Quality Analysis Of Traditional Medicinal Plants With Potential In Management Of Liver Cancer

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Abstract

Liver and its dysfunctions are well defined in Indigenous systems of medicine. Various herbal drugs are documented for effective treatment of liver disorders. Some of these are highly effective and have highpotential for application in the treatment of hepatocellular carcinoma. Standardization of herbal drugs is an essential requirement to persuade scientific studies. In this study, we have provided quality parametersfor fifteen selected constituent herbs based on their physicochemical and thin layer chromatographic parameters and their correlation with cytotoxic effect on HepG2 cell line.Quality standards for these 15 plant ingredients will contribute to further develop a standardised product.

Methods Fifteen plants sampleswere procured from at least three different sources. These were authenticated and analysed for organoleptic, physicochemical properties and TLC.The aqueous extracts of all plant drugs were evaluated for cytotoxic effect on HepG2 cell line using MTT assay.

Results The physicochemical parameters and Rf values were compared with the standards.The results were correlated with cytotoxic potential of the drug on HepG2 cell line. The study determined quality profile of plant drug samples matching with pharmacopeial standards and having maximum efficacy for cytotoxic potential on HepG2 cell line.

Conclusion The study is essentialto determine the quality and purity of the crude plant drug (raw material) as standards for selection of plants and for development of a monograph for the product formulation,to be used in the management of hepatocellular carcinoma. It is also of value in laying down of standards for a multi-ingredient formulation.

Key words: Standardization, physicochemical, TLC, cytotoxicity

INTRODUCTION

Traditional herb-based medicines have universal acceptance owing to their preventive, curative, affordable and accessible potential. Millions of people around the world prefer to use traditional medicines as first level of medication for treatment of various diseases. World health organization has estimated that around 80% of the world's population use traditional medicines. Over 170 WHO member states have reported the use of traditional medicines and emphasized on creating of reliable evidence on traditional medicinal practices and products [1]. Traditional medicines are also increasingly prominent in the world of modern science. About 40% of the approved pharmaceutical products in use today are derived from natural substances [2]. The global demand for natural medicines highlights the importance of natural biodiversity, traditional knowledge and the requirement of high quality sustainable natural resources. This also emphasizes the need for determination of quality parameters to ensure efficacy and safety of herbal drugs.

India owns a rich legacy of well recorded and well-practiced knowledge of traditional systems of medicine. Knowledge relating tomedicinal herbs used in liver disorders is probably the best gift that Indian traditional medicinal systems have offered to the current society [3,4]. The liver is a vital organ having wide range of functions including detoxification, protein synthesis and production of biochemicals necessary for digestion. There has been an alarming increase in the incidence of liver diseases in recent years. Contribution of chronic liver diseases to the mortality in India have been

increasing progressively in last two decades. The burden of liver diseases in India contributes to about 18.3% of the total two million liver related deaths occurring in the world [5]. Intervention with new generation of treatment regimen involving combinations of direct acting antiviral drugs (DAAs) and/or hepatoprotective, chemotherapeutic drugs is unable to produce its anticipated impact due to resistance imposed by chronic liver conditions to these drugs [6]. Moreover, limited access to sophisticated drugs, high cost, unawareness and emergence of drug resistant hepatitis virus strains impedes the impact of these drugs. They highlight the need for development of highly acceptable, cost effective and safe treatment modalities suitable for long term treatment. Natural products are the best resource for development of effective and safe pharmaceuticals.

Hong M.*et. al.* (2015) and Siddiqui MA *et. al.* (2015) have reviewed medicinal plants/ formulations used in treatment of various liver disorders and case studies demonstrating a significant decrease in fibrosis and improvement in liver functions due to systematic treatment in patients with decompensated liver cirrhosis [7,8]. In the systematic review of herbal formulations used in treatment of liver disorders (2021), we have shortlisted the herbal drugs which are likely to be effective in the treatment of hepatocellular carcinoma [9]. In present study, we have attempted quality standard determination of fifteen selected plants having role in treatment of chronic liver conditions including hepatocellular carcinoma. They were evaluated for physicochemical parameters, thin layer chromatography and cytotoxic effect on HepG2 cell line.The referential information will be of value in selection of crude plant drugs for designing of new cost effective, efficacious and safe drug regimens for management of chronic liver diseases.

2. MATERIAL AND METHODS

2.1 The collection of samples

The selected plant samples were procured from minimum three reliable sources (authentic herbal drug suppliers from Mumbai, Pune and other parts of India). Saffron and *Cymbopogon jwarancusa* were obtained from two sources only. All the samples were cleaned, segregated and preserved in airtight containers. Herbarium for each plant was prepared and submitted for authentication to Dept. of Botany, University of Pune (SPPU). The list of fifteen plants selected for present study, part of plant used and their abbreviations based on source of procurement are detailed in Table 1 [Figure 1].

Table 1: The List Of Plants Selected For Present Study, The Part Of Plant Used And Their Abbreviations Based On The Source Of Procurement.

S. No.	Botanical name	Parts used	Sources 1	Source 2	Source 3
1.	Berberis aristata DC.	Bark	BA1	BA2	BA3
2.	Cinnamom cassia	Bark	CC1	CC2	CC3
3.	Cinnamomum zeylanicum Blume	Bark	CZ1	CZ2	CZ3
4.	Comminifera myrrha	Gum resin	CM1	CM2	CM3
5.	Crocus sativus	Stamens	-	-	CS3
6.	Cymbopogan jwarankush	Flower	CJ1	-	CJ3
7.	Glycyrrhiza glabra Linn	Root	GG1	GG2	GG3
8.	Eclipta alba Hussk	Whole plant	EA1	EA2	EA3
9.	Leucas cephalotes	Whole plant		LC2	LC3
10.	Nardostachys jatamansi DC	Rhizomes	NJ1	NJ2	NJ3
11.	Nigella sativa	Seeds	NS1	NS2	NS3
12.	Saussurea leppa C.B. Clarke	Root	SL1	SL2	SL3
13.	Tecomella undulate (Amoora rohitaka)	Bark	-	AR2	AR3
14.	Tephrosea purpurea	Root	TP1	TP2	TP3
15.	Tinospora cordifolia (Wild) miers	stem	TC1	TC2	TC3

Figure 1 Photographs Of Collected Samples

Commiphora myrrha

CM1

CM2

CM 3

Crocus sativus

CS3

Cymbopogan jwarankush

CJ2

CJ3

Eclipta alba Hassk

EA1

EA2

EA3

Glycyrrhiza glabra Linn

GG1

GG2

GG3

Leucas cephalotes

LC2

Nardostachys jatamansi DC

NJ1

NJ2

NJ3

Nigella Sativa Linn.

NS1

NS2

NS3

Saussurea lappa C.B. Clarke

SL1

SL2

SL3

Tecoma undulate [Amoora rohitaka]

AR2

AR3

Tephrosea purpurea

TP1

TP2

TP3

Tinospora cordifolia (Wild) miers

TC1

TC2

TC3

2.2 Macroscopic study

The selected plant samples were analysed for macroscopic characteristics such as appearance, shape, size, colour, surface characteristics, texture, odour, taste and compared with API/UPI guidelines [10].

2.3 Physicochemical analysis

Physicochemical analysis involved measurement of ash content, moisture content, water soluble extractive value, alcohol soluble extractive value, and pH of all collected samples[11,12].

Moisture content: -Moisture content of the plant samples was tested to determine the quality of crude drug. 2gm of powdered drug was taken in tarred foil and weighed. It was dried in hot air oven at 100ºC. After cooling, the weight loss was recorded as moisture content. The procedure was continued till constant readings and percentage of moisture content was determined.

Ash value: The ash value content determines the quality and purity of drug.2gm of each powdered drug was taken in tarred crucible and subjected to heating in the muffle furnace at 450ºC for 3.0 hrs. After cooling, weight of crucible was measured at an interval of 2.00 hrs till the constant reading was observed. Percentage of total ash and acid-insoluble ash values were determined from initial and time interval readings.

Extractive value: Alcohol and water-soluble extractive values are valuable testsfor denoting the quality of drug. Any variation in the chemical constituents may cause a change in the extractive values. It helps in the determination of the adulteration and is an index of the purity of drug.5gm of powdered *drug* was suspended in 100ml of water/ alcohol for extraction. The flask was kept on shaker (150 rpm, 6.00 hrs, at ambient temperature) and then it was allowed to stand for 18.00 hrs at room temperature. The filtrate obtained was subjected to evaporation and % extractive value was calculated.

2.4 Thin Layer Chromatography

The selected plant samples were analysed qualitatively by thin layer chromatography [13]. Methodology and solvent systems described in Reviews on Indian Medicinal Plants [14] were used as reference for each plant. Thin layer chromatography was carried out on TLC plastic sheet of silica gel pre-coated with a layer thickness of 0.2 mm. 2 g of plant samples were extracted using alcohol, methanol, ethanol and hexane successively using Soxhlet apparatus.Spots were applied manually using capillary tube; plates were dried using air blower and developed at room temperature. The plates were visualized in a UV chamber at wavelengths 254 and 366 nm. Spots on TLC plates were visualised by spraying with acetaldehyde sulphuric acid for the development of the separated bands the movement was expressed by its retention factor (Rf) values were calculated for different samples.

2.5 Cytotoxic effect of plant samples on HepG2 cell line

Preparation of aqueous extracts

The selected plant drug samples were washed, shed dried and powdered. 5 gm of powder was extracted with 50 ml of water for 48 hrs. at room temperature. The extracts were filtered and freeze dried. 10 mg of freeze-dried sample was dissolved in 1 ml of sterile PBS and tested for sterility. Sterile extracts were diluted to various concentrations for treatment on cells.

MTT ASSAY

HepG2 (Human heapatocellular carcinoma) cell line was procured from National Animal Cell Repository at National Centre for Cell Science, Pune, India. Cells were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS (Gibco), 2mM L-glutamine, 50 IU/ml penicillin, 50 µg/ml streptomycin and maintained at 37^0C in humidified 5% CO_2 atmosphere. About 1 x 10^4 cells were seeded into 96 well microtitration plates. The plates were incubated in 5% CO_2 atmosphere at 37°C for 24 hrs to allow the adherence of cells. Varied concentrations (2µg/ml - 1000 µg/ml) of plant drug aqueous extracts were treated in micro-test plates. Equal amount of PBS was added to wells which served as control. After addition of all test samples, plates were incubated in 5% CO_2 atmosphere for next 48 hrs.10 µl of 5 µg/ml MTT was then added to all the wells and plates were incubated in dark for 5 - 6 hrs. About 100 µl of DMSO and 25 µl of glycine buffer were added to dissolve the formazan crystals resulting from the reduction of the tetrazolium salt by the metabolically active cells. The absorbance was measured at 540 nm using a micro-plate reader (BIO-RAD, Model 680). The cell survival was measured as absorbance (OD) of the mean of the replicate wells compared to that of control. IC_{50} values, defined as the concentration of the drug that killed 50% of cells in comparison with the untreated cultures, were estimated by plotting OD readings versus the drug concentrations.

3. RESULTS

3.1 The collection of samples from various sources and their authentification

The samples of 15 selected plants were procured from three different sources. They were cleaned, segregated, codified and stored in appropriate conditions. Voucher specimens of each of these was prepared, authenticated and preserved for any future reference.

3.2 Organoleptic Examination

A total of 40 samples were subjected to organoleptic examination.The sensory parameters such as colour, odour, taste, appearances, size, and shape of all collected specimens were examined.The observations of each plant sample are presented in Table 2

Table 2: Organoleptic Examination Of Ingredients Of Formulations

Plant name and parts	Colour	Shape	Size	Peculiar features Appearance	Odour	Taste
Berberis aristata DC. Bark	Pale yellowish-brown	Rough cylindrical	0.2 cm long 0.1 wide	Flattened, oblong, angular	Not specific	Bitter
Cinnamom cassia Bark	Outer and inner surface Brownish	Cylindrical	4-6 cm long	Thick , woody, hard	Characteristic	Bitter
Cinnamomum Zeylanicum Blume Bark	Yellowish brown	Cylindrical, Outer bark thick, woody, hard	less than 16 mm	cambium ring about one-third of radius from outer surface and a small central pith	Characteristic	Sweet
Comminifera myrrha Gum resin	Golden	Vermicular	Irregular	Not much viscid	Aromatic	Bitter , Astringent
Crocus sativus Stamens	Stigma dark red to reddish brown. Style-yellowish brown to yellowish orange	stigma trifold and styles cylindrical	stigmas 25-mm long, and styles are about 10-mm long	When mixed with water gave golden colour. After taken threads out of water , threads remain same colour it wouldn't have lost its original colour	Characteristic	Bitter
Cymbopogan jwarankush Flower	Dried buff colour specimens	Slender grouped together at base	8-10 cm long	Easily broken	Characteristic	Bitter
Glycyrrhiza glabra Linn Root	Yellowish brown	Cylindrical Outer bark thick, woody, hard, inner light yellow in colour	5-6 cm	Cambium ring Present	Characteristic	Sweet
Eclipta alba Hassk Whole plant	Greenish	Root- Greyish Stem- Greenish with all parts	All parts available	Root- 7 mm in diameter Leaves 2.2 - 8.5 cm long, 1.2 - 2.3 cm wide	Characteristic	Bitter
Lecus cephalotes Whole plant	Stem- light green, Leaves- yellowish green, Flowers - white in colour, and tasteless	Stems -stout, slightly fibrous Leaves -ovate, serrate Flowers- sessile, globose, Roots - tapering, fibrous nature Fruits- brown colour , smooth 3-4 mm long legus, Seed - 0.2 cm long , smooth , dark brown	Stem- 3 mm thick, Leaves- 3-6 cm long, 1-2.5	All parts present, nodes present	Characteristics	Bitter
Nardostachys jatamansi Rhizomes	Brown externally, Internally reddish brown	Cylindrical, brown fibers	3.5 – 4 cm	Fibrous	Strongly aromatic	Bitter
Nigella sativa Linn Seeds	Black	Small funnel shaped	0.2 cm long0.1 wide	flattened, oblong, angular	Aromatic	Bitter
Saussurea leppa C.B. Clarke Root	Dark brown	Cylindrical	7-8 cm long	Fibrous	Strongly aromatic	Bitter
Tecomella undulata (Amoora rohitaka)Bark	Greyish brown	Curved Surface with dark brownish patches	5-6 mm thick	Rough surface	Odourless	Pungent
Tephrosea purpurea Root	Yellowish green	Irregular Mixture of all the parts	1-4 cm long	All parts visible and in dry condition	Characteristics	Bitter
Tinospora cordifolia stem	Green with smooth surface Brown,	Cylindrical pieces	1-2 cm	Light brown surface marked due to circular swelling at nodes	Not specific	Bitter

The organoleptic parameters of thirty four selected plant samples matched with the standard parameters determined by Ayurveda/Unani Pharmacopeia of India. The data thus confirmed the authenticity of the collected plant samples. *Eclipta alba* Hassk, *Glycyrrhiza glabra Linn, Leucas cephalotes, Nardostachys jatamansi DC, Tecomella undulata (Amoora rohitaka*) of sources 3 and *Cinnamon cassia* source 2 however, did not satisfy UPI/ API guidelines and hence not considered for further study.

3.3 Analysis of collected samples

The samples approved through organoleptic study were further analysed for physico-chemical parameters and thin layer chromatography (TLC). Thirty four plant samples were thus evaluated for moisture content, ash values, water and alcohol soluble extractive values. The results of each of these tests are given in Table 3.

Table 3: Physicochemical Evaluation Of The Samples For Identification

Samples	Moisture content in %	Total ash in %	Acid insoluble ash in %	Alcohol soluble extractive in %	Water soluble extractive in %
Berberis aristata DC.					
BA1	7.16	11.89	0.33	14.60	18.65
BA2	6.36	4.58	2.93	1.31	6.47
BA3	2.21	0.86	0.15	0.6	1.87
Cinnamom cassia					
CC1	8.96	3.67	0.11	6.98	6.95
CC3	5.355	4.647	0.2108	5.996	10.1214
Cinnamomum zeylanicum Blume					
CZ1	7.06	2.63	0.14	8.01	6.90
CZ2	9.62	2.59	0.26	7.50	7.13
CZ3	8.35	2.41	0.14	7.21	6.04
Comminifera myrrha					
CM1	12.23	4.06	0.57	17.27	58.45
CM2	11.1847	6.99	2.929	25.026	42.966
CM3	8.30	4.03	0.49	16.92	67.21
Crocus sativus					
Cs3	7.013	4.517	1.00	39.07	42.16
Cymbopogan jwarankusa					
CJ2	5.466	6.6473	4.542	1.213	4.9057
CJ3	4.5	6.43	4.47	1.213	4.5032
Eclipta alba Hassk					
EA1	8.59	24.99	8.99	1.18	9.35
EA2	7.97	19.51	14.56	8.90	3.30
Glycyrrhiza glabra Linn					
GG1	5.67	5.14	0.34	3.72	23.11
GG2	7.73	6.00	0.06	57.19	26.83
Leucas cephalotes					
LC2	5.85	10.69	1.42	2.78	3.16
Nardostachys jatamansi					
NJ1	9.03	10.46	6.20	3,21	3.34
NJ2	7.21	9.56	5.31	5.62	5.22
Nigella sativa Linn					
NS1	5.42	32.70	0.96	33.75	11.66
NS2	4.42	4.18	0.09	34.81	13.20
NS3	5.68	3.75	0.09	38.13	13.45
Saussurea leppa C.B. Clarke					
SL1	7.69	3.390	0.9554	2.3882	33.13
SL2	8.76	5.20	0.14	1.00	13.22
SL3	6.25	4.04	0.12	5.75	12.29
Tecomella undulata (Amoora rohitaka)					
AR2	7.16	11.89	0.33	14.60	18.65
Tephrosea purpurea					
TP1	5.48	5.18	0.40	12.94	3.97
TP2	4.4866	6.004	0.7424	3.292	10.451
TP3	7.05	6.8	0.85	4.29	12.99
Tinospora cordifolia (Wild) miers					
TC1	5.09	6.92	0.29	2.66	11.30
TC2	8.10	8.9	0.25	2.60	7.93
TC3	5.589	8.704	0.5649	2.083	10.213

Out of 40 samples, total 34 samples were analysed and satisfied the all the criteria.
The moisture content, ash values, water and alcohol extractive values of each plant sample were compared with standard approved values in Ayurvedic Pharmacopoeia of India (API) and Unani Pharmacopoeia of India (UPI). The values of all study samples were within limits of values prescribed in API/UPI. The data thus suggested satisfactory level of purity and/or quality of the selected plant drug samples.

3.4 Thin Layer Chromatography

The plant samples were next analysed for presence of phytochemical constituents using TLC. The non-volatile compounds are separated on the basis of their affinity to the mobile and stationary phase. The compounds in the mixture show different degrees of affinity for the solid support (or adsorbent) and as the adsorbent is washed with the fresh solvent, the compounds move and arrange themselves in the order of affinity to the adsorbent. Those with the least affinity move down the column at a faster rate than those with greater affinity resulting in definitive separation of phytoconstituents on the plate. The details of solvent extract, mobile phase used, number of spots obtained and RF values for each plant sample are presented in Table 4.

Table 4 Result Of Thin Layer Chromatography (TLC)

Drug	Extract	Mobile Phase	Number of spots	Rf value
Berberis aristata DC.				
BA1	Alcoholic	Toluent : Ethyl acetate: Formic acid [5:4:4]	02	0.04 Orange, 0.12 Brown
BA2			05	0.05, 0.09, 0.36, 0.58, 0.83 [Greenish]
BA3			03	0.07 [Yellow], 0.57 [Flu. Blue], 0.84 [Blue]
Cinnamom cassia				
CC1	Alcoholic	Toluene Ethyl acetate [9:1]	04	0.12, 0.16, 0.96 [Blue], 0.90 [Pink Violet]
CC3			05	0.17 [Brown], 0.48, 0.64, 0.75 [Violet], 0.9 [Blue]
Cinnamomum zeylanicum Blume				
CZ1	Alcoholic	Toluene Ethyl acetate [9:1]	06	0. 02 [Brown], 0.06, 0.21, 0.62 [Grey], 0.91 [Green], 0.97 [Blue]
CZ2			08	0.04 [Brown], 0.09, 0.14 [Grey], 0.26, 0.61, 0.75, 0.97 [Pink Violet], 0.35 [Blue],
CZ3			08	0.04, [Brown], 0.22, 0.84, 0.94 [Pink Violet], 0.27, 0.37, 0.59, 0.71[Grey]
Comminifera myrrha				
CM 1	Methanolic	Toluene: Ethyl Acetate: Formic acid [5:4:1]	03	0.04 [Yellow], 0.39 [Pink], 0.8 [Violet]
CM 2	Ethanolic	60-80 Ether + Ethyl Acetate [3;1]	02	0.33 [Grey Brown], 0.51 [Grey Brown]
CM 3	Alcoholic	Toluene: Ethyl Acetate: Formic acid [5:4:1]	03	0.05 [Yellow], 0.42 [Pink], 0.83 [Violet]
Crocus sativus				
CS3	n- Hexane	Toluene Ethyl acetate [9.7: 0.3]	09	0.08, 0.95 [Blue], 0.16, 0.87 [Green], 0.64 [Violet], 0.58 , 0.53, 0.31,0.22 [Ash colour]
Cymbopogan jwarankusa				
CJ2	Ether	Benzene : chloroform [1:1]	05	0.0624 [Dark Grey], 0.45 [Dark Violet], 0.725 [Violet], 0.912 [Light Orange], 0.97 [Dark Orange]
CJ3	n- hexane	Toluene: Ethyl Acetate [93:7]	05	0.12, 0.60, 0.78 [Blue], 0.22 [Pink], 0.55 [Pink Violet]
Eclipta alba Hassk				
EA1	Methanolic	Toluene : Acetone : Formic acid(11:6:1)	08	0.52, 0.75 [Brown], 0.56, 0.62 [Flu. Blue], 0.69, 0.77, 0.85, 0.94 [Flu. Yellow]
EA2			09	0.1 [Flu. Blue], 0.55 [Brown], 0.62 , 0.69, 0.79 [Red], 0.75, 0.90,0.94 [Flu. Yellow], 0.97 [Grey]
Glycyrrhiza Glabra Linn				
GG1	Alcoholic	Toluene Ethylacetate : Formic acid [5:4:1]	11	0.05, 0.12 [Both Yellow], 0.26, 0.94 [Blue], 0.42, 0.52, 0.59, 0.62, 0.67 [All Pink], 0.72, 0.84 [Both Red]
GG2			08	0.04 [Yellow], 0.40, 0.61 [Blue], 0.51, 0.8,0.69, 0.87 [Pink], 0.79 [Red]
Leucas cephalotes				
LC2	Methanolic	Toluene :EthylAcetate [9:1]	05	0.16 [Grey], 0.30, 0.39 , 0.62, 0.95 [Violet]
Nardostachys jatamansi				
NJ1	Alcoholic	Toluene Ethyl acetate [9: 1]	06	0.62, 0.30, [Blue], 0.42 [Grey] 0.60, 0.69, 0.91[Pink violet]
NJ2			09	0.04, 0.10 [Brown],0.19 [Pink violet]0.29[Pink], 0.35, 0.44, 0.54 [Blue], 0.49, 0.61 [Grey]
Nigella Sativa Linn.				
NS1	Alcoholic	Toluene Ethyl acetate [9: 1]	09	0.1, 0.26, 0.65 [Green], 0.32, 0.39, 0.51, 0.74, 0.84, 0.92 [Grey]
NS2			05	0.04, 0.09 [Brown],0.25 [Pink Violet], 0.35, 0.52, 0.85 [Blue]
NS 3			05	0.04, 0.86 [Green], 0.06 [Light Yellow], 0.12 [Grey], 0.56 [Yellow]
Saussurea lappa C.B. Clarke				
SL1	Alcoholic	Toluene: Ethyl acetate: Formic acid [5: 4.5: 0.5]	07	0.22[Grey], 0.42, 0.66, 0.74 [Violet], 0.82, 0.9 [Pinkish Red]
SL2			08	0.05, 0.11,.60, 0.66, 0.81 [Grey], 0.42, 0.71, 0.95 [Violet]
SL3			10	0.04 [Brown], 0.12, 0.34, 0.42 [Blue], 0.22 0.26 [Violet], 0.54, 0.65, 0.87 [Grey], 0.75 [Yellow]
Tecomella undulate (Amoora rohitaka)				
AR2	Alcoholic	Toluene :Ethyl Acetate :Formic acid [5:4:1]	02	0.04 [Orange], 0.12 [Brown]
Tephrosea purpurea				
TP1	Methanolic	n- Hexane: Ethyl acetate: Methanol [9:1;1]	07	0.06, 0.41 [Yellow], 0.15 [Grey], 0.55,0.94 [Blue], 0.70 [Pink Violet], 0.85 [Violet]
TP2			06	0.046 [Brown Green], 0.1 [Greenish Violet], 0.146, 0.493 [Violet Blue] 0.3, 0.42 [Violet], 0.6 [Blue]
TP3			08	0.06,0.33 Green, 0.13, 0.21, 0.44, 0.48 [Blue], 0.56, 0.82 [Violet]
Tinospora cordifolia (Wild) miers				
TC1	Alcoholic	Choloroform : Methanol [9: 1]	07	0.03, 0.08, 0.19 [Yellow], 0.58, 0.64 , 0.73, 0.81 [Blue]
TC2			08	0.14, 0.25, 0.44, 0.47, 0.54, 0.67 [Violet], 0.80 [Blue], 0.89 [Brown]
TC3			12	0.04, 0.75 [Brown], 0.18, 0.29, 0.38, 0.48, 0.54 [Grey Blue], 0.65, 0.766, 0.883, 0.91, 0.96 [Violet]

Table 4: Presents solvent extract of plant drug, mobile phase used, number and colour of spots and the Rf values of each plant sample analysed by thin layer chromatography (TLC)

3.5 MTT Assay

The aqueous extracts of 34 selected plant samples were tested for cytotoxic effects on HepG2 cell line using MTT assay. Various concentrations (2µg/ml – 1000 µg/ml) of each plant extract was treatedon HepG2 cell line for 48 hours and treated with MTT dye. The MTT is reduced by cellular dehydrogenase enzyme to purple product formazan which is read at 570 nm wavelength. Percent cell viability for each drug sample was plotted against the drug concentration. The data were statistically analysed and IC_{50}values were determined using Prism VI software (Table 5).

Table 5 Comparison Of IC50 Value Of Study Drug Samples From Different Sources

Sr. No	Samples	IC50 vales µg/ml Source 1	IC50 value µg/ml Source 2	IC50 value µg/ml Source 3
1.	Barberis aristata	147.3	67.72	36.24
2.	Cinnamomum cassia	8.726	65.04	62.10
3.	Cinnsmomum zeylanicum	111	114	97.13
4.	Commiphora myrrha	294.8	392	400
5.	Crocus sativus	44.55	679	36.15
6.	Cymbopogon jwarancusa	179.5	10.20	171.1
7.	Glycirrhiza glabra	36.28	83.93	163.2
8.	Nardostachys jatamanasi	63.43	163	102
9.	Nigela sativa	227.8	382	270
10.	Sausurria lappa	112	126	62.95
11.	Tecomella undulata (Amoora rohitaka)	138.1	201	137.7

The results showed that all of the selected plant samples have some level of toxicity to HepG2 cell line. The extent of toxicity however differed in *Cymbopogon jwarancusa, Commiphora myrrah, Cinnamonum cassia, Crocus sativus, Nardostachys jatamanasi and Berberis aristata* respectively. CC, GG, NS, and NJ procured from source 1 demonstrated maximum effect against HepG2 cell line. Whereas, CJ and AR of source 2 and BA, CZ, CM3, CS, SL and AR from source 3 were the mediators of effectivity. Efficacy of herbal drugs is correlated to the phytochemical constituents present in the plant. It was observed that the plant drug samples obtained from different sources varied in the IC_{50} values against $HepG_2$ cell line. It suggests that the samples of same species may vary in their phytochemical content and efficacy. The plant drug showing minimum and pharmacologically significant IC_{50} values would be efficient for use in cancer treatment. In accordance with the objective of present study, the samples showing cytotoxic effect at minimum concentration (minimum IC_{50} value) were identified and correlated with analytical parameters. Table 6 presents analytical parameters of drug samples showing maximum cytotoxic effect on HepG2 cell line. It is of value to determine the quality standards of plant drugs for use in hepatocellular carcinoma.

Table 6: Correlation Of IC50 Value With Analytical Parameters

Sr No	Sample	IC50 value	Physicochemical evaluation in %					Thin Layer Chromatography	
			Moisture content	Total ash	Acid insoluble ash	Alcohol soluble extractive	Water soluble extractive	Spots	Rf Values
1	BA3	36.24	2.21	0.86	0.15	0.6	1.87	03	0.07 [Yellow], 0.57 [Flu. Blue], 0.84 [Blue]
2	CC1	36.18	8.96	3.67	0.11	6.98	6.95	04	0.12, 0.16, 0.96 [Blue], 0.90 [Pink Violet]
3	CZ1	97.13	7.06	2.63	0.14	8.01	6.90	06	0 .02 [Brown], 0.06, 0.21, 0.62 [Grey], 0.91 [Green], 0.97 [Blue]
4	CM1	145	12.23	4.06	0.57	17.27	58.45	03	0.04 [Yellow], 0.39 [Pink], 0.8 [Violet]
5	Cs3	36.15	7.013	4.517	1.00	39.07	42.16	09	0.08, 0.95 [Blue], 0.16, 0.87 [Green], 0.64 [Violet], 0.58 , 0.53, 0.31,0.22 [Ash colour]
6	CJ2	126.9	5.466	6.647 3	4.542	1.213	4.905	05	0.0624 [Dark Grey], 0.45 [Dark Violet], 0.725 [Violet], 0.912 [Light Orange], 0.97 [Dark Orange]
7	GG1	36.28	5.67	5.14	0.34	3.72	23.11	11	0.05, 0.12 [Both Yellow], 0.26, 0.94 [Blue], 0.42, 0.52, 0.59, 0.62, 0.67 [All Pink], 0.72, 0.84 [Both Red]
8	NJ1	63.43	9.03	10.46	6.20	3,21	3.34	06	0.62, 0.30, [Blue], 0.42 [Grey] 0.60, 0.69, 0.91[Pink violet]
9	NS1	227.8	5.42	32.7	0.96	33.75	11.66	09	0.1, 0.26, 0.65 [Green], 0.32, 0.39, 0.51, 0.74, 0.84, 0.92 [Grey]
10	SL3	62.95	6.25	4.04	0.12	5.75	12.29	10	0.04 [Brown], 0.12, 0.34, 0.42 [Blue], 0.22 0.26 [Violet], 0.54, 0.65, 0.87 [Grey], 0.75 [Yellow]
11	AR3	137.7	7.16	11.89	0.33	14.60	18.65	02	0.04 [Orange], 0.12 [Brown]

4. DISCUSSION

Assessment of quality parameters of herbal drugs is of profound importance in determination of the efficacy and safety of the herbal medicines. Selection of appropriate raw material is crucial for the desired effect of herbal drugs. Establishment and validation of analytical parameters are important requirements of quality assurance. The present study was aimed to evaluate the traditional medicinal plants used in treatment of liver dysfunctions for quality standards. The selected plant samples comprised of group of plants having anti-cancer effect against hepatocellular carcinoma. The plants

thus are likely to be of great value in exploring their application in treatment of hepatic cancers. The plant samples selected from three sources were analysed for organoleptic, physicochemical parameters and thin layer chromatography. Aqueous extracts of these plants were evaluated for cytotoxic effects on the hepatocellular carcinoma cell line. Choice of right raw material would be of profound value in determination of its efficacy.

The authentication report of the selected plant samples approved of 42 samples out of 45 submitted to them. Out of the three samples of *Cinnamom cassia*, only one was authentic,whereas the other two were identified as *Cinnamomum zeylanicum.* Taxonomic evaluation is the most important toolfor quality. Medicinal plants are generally collected by professionals who may not be botanists or taxonomists. Similarly, the identity of crude drugs purchased from the market is often not subjected to a stringent method of botanical identification. It is also observed that, in addition to nomenclatural ambiguity, the traditional drugs sold in herbal markets in various places are adulterated or substituted with quite unrelated plant materials unintentionally. This may be due to the confusion in vernacular names between indigenous systems of medicine and local vendors, lack of knowledge about the authentic plant, unavailability of the authentic plant, similarity in morphological characters and careless collection, etc [15]. The authentication study disqualified only three plants in our collection of plants.

Presence of adulterants in plant material can be detected by evaluation of physicochemical characteristics. Ash values, especially in powder form, are significant quantitative benchmarks for verifying the identification and purity of crude medicines. The purity of the crude and prepared drugsis reflected in the total ash of a crude drug. The values of total ash and acid insoluble ash for all the samples were in accordance with the Ayurveda/Unani Pharmacopoeia guidelines. The values for samples NJ1, CM2, NS1, EA1 were however, more than as mentioned in the guidelines.The Pharmacopoeia of India is an official document published by the authority for guiding the users for standardisation and purity aspects of single drugs used in traditional ASU systems of medicine.

Moisture content aids in the calculation of the real weight of medicinal material, reducing mistakes. As per the guidelines the moisture content should be around 8-10% to avoid fungal and other pests' contamination.Result suggested that all the drugsexcept CM1 and CM2 have moisture content between 2 to 9 %. The weights of the extractable chemical elements of crude medicines in various solvent conditions are referred to as extractive values. It gives an idea about the nature of chemical constituents present in it [16].The water-soluble extractive value was indicates the presence of sugar, acids and inorganic compounds and alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids and secondary metabolites present. Most of the plants in present study showed higher water extractive values. Seeds of *Nigella sativa* however showed much higher alcohol soluble extractive values. In other cases viz., EA, GG, TP there were variations among the three samples collected. It is observed that even when correctly authenticated, the same herbal ingredient may differ in quality due to a number of factors such as inter-or intra-species variation, environmental factors like climate and altitude under which it was cultivated.

The thin layer chromatography (TLC) study of selected plant samples were conducted using aluminium sheets of silica gel F254 (Merck®). All chromatograms were developed in a saturated chamber. The mobile phases employed in this study were selected from the reference available in Reviews of Medicinal Plants (Vol I to Vol 12). The criterion for acceptance of the TLC procedure was adequate detection of the specific marker with respect to the colour and relative position of the band. The TLC of alcoholic extracts of BA, CC, CZ, CM, GG, NJ, NS, *SL, ARs*amplesinvolved the solvent system of Toluent : Ethyl acetate: Formic acid in various concentrations and resulted in formation of different coloured spots.

N-hexane etherextract of CJ1 sample with Benzene : chloroform [1:1] and CJ2 with Toluene: Ethyl Acetate [93:7] solvent system resulted in generation of 05 spots for each, while N-hexane extract of *Crocus sativa* Toluene Ethyl acetate [9.7: 0.3] solvent system resulted in 9 spots having Rf values 0.08, 0.95, 0.16, 0.87, 0.64, 0.58 , 0.53, 0.31, 0.22 respectively. The methanolic extract of EA1 and EA3 in the presence of toluene: Acetone : Formic acid(11:6:1) solvent system resulted in formation of 08 &09 spots respectively, while methanolic extract of *Leucas cephalotes* with Toluene: Ethyl Acetate [9:1] as solvent system produced 5 grey and violet coloured spots with Rf values of 0.16, 0.30, 0.39, 0.62, and 0.95 respectively. The alcohol extract of *Tinospora cordifolia* with solvent system of Chloroform: Methanol [9: 1] gave 08,07 and 12 spots for TC1, TC2& TC3 samples respectively with violet, blue and brown colours.

These Rf values are indicative of the phytochemicalconstituents of the respective plant species and provide important information about their polarity and basis of separation of these phytochemicals in the separation process. This information will help in the selection of an appropriate solvent system for further separation of compounds from these plant extracts.

The MTT assay determined that all of the selected plant samples possess some level of toxicity to liver cancer derived HepG2 cell line. The extent of toxicity however differed. There was significant variation in the IC_{50} values of the drug obtained from three different sources. Plant sample showing least IC_{50} value i.e. lowest concentration at which it is toxic to 50% of hepatocellular carcinoma cell line is of value for use in anticancer treatment. The physiochemical characteristics of these plants may serve as quality parameters of these plants. The efficacy of herbal drugs is correlated to the presence of phytochemical constituents in them. Various parameters may vary the phytochemical constitution of the plant species. Random collection of plant material may result in significant variation in its effectivity and safety. Determination of analytical parameters and its correlation with desired application is of great value in selection of right raw material for the development of drugs. Analytical data and quality parameters of medicinal plants will have great contribution in application of traditional medicine to global health and sustainable development.

5. CONCLUSION

The present study evaluated organoleptic, physicochemical, TLC, and cytotoxic effect of fifteen plant samples procured from three different sources. The tests were used to standardise fifteen medicinal plants used to treat diverse liver ailments. The results of this investigation were dependable, rapid and repeatable. They may be utilised for routine formulation quality control examination. It is also of value in laying down of standards for a multi-ingredient formulation. To meet the rising demand for traditional medicines, an integrated method must be developed to standardise traditional medicines for their efficacy and safety. The quality standards will be used as diagnostic parameters for its identification and will aid in the maintenance of medicine quality.

CONFLICT OF INTEREST- NIL

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