Original Research Article

Profiling and determination of phenolic compounds in Indian marketed hepatoprotective polyherbal formulations and their comparative evaluation

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Background: Nowadays, plants have been considered as powerful agents for treatment of disorders due to their traditional use. Plants have a special role in the treatment of various diseases in Ayurveda. Liver disorders with their devastating outcomes have been discussed in Ayurveda as well. Objectives: In the present study, polyherbal products (L52 and L38) were retrieved from Ayurveda and its pharmacognostic standardization was performed. Materials and methods: Quality control test for the Ayurveda tablets were performed as per Indian Pharmacopoeia. Dissolution studies of polyherbal Ayurveda marketed formulations were assessed based on the phenolic content. Fingerprinting of phytochemical constituents of L52 and L38 was performed using spectroscopical (like IR and UV) and chromatographic techniques like HPLC, HPTLC and TLC. Results: The results showed that L52 and L38 successfully passed quality control tests. Moreover, L52 and L38 exhibited different pharmacognostic behavior of all herbs present in the product. In addition, TLC, IR, HPTLC and HPLC fingerprinting of L52 and L38 demonstrated the presence of several phenolic constituents corresponding to the polyherbs. Conclusion: Regarding the role of phenolic compounds in the treatment of hepatitis, L52 and L38 could be appropriate candidates for hepatitis with respect to their traditional use in Ayurveda formulation. Moreover, HPTLC and HPLC fingerprinting could be utilized as an applicable method for quality control of the prepared formulation.

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1. Introduction

India can emerge as a major country and play the lead role in production of standardized, therapeutically effective Ayurveda formulations. India needs to explore the medicinally important plants and this can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization. Liver disorders are considered among the major world health problems [1]. Polyherbal tablet formulation 1 (L52) and polyherbal tablet formulation 2 (L38) are Ayurveda proprietary drugs from Himalaya Herbal Healthcare Products, India and Patanjali Ayurved Limited, India respectively. L52 and L38 are the combination of 8 and 9 polyherbal materials respectively, and control liver disorders effectively. Chemical and instrumental analysis is routinely used for analyzing single herbal ingredient drugs for the purpose of standardization [2]. A single herbal drug extract was standardized on the basis of its active principles. As per literature review, only very few chemical or analytical methods are available for polyherbal drug standardization [3]. So there is a need to develop a novel scheme for the standardization of the finished Ayurveda product, made up of more than one polyherbal material. Standardization of Triphala, a mixture of Emblica officinalis, Terminalia chebula and Terminalia belerica in equal proportions, has been reported by the HPLC method by using the RP18 column with an acidic mobile phase. Complete extraction of phenolic compounds was also studied, which enabled the efficient separation of total phenol compounds, that is, gallic acid, tannic acid, syringic acid and epicatechin along with ascorbic acid, within a 20 min analysis. Validation of the method was also performed in order to demonstrate its selectivity, linearity, precision, accuracy and robustness.

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[4]. Vasudevan et al. [5] conducted pharmacognostical and phytochemical standardization for *Tila Kwatha* which is a polyherbal formulation. There is no method for dissolution studies for polyherbal formulations due to the presence of poly-constituents. Our aim is to develop dissolution studies for L52 and L38 based on the presence of phenolic compounds. In the present study, the scheme for the standardization of polyherbal formulations were developed, which will give answers for almost all the requirements for polyherbal medicine standardization. Our main objectives are to standardize the herbal formulations (L52 and L38) based on pharmacognostical evaluation and quality control evaluation and to identify and estimate of phenolic compound in both formulations using different chromatographic and spectroscopic technique and their comparative evaluation includes dissolution studies.

2. Materials and methods

A packet of 120 tablets of L52 and L38 each was been taken from local market. L52 is a mixture of the following 8 polyherbal materials: Caper brush (*Himsra* — *Capparis spinosa*) 65 mg; Wild chicory (*Kasani* — *Chichorium intybus*) 65 mg; Mandur Bhasma (*Calx — Ferric oxide*) 33 mg; Black Night Shade (*Kakimanchi* — *Solanum nigrum*) 32 mg; Arjuna (*Terminalia arjuna*) 32 mg; Negro Coffee (*Kasamandra — Cassia occidentalis*) 16 mg; Yarrow (*Bninjasipha — Achillea millefolium*) 16 mg; and Tamarisk (*Jhavuka — Tamarix gallica*) 16 mg. L38 is a mixture of the following 9 polyherbal materials: Bhumi Amla (*Phylanthus niruri*) 100 mg; Bhringraj (*Eclipta alba*) 75 mg; Kutki (*Picrorhiza kurroa*) 75 mg; Giloy (*Tinospora cordifolia*) 50 mg; Kal megah (*Andrographis paniculata*) 50 mg; Makoy (*S. nigrum*) 50 mg; Punarnava (*Boerhavvia diffusa*) 50 mg; Arjuna (*T. arjuna*) 25 mg; and Daruhaldi (*Berberis aristata*) 25 mg.

2.1. Organoleptic evaluation

Organoleptic evaluation refers to evaluation of formulation by color, odor, taste, texture etc. The organoleptic characters of the tablets were carried out based on the method described by [6].

2.2. Quality control test for tablet formulations

The general appearance involved measurement of size, shape, color presence (or) absence, powder taste and surface texture. Standard physical tests for the marketed Ayurveda formulation tablets were performed and average values calculated. Mass variation was determined by weighing 20 tablets individually, and the average mass and percent variation of each tablet was calculated. Hardness was determined by taking 6 tablets from each formulation using a Monsanto hardness tester (Electrolab Pvt. Ltd., India) and the average pressure (kg cm$^{-2}$) applied to crush the tablet was determined. Friability was determined by first weighing 20 tablets after dusting and then placing them in a Roche Friabilator, which was rotated for 4 min at 25 rpm. After dusting, the total remaining mass of the tablets was recorded and the percent friability calculated. Thickness was determined by digital Vernier calipers and expressed in mm. (Leon Lachmann et al., 1987). Disintegration test was determined by inserting one tablet into each tube of basket rack assembly of disintegration apparatus and cylindrical discs were placed on the top of tablets. The apparatus was operated by using water as an impression liquid at 37 ± 2 °C. Disintegration time for both formulations was noted.

2.3. Pharmacognostical evaluation

Tablets were powdered using mortar and pestle. For microscopical study, finely powdered tablets were taken and stained with phoroglucinol and HCl. Physicochemical studies like total ash, water soluble ash, acid insoluble ash, sulfated ash, water and alcohol soluble extract, loss on drying at 105 °C, and extractive values by Soxhlet extraction method were carried out as per the WHO guidelines [7]. 1 mg of powdered drugs of each formulation was exposed to ultraviolet light at wavelength of 254 nm and 366 nm and in daylight while wet after being treated with different reagents [8].

2.4. Extraction

The extracts of L52 and L38 tablets were prepared by soxhlation with ethanol and water. The shade dried whole tablet powder was packed in thimble kept in the Soxhlet apparatus and extraction was allowed to run separately using ethanol and water. Finally, the Marc was dried. Ethanol and aqueous extract were concentrated by evaporating the solvent and the obtained extracts were weighed. The physical characteristics and percentage yield of various extracts were reported. The dried extracts of all solvents were kept in desiccator prior to analysis.

2.5. Phytochemical screening

All the extracts of polyherbal tablets were subjected to preliminary phytochemical screening for the detection of various chemical constituents. The presence or absence of different phytoconstituents viz. carbohydrates, proteins and amino acids, glycosides, saponins, alkaloids, phenolic contents and tannins were detected by usual prescribed methods [9,10].

2.6. Preliminary thin layer chromatography

Qualitative determination of phytoconstituents like phenolic content, tannins and flavonoids were determined by thin layer chromatography (TLC) technique. Two extracts were dissolved in their respective solvents and spotted on TLC plates (silica gel GF plates). The plates were developed in toluene-acetone-formic acid (4.5:4.5:1) for the determination of phenolic compounds; n-butanol—glacial acetic acid—water (4:1:5) for the determination of tannins; toluene-ethyl acetate-glacial acetic acid (30:40:5) for the determination of flavonoids. After developing the plate, they were dried and the resolution of components of extracts was studied by locating various spots on chromatogram using Folin—Ciocaleu reagent and sodium carbonate solution for phenolic content; UV light for tannins; and mixture of 1% FeCl3 and 1% potassium ferric cyanide for flavonoids. The distance of each spot from the point of its application was measured and recorded and the RF value was calculated [11,12].

2.7. Total phenolic content

Total phenolic content was analyzed spectrophotometrically by a modified Folin—Ciocaleu colorimetric method ([20]: Singleton VL. 1999). 0.125 ml of all the extracts (1:10 g/ml) was taken in each test tube. 1.5 ml of water and 0.125 ml of Folin—Ciocaleu reagent were added and allowed to stand for 6 min. 1.25 ml of 7% sodium carbonate and 3 ml of water were added in to each mixture and then allowed to stand for 90 min at room temperature. After the color formation, the absorbance was measured at 550 nm using Labindia UV—Visible spectrophotometer. Gallic acid was used to prepare a standard curve (1–10 µg/ml; y = 0.1071x + 0.007829; r² = 0.9987 ± 0.0016; y is the absorbance; x is the solution concentration). The results were expressed as milligrams of Gallic acid equivalents (GAE) per gram of powdered crude drug.