Evaluation of Ayurvedic compound formulations III- *Laghugangadhar Churna*

Anshu Rathi*, Vartika Rai, Sayyada Khatoon, Subha Rastogi, Ajay Kumar Singh Rawat, Shanta Mehrotra & M M Pandey
Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow 226 001, Uttar Pradesh
E-mail: anshurathi@rediffmail.com

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Standardization of a compound Ayurvedic formulation is essential for establishing the authenticity, quality and efficacy of Ayurvedic medicines/finished herbal product. *Laghugangadhar Churna* is a compound formulation containing *Cyperus rotundus* (rhizome), *Symplocos racemosa* (stem bark), *Woodfordia fruticosa* (flower), *Aegle marmelos* (fruit pulp) and *Holarrhena antidysenterica* (seeds) as ingredients. It is one of the most effective and widely used remedy for diarrhoea and dysentery. To achieve the desired aim four samples of *Laghugangadhar Churna* were procured from different Ayurvedic pharmacies and were investigated by microscopy, physico-chemical parameters and high performance thin layer chromatography (HPTLC). The microscopic analysis of samples revealed starch grains in three ingredients viz. *C. rotundus*, *S. racemosa* and *W. fruticosa*. These plant species could be differentiated with the help of other diagnostic characters. HPTLC analysis of all four samples revealed the presence of all the ingredients in compound formulations as determined by simultaneous application of authentic ingredients. The diagnostic spots would be utilized as standardization profile. The physico-chemical data of the formulation assists in maintaining standard limits of *Laghugangadhar Churna*.

**Keywords**: Ayurvedic drugs, Standardization, *Laghugangadhar Churna*, HPTLC

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In recent years, greater global interest has inclined towards non-synthetic natural drugs, derived from plant sources, due to their better tolerance and negligible adverse drug reactions. The World Health Organization (WHO) has also considered phyotherapy in its health programs and suggested basic guidelines and procedures for the validation of drugs from plant origin both for developed western countries and developing countries like India and China. Despite various efforts by WHO, there is lack of supporting studies regarding the scientific evaluation of formulation and preparation related parameters. However, India’s ancient system of plant based medicine, *Ayurveda* is gaining recognition throughout the world and many *Ayurvedic* drugs are now clinically tested and accepted for manufacture. The *Ayurvedic* formulations are mainly available in the form of solid dosage (*vati, ghan vati, and churna*), liquid dosage (*asavas, arishtas*) and semisolid dosage (*ghritas, avlehas*). These forms are often referred to as drugs. These drugs contain more than one plant ingredients and each has complementary effects, which seemingly work together to enhance the therapeutic value or other properties of the mixture.

Various parameters are considered to standardize these medicinal preparations as safe drugs besides adhering to quality and efficacy as per standards of the *Ayurvedic* formulations. As most of the tests described in ancient literature appear to be based on observation and seem subjective without valid scientific backing therefore standardization and development of reliable quality protocols for *Ayurvedic* formulations using modern techniques of analysis is extremely important. The study deals with *Lagugangadhar Churna* (LGC), a common compound preparation used for treatment of diarrhoea and dysentery. This compound preparation is composed of six medicinal plant ingredients like exudates of *S. malabarica*, rhizome of *C. rotundus*, stem bark of *S. racemosa*, flower of *W. fruticosa*, fruit pulp of *A. marmelos* and seeds of *H. antidysenterica*. The investigation was carried out with the aim to develop standard pharmacopoeial parameters for LGC. The objectives included microscopic identification of plant drug constituents of commercial formulation procured from different *Ayurvedic* pharmacies; determination of the analytical values for defining the limits of physico-chemical parameters, and development of HPTLC finger print profile as a rapid analytical tool for authentication of commercial samples.

*Corresponding author*
Methodology

All the solvents of analytical grade were purchased from SD Fine Chemicals, Mumbai. Solvents used for extraction and HPTLC studies were distilled before use. Pre-coated silica gel GF-254 plates for HPTLC studies were from E Merck, Mumbai. HPTLC studies were carried out using CAMAG LINOMAT IV applicator and analyzed by Desaga Video documentation Unit III. All the samples of LGC were prepared according to Ayurvedic formulary by mixing equal parts by weight of each of the six ingredients in the form of exudates of S. malabarica, rhizome of C. rotundus, stem bark of S. racemosa, flower of W. fruticosa, fruit pulp of A. marmelos and seeds of H. antidysenterica. All ingredients and prepared LGC formulation were procured from Indian Ayurvedic Pharmacy of Indian Institute of Kaya Chikitsa, Patiala, (LGC I); Gujarat Ayurvedic University, Jamnagar, (LGC II); Department of Rasa Shastra, Jaipur, (LGC III); Indian Medicines Pharmaceutical, Almora (LGC IV). The organoleptic and microscopic studies of LGC were made. LGC sample (100 mg powder of each) and its ingredients were mounted separately in glycerin, iodine, water and chloral hydrate. The diagnostic characters of the ingredients were noted both in individual and in compound formulations under microscope by careful examination. The authenticities of the procured LGC ingredients were compared. The physiochemical parameters of LGC were carried out by quantitative analysis for loss on drying, total ash content, acid insoluble ash, sulphated ash, pH at 1% and 10% w/v aqueous solution, extractive values in petroleum ether, ethyl acetate, acetone, ethanol and water, successive soxhlet extractions in n-hexane, alcohol and water were also determined by standard pharmacopoeia(s) methods. Total percentage of sugar, starch and tannins were also determined in all the samples. Samples of LGC and each of its ingredients in 25 ml methanol were separately refluxed three times over water bath for 25 minutes by following the standard method. The extracts were filtered, pooled and concentrated on rotavapour and dried in lyophilizer under reduced pressure to obtain 10% of solid residue. 10 mg dried extracts were redissolved in 1 ml of methanol. Ten µl of extract was applied on a precoated silica gel 60 F254 TLC plate of uniform thickness (0.2 mm). The finger print profile was developed inToluene: Ethyl acetate (90: 10 v/v) solvent system to a distance of 8 cm and dried in a current of hot air. The plate was visualized under UV 254 nm. The spots were also developed after spraying with anisaldehyde-sulphuric acid reagent followed by brief heating at 110° C for 10 minutes.

Results and discussion

The organoleptic characters of all LGC samples from four different manufacturers were reddish brown in colour with spicy odour and had bitter taste. About 95% of the LGC samples from Patiala, Jamnagar and Jaipur passed through 60 mesh sieve and only 85% sample of Almora passed through the same mesh size. It was also observed that approximately 50% of all the four samples passed through 85 mesh size. The diagnostic cellular structures and cell contents with their shape and size for all ingredients areas follows:

Cyperus rotundus

The rhizome shows patches of loosely arranged oval to oval shaped parenchymatous cells measuring 70-140 µm in diameter with intercellular spaces, the cell are filled with starch grains, starch grains round to oval in shape measuring 5-20 µm in diameter; pitted stone cells of different shape, size and thickness, measuring 70-225 µm in diameter; brown coloured oil cells measuring 45-60 µm in diameter, fragment of vessels 10-30 µm broad with annular, spiral, scalariform and reticulate, secondary wall thickening; trachieds with bordered pits measuring 100-250 µm in length and 15-25 µm in width and groups of polygonal suberized cork cells (Fig.1).

Fig. 1— Powder microscopy of C. rotundus rhizome a. Parenchymatous cells with starch grains. b. Polygonal suberised cork cells. c. Stone cells. d. Oil cells. e. Scalariform vessels, vessels-pitted, reticulate, spiral. f. Tracheids cork from root in surface view.
**Symplococcus racemosa**

The bark shows prismatic crystals measuring 20-30 µm in diameter; fragments of bast fibres measuring 30-70 µm with very thin lumen; stone cells solitary or in a group of 2-5, some stone cells with broad lumen and simple pits and filled with brown coloured content; starch grains simple and compound up to 10 µm in diameter (Fig. 2).

**Woodfordia fruticosa**

The flowers shows groups of angular cells filled with rosette crystals of calcium oxalate; patches of angular parenchymatous cells filled with small starch grains measuring up to 5 µm in diameter; prismatic crystals of calcium oxalate measuring 5-70 µm in diameter; crushed pieces of anther lobes containing pollen grain; pollen grains tetracolpate measuring 20-25 µm in diameter; red coloured oil globules; trichomes unicellular, thin walled, varying in length 45-200 µm and width 10-20 µm (Fig. 3).

**Aegle marmelos**

The fruit pulp shows groups of yellowish orange coloured parenchymatous cells filled with yellowish orange content which dissolves in concentrated H₂SO₄ and gives bright magenta colour, fragments of vessels with spiral, scalariform and reticulate secondary wall thickenings (Fig. 4).

**Holarrhena antidysenterica**

The seeds show patches of yellow coloured polygonal sclerenchymatous cells measuring 30-60 µm in diameter, orange coloured oil globules measuring 25-60 µm in diameter, prismatic crystals of calcium oxalate, 15-20 µm in diameter, and fragments of thin vessels with spiral secondary wall thickening (Fig. 5).

The microscopic examination was carried out to see the presence of all the above ingredients in four samples of LGC. Microscopic examination showed the presence of all the ingredients in samples LGC II, LGC III and LGC IV. The analytical parameters estimated in physico-chemical studies showed variations in most of the parameters studied. The total ash of LGC III was found to be higher than remaining samples. Similarly, the acid insoluble ash and sulphated ash values were also relatively higher in case of LGC III. The extractive values of LGC III in
petroleum ether, acetone, ethyl acetate, alcohol and water were found to be higher than other LGC samples. When subjected to successive soxhlet extractions, the hexane soluble extractive for LGC III (2.93%) was found to be much lower than other three samples (9.25-9.37%). However, the alcohol soluble extractive value for LGC II and LGC III were found to be much higher when compared to LGC I and LGC IV. The loss on drying, pH, tannin, sugar and starch % were nearly same for all the samples. These variations may be due to differences in quality of raw materials used, their season of collection, region of collection and storage time. The Physico-chemical data of four different LGC’s were utilized for adjusting the upper and lower limits of various parameters on similar pattern as applicable for single drug pharmacopoeial parameters.

The test samples of LGC I, LGC II, LGC III and LGC IV were compared with the ingredients C. rotundus, S. racemosa, W. fruticosa, A. marmelos and H. antidysenterica (Figs. 6 & 7). The tracks 1-9 (Fig. 6) represent the 254 nm finger print profile. The track five shows finger print profile of C. rotundus in the form of four black spots at Rf 0.03, 0.07, 0.28 and 0.56. Out of these four spots, a maximum of three were present in LGC II (track 2) at Rf 0.03, 0.07 and 0.56. The test sample of LGC IV showed only two spots at Rf 0.07 and 0.56. The test samples of LGC IV showed only two spots at Rf 0.03, 0.12, 0.28 and 0.56. Out of these four spots, the spot at Rf 0.56 was found to be present in all samples of LGC I, LGC II, LGC III and LGC IV. In addition to this spot, LGC II and LGC IV showed spot at Rf 0.26. However none of the other spots of A.marmelos were visualized in any of the LGC test samples. Track eight shows fingerprint profile of S. racemosa showing two black spots at Rf 0.03 and 0.91. The test sample of only LGC II contained both these spots. Nevertheless, all four LGC samples contained a common spot of S. racemosa at Rf 0.91. Track nine shows fingerprint profile of W. fruticosa with only one black spot at Rf 0.91. All the test sample of LGC contained this spot. The same plate was sprayed with anisaldehyde-sulphuric acid and heated at 110°C for 10 minutes and visualized in visible light (Fig. 7). The track of C. rotundus showed seven spots at Rf 0.09 (purple), 0.12 (purple), 0.38 (blue), 0.45 (purple), 0.56 (orange), 0.63 (purple) and 0.89 (purple). The track of H. antidysenterica showed nine spots at Rf 0.09 (blackish brown), 0.16 (yellow), 0.17 (purple), 0.38 (blue), 0.45 (purple), 0.47 (orangish brown), 0.56 (purple), 0.63 (purple), 0.74 (yellow orange), 0.89 (orange brown) and 0.91 (purple). The spots at Rf 0.91, 0.89, 0.74 and 0.56 were present in all four test samples of LGC. The spots at Rf 0.45, 0.38, 0.17, 0.16 and 0.09 were present in only LGCI, III and IV. The spot at Rf 0.47 was present in LGC III and IV only. The track of A.marmelos showed six spots at Rf 0.26 (blue), 0.38 (purple), 0.45 (blue), 0.56 (sea blue), 0.63 (purple) and 0.79 (blue). Except for the spot at Rf
0.56, test samples of all LGC showed the presence of five spots of A. marmelos. The track of S. racemosa showed no spots after spraying. The track of W. fruticosa showed four spots at Rf 0.17 (purple), 0.38 (blue), 0.45 (purple), and 0.47 (purple). Except spot at Rf 0.45 and 0.47, the test samples of all LGC showed the presence of spots of W. fruticosa at Rf 0.17 and 0.38.

Conclusion
The development of pharmacopoeial standards of Laghugangadhar Churna was based on outcome of microscopical, physicochemical and HPTLC finger print profiles. The results were found to be highly accurate, quick and reliable for routine monitoring based on quality control of raw material, processed powder and compound preparations. The ingredients C. rotundus and H. antidysenterica are also used in other compound formulations like Ashwagandhadi churna, Katphaladi churna and Palashbijadi churna and these preparations can also be standardized by similar protocols. With the growing demand of herbal drugs in the herbal drug market, it is suggested that this standardization tool will help in maintaining the quality and batch to batch consistency of many important Ayurvedic preparations including Laghugangadhar Churna.

References