Evaluation of an Ayurvedic compound formulation—Agnimukha Cūrna

Ariamuthu Saraswathy*, Joy Suganthan, R Vijayalakshmi & P Brindha

Captain Srinivasa Murti Drug Research institute for Ayurveda (CCRAS), Arumbakkam, Chennai 600 106, Tamil Nadu
Email: csmdria@rediffmail.com

Received 1 July 2005; revised 23 October 2006

India has a vast heritange of traditional systems of medicine for various ailments. An attempt has been made to scientifically evaluate Agnimukha Cūrna, an Ayurvedic compound formulation for laying down standards. Four different samples were procured from different Ayurvedic product manufacturers and subjected to pharmacognostic studies, physicochemical analysis, and TLC/HPTLC finger printing using authentic ingredients as reference standards. Volatile oils of Ferula foetida and Trachyspermum ammi were also used as control for TLC investigation of the volatile oils of four samples of Cūrna. The evolved microscopic features and chromatographic finger print profiles were found to compliment each other and are sufficient for establishing the presence of the ingredients in the compound formulation Agnimukha Cūrna.

Keywords: Agnimukha Cūrna, Ayurveda drugs, Fingerprint profiles, Volatile oils

IPC Int. Cl.: A61K36/00, A61P9/14, A61P11/00, A61P11/06, A61P11/10, A61P25/00, A61P25/02, A61P29/00, A61P31/02, A61P35/00

Agnimukha Cūrna is an Ayurvedic compound formulation prescribed for udāvarta (upward movement of gases), ajīna (dyspepsia), gulma (abdominal lump), phîlaroga (splenic diseases), udararoga (diseases of abdomen), arṣa (piles), sāla (pain), kāsa (cough), śvāsa (asthma) and knaya (phthisis). It is digestive, carminative, appetizer, and useful in peptic ulcer and bronchial asthma. There is a lack of data regarding the parameters and testing methods to be employed for assessing the quality of traditional system of medicines. As the global market for herbal medicinal product is increasing tremendously, need for quality control parameters which are accepted globally, is being felt. Therefore, an attempt has been made in developing standardization parameters covering pharmacognostic characterization, physicochemical values and TLC/HPTLC profile for the Ayurvedic compound formulation Agnimukha Cūrna.

Methodology

The raw drugs used in the preparation (Table 1) were procured from the local market and identified. Fruits of Apium leptophyllum are often found mixed with the commercially available samples of Trachyspermum ammi and Trachyspermum roxburghianum. In order to identify whether Trachyspermum ammi has been used in the cūrna samples by the manufacturers, authentic samples of Apium leptophyllum and Trachyspermum roxburghianum were also procured from Jamnagar and Chennai, respectively and were used during the course of study. Samples of Agnimukha Cūrna of one batch each prepared by four different Ayurvedic pharmacies (Almora AC-1, Jaipur AC-2, Jamnagar AC-3, Patiala AC-4) were procured. Test for foreign matter was done by spreading 10 gm of the sample (each) on petri dish. Particle size of the all samples was done in 60 and 85 mesh sieve.

For microscopic analysis, a few mg of the sample was warmed with chloral hydrate, cooled, washed and mounted in glycerin; a few mg was cleared in 4% KOH, washed and mounted in glycerin; a few mg was washed in plain water and mounted in glycerin, to identify the diagnostic microscopic features of the ingredients. Physicochemical analysis of total ash, acid-insoluble ash, sulphated ash, extractive values in alcohol and water separately, Soxhlet extractive value in n-hexane, loss on drying at 105°C, pH of filtrate of 10% w/v aqueous solution were done in triplicate according to the standard methods in all the four samples. For TLC 4 gm of each cūrna sample was soaked in 50 ml of chloroform for 18 hrs and boiled

*Corresponding author
for 10 min and filtered. The filtrate was concentrated and made up to 5 ml in a standard flask. Similarly, chloroform extracts were prepared for all the ingredients as well as for fruit of *Trachyspermum roxburghianum* and *Apium leptophyllum* (to correctly identify whether *Trachyspermum ammi* has been used in the samples of *cūrna* procured)\(^{15}\). TLC of the chloroform extracts of the all the samples and the reference ingredients were developed on aluminum plates precoated with silica gel G F\(_{254}\) (0.2 mm thickness; Merck). Camag Linomat IV applicator was used for sample application and Desaga photo documentation unit was used for documentation of finger print profiles. The mobile phase used was toluene: ethyl acetate (10:1), (10:3) and toluene: chloroform (1:1) for volatile oil. The plates were dried and visualized under UV 254 and 366 nm and under visible light, after exposing to iodine vapours and derivatization with vanillin-sulphuric acid\(^{10-13}\). For volatile oil separation, 15 gm of each *cūrna* sample was steam distilled and the distillate was shaken with ether and used for TLC. The volatile oil of *Ferula foetida* resin and *Trachyspermum ammi* fruits and ingredients were prepared by similar procedure\(^{14}\).

**Results and discussion**

*Agnimukha Cūrna* samples AC-1, AC-2, AC-3, AC-4 procured from different Ayurvedic pharmacies were brown in colour, moderately fine powder possessing astringent taste and pleasant odour. It was observed that all of the samples passed through 60 meshes and not less than 50% passed through 85mesh sieve. Microscopic characterization (Fig 1a to 1c) revealed groups of annular vessels; fragments of reticulate vessels; row of circular parenchymatous cells forming a network enclosing large air spaces, and packed with starch grains, some also containing oil (*Vacā-Acorous calamus* Linn.); spiral vessels; yellow or orange coloured particles; perisperm cells packed with starch grains and minute crystals of calcium oxalate; stone cells upto 150 \(\mu\) length and without a broad lumen (*Pippali-Piper longum* Linn.). Large starch grains, oval shaped up to 55 \(\mu\) in size with eccentric hilum; reticulate and spiral vessels; septate fibres with thin wall, broad lumen and sharp tip (*Śunthī-Zingiber officinale* Rosc.). Epidermis with stomata and papillose and glandular outgrowth; polygonal parenchyma cells containing aleurone grains with numerous minute calcium oxalate crystals (*Yavānī-Trachyspermum ammi* (Linn.) Sprague). Thick walled polygonal parenchyma cells; elongated stone cells up to 350 \(\mu\) in length, with pitted walls and broad lumen; fibres with thin wall and broad lumen and pegged tip; criss cross layers of fibres; crystals fibres with prismatic crystals; isolated druses up to 60 \(\mu\); elongated lignified cells with pitted walls (*Haritaki-Terminalia chebula* Retz.). Cork cells; lignified cells with pitted walls; pitted fibres with oblique pointed ends, thin walled and broad lumen upto 500 \(\mu\) in length and 35 \(\mu\) in width (*Citraka-Plumbago zeylanica* Linn.). Fibres up to 400 \(\mu\); storage parenchyma filled with masses of inulin (A few mg of *Cūrna* treated with \(\alpha\)-naphthol and concentrated sulphuric acid, gently warmed, develops dark violet colour indicating the presence of inulin); xylem vessels with scalariform and reticulate thickening (*Kushta-Saussurea lappa* C.B. Clarke).

The physicochemical analysis of ash values, extractive values, loss on drying at 105\(^{\circ}\)C, pH are presented (Table 2). The total ash value is comparable in all the four samples. Acid- insoluble ash was slightly high in AC-2 on the contrary sulphated ash was less in AC-2. The n-hexane soluble extractive value was found to be relatively high in AC-1. Alcohol and water-soluble extractive values were comparatively low in AC-4 and the same were comparable in AC-1, AC-2 and AC-3. These variations may be due to the change in the quality of the raw materials used in terms of place and period of collection and storage period and conditions. TLC finger print profiles of the four samples are depicted (Fig.2). The chromatogram was observed similar excepting minor changes in the concentration of a few spots. TLC of chloroform extract of *cūrna* using toluene: ethyl acetate (10:1) v/v shows under UV (254 nm) 12 spots at Rf. 0.04, 0.06, 0.12 (all blue), 0.16, 0.26, 0.34, 0.51, 0.52 (all green), 0.60 (blue green), 0.66, 0.80 and 0.94 (all green). Under UV (366 nm) 18 spots are seen at Rf. 0.04, 0.08,

<table>
<thead>
<tr>
<th>Name</th>
<th>Botanical Name</th>
<th>Anatomic part</th>
<th>Quantity (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hingu</td>
<td>Ferula foetida Regel.</td>
<td>Exudates</td>
<td>100</td>
</tr>
<tr>
<td>Vacā</td>
<td>Acorous calamus L.</td>
<td>Rhizome</td>
<td>200</td>
</tr>
<tr>
<td>Pippali</td>
<td>Piper longum L.</td>
<td>Fruit</td>
<td>300</td>
</tr>
<tr>
<td>Śunthī</td>
<td>Zingiber officinale Rosc.</td>
<td>Rhizome</td>
<td>400</td>
</tr>
<tr>
<td>Yavānī</td>
<td>Trachyspermum ammi (Linn. Sprague)</td>
<td>Fruit</td>
<td>500</td>
</tr>
<tr>
<td>Haritaki</td>
<td>Terminalia chebula Retz.</td>
<td>Fruit rind</td>
<td>700</td>
</tr>
<tr>
<td>Citraka</td>
<td>Plumbago zeylanica L.</td>
<td>Root</td>
<td>800</td>
</tr>
<tr>
<td>Kushta</td>
<td>Saussurea lappa C.B. Clark</td>
<td>Root</td>
<td>600</td>
</tr>
</tbody>
</table>

Table 1—Ingredients of *Agnimukha Cūrna*
On exposure to iodine vapour 6 spots at Rf. 0.44, 0.54, 0.58, 0.60, 0.79 and 0.84 (all yellowish brown) are seen. On dipping the plate in vanillin-sulphuric acid and heating at 105˚C for 5 minutes, 18 spots appear at Rf. 0.04, 0.06 (both brown), 0.08 (blue), 0.12 (violet), 0.16, 0.19 (both grey), 0.29 (blue), 0.39, 0.44, 0.51, 0.54 (violet), 0.58 (blue), 0.60 (violet), 0.64 (dark blue), 0.71 (magenta), 0.73 (blue), 0.89 (violet) and 0.94 (blue). TLC profile of ingredients was compared with the profiles of all the samples of Agnimukha Ćũrna (Fig.3). TLC of chloroform extract in the solvent system toluene: ethyl acetate (10:3) v/v shows under UV (254 nm) 13 spots at Rf. 0.13 (blue), 0.19 (green), 0.21 (blue), 0.25 (green), 0.29 (blue), 0.31, 0.35, 0.45, 0.52, 0.64, 0.72,
Fig. 1a—Microscopic characters of Agnimukha Cūrṇa
Fig. 1b—Microscopic characters of Agnimukha Cūrṇa
Fig. 1c Microscopic characters of Agnimukha Ĉūrna
Fig. 2 TLC profile of chloroform extracts of Agnimukha Cuma samples
(a) Under UV 254 nm. (b) After derivatization under visible light

Fig. 3 TLC profile of chloroform extracts of Agnimukha Cuma (a) Under UV 254 nm.
(b) Under 366 nm. (c) In iodine (d) After derivatization under visible light

Fig. 4 HPTLC of volatile oil of Agnimukha Cuma and Ferula foetida and
Trachyspermum ammi (a) Under UV 254 nm (b) Under UV 366 nm
(c) After derivatization under visible light
0.80 and 0.94 (all green). Under UV (366 nm) 18 fluorescent spots appear at Rf. 0.05, 0.07, 0.13 (all white), 0.17 (pink), 0.23, 0.25, 0.31, 0.33, 0.39, 0.45, 0.50, 0.58, 0.64, 0.70 (all white). On exposing the plate to iodine vapour 3 yellowish brown spots appear at Rf. 0.58, 0.72 and 0.80. On dipping the plate in vanillin-sulphuric acid and heating at 105°C till the spots of colour appeared, 16 spots were observed at Rf. 0.05, 0.07 (dark grey), 0.15, 0.19, 0.23, 0.23 (brown), 0.31 (grey), 0.33 (dark grey), 0.37 (brown), 0.58, 0.64 (pink), 0.72 (purple), 0.78 (grey), 0.80 (pink), 0.92 (violet) and 0.94 (dark grey). The observed spots at Rf 0.13 (F.foetida), 0.78 (T.chebula) under UV 366 nm; 0.52 (P.longum) and 0.72 (A.calamus) under UV 254 nm; 0.50 (P.zeylanica), 0.64 (Z.officinale) and 0.78 (S.lappa) after derivatization with vanillin-sulphuric acid and 0.80 (T.ammi) on exposure to iodine were found to be present in all the four samples. The volatile oil from the ĉūrna in the solvent toluene: chloroform (1:1) v/v showed under UV (254 nm) 4 spots at Rf. 0.08, 0.49, 0.65 and 0.71 (all green). Under UV (366 nm) 5 spots appeared at Rf. 0.11, 0.25, 0.35, 0.49 and 0.58 (all pale blue). On exposing the plate to iodine vapour, 5 yellowish brown spots appeared at Rf. 0.08, 0.25, 0.35, 0.49, 0.65, and 0.71. On dipping the plate in vanillin-sulphuric acid and heating at 105°C for 2 minutes 6 spots at Rf. 0.08, 0.25, 0.35, (all grey), 0.49 (violet), 0.65 (magenta) and 0.71 (grey) were seen. Since the spots at Rf. 0.08, 0.25, 0.35, 0.71 (all grey) corresponding to F. foetida and 0.65 (magenta) corresponding to T. ammi (vanillin-sulphuric acid)
were present in the cūrna, it was concluded that F. foetida and T. ammi were included in the compound preparation (Fig. 4).

The HPTLC fingerprint profiles are depicted in Table 3. There were 12, 10, 10 and 13 spots respectively in the all the AC-1, AC-2, AC-3, AC-4 samples of Agnimukha Cūrna. The four major spots at Rf 0.10, 0.47, 0.62 and 0.72 were observed in the four samples. The similar pattern of the chromatograms with these four common spots (Rf 0.10, 0.38, 0.49 and 0.72) also indicated that all the samples contained the ingredients. Further the presence of the marker chemical, ferulic acid (F. foetida) at Rf 0.39 in all samples (Figs. 5a to 5e) was confirmed by superimposing the UV spectra with that of spots with same Rf value (Fig. 6). The small variation in matching of the spectra may be due to the changes in the concentration. Thus, on the basis of these data the presence of different ingredients in Agnimukha Cūrna can be tested. Hence, the microscopic features, physicochemical parameters, TLC/ HPTLC fingerprint profiles together may be used for quality evaluation and the standardization of the compound formulation Agnimukha Cūrna.

Acknowledgement
Authors are grateful to the Director, Central Council for Research in Ayurveda and Siddha, New Delhi for providing the facilities and Department of AYUSH, Ministry of Health and Family Welfare, Govt. of India for financial support.

References