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Original Research Article (Experimental)

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

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ABSTRACT

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 pharmacognostic 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 (*Kasamarda* – *Cassia occidentalis*) 16 mg; Yarrow (*Biranjasipha* – *Achillea millifolium*) 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; *Kalmegh* (*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–Ciocalteu reagent and sodium carbonate solution for phenolic content; UV light for tannins; and mixture of 1% $FeCl_3$ 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–Ciocalteu 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–Ciocalteu 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; $y = 0.1071x + 0.007829$; $r^2 = 0.9987 \pm 0.0016$; 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.

2.8. Total flavonoid contents

Total flavonoid contents of the L52 and L38 extracts were determined according to the described method using quercetin as a reference compound. Total flavonoids were determined by the following method. 1 ml of an extract in methanol (10 g/l) was mixed with 1 ml aluminum trichloride in ethanol (20 g/l) individually and diluted with ethanol to 25 ml. The absorption of 400 nm was read after 40 min at 20 °C. Blank samples were prepared from 1 ml tablet extract and 1 drop acetic acid, and diluted to 25 ml. The quercetin calibration curve was prepared in ethanolic solutions with same procedure. Different concentrations of quercetin were prepared with ethanolic solutions. The total flavonoid content in tablet extracts in quercetin equivalents was calculated. Quercetin was used to prepare a standard curve (5–25 μg/ml; $y = 0.03524x + 0.000093$; $r^2 = 0.9941 \pm 0.0071$; y is the absorbance; x is the solution concentration). The results were expressed as milligrams of quercetin equivalents (QE) per gram of powdered crude drug. Linearity curve of gallic acid and quercetin are presented in Fig. 1.

2.9. FT-IR study

Infrared spectrum was taken (FT-IR, Spectrum RX 1, Perkin Elmer Ltd, Switzerland) by scanning the sample in potassium bromide discs. The samples of both formulations and standards were scanned individually to find the common bands of the vibrational spectra of standards of phenolic compounds and formulations for ensuring the presence of phenolic group [25,26].

2.10. HPTLC analysis

HPTLC analysis of alcoholic and aqueous extracts of polyherbal formulations (L52 and L38) for phenolic profile was performed on pre-coated silica gel 60F254 TLC plate (0.2 mm, Merck 60 F-254, Germany) as the stationary phase and toluene: acetone: formic acid (4.5:4.5:1) as a mobile phase. The dried extracts were dissolved in appropriate solvent (100 mg/ml) and filtered the solutions. The samples (10 μl) of extracts and standard quercetin, rutin, gallic acid and kaempferol were spotted in the form of bands of width 5 mm with a 2 μl Hamilton syringe on pre-coated silica gel aluminum plate (5 cm × 10 cm) with the help of Linomat 5 applicator. The applicator was attached to HPTLC system CAMAG which was operated through winCATS software (CAMAG Scientific Inc., USA). The linear ascending development was carried out in a 20 cm × 10 cm twin through glass chamber saturated with the mobile phase. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was placed in an ultraviolet (UV) chamber and observed at 254 and 366 nm. The plate was kept in densitometer (CAMAG Scanner 3) under UV light at 254 and 366 nm. The Rf values and finger print data were recorded by winCATS software. The peak table, peak display and peak densitogram were noted. The developed plate was then sprayed with 20% sodium carbonate solution and briefly dried followed by Folin–Ciocalteu reagent and dried at 100 °C in hot air oven. The plate was photo-documented at day light using photo-documentation chamber (CAMAG Reprostar 3) [11,12].

2.11. Dissolution studies based on the phenolic content

The dissolution studies of both formulations were monitored by measuring the content of phenols released at different time intervals using a Dissolution Apparatus Type II of USP (Paddle) at 50 rpm. The dissolution was studied using 900 ml of 5.8 pH phosphate buffer solution. The temperature was maintained at 37 ± 0.5 °C. 5 ml of the sample was withdrawn at different time intervals, i.e., 5, 15, 30, 45, 60 and 90 min, filtered through Whatman filter paper (AurocoPvt Ltd, Thailand) and replaced by an equal volume of dissolution medium. Filtrate was placed in 50 ml volumetric flask individually. Then 10 ml of water and 1.25 ml of Folin–Ciocalteu reagent were added and allowed to stand for 6 min. 12.5 ml of 7% sodium carbonate was added in to each mixture then made upto the mark with water. The mixtures individually were allowed to stand for 90 min at room temperature. After the color formation, the absorbance was measured at 550 nm using Elico UV–Visible spectrophotometer. Gallic acid was used to prepare a standard curve (1–10 μg/ml; $y = 0.29146x - 0.33607$; $r^2 = 0.997695 \pm 0.0015$; y is the absorbance; x is the concentration). The percentage of phenolic content release was calculated.

2.12. HPLC analysis

HPLC analysis of alcoholic and aqueous extracts of polyherbal tablets (L52 and L38) for phenolic profile was analyzed on a Gracesmart RP18 with 5μ (250 mm × 4.6 mm) as the stationary phase and acetonitrile: water: concentrated phosphoric acid (400:600:5) as a mobile phase. Mobile phase was filtered and degassed prior to use and flowing at the rate of 0.8 ml/min. The

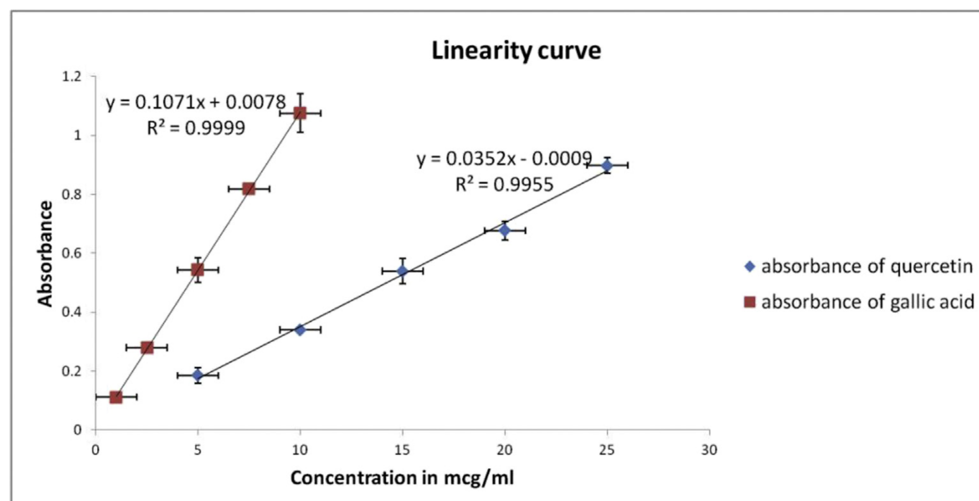

Fig. 1. Linearity data for Gallic acid and quercetin.

dried extracts were dissolved in 70% aqueous methanol (2000 mg/ml) and the solutions were filtered using 0.45 μ filter porosity membrane filter prior to injection. 20 μl of extract samples were spotted by injecting on column. The photo diode array detector (SPD-M20A, Shimadzu, USA) was attached to HPLC system (Shimadzu Prominence Modular HPLC) which was operated through Shimadzu LC solution software (Shimadzu, USA). The detector wavelength was set at 254 nm. The duration of each analysis was 50 min. The sample components were identified by comparison of their retention times to those observed in the chromatograms of reference solutions which were collected from the library data. The relative content of each component was determined by measuring the area under the corresponding peak and using the method of internal normalization [24].

3. Results

3.1. Quality control test

L52 tablet was red in color and L38 tablet was buff to brown in color. Both tablets had slightly bitter taste, characteristic odor and were smooth and soft to touch and round in shape. Diameter and width of both L52 and L38 tablets were 10 mm and 0.2 mm. In a weight variation test, mass values of the L52 tablets were between 0.48 and 0.51 gm; for L38, between 0.53 and 0.57 gm. The average percentage deviation of both tablet formulations was found to be within the limit, and hence all formulations passed the test for uniformity of weight as per official requirements. The hardness of L52 and L38 tablets were 2.33 ± 0.2581 kg/cm^2 and 4.6 ± 0.2581 kg/cm^2. Hardness studies indicated the strength of tablets. In general, tablets should be sufficiently hard (standard range from 4 to 8 kg/cm^2) to resist breaking during normal handling and yet soft enough to disintegrate properly after swallowing. A force of minimum 4 kg is considered as minimum requirement for a satisfactory tablet. For L52 tablets the hardness was not satisfactory; this may be due to less binder usage, punching pressure difference and less compressive force [13]. The percentage friability of L52 and L38 tablets were 0.62 and 0.78% w/w respectively. Conventional compressed tablets that lose less than 1% of their weight are generally considered acceptable. In the present study, the percentage friability for both formulations was below 1%, indicating that their friability was within the prescribed limits. Deterioration time of the polyherbal formulations depends upon the amount of water present in tablet material. If the water content is high, the formulations can be easily deteriorated due to fungus. The percentage of loss on drying at 105 °C in L52 and L38 tablets were found to be 0.01 ± 0.000 and 0.016 ± 0.0051% w/w respectively. Values of the hardness test and percent friability indicate good handling properties of the Ayurvedic marketed tablets. Disintegration time for L52 was between 33 min 23 s and 33 min 43 s; for L38 was between 60 min 0 s and 60 min 45 s. The standard set for this experiment was to have the tablet disintegrate not more than half an hour in water medium. Time taken by L38 tablets was more than 44 min. If the disintegration time is too high; it means that the tablet is too highly compressed. But this result did not really imitate how the preparation would disintegrate in human body. Multiple parameters to really imitate our body system upon drug intake were not provided. L52 and L38 gave successful results [14,15].

Table 1
Phytochemical analysis data.

Quantitative parameter	L52	L38
Xylem fibers (width and length)	21.87 μ and 68.75 μ	25 μ and 81.25 μ
Starch grains (width and length)	6.25 μ and 12.5 μ	3.12 μ and 9.37 μ
Mucilage (width and length)	29 μ and 45 μ	22 μ and 29 μ
Calcium Oxalate Crystal (width and length)	12.5 μ and 15.62 μ	15.62 μ and 21.87 μ
Trichomes (width and length)	6.25 μ and 131.25 μ	6.52 μ and 31.25 μ
Total ash value	15.5% w/w	10% w/w
Acid insoluble ash	0.5% w/w	0.5% w/w
Water soluble ash	6.0% w/w	1.0% w/w
Sulfated ash	2.0% w/w	1.5% w/w
Moisture content	0.12% w/w	0.1% w/w
Alcohol soluble extract value	27.58% w/w	19.7% w/w
Water soluble extract value	25.07% w/w	35.62% w/w

3.2. Pharmacognostic evaluation

The powdered microscopy of L52 and L38 revealed the presence of the following: Cork cells were found to be thin-walled and polygonally arranged, unicellular trichomes, lignified xylem fibers

Fig. 2. Powder microscopical image.

and circular to oval shaped mucilage. Starch grains present were circular to oval in shape. Stone cells were observed in L52 powder microscopy. The results of quantitative microscopy and physiochemical parameters of whole powder of L52 and L38 are presented in Table 1. Fig. 2 expressed the powder microscopical report of L52 and L38. Total ash value of tablet material indicated the amount of minerals and earthy materials present in the tablet material. Analytical results showed that total ash value of L52 and L38 were 15.5 and 10% w/w respectively. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compounds present in drugs. The acid insoluble ash mainly consists of silica and indicates contamination with earthy material. Moisture content of drugs should be at minimal levels to discourage the growth of bacteria, yeast or fungi during storage. Water-soluble extractive value indicated the presence of sugar, acids and inorganic compounds. The alcohol soluble extractive values indicate the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids while n-hexane (hot) extractive values indicate the non-polar secondary metabolites present in the formulations [16]. The extractive values of L38 in water were found to be higher than alcohol extractive values. But in L52 alcohol soluble extract, values were slightly higher than water soluble extractive values. In fluorescence analysis the powder samples were exposed to ultraviolet light at wavelength of 254 nm and 366 nm and day light after being treated with different reagents. Fluorescence analysis results show whether any fluorescent ingredients are present or not. It acts as a tool to detect adulterants and substituent and helps in maintaining the quality, reproducibility and efficacy of natural drugs.

3.3. Extraction and phytochemical screening

After extraction with ethanol and water solvents, the residues were dried and measured. The percentage yield obtained was 18.2% and 38.65% w/w for alcohol and aqueous extracts of L52 respectively; 15% and 59.62% w/w for alcohol and aqueous extract of L38 respectively. The brown residues were for alcohol and aqueous extracts of L52 and L38 respectively. All the extracts were sticky in nature. The extractions of any crude drug with a particular solvent yielded a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gave an indication whether the crude drug is exhausted or not [17]. Phytochemical screening of the ethanolic and aqueous extracts of L52 and L38 gave general ideas regarding the nature of chemical constituents present in the drug [18]. Preliminary phytochemical studies confirmed the presence of carbohydrates, glycosides, flavanoids, saponins, alkaloids, phenols and tannins in ethanolic and aqueous extracts of L52 and L38. The reports of phytochemical analysis reported that all four extracts didn't consist phytosterols.

3.4. Preliminary thin layer chromatography

Preliminary TLC separation and identification of phenolic compounds, flavonoids and tannins in alcoholic and aqueous extracts of L52 and L38 was performed using the chromatographic system with silica gel GF as stationary phase and the corresponding mobile phase mentioned above. Gallic acid, tannic acid and quercetin were used as a standard for phenolic compounds, tannins and flavonoids respectively. Spots of standards were easy to detect and were compared with samples' spots. After using visualizing agents, the spots' color dominantly appeared for above mentioned constituents. Rf values of gallic acid, tannic acid and quercetin were 0.6, 0.88, and 0.53 respectively for corresponding mobile phase mentioned earlier. The TLC analysis in various solvent systems for each solvent type revealed the presence of spots. Each spot was presumably due to a pure natural product or phytochemical. Each spot also had a specific Rf value. One of the Rf values of sample spot coincided with Rf values of standards used. Aqueous extract of L52 and L38 and ethanolic extract of L52 showed the presence of gallic acid, tannic acid and quercetin, but in ethanolic extracts of L38 formulations, except gallic acid, tannic acid, remaining quercetin was present. These results are preliminary studies to confirm the presence of phytoconstituents.

3.5. Total phenolic content and flavonoid content

The milligrams of GAE per gram of ethanol and aqueous extract of L52 were found to be 5.65 ± 0.17 and 1.22 ± 0.021 respectively. The milligrams of GAE per gram of ethanol and aqueous extract of L38 were found to be 3.16 ± 0.09 and 0.645 ± 0.161 respectively. The ethanol extract of L52 product contained maximum total phenolic content (5.65 mg GAE/g) than other extract. The milligrams of quercetin equivalents (QE) per gram of ethanol and aqueous extract of L52 were found to be 3.21 ± 0.44 and 3.37 ± 0.198 respectively. The milligrams of quercetin equivalents (QE) per gram of ethanol and aqueous extract of L38 were found to be 2.29 ± 0.19 and

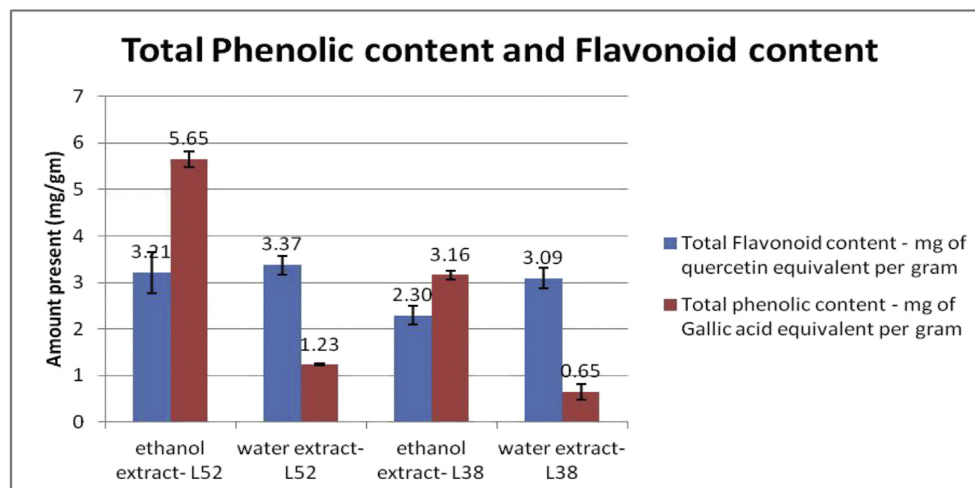

Fig. 3. Total phenolic content and flavonoid content.

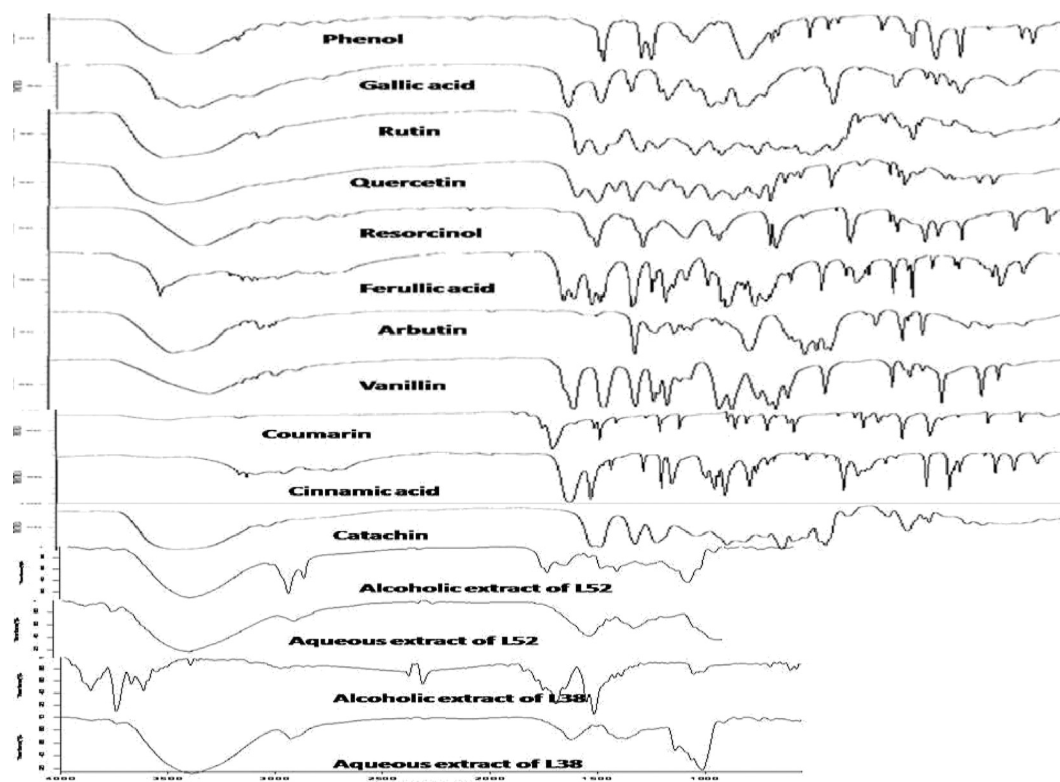

Fig. 4. Overlap FTIR profile.

3.091 ± 0.22 respectively. The aqueous extract of L52 product contained maximum total phenolic content (3.37 mg QE/g) than other extract. Total phenolic and flavonoid content estimation of the alcoholic and aqueous extracts of L52 and L38 were evaluated and reported in Fig. 3. Shahidi and Naczk [20] reported that the usage of Folin–Ciocalteu reagent was also measured based on the color measurement which was non-specific on phenol. Perhaps there were other components that can react with the reagent such as ascorbic acid. Besides, various phenolic compounds have different response to this assay. However, the measurement of color changes after 2 h storage could be used to determine the existence of phenol in samples. This may due to the antioxidant properties of polyherbal tablet extract that react as reducing agent which are known as redox action.

3.6. FTIR studies for phenolic compounds

IR spectra of standards like phenol, gallic acid, rutin, quercetin, resorcinol, ferulic acid, arbutin, vanillin, coumarin, cinnamic acid, and catechin were used for comparison studies. IR spectra of all extracts of both tablets were compared with standard IR spectra using KBr Disc method. Overall results of FTIR spectra of alcoholic and aqueous extracts of two different marketed formulations showed the presence of alcoholic group (at 3390/cm) and ethylenic group (at ~1630/cm for C=C). It was compared with standards and the reports suggested that presence of phenolic groups in the alcoholic and aqueous extracts of L52 and L38. IR spectrum of alcoholic extract of L52 products showed the presence of carboxylic acid (at 2854/cm for COOH and at 1710/cm for C=O). From the results, the alcoholic extract of DBC product may consist cinnamic acid or ferulic acid or gallic acid etc., Fig. 4 expressed the overlap image of FTIR spectra of standard phenolic content and extracts of L52 and L38.

3.7. HPTLC analysis for phenolic compounds

HPTLC coupled with CAMAG TLC SCANNER 3 was employed to separate, identify and quantify phenolic compounds in the alcoholic and aqueous extracts of L52 and L38. Blue colored zone at day light mode present in the given standard and sample tracks observed in the chromatogram after derivatization, may be the presence of phenolic compounds in the given samples. The concentrations were determined by calculating the spot areas which are proportional to the amount of analyte in a peak presented. In

Table 2
HPTLC data profile of L52, L38 and standard phenolic compounds.

Sample/Standard		No of spots found	No of spots identified as phenol	Rf values of identified spots	Peak area of identified spots	Identified compounds
Alcoholic extract	L52	8	2	0.55 and 0.74	6498.3 and 17657.8	Gallic acid and kaempferol derivatives
	L38	8	2	0.55 and 0.73	1641.5 and 24088.8	Gallic acid and kaempferol derivatives
Aqueous extract	L52	8	1	0.54	2079.4	Gallic acid derivatives
	L38	5	0	–	–	–
Standards	Quercetin	1	1	0.68	11711.0	Quercetin
	Rutin	1	1	0.12	8282.3	Rutin
	Gallic acid	1	1	0.51	19467.8	Gallic acid
	Kaempferol	1	1	0.71	8957.7	Kaempferol

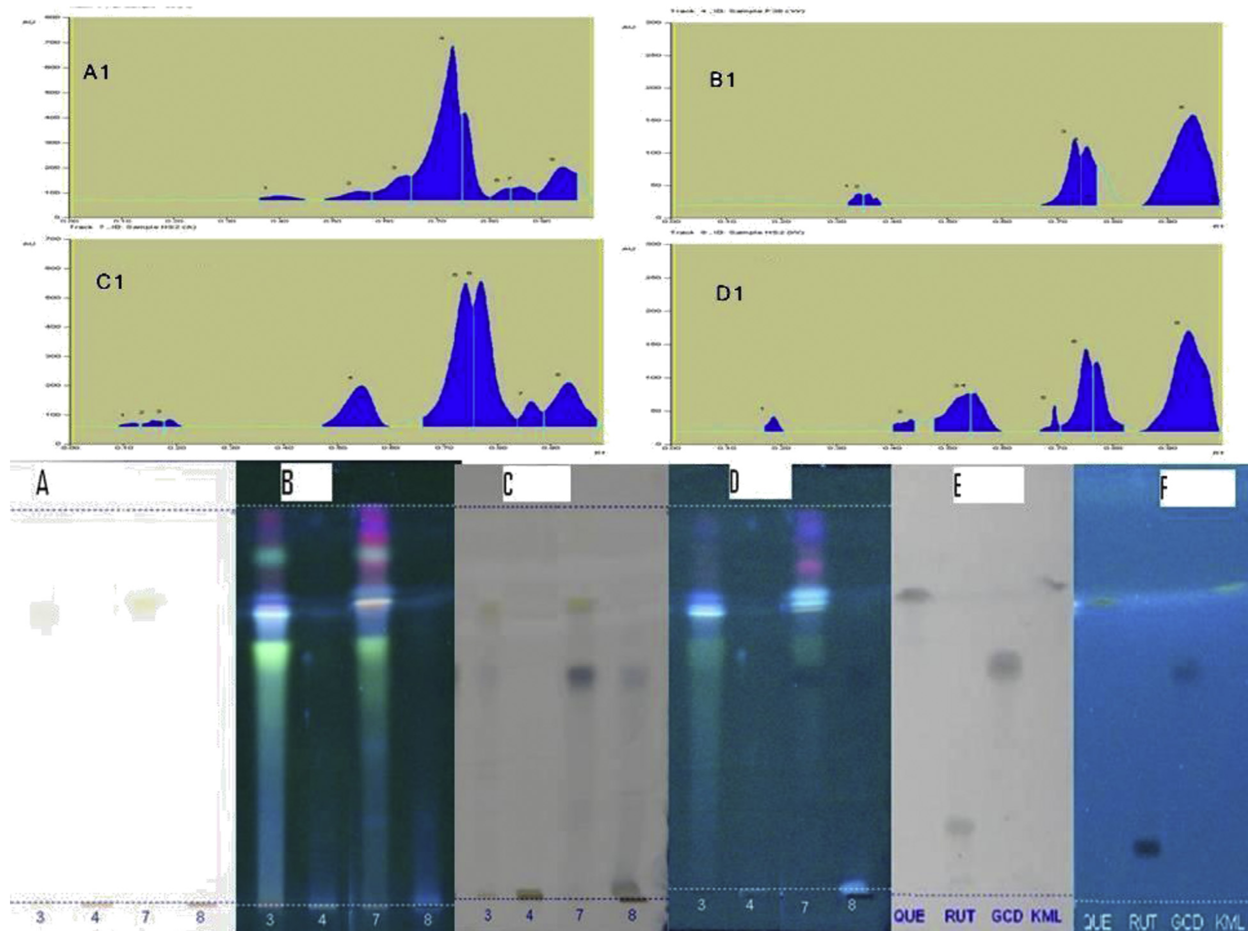

Fig. 5. HPTLC Profile; A1 and B1 are peak densitogram of alcoholic and aqueous extracts of L38; C1 and D1 are peak densitogram of alcoholic and aqueous extracts of L52; A and B are sample chromatograms under visible light and UV light (before derivatization); C and D are sample chromatograms under visible light and UV light (after derivatization); E and F are standard chromatograms under visible light and UV light (after derivatization); 3 and 4 denotes the alcoholic and aqueous extracts of L38 respectively; 7 and 8 denotes the alcoholic and aqueous extract of L52 respectively; QUE- Quercetin; RUT-Rutin; GCD-Gallic acid; KML- Kaempferol.

ethanolic extract of L38 product, total 8 spots appeared; among those two were found to be phenols which are similar to the Rf value of gallic acid and kaempferol. In aqueous extract of L38 product, total 5 spots appeared; among those none was found to be phenols. In aqueous extract of L52 product total 8 spots appeared; among those one was found to be phenols which is similar to the Rf value of gallic acid. In ethanol extract of L52 product, total 8 spots appeared; among those two were found to be phenols which are similar to the Rf value of gallic acid and kaempferol. Table 2 expresses the results of HPTLC for the determination of phenol in L52 and L38 compare with standards. Fig. 5 shows the peak densitogram display and HPTLC chromatogram of L52 and L38 ethanol and aqueous extracts respectively. HPTLC fingerprinting of L52 and L38 demonstrated the presence of several phenolic compounds corresponding to the plants present in polyherbal tablets extracts. Moreover, characteristic peaks were observed in L52 and L38 profile, so HPTLC fingerprint could be used as an applicable method for quality control of the prepared formulation.

3.8. Dissolution studies for tablet formulations

Dissolution studies were performed for L52 and L38 tablets based on the presence of phenolic compounds. Both tablets and pure gallic acid were placed in dissolution apparatus and the results were analyzed and calculated. Dissolution studies' data of amount (mg of gallic acid equivalent/tablet), % of gallic acid equivalent release and log% drug undissolved were evaluated and reported in Table 3 and Fig. 6. Maximum percentage of gallic acid equivalent

Table 3
Dissolution studies for L52 and L38.

	Time	5 min	15 min	30 min	45 min	60 min	90 min
Amount (mg of gallic acid/tablet)equivalent	L52	11.36 ± 0.01	10.96 ± 0.002	11.70 ± 0.03	10.96 ± 0.08	10.81 ± 0.07	10.65 ± 0.06
	L38	11.21 ± 0.01	10.84 ± 0.02	10.87 ± 0.01	12.23 ± 0.001	11.49 ± 0.09	12.66 ± 0.08
	Gallic acid	14.39 ± 0.03	16.27 ± 0.01	16.15 ± 0.01	14.98 ± 0.01	14.51 ± 0.01	13.74 ± 0.01
% of gallic acid equivalent release	L52	69.87 ± 0.01	67.4 ± 0.002	71.95 ± 0.03	67.4 ± 0.08	66.45 ± 0.07	65.5 ± 0.06
	L38	68.92 ± 0.01	66.64 ± 0.02	66.83 ± 0.01	75.18 ± 0.001	70.63 ± 0.09	77.84 ± 0.08
	Gallic acid	88.47 ± 0.03	100 ± 0.014	99.29 ± 0.01	92.08 ± 0.01	89.23 ± 0.01	84.48 ± 0.01
Log % drug undissolved	L52	1.47 ± 0.01	1.51 ± 0.002	1.44 ± 0.03	1.51 ± 0.08	1.52 ± 0.07	1.53 ± 0.06
	L38	1.49 ± 0.01	1.523 ± 0.02	1.52 ± 0.01	1.39 ± 0.001	1.46 ± 0.09	1.34 ± 0.08
	Gallic acid	1.06 ± 0.03	0 ± 0.014	−0.14 ± 0.01	0.898 ± 0.01	1.03 ± 0.01	1.19 ± 0.01

Mean ± standard deviation; n = 6.

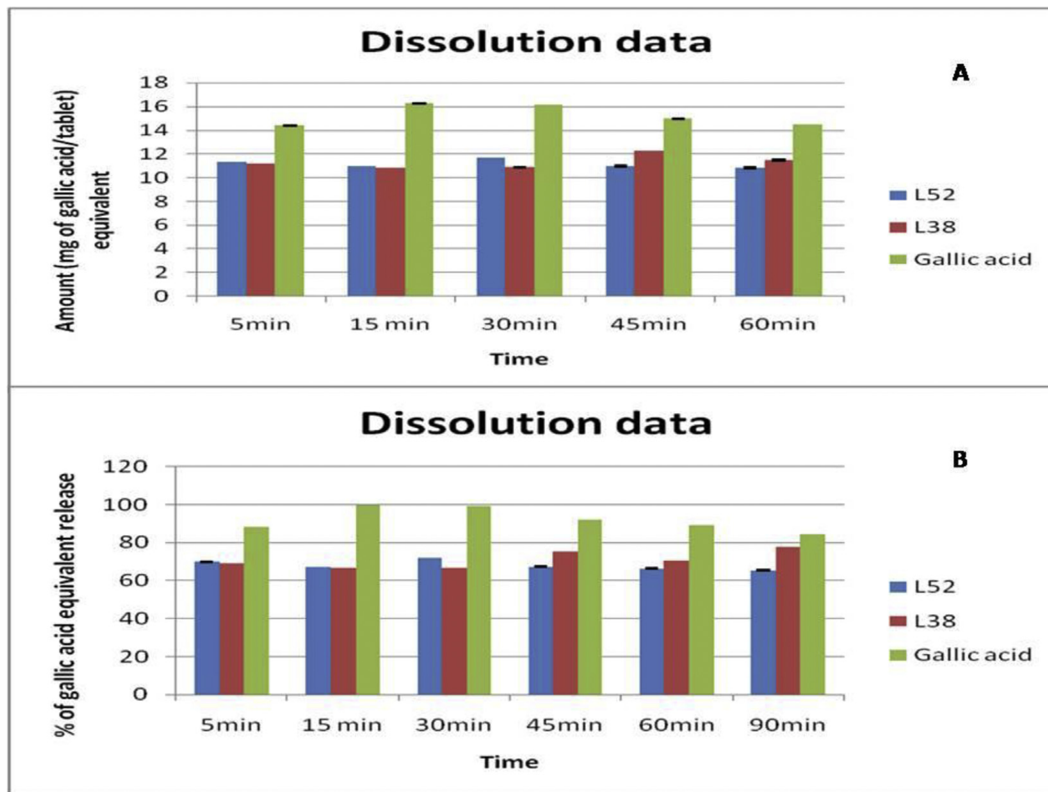

Fig. 6. Dissolution data; A is an amount equivalent vs time; B is Percentage of gallic acid equivalent release vs time.

release was 71.95% and 75.18% for L52 and L38 respectively. Maximum gallic acid equivalent release was achieved at 15 min for L52; and at 45 min for L38. Pure gallic acid reached 100% GAE release at 15 min [13]. Fig. 6 expressed the amount (mg of gallic acid/tablet) equivalent and % of GAE release in L52 and L38.

3.9. HPLC analysis

RP-HPLC coupled with PDA detector was employed to separate, identify and quantify phenolic compounds in the ethanol and aqueous extracts of L52 and L38. The ethanol and aqueous extract of L52 under study showed little variations in their content of the different phenolic compounds and nearly 5 compounds were identified. In general, ferulic acid, cinnamic acid, robinin, ellagic acid and quercetin were major phenolic compounds in the L52 of ethanol and aqueous extracts; other than identified peaks, some other peaks were also observed in both extracts. In ethanolic extract, retention time (relative content) of 11.07 (7.77); 23.097 (0.037); 24.99 (5.72); 28.38 (14.158); 30.247 (22.172); 32.664 (3.597) and 34.121 (1.056) were found for other unidentified peaks. In aqueous extract, retention time (relative content) of 11.03 (10.97); 13.17 (0.513); 13.7 (0.274); 14.348 (1.638); 24.918 (5.199); 28.58 (13.38); 33.46 (1.92) and 34.98 (22.17) were found for other unidentified peaks.

The ethanol and aqueous extract of L38 under study showed little variations in their content of the different phenolic compounds and nearly 6 compounds were identified. In general, ferulic acid, rutin, cinnamic acid, robinin, ellagic acid and quercetin were major phenolic compounds in the L38 of ethanol and aqueous extracts; Other than identified peaks, some other peaks were also

Table 4
HPLC analysis.

Minutes	Retention time	L52 aqueous extract		L38 aqueous extract		L52 alcoholic extract		L38 alcoholic extract	
		Retention time	Relative content %	Retention time	Relative content %	Retention time	Relative content %	Retention time	Relative content %
Ferulic acid	4.37	4.199	2.815	4.115	1.739	4.214	1.236	4.220	2.068
Rutin	17.58	–	–	–	–	–	–	17.60	1.454
Cinnamic acid	19.76	22.178	8.780	22.082	6.204	–	–	19.618	0.868
Robinin	22.55	–	–	–	–	22.376	8.605	22.258	1.972
Ellagic acid	27.16	27.551	7.455	27.511	4.894	27.365	4.339	27.260	9.3
Quercetin	35.54	36.060	24.872	36.091	68.366	35.196	31.295	35.059	43.381
Other peaks		11.030	10.974	14.512	3.098	11.073	7.775	11.065	3.176
		13.174	0.513	23.184	0.706	23.097	0.037	26.577	1.005
		13.709	0.274	24.694	1.944	24.990	5.728	28.35	15.458
		14.348	1.638	28.530	9.268	28.387	14.158	29.686	14.301
		24.918	5.199	33.404	2.721	30.247	22.172	32.550	4.719
		28.582	13.383	34.943	1.060	32.664	3.597	34.008	2.298
		33.464	1.927			34.121	1.056		
		34.987	22.170						

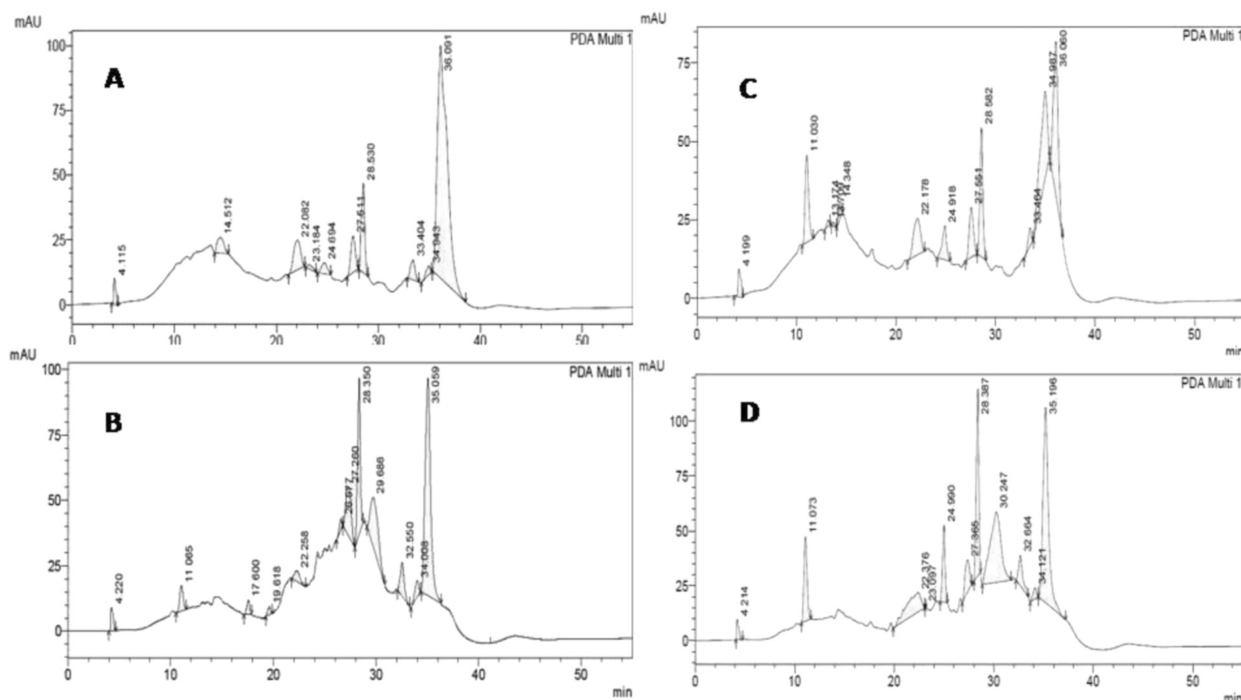

Fig. 7. HPLC Profile; A and B are HPLC chromatogram of aqueous extract and alcoholic extract of L38. C and D are PLC chromatogram of aqueous extract and alcoholic extract of L52.

observed in both extracts. In ethanolic extract, retention time (relative content) of 11.06 (3.17); 26.57 (1.005); 28.35 (15.45); 29.68 (14.301); 32.55 (4.719) and 34.008 (2.29) were found for other unidentified peaks. In aqueous extract, retention time (relative content) of 14.51 (3.098); 23.18 (0.706); 24.69 (1.944); 28.53 (9.268); 33.404 (2.721) and 34.943 (1.06) were found for other unidentified peaks. Table 4 depicts the retention time and relative content of identifying phenolic compounds by HPLC while Fig. 7 depicts the HPLC of both extracts of L52 and L38.

4. Discussion

TLC method is the most basic method for confirming the presence of phenolic compound. Results of Rf value of TLC and HPTLC expressed the presence of phenolic compound in the ethanol and aqueous extracts of L52 and L38. From the results of TLC and UV, the level of these components in various solvent extracts of L52 and L38 and also showed differences. Perez et al. [19] found that rosemary methanol extract had higher phenolic contents than its aqueous extract. Different levels reported in these studies may be attributed to the procedures and standards used to express as total phenolic contents used by individual groups of investigator. Based on the report of TLC, UV, IR, HPTLC and HPLC, ethanol and aqueous extracts contain remarkable levels of phenols. HPTLC and HPLC analyses showed that these phenolic compounds belong to the most part to flavonoids and derivatives of phenol carboxylic acid. Both L52 and L38 contain a rich complex of biologically active compounds of phenolic nature. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Enhanced lipid peroxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis. Among plant metabolites, phenolics are reputed to play a noticeable protective role against several health disorders [21]. Phenolics possess various biological activities, for instance, anti-ulcer, anti-inflammatory, antidiabetic, antioxidant, cytotoxic and anti-tumor [22]. It was found that phenolic, especially polyphenolic, compounds such as flavonoids are very efficient scavengers of free radicals [23] because of their molecular structures, which include an aromatic ring with hydroxyl groups containing mobile hydrogen. All analytical reports suggest that L52 and L38, rich in flavonoids and phenolics have potential to contribute to the management of diabetes.

5. Conclusion

In this investigation, standardization of L52 (from Himalaya Herbal Healthcare Products, India) and L38 (from Patanjali Ayurved Limited, India) was performed based on pharmacognostic evaluation and quality control evaluation. Identification and estimation of phenolic content in both formulations were done using different chromatographic and spectroscopic techniques. Results of analytical reports suggested that L52 and L38 consist of rich amount of phenolic content. Even, water and ethanol could extract the highest concentration of polyphenols from the marketed Ayurvedic formulation. L52 and L38 may have a good pharmacological potency due to the presence of polyphenols. Regarding the role of phenolic compounds in hepatoprotective activity, L52 and L38 could be an appropriate candidate for protecting the liver with respect to its traditional use in Ayurveda.

Sources of funding

None.

Conflict of interest

None.

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