QUALITY ASSESSMENT OF SOME MARKETED HEPATOPROTECTIVE POLYHERBAL FORMULATIONS

Rajendra O. Ganjiwale[a]*, Pramod G. Yeole[b] and Dilesh J. Singhavi[c]

(Received 20 December 2020) (Accepted 01 January 2022)

ABSTRACT

The present study was proposed for assessing the variation in quality of some hepatoprotective polyherbal products that are widely available in the market. Different brands of marketed hepatoprotective formulations were evaluated in the present investigation with respect to qualitative analysis by HPTLC, phytochemical evaluation and heavy-metal detection. Among all formulations, it was observed that formulation of Brand C showed maximum amount of phenolic content, tannin content, and flavonoid content. In HPTLC chromatograms of formulations of various brands, spots with RF values (0.46 ±0.3 and 0.86±0.3, respectively) were found, which confirmed the presence of andrographolide and phyllanthin in them. The increased risk of harmful side effects to the patients was indicated by the presence of heavy metals in some formulations above the permissible limits. The present study gives an insight into the fact that there is a requirement to prepare stricter quality control procedures and parameters for formulations for consumer safety.

Keywords: Hepatoprotective, Phytochemical, HPTLC, Heavy metal, Marketed formulation, andrographolide, phyllanthin

INTRODUCTION

As is known to all, the liver is the most dynamic and essential organ and is, required in a large number of functions, that is, the digestion and alteration of nutrients after the gastrointestinal tract absorption so that it could become an extra helpful energy form, as well as the complete elimination of various drugs, foreign materials, and numeral other substances from the body[1]. The liver is an energetic body part in the human body as it helps in performing many complicated mechanisms. It plays the crucial work of clear-up of several harmful and unwanted substances[2]. Various hepatic disorders can occur due to regular exposure of the liver to toxic elements (like drugs). If the situation worsens, hepatic failure and eventual death can occur due to liver damage[3]. Owing to the elevated numbers of adverse effects, it is opined that synthetic drugs are not safe instead of appropriate for liver diseases. Therefore, it is, required to look for another remedies[4].

There are a small number of natural plants showing superiority for curing liver diseases, and at the same time imposing only very less side effects. It is observed that about 600 herbal drugs are being marketed worldwide for hepatoprotective effects[5,6]. It is also noted that herbal products are launched in the market, in most of the countries, without conducting any proper scientific evaluation as well as without any detailed toxicological studies and mandatory safety. There is no effective machinery for regulating quality standards and manufacturing process. Consumers can easily purchase the formulations in the absence of any prescription and might not even be familiar with the possible hazards adding up with the low-grade products. It is shown by the studies on phytomedicines that purchasers have almost fewer than 50% probability of actually benefiting from what is given on the formulation label. Moreover, analyses report for ayurvedic products have distinctly observed that noteworthy variations exist between what is in the actual products and what are listed on the products label[7].

However, mainly for the prescribed use, only few formulations have been technically validated. Although some information is available about the individual formulation, the studies on their chemoprofiling and phytochemical evaluation are limited and subsequently scarce in the literature. In the light of the above mentioned circumstances, it was believed to be worthwhile to conduct

[a] Dept. of Pharmacognosy, Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha - 442 001, Maharashtra, India
[b] Dr. Babasaheb Ambedkar Marathwada University, Aurangabad – 431 004, Maharashtra, India
[c] Department of Pharmaceutics, Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha - 442 001, Maharashtra, India
*For Correspondence: E-mail: ro_ganjiwale@rediffmail.com

https://doi.org/10.53879/id.59.05.12808

Table I: Results of phytochemical screening tests of different marketed hepatoprotective formulations

Test	A	B	C	D	E	F	G	H	I	J
Sterol										
Salkowaski	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoid										
Shinoda	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve
Sulphuric acid	-ve	+ve	-ve	+ve	+ve	+ve				
Saponin Foam test	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve
Alkaloid										
Dragendorff	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
Mayers	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
Wagners	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
Hagers	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
Tannins										
Lead acetate	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Ferric chloride	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Potassium dichromate							+ve	-ve	+ve	+ve
Amino acid										
Ninhydrin test	-ve	-ve	-ve	-ve	-ve	-ve				
Triterpeoid										
Lieberman test							+ve	+ve	-ve	-ve
Proteins										
Biuret							+ve	+ve	+ve	+ve
Xanthoproteic							+ve	+ve	-ve	+ve
Sugar										
Molish test							+ve	+ve	+ve	+ve

proper studies and scientific validation of a number of polyherbal hepatoprotective phytomedicines that are available in the Indian market. A total of ten different polyherbal hepatoprotective formulations of specifically varied companies were selected and coded in the present study, and their chemoprofiling and phytochemical evaluation were carried out.

MATERIALS AND METHODS

Materials

Standard phyllanthin and andrographolide, basically utilized as a standard authentic marker, were procured from the authorized supplier—Yucca Laboratories, Mumbai and SPIC, Chennai, respectively. All reagents and chemicals used were of analytical grade. Marketed formulation Brand A (Capsule), Brand B (Capsule), Brand C (Capsule), Brand D (Tablet), Brand E (Tablet), Brand

Table II: Total phenolic, flavonoid and tannin contents of different marketed formulations

Sr. No.	Formulation	Total Content*		
		Phenolic	Flavonoid	Tannin
1	Brand A	30.67 ± 0.12	15.53± 0.12	10.99 ± 0.24
2	Brand B	32.60 ± 0.99	14.23± 0.22	12.14 ± 0.37
3	Brand C	44.02 ± 0.31	18.63 ± 0.24	13.86 ± 0.37
4	Brand D	41.74 ± 1.58	13.23 ± 0.54	11.26 ± 0.23
5	Brand E	40.97 ± 0.25	12.73± 0.14	10.33 ± 0.12
6	Brand F	33.17 ± 0.45	18.41 ± 0.45	9.60 ± 0.13
7	Brand G	40.94 ± 0.40	16.25 ± 0.44	11.60 ± 0.20
8	Brand H	36.94 ± 0.53	15.86 ± 0.33	10.56 ± 0.12
9	Brand I	42.54 ± 0.20	14.28 ± 0.23	12.56 ± 0.29
10	Brand J	43.81 ± 0.42	14.75± 0.13	10.60 ± 0.17

**Mean ± SD (n = 3)*

Table III: Percent recovery of phenolic, flavonoid and tannin compounds in their tests of analysis

Sr. No.	Formulation	% Recovery*		
		Phenolic	Flavonoid	Tannin
1	Brand A	96.63 ± 1.22	95.56 ± 0.87	93.92 ± 0.48
2	Brand B	95.23 ± 0.89	96.23 ± 1.56	95.28 ± 1.23
3	Brand C	96.25 ± 1.37	95.45 ± 1.83	95.16 ± 1.82
4	Brand D	94.52 ± 0.81	96.48 ± 0.78	96.45 ± 0.89
5	Brand E	96.51 ± 1.42	94.81 ± 1.93	96.38 ± 0.37
6	Brand F	96.26 ± 2.09	93.95 ± 2.23	95.86 ± 1.46
7	Brand G	95.27 ± 1.64	94.98 ± 0.45	94.92 ± 1.23
8	Brand H	94.80 ± 1.44	95.23 ± 1.23	94.77 ± 1.85
9	Brand I	95.71 ± 0.67	96.14 ± 1.29	94.14 ± 1.63
10	Brand J	95.83 ± 0.56	95.57 ± 0.76	95.32 ± 1.51

**Mean ± SD (n = 3)*

Table IV: Heavy elements concentration estimated by atomic absorption spectroscopy

Sr. No.	Formulation	Heavy metal content in ppm*				
		Cd	Cu	Pb	As	Hg
	Limit prescribed by WHO	0.3	20	10	3	1
1	Brand A	0.290 ± 0.002	0.855 ± 0.013	10.864 ± 0.107	0.229 ± 0.004	1.408 ± 0.003
2	Brand B	0.305 ± 0.005	1.002 ± 0.013	7.777 ± 0.016	0.902 ± 0.021	1.468 ± 0.61
3	Brand C	0.206 ± 0.023	1.537 ± 0.043	9.222 ± 0.067	0.812 ± 0.031	1.241 ± 0.012
4	Brand D	0.319 ± 0.002	5.026 ± 0.033	9.111 ± 0.035	0.762 ± 0.041	0.569 ± 0.022
5	Brand E	0.502 ± 0.013	0.855 ± 0.053	8.024 ± 0.061	0.820 ± 0.028	0.843 ± 0.090
6	Brand F	0.253 ± 0.004	0.802 ± 0.008	7.777 ± 0.061	3.617 ± 0.023	1.023 ± 0.045
7	Brand G	0.221 ± 0.002	0.374 ± 0.061	10.123 ± 0.023	1.025 ± 0.012	1.022 ± 0.002
8	Brand H	0.381 ± 0.007	0.361 ± 0.002	7.037 ± 0.021	0.910 ± 0.010	0.947 ± 0.006
9	Brand I	0.154 ± 0.002	0.387 ± 0.002	8.888 ± 0.002	0.918 ± 0.002	0.907 ± 0.002
10	Brand J	0.485 ± 0.002	0.427 ± 0.002	7.530 ± 0.002	0.951 ± 0.002	0.938 ± 0.002

**Mean ± SD (n = 3)*

F (Tablet), Brand G (Syrup), Brand H (Syrup), Brand I (Syrup), and Brand J (Syrup) were purchased from the local pharmacy.

Preliminary phytochemical evaluation

The alcoholic and aqueous extracts of the respective formulations were prepared and then were subjected to preliminary phytochemical screening.

Total phenolic content (TPC)

1.0 mL of test solution of each formulation extract was transferred to a 25 mL volumetric flask. Then, in the next step, 10 mL water, 4.0 mL of sodium carbonate (7.5% w/V), and Folin–Ciocalteu reagent (1.5 mL, tenfold diluted) were added. Then at room temperature, the reaction blend was incubated for 30 min. The absorbance of the resulting blue complex was analyzed at 765 nm.

By Equation 1, the TPC of each formulation was determined as gallic acid equivalent (GAE)[8]. By spiking standard gallic acid at two levels, the recovery experiments were conducted in triplicate.

$$GAE = \frac{[(CXV)\ ]}{M} X\ 100 \quad (1)$$

where,

C = concentration of gallic acid established from calibration curve in µg mL^{-1}

V = volume of formulation extract taken in mL

M = weight of dried formulation extract taken in mL

Total flavonoid content (TFC) (Aluminum chloride colorimetric method)

Mainly by dissolving formulation extract in methanol (100 mL), test solution was formulated. Then test solution (1 mL) extracted from each formulation extract was transferred to a 25 mL volumetric flask. To this solution, 2.0 mL of 2% w/V aluminum trichloride in methanol was added. Then, 25 mL of distilled water was added to make up the volume. After half an hour, absorbance of the resultant solution was determined at 415 nm.

The result is expressed as percent TFC. With the help of Equation 2, the TFC of each formulation was calculated as quercetin equivalent[9-11]. By spiking standard flavonoid at two levels, the recovery experiments were performed in triplicate.

$$TFC\ (\%) = \frac{Absorbance\ X\ Dilution\ Factor\ X\ 100}{E^{1\%}_{1cm}\ X\ Weight\ of\ extract\ (g)} \quad (2)$$

where,

E 1%, 1 cm = specific absorption of the quercetin $ALCl_3$ complex

Total tannin content (TTC) (Folin–Denis method)

Liquid formulation was evaporated to obtain solid extract, which was mainly utilized for sample preparation. By adding extract (10 mg) into 100 mL methanol, test solution was prepared.

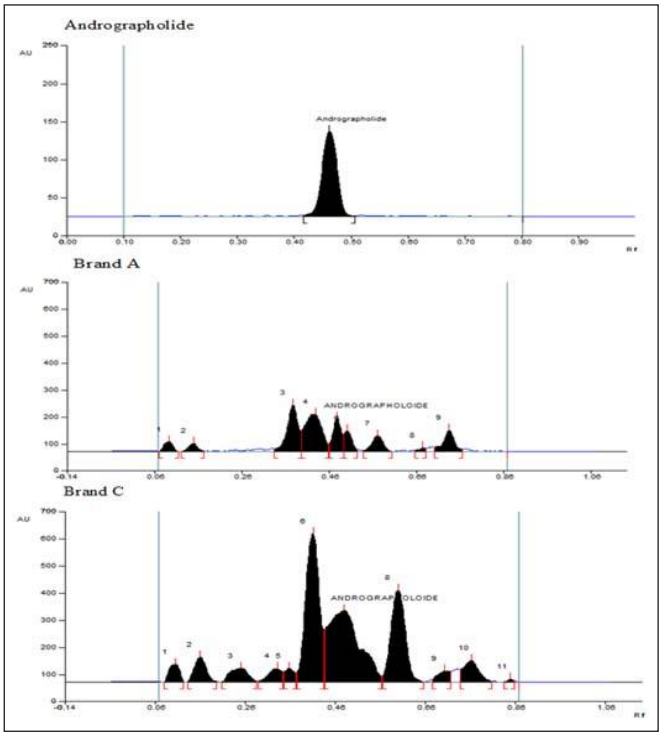

Fig. 1a: HPTLC chromatogram of standard andrographoloide, brand A and brand C

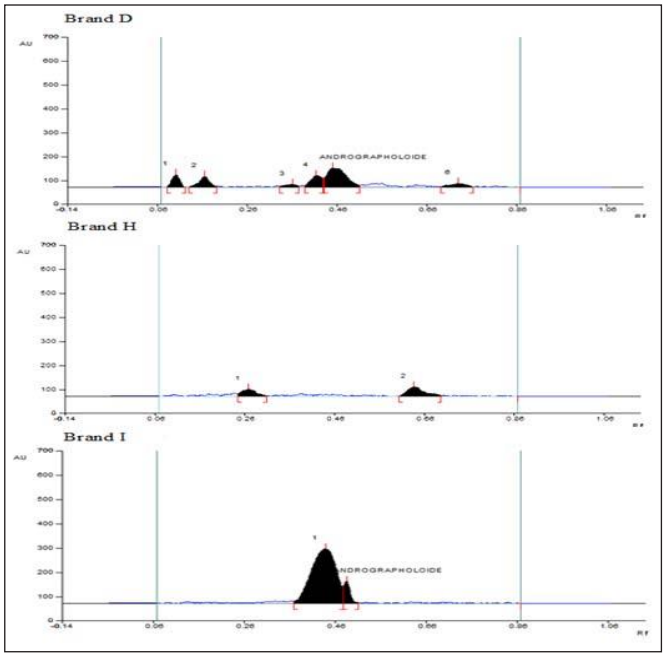

Fig. 1b: HPTLC chromatogram of brands D, H and I

In the next step, 1 mL of test solution of each formulation extract was transferred to 10 mL capacity volumetric flasks. To every flask, sodium carbonate solution (1.0 mL) and Folin–Denis reagent (0.5 mL) were added; then the volume was adjusted with distilled water. Immediately within 30 min, resulting solution was measured for absorbance at 700 nm[12].

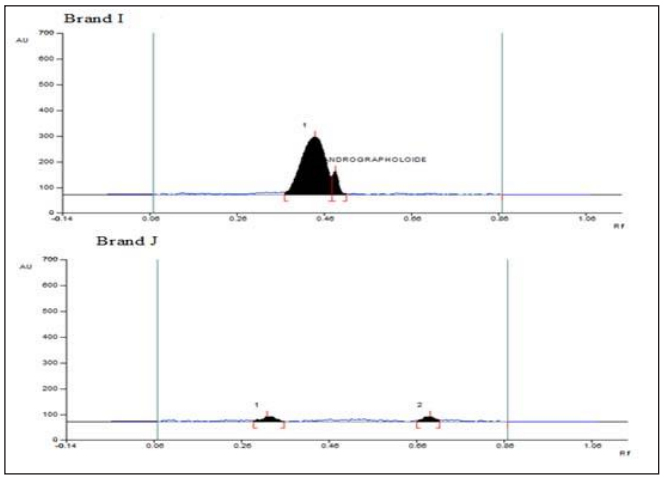

Fig. 1c: HPTLC chromatogram of brands I and J

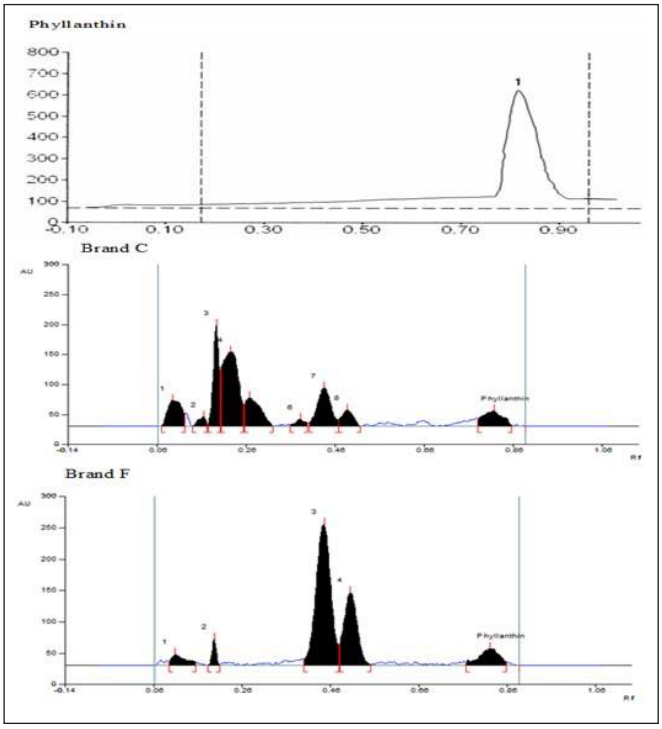

Fig. 2a: HPTLC chromatogram of standard phyllanthin, brand C and brand F

The TTC of each formulation was calculated as tannic acid equivalent (TAE). Then, by spiking standard tannin at two levels, the recovery experiments were done in triplicate.

Qualitative chemoprofiling of andrographolide by HPTLC[3, 13-15]

Preparation of standard solution of andrographolide

An accurately weighed quantity of andrographolide (1 mg) was added to a volumetric flask of 10 mL capacity.

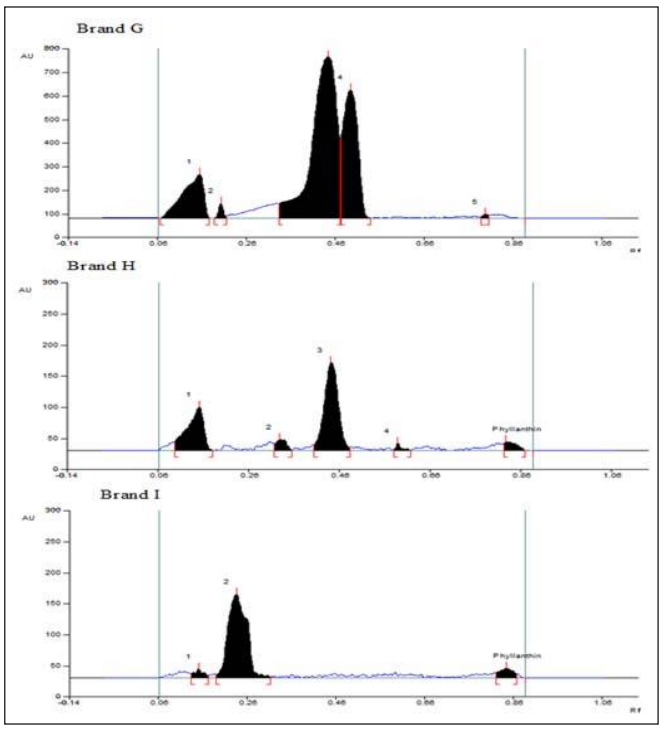

Fig. 2b: HPTLC chromatogram of brands G, H and I

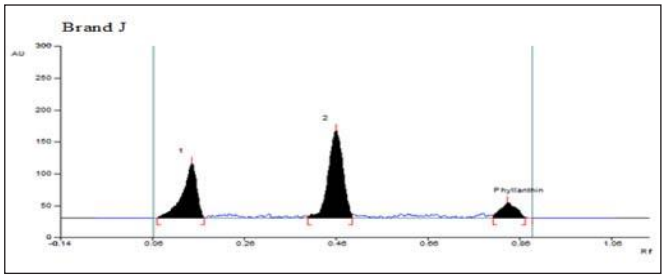

Fig. 2c: HPTLC chromatogram of brands J

Then, methanol was added to dissolve it and the volume was made upto 10 mL (100 µg mL^{-1}). In the next step, filtration of resulting solution was achieved using Whatman filter paper no. 41 and used for the further experimentation.

Preparation of test sample (polyherbal formulations)

Brands A, B, and C were crushed (tablets) and dispersed in 75 mL methanol, sonicated for the period of 10 min and volume was made upto 100 mL with methanol. Brands D, E and F (capsules) were removed and the powdered drug was sonicated with 75 mL methanol. Brands G, H, I and J (syrups) were kept for drying, and the residues obtained were sonicated with addition of methanol. All the methanolic dispersions were filtered and evaporated until dryness was attained. Dried residue was once again reconstituted in methanol and then was utilized for further experimentation.

Selection of mobile phase

A number of mobile phases were tried to get good separations on the basis of sample solubility and suitability. Standard solution of andrographolide and andrographolide containing extract of marketed formulations were run in different mobile phases. Among the various mobile phases that were taken into consideration, mobile phase containing chloroform and methanol ratio as 9:1 (V/V) showed appreciable separation. Therefore, this mobile phase was selected and used throughout the further experimentation.

Development of HPTLC chromatogram of andrographolide

During the procedure, 10 µL samples of methanolic extract of standard andrographolide and andrographolide containing extracts of marketed preparations were distinctly marked on precoated silica gel 60 F_{254} plate in the form of band (width 6 mm) with the help of CAMAG Hamilton syringe using CAMAG Linomat 5 Applicator (Switzerland). The plates were thoroughly prewashed with methanol and then were activated at 60°C for 5 min prior to chromatography.

HPTLC chromatogram was developed and evaluated in the scanner. Using CAMAG TLC Scanner 3, scanning was done in the reflectance absorbance mode at 232 nm. The compound with RF value 0.46±0.3 was identified as andrographolide.

HPTLC conditions

Samples used: Polyherbal hepatoprotective formulations

HPTLC Applicator: CAMAG LINOMAT – 5

HPTLC Scanner: CAMAG TLC SCANNER– 3

Volume of injection: 10 µL

Mobile phase: Chloroform:Methanol (9:1V/V)

Lambda max: 232 nm

Lamp: Deuterium

Stationary phase: TLC silicagel 60 F_{254} (Merck)

Qualitative chemoprofiling of phyllanthin in formulations[3,16,17]

Preparation of standard solution of phyllanthin

An accurately weighed quantity of phyllanthin (1 mg) was dissolved in methanol in a 10 mL volumetric flask. Then, the volume was made upto 10 mL (100 µg mL^{-1})

and filtered using Whatman filter paper No. 41 and was utilized for the further experimentation.

Preparation of test sample (polyherbal formulations)

Brands A, B, and C were crushed (tablets) and dispersed in 75 mL methanol. Then, it was sonicated for 10 min and volume was made up to 100 mL with methanol. Brands D, E, and F (capsules) were removed and the powdered drug was sonicated with 75 mL methanol. Brands G, H, I, and J (syrups) were evaporated to dryness.Then the residues obtained were sonicated with methanol. All the methanolic dispersions were filtered and evaporated until the dry state was achieved. Dried residue was again reconstituted in methanol and used for further experimentation.

Selection of mobile phase

Various mobile phases were tried to acquire good separations based on the sample solubility and suitability. Standard solution of phyllanthin and phyllanthin containing extracts of a number of brands of marketed formulations were run in different mobile phases. From the various mobile phases tried, mobile phase containing toluene: ethyl acetate in ratio of 9:1 (V/V) showed appreciable separation or elution and was selected and used throughout while experimenting further.

Development of HPTLC chromatogram of phyllanthin

An amount of 10 µL samples of methanolic solution of standard phyllanthin and phyllanthin extracts obtained from various brands of marketed preparations were marked on precoated silica gel 60 F_{254} plate in the form of band (width 6 mm) with the help of CAMAG Hamilton syringe using CAMAG Linomat 5 Applicator (Switzerland). With methanol, the plates were carefully prewashed. Then they were activated at 120 °C for 5 minutes prior to chromatography. HPTLC chromatogram was developed and evaluated in the scanner. On CAMAG TLC scanner 3, scanning was performed adopting the reflectance absorbance mode at 254 nm. The compound with RF value 0.86 was recognized as phyllanthin.

HPTLC conditions

Samples used: Polyherbal hepatoprotective formulations

HPTLC Applicator: CAMAG LINOMAT – 5

HPTLC Scanner: CAMAG TLC SCANNER – 3

Volume of injection: 20 µL

Mobile phase: Toluene:ethyl acetate (9:1V/V)

Lambda max: 254 nm

Lamp: Deuterium

Stationary phase: TLC silicagel 60 F_{254} (Merck)

Determination of Heavy metal content

For heavy metal analysis, each formulation was weighed and dried at 60 °C to a constant weight. An amount of 10 µL of nitric acid was transferred to 100 mL beaker containing accurately weighed dried samples and heated upto 95°C on hot plate for 15 minutes. The wet digested formulations were cooled and concentrated nitric acid (5 mL) was transferred to them. The resulting mixtures were heated for a further half an hour at 95°C. In the next step, digested formulations were cooled and filtered through Whatman Filter Paper No. 42.Then, these digested samples were analyzed for heavy metals by using Atomic Absorption Spectrophotometer (AAS Lab India Model No. AA 7000) equipped with hydride generator. The hollow cathode lamps for Copper (Cu), Cadmium (Cd), Lead (Pb), Arsenic (As), and Mercury (Hg) were employed as a source of radiation. The fuel was acetylene, and nitrogen was used as carrier gas. A quantitative analysis was properly conducted by using calibration curve technique[18,19]. All the measurements were done in triplicate.

RESULTS AND DISCUSSION

Selection of formulations

In the present study, ten polyherbal hepatoprotective formulations of different manufacturers were selected. Formulations were randomly selected on the basis of 1) claim as an ayurvedic medicine; 2) commercial availability; 3) availability in all dosage forms i.e., tablets, capsules, and syrups; and 4) hepatoprotective activity as claimed by the company.

Preliminary phytochemical screening

All the 10 polyherbal formulations were subjected to phytochemical screening by standard qualitative chemical tests. Phytochemical evaluation elaborates the idea regarding the phytoconstituents present in the formulations. Findings of phytochemical screening revealed the presence of various phytoconstituents like tannins, phenolics, flavonoids, alkaloids and others. The results are shown in Table I.

All 10 formulations were purchased from the local market in Wardha. Then, these were subjected to phytochemical, pharmacological, safety, and physicochemical evaluations.

Quantitative evaluation

Preliminary phytochemical screening revealed the presence of phenolics, flavonoids and tannins as well as phenolic compounds. Literature survey revealed that owing to the presence of phenolic, flavonoids and tannins like compounds, plants used in the polyherbal hepatoprotective formulations exhibits antioxidant activity. Hence, the work was extended for performing the quantitative evaluation of all polyherbal formulations[20-22]. Phenolics are ubiquitous secondary metabolites present in the plant materials. Phenolic compounds possess wide spectrum of activities such as antioxidant activity. Percent TPC ranged from 30.67 ± 0.12% to 44.02 ± 0.31% in all brands (Table II). There is a marked difference in the phenolic content in the variety of brands with the highest content in Brand C and least content in Brand A. TFC was calculated as quercetin equivalent gram%. The percent TFC of all formulations were found ranging between 12.73± 0.143% to 18.41 ± 0.45% (Table II). Brand F showed maximum amount of TFC content amongst all formulations. Tannin content was expressed as TAE gram%. The percent TTC of all brands was found to range from 9.60 ± 0.13% to 13.86 ± 0.37% (Table II). Maximum and minimum percent TTC was found in brands C and F, respectively. From the Table III, it was observed that the percent recoveries of all brands for TPC, TFC and TEC were found satisfactory, which indicates the good accuracy of the methods used for their detection.

Qualitative chemoprofiling by HPTLC

HPTLC is one of the very important analytical tools for the determination of finger print profiles of the herbal formulations. In the present research, polyherbal hepatoprotective formulations that were selected chiefly contain extracts or fractions of hepatoprotective medicinal plants such as *Andrographis paniculata*, *Phyllanthus niruri* or *Amarus*, and many other medicinal plants extracts having potent hepatoprotective potential. In the current study, qualitative analysis of specific biologically actives, i.e., andrographolide and phyllanthin was conducted on polyherbal formulations (to be precise, formulations containing and rographolide and phyllanthin were selected for the present study) by using HPTLC. In the literature, it was reported that andrographolide exerts anti-hepatotoxic actions[23-25].

To confirm the quantitative presence of andrographolide and phyllanthin (RF value 0.76), HPTLC analysis was performed employing chloroform and methanol of ratio 9:1 (V/V) as well as toluene and ethyl acetate ratio of 9:1 (V/V) as a solvent system at wavelengths of 232 nm and 254 nm, respectively. The chromatograms thus obtained are shown in Fig. 1a, 1b and 1c and Fig. 2a, 2b and 2c. Chromatographic pattern of reference compounds in the formulations is clearly present in the chromatograms. The compound showing RF value 0.46 ±0.3 was recognized as andrographolide and the compound showing RF value 0.86±0.3 was recognized as phyllanthin. Andrographolide band can be easily traced in the Tracks 5, 7, 5, and 2 with RF value found in the range of 0.46 ±0.3 in the formulations of Brands A, C, D, and I, respectively. But it is absent in Brands H and J. Similarly, phyllanthin band is visible in the Tracks 9, 3, 5, 5, 5, 3, and 3 with RF value in the range of 0.86±0.3 in the formulations of Brands C and F, respectively. In the formulations chromatographic pattern, the identification of a chromatographic pattern of reference compounds suggests their occurrence in the formulation. Thus, by this means it establishes the chemical standardization method using HPTLC for the formulation.

The RF values 0.46 ± 0.3 and 0.86 ± 0.3 of andrographolide and phyllanthin in both sample and reference standard were found comparable under UV light at 232 nm and 254 nm, respectively.

Determination of heavy metal content

Heavy metals such as Cd, Cu, Pb, Hg and As are the natural elements of environments. In addition, they are generated by industrial and technical processes. Therefore, these have earned attention as contaminants. Medicinal plants flourishing in natural environment can absorb heavy metals to a certain degree, depending on their characteristics and amount of heavy metals present in the surrounding environment. Because heavy metals are hazardous to living beings, their presence in medicinal plants and products used for therapeutic purposes must be restricted and kept under control. Therefore, limit of heavy metal has been set by health authorities. The World Health Organization (WHO) recommended the limits of heavy metals for different medicinal plants and final dosage forms of the plant materials[26].

In the present investigation, the determination of heavy metal content of hepatoprotective polyherbal formulations are dealt with. With the help of Atomic Absorption Spectrophotometry (AAS), heavy metal content was determined.The results showed that some of the formulations exceeded the WHO permissible limits of Cd, Cu, Pb, As and Hg. The results also showed that heavy metal content of the products varied to a considerable extent. The results are mentioned in Table IV. The Cd concentration in all formulations was found

to be in the range of 0.154 ± 0.002 to 0.502 ± 0.013. Formulations of Brands A, C, F, G, and I showed Cd concentration within the limits 0.290 ± 0.002, 0.206 ± 0.023, 0.253 ± 0.004, 0.221 ± 0.002, and 0.154 ± 0.002, respectively. On the other hand, formulations of Brands B, D, E, H, and J showed Cd concentration beyond the limit. The elevated amounts of Cd have a severe harmful effect on human health. Within the exposed population, kidney is the seriously affected organ. Due to very slow excretion of Cd, it is retained in the kidney for a comparatively longer duration which lead to irreversible damage of the renal tract[27]. The Cu concentrations varied in a wide range between 0.361 ± 0.002 mg $kg^{-1}$and 5.026 ± 0.033 mg kg^{-1}. In the current study, all of the formulations of Brands A to J had not exceeded the limit of 20 $mgkg^{-1}$. Cu plays a vital function in a broad range of physiological processes such as melanin production, elimination of free radicals, iron utilization, development of bone and connective tissues, and so on. However, intake of large amount of Cu can lead to skin inflammation, abdominal pain, upper respiratory tract irritation, diarrhea, vomiting and liver dysfunction[27].The Pb content in the tested formulations varied from 7.037 ± 0.021 mg $kg^{-1}$to 10.864 ± 0.107 $mgkg^{-1}$. The found data showed that formulations of the Brands A and G exceeded permissible limits. However, the formulations of remaining brands showed amounts of Pb lower than 10 mg kg^{-1}. It is a fact that Pb is considered as one of the extremely toxic pollutants present in the environment till date. It can form complexes with a variety of biological molecules and can badly affect their functions to a great extent. Exposure to Pb may lead to harmful effects on the internal and external parts of the body which may cause various defects such poor muscle response, gastro -in- testinal side effects, brain and kidney damage, and reproductive defects[28]. The concentration of As in the test formulations varied between 0.229 ± 0.004 mg $kg^{-1}$and 3.617 ± 0.023 mg kg^{-1}. At an overall glance, the results revealed that formulations of all brands analyzed had concentrations lower than 3 mg kg^{-1}, except Brand F. Acute As poisoning is associated at first with vomiting, nausea, abdominal discomfort and severe diarrhea. Persistent arsenic exposure results in multiorgan disease. The metal As is a well-reported carcinogenic element affecting various organs[28]. The obtained range of Hg in the present investigation was found between 0.569 ± 0.022 mg kg^{-1} and 1.468 ± 0.61 mg kg^{-1}. The levels of Hg in formulations of Brands A, B, C, F, and G were above the permissible limits (1 mg kg^{-1}). The metal Hg is a well-known toxic heavy metal, securing a high score on the CDC toxic substances List. Hg content found in traditional medicines genuinely scares the layman. Hence, a number of Hg-containing traditional medicines have been banned for the same reason. The obtained data of the heavy metal study suggest a wide fluctuation in heavy metal content in different formulations.

CONCLUSION

In the present work, standardization of marketed hepatoprotective formulations was performed on the basis of phytochemical and analytical evaluation. Identification, estimation and recovery of phenolic, flavonoid, and saponin compounds in all formulations of various brands were performed by using spectroscopic techniques. The results obtained from analytical reports suggested that Brand C of marketed hepatoprotective formulation consists of rich amount of phenolic content (44.02 ± 0.31mg g^{-1}), flavonoid content (18.63 ± 0.24), and tannin content (13.86 ± 0.37). Qualitative HPTLC analysis confirmed the presence of andrographolide and phyllanthin in most of the marketed hepatoprotective formulation brands. It is critical to examine heavy metals in herbal medicine to ensure that their levels do not go beyond the requisite limits recognized by regulations. The concentrations of Cd, Pb, As, and Hg was found higher than permissible limit in some of the marketed hepatoprotective brands. The results suggested that herbal formulations should be subjected to enough quality control requirements in the company which are suggested by WHO, to make sure about their potency, efficacy and safety. Moreover, the current results increase doubt over the safety of these formulations. It is concluded that the production of these formulations should be conducted under strict regulated protocol before introducing them into the market. Elaborative scientific investigation is required to be performed to establish safety for patient use.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Balasaheb Ghule, Associate Professor, Government College of Pharmacy, Amravati for his support for HPTLC studies."

REFERENCES

1. Nadeem M., Dandiya P.C., Pasha K.V., Imran M. and Balani D.K.: Hepatoprotective activity of Solanumnigrum fruits. **Fitoterapia**, 1997, 68, 245-251.
2. Yang J., Li Y., Wang F. and Wu C.: Hepatoprotective effects of apple polyphenols on CCl4-induced acute liver damage in mice. **J. Agric. Food Chem.,** 2010, 58, 6525-6531.
3. Mukherjee P.K., Wahile A., Kumar V., Rai S., Mukherjee K.and Saha B.: Marker profiling of botanicals used for

hepatoprotection in Indian system of medicine. **Drug Inf. J.,** 2006, 40, 131-139.

4. Kang K.: Mechanism of hepatic ischemia/reperfusion injury and protection against reperfusion injury. Paper presented at: Transplantation Proceedings (Elsevier). 2002.
5. Sharma A., Chakraborti K.K. and Handa S.S.: Antihepatotoxic activity of some Indian herbal formulations as compared to silymarin. **Fitoterapia,**1991, 62(3), 229.
6. Fiaz M., Fiaz N., Shakir L., Alamgeer, Mehmood W., Mustafa G., Rauf A. and Najam K.: Hepatoprotective effect of a polyherbal formulation and ascorbic acid in paracetamol induced hepatic damage in rabbits. **Biomed. Res.Ther**., 2017, 4(4), 1261-1277.
7. Bandaranayake W. M.: Quality control, screening, toxicity, and regulation of herbal drugs. In: Ahmad I., Aqil F., Owais M. and Dr. Mohammad Owais, editors. **Modern Phytomedicine,** Wiley, Weinheim 2006, pp.25-57.
8. Singh C.B., Devi M.C., Thokchom D.S., Sengupta M. and Singh A.K.: Phytochemical screening, estimation of total phenols, total flavonoids and determination of antioxidant activity in the methanol extract of *Dendrobium denudans* D. Don stems. **J. Pharmacogn. Phytochem.,** 2015, 4(4), 6.
9. Kalita P., Tapan B.K., Pal T.K. and Kalita R.: Estimation of total flavonoids content (TFC) and anti-oxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. **J. Drug Deliv.Ther.,** 2013, 3(4), 33-37.
10. Fernandes A.J., Ferreira M.R., Randau K.P., de Souza T.P. and Soares L.A.: Total flavonoids content in the raw material and aqueous extractives from *Bauhinia monandra* Kurz (Caesalpiniaceae). **Sci. World J.,** 2012, 2012.
11. Chandra S., Khan S., Avula B., Lata H., Yang M.H., ElSohly M.A. and Khan I.A.: Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. **Evid. Based Complement Alternat. Med.,** 2014, 2014.
12. Nasser M., Cheikh-Ali H., Hijazi A., Merah O., Al-Rekaby A.E. and Awada R. Phytochemical profile, antioxidant and antitumor activities of green grape juice. **Processes,** 2020, 8(5), 507.
13. Zade S.R., Priya B., Bagmar U.R. and Ganjiwale R.O.: Development and validation of HPTLC method for estimation of hepatoprotective diterpenoid and rographolide in polyherbal formulations. **Int. J. Pharm. Pharm. Sci.,** 2013, 5(3), 976-980.
14. Vijaykumar K., Murthy P.B., Kannababu S., Syamasundar B.and Subbaraju G.V.: Estimation of Adrographolide in *Andrographi spaniculata* herb, extracts and dosage forms. **Int. J.Appl.Eng. Res.,** 2007, 5(1), 27-39.
15. Chauhan S.K., Singh B.P., Kimothi G.P. and Agrawal S.: Determination of andrographolide in *Andrographi spaniculata* by high performance thin layer chromatography. **Indian Drugs,** 1999, 36, 130-132.
16. Arsul V.A., Ganjiwale R.O. and Yeole P.G.: Development and validation of phyllanthin by HPTLC in hepatoprotective polyherbal tablet dosage form. **Asian J.Res. Chem.,** 2011, 4(5), 815-817.
17. Aeri V., Zafar R., Ahamad S. and Kham P.I.: Quantitative estimation of phyllanthin in *Phyllanthus niruri* and some polyherbal *hepatoprotective* formulation by HPTLC. **Indian J. Nat. Prod.,** 2012, 21 (1), 12-13.
18. Sukender K., Jaspreet S., Sneha D. and Munish G.: AAS estimation of heavy metals and trace elements in Indian herbal cosmetic preparations. **Res. J. Chem. Sci.,** 2012, 2231, 606X.
19. Garg M. and Singh J.: Quantitative AAS estimation of heavy metals and trace elements in marketed ayurvedic churna preparations in India. **Int. J. Pharm. Sci. Res.,** 2012, 3(5), 1331.
20. Lijitha L.:*In vitro* screening of antioxidant, free radical scavenging and antimicrobial potential of *Micrococca mercurialis* whole plant extracts. **Int. J. Res. Pharm. Sci.,** 2019, 10(4), 3251-3262.
21. Ali S.A., Sharief N.H. and Mohamed Y.S.: Hepatoprotective activity of some medicinal plants in Sudan. **Evid. Based Complement. Alternat. Med.,** 2019, 2019.
22. Hiraganahalli B.D., Chinampudur V.C., Dethe S., Mundkinajeddu D., Pandre M.K., Balachandran J. and Agarwal A.: Hepatoprotective and antioxidant activity of standardized herbal extracts. **Pharmacogn. Mag.,** 2012, 8(30), 116.
23. Chao W.W. and Lin B.F.: Hepatoprotective diterpenoids isolated from *Andrographi spaniculata* **Chin. Med.,** 2012, 3(3), 22713.
24. Chirdchupunseree H. and Pramyothin P.: Protective activity of phyllanthin in ethanol-treated primary culture of rat hepatocytes. **J.Ethnopharmacol.,** 2010, 128, 172-176.
25. Krithika R. and Verma R.J., Shrivastav P.S. and Suguna L.: Phyllanthin of standardized *Phyllanthu samarus* extract attenuates liver oxidative stress and exerts cytoprotective activity on human hepatoma cell line. **J.Clin. Exp. Hepatol.,** 2011, 1, 57–67.
26. Dghaim R., Al Khatib S., Rasool H. and Ali Khan M.: Determination of heavy metals concentration in traditional herbs commonly consumed in the United Arab Emirates. **J. Environ. Public Health Complement,** 2015, 2015.
27. Ratnaike R.N.: Acute and chronic arsenic toxicity. **Postgrad. Medical J.,** 2003, 79(933), 391-396.
28. Gholap A.H., Mahajan M.P., Kakade R., Rokade S. and Gupta M.K.: Review article on mercury containing compounds and its toxicity. **Int. J. Rec. Sci. Res.,** 2015, 6(8), 5987-5990.