Chapter 1: Abstract

In the last few decades there has been an exponential growth in the field of herbal medicine. India has a very long history of safe and continuous usage of many herbal drugs, which are included in officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, homeopathy and Naturopathy. India can emerge as the major source of therapeutically active herbal formulation. This can be achieved only if the herbal products are evaluated and analyzed using reliable and reproducible techniques of standardization such as UV-visible spectroscopy, TLC, HPLC, HPTLC, GC-MS, Spectrofluorimetric and other methods. With this background in mind Rasayana churna and Amalakyadi churna were selected for the present study. Rasayana churna is an Ayurvedic formulation, well-known for its adaptogenic and immunomodulatory activities. It consists of three ingredients, viz. galo (dried stem of Tinospora cordifolia), gokharu (dried fruits of Tribulus terrestris) and amla (dried pericarp of Emblica officinalis) in equal proportions. The Amalakyadi churna is well known polyherbal formulation official in Ayurvedic Pharmacopoeia. Traditionally it is used in the treatment of anorexia, dyspepsia, fever and indigestion. It consists of amla (dried pericarp of Emblica officinalis), harde (dried pericarp of Terminalia chebula), chitrak (dried roots of Plumbago zeylanicum), pippali (dried fruits of Piper longum) and sindhava. In the present study, Rasayana churna and Amalakyadi churna were standardized by means of pharmacognostical and physicochemical parameters along with chromatographic fingerprinting and quantification of the marker compounds by UV-visible spectroscopy, HPLC and HPTLC techniques. Rasayana churna was also evaluated for its immunomodulatory activity by carbon clearance assay.

Rasayana churna appears as a fine powder of greenish brown colour with characteristic odour with bitter and astringent taste. Rasayana churna was also found to have diagnostic microscopical characters like isodiametric to elongated stone cells; calcium oxalate clusters and prisms; abundant lignified fibers; simple, unicellular narrow trichomes with bulbous base; simple, ovoid starch grains; pitted and bordered pitted xylem vessel, So Phytochemical screening of Rasayana churna showed the presence of alkaloids, flavonoids, saponins, phenolics, carbohydrates, tannins, steroids and
triterpenoids. The content of total phenolics in the alcoholic extract of *Rasayana churna* was found to be 7.19 mg of gallic acid eq. /gm of dried powder. Berberine content in *Rasayana churna* was found to be 0.102 ± 0.004% w/w by UV spectrophotometric method.

Berberine, diosgenin and gallic acid were selected as unique markers for *Tinospora cordifolia*, *Tribulus terrestris* and *Emblica officinalis*, respectively. The analysis was done on precoated silica gel 60 F254 TLC plates using a mobile phase composed of chloroform: methanol : ammonia (8 : 2 : 0.1) for berberine; hexane : acetone (7 : 3) for diosgenin and toluene : ethyl acetate : formic acid (3 : 3 : 0.8) for gallic acid. The contents of berberine, gallic acid and diosgenin were found to be 0.115 ± 0.02 % w/w, 0.719 ± 0.02%w/w and 0.023 ± 0.001 % w/w, respectively, in *Rasayana churna*. The proposed methods were validated by ICH guidelines and found to be accurate, reproducible and sensitive.

HPLC method was developed and validated for simultaneous estimation of gallic acid and berberine in *Rasayana churna*. The amount of berberine and gallic acid in *Rasayana churna* was found to be 0.133 ± 0.004 % w/w and 0.823 ± 0.012 % w/w respectively. HPLC method was also developed for quantification of Diosgenin in *Rasayana churna*. It was found to contain 0.025 ± 0.004 % w/w of Diosgenin.

Immunomodulatory activity of *Rasayana churna* was evaluated for its immunostimulant activity in rats by carbon clearance assay. *Rasayana churna* treated groups, exhibited significantly high phagocytic index as compared to that of control group. The results suggest the probable stimulation of the reticulo-endothelial system by treatment with *Rasayana churna*.

*Amalakyadi churna* is a fine powder with brown colour, characteristic pleasant odour and salty and spicy taste. *Amalakyadi churna* was found to have diagnostic microscopical characters like brownish cork cells; perisperm packed with starch grains, uniseriate multicellular trichomes, groups of sclereids with pitted wide lumen, prisms of calcium oxalate and silica crystals in epidermal cell. Phytochemical screening of *Amalakyadi churna* showed the presence of alkaloids, flavonoids, saponins, phenolics,
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carbohydrates, tannins, steroids, triterpenoids and coumarins. Sodium and potassium estimation was found by flame photometric method and contents of sodium and potassium were found to be 8.24 ± 0.108 % w/w and 0.0123 ± 0.123 % w/w, respectively. Total phenolic content of alcoholic extract of *Amalakyadi churna* was found to be 18.80 mg of gallic acid eq. /gm of dried powder.

Simultaneous HPTLC analysis of piperine and gallic acid in *Amalakyadi churna* was performed on precoated silica gel 60 F$_{254}$ TLC plates using a mobile phase composed of toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2). The contents of piperine and gallic acid were found to be 0.139 ± 0.001% w/w, and 0.443 ± 0.008 %w/w, respectively, in *Amalakyadi churna*. Validation of the proposed method was done using the ICH guidelines and the method was found to be accurate, reproducible and sensitive for quantification of piperine and gallic acid in *Amalakyadi churna*.

HPLC method was also developed for quantification of gallic acid, plumbagin and piperine in *Amalakyadi churna*. It was found to contain 0.475 ± 0.008 % w/w of gallic acid, 0.126 ± 0.004 % w/w of piperine and 0.042 ± 0.005 % w/w of plumbagin.