pitted walls, single layer of flattened polygonal pigment cells with reddish-brown contents, aleurone grains in the cotyledons, abundant globule of fixed oil (Figs. 2-4).

**Powder microscopy**

Microscopical characteristics of powdered drug observed were yellow coloured fibre, pitted walls, lignified sclerides, rounded collenchymatous cells in hypodermis and polygonal epidermis cells filled with mucilage, pigment layer, square cells with orange brown mass, aleurone grains, fatty oil globules, etc. (Figs. 5-10).

**Histochemical evaluation**

Transverse sections of flax seed were subjected to various Histochemical tests\(^5\) (Table 2).

**Physico-chemical Parameters**

The sample of flax seed was first subjected to Fixed oil content\(^7\) parameter and the result showed that it contains 42.05% fixed oil which confirms the genuinity of the drug as per API standards. After that the oil was expressed from the same sample...
Table 2—Histo-chemical tests

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Reagent</th>
<th>Observation</th>
<th>Characteristics</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phloroglucinol+Conc. HCL</td>
<td>No effervescence</td>
<td>Calcium oxalate</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Phloroglucinol+Conc. HCL</td>
<td>Pink</td>
<td>Lignified sclerides</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Sudan Red III</td>
<td>Red</td>
<td>Oil globules in the cells of endosperm and cotyledon</td>
<td>++</td>
</tr>
</tbody>
</table>

Table 3—Physico-chemical parameters of flax seed oil

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Test</th>
<th>Sample</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss on drying⁴</td>
<td>Flax seed oil</td>
<td>0.19% w/w</td>
</tr>
<tr>
<td>2</td>
<td>Refractive Index at 40⁰C⁴</td>
<td></td>
<td>1.4835</td>
</tr>
<tr>
<td>3</td>
<td>Specific Gravity 40/40⁰C⁴</td>
<td></td>
<td>0.9215</td>
</tr>
<tr>
<td>4</td>
<td>Acid value⁴</td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>5</td>
<td>Iodine value⁴</td>
<td></td>
<td>170.90</td>
</tr>
<tr>
<td>6</td>
<td>Saponification value⁴</td>
<td></td>
<td>192.77</td>
</tr>
</tbody>
</table>

Table 4—TLC Profile of Flax seed oil on Silica Gel GF 254

- **Flax seed Oil**
  - **Solvent System**: Hexane: Diethyle ether: Gl.Acetic acid – 5:4.5:0.5 (V/V)
  - **Short UV – 254nm**
    - Spot no. 1: Rf 0.27
    - Spot no. 2: Rf 0.41
    - Spot no. 3: Rf 0.5
    - Spot no. 4: Rf 0.7
    - Spot no. 5: Rf 1.03
    - Spot no. 6: Rf 1.32
    - Spot no. 7: Rf 1.47
  - **Long UV – 366nm**
    - Spot no. 1: Rf 0.34
    - Spot no. 2: Rf 0.53
    - Spot no. 3: Rf 0.68
    - Spot no. 4: Rf 1.33
    - Spot no. 5: Rf 0.62
    - Spot no. 6: Rf 0.89
  - **After sprayLibberman Buchard reagent**
    - Spot no. 1: Rf 0.04
    - Spot no. 2: Rf 0.12
    - Spot no. 3: Rf 0.28
    - Spot no. 4: Rf 0.44
    - Spot no. 5: Rf 0.62
    - Spot no. 6: Rf 0.89

by grinding and expression pressing method and Physicochemical parameters of the flax seed oil subjected to various parameters, i.e. Refractive index, loss on drying, acid value, specific gravity, etc. (Table 3).

**Thin layer chromatography**

Thin Layer Chromatographic study of the Unsapofiable matter of Flax seed oil was carried out on Silica gel G plate using Hexane: Diethyle ether: Gl.Acetic acid – 5:4.5:0.5 (V/V) as solvent system. The details of the chromatogram, i.e. Rf values have been presented in Table 4 (Fig. 11).

**Traditional significance of study and constructive recommendations**

Flax seed oil, being a rich source of Omega 3 fatty acids, has a potential role in brain and neurological health. The present study is a pilot study that explores pharmacognostic and histo-chemical analysis of flax seed and physico-chemical properties and TLC profile of flax seed oil which can serve as a preliminary step towards standardization of flax seed oil. Further study is necessary to explore other parameters related to standardization of flax seed oil to be carried out in different batches to set the limit for the reference standards for the quality control and quality assurance. Since flax seed oil is highly prone to rancidity and light & oxygen can break down the essential fatty acid, it should be kept refrigerated & should prevent from light and should be packed in opaque plastic bottles. Based on this fact future researchers can discover the shelf life of flax seed oil by doing quantitative analysis of ALA in it and can also look for promising preservative methods to increase its shelf life.

**Conclusion**

Flax seed (Atasi) is the best plant source of omega 3 fatty acids. It is a richest source of Alpha-linolenic acid (ALA) which can be endogenously converted to longer chain Omega 3 fatty acids; EPA and DHA. Flax seed oil deliver greater amount of Omega 3s compared to ground flax seed and it is the easiest form to take. The present study pharmacognostically fibres, lignified sclerides, pigment layer, aleurone grains, the phytochemical study shows that 42.05% of fixed oil content of flax seed proves the purity and genuineness of the
drug, supports the fact that Omega-3 fatty acids affect brain derived neurotrophic factor, which encourages synaptic plasticity, provides neuroprotection, enhances neurotransmission and has antidepressant effects. The TLC reports 7 spots in 254 nm, 5 spots in 366 nm and 6 spots after spray. The strength of single drugs and those of formulations at a later stage assumes importance for the effective enforcement of the provision of the Act. Though the groundwork requisites for the standardization of Flax seed oil are covered in the current study, quantitative analysis of Omega 3 fatty acids which is the active chemical constituents of flax seed oil is required to be done additionally to substantiate the clinical study.

References