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Dr. RB Sharma
L.R. Institute of Pharmacy,
Solan, Himachal Pradesh, India

Rachna Verma
L.R. Institute of Pharmacy,
Solan, Himachal Pradesh, India

Corresponding Author:
Dr. RB Sharma
L.R. Institute of Pharmacy,
Solan, Himachal Pradesh, India

Development of hepatoprotective polyherbal formulation from methanolic extracts of stems of *Berberis aristata,* fruits of *Piper longum and* roots of *Boerhaavia diffusa*

Dr. RB Sharma and Rachna Verma

Abstract
Liver plays a vital role in the metabolism of various exogenous and endogenous compounds. As a result of its, continuous involvement, is susceptible to toxic injuries caused by certain agents and any damage to hepatic cells disturb body metabolism. Indiscriminate use of systemic agents like tetracycline, paracetamol, anti-tubercular drugs, oral contraceptives of hormonal origin, chemicals used as food preservatives and agrochemicals are threatening the integrity of the liver. The present study is therefore undertaken to explore the effects of methanolic extracts of stem of *Berberis aristata*, roots of *Boerhaavia diffusa* and fruits of *Piper longum*. Development and evaluations of Hepatoprotective Polyherbal Formulation showing significant activity. The objective of present study is development of Hepatoprotective Polyherbal Formulation.
Methanolic extracts of stem of *Berberis aristata*, roots of *Boerhaavia diffusa* and fruits of *Piper longum* were formulated for hepatoprotective formulation. Polyherbal formulation was prepared by dissolving extracts in 100ml purified water containing solubilizer, hydrotropic agent, suspending agent, 66.7%w/w sucrose and heated until complete dissolved with occasional shaking. Formulation F3 Formulated Polyherbal Formulation was evaluated for organoleptic characters (colour, odour and taste), density, viscosity, pH, specific gravity, turbidity and homogeneity. Evaluation parameters for formulations have shown good results within the specification of I.P. Polyherbal Formulation was formulated containing mixture of methanolic extracts of different experimental plants.
Acceleratory stability study has indicated that the formulation was stable, homogeneity was maintained and no turbidity was found for long time of storage was formulated containing mixture of methanolic extracts of different experimental plants.
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Keywords: Polyherbal formulation, hepatoprotective activity, *Berberis aristata*, *Boerhaavia diffusa*, *Piper longum*

Introduction
Now-a-days natural products are an integral part of human health care system, because there is a now popular concern over toxicity and side effects of allopathic drugs. There is also a realization that natural medicines are safer and allopathic drugs are often ineffective in several ailments. Medicinal plants existed even before human being made their appearance on the earth. Man`s existence on this earth has been made possible only because of the vital role played by plant kingdom in sustaining his life. Since the down of civilization, in addition to food crops, man cultivated herbs for his medicinal needs [1].
In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. More than 700 mono and polyherbal preparation in the form of decoction, tincture, tablets and capsules from than 100 plants are in clinical use [2].

Experimental Methods
Soxhlet extraction (continuous hot extraction) [3].
The drugs were cleaned and died at room temperature in a shade. They were then powdered and used for extraction. About 25gm of dried powdered was extracted with 250ml of methanol. The extraction was continued until solvent in the thimble became clear. After complete extraction, the extract was collected and the solvent was distilled off.

Then it was concentrated to dry residue on water bath and extract (dry residue) was weighed. The dried extract was stored carefully for Hepatoprotective screening and development of Polyherbal Formulation.

Development of hepatoprotective polyherbal formulation [4].

In the present investigation, mixed-solvency approach has been utilized for solubility enhancement of poorly water-soluble drug. In this technique solubilizing agents from the category of hydrotropes (sodium benzoate, sodium citrate), solubilizers (PEG 400, PEG 800) and wetting agents (Tween 80) are employed.

Method of preparation of simple polyherbal formulation

66.7% w/w of sucrose was weighed and added to purified water and heated until dissolved with occasional stirring. Sufficient boiling water was added to produce 100ml. All trial formulations were prepared as 100ml quantity by varying concentration of PEG 400, PEG 800 (solubilisers), sodium citrate (hydrotropic agents) and Tween 80 (wetting agents). The formulation development with mixture of extracts of herbal drugs is shown in Table 1

Optimization of polyherbal formulation

Table 1: Formulation development with extracts of herbal drugs

Sr. No.	Ingredients	Formulation Code				
		A	B	C	D	E
1.	Mixture of extracts	750mg	750mg	750mg	750mg	750mg
2.	Sucrose	66.7gm	66.7gm	66.7gm	66.7gm	66.7gm
3.	PEG 400	-	1ml	-	-	-
4.	PEG 800	-	-	1ml	-	-
5.	Tween 80	-	-	-	-	0.5ml
6.	Sodium citrate	-	-	-	1gm	-
7.	Purified water	Up to 100ml	Up to 100ml	Up to 100ml	Up to 100ml	Up to 100ml

Composition of hepatoprotective polyherbal formulation

Extracts was selected based upon their significant hepatoprotective activity. All the ingredients were mixed thoroughly in three different compositions as Formulation F1, Formulation F2 and Formulation F3. Composition of three designed Formulations (F1, F2 & F3) containing extracts combination along with excipients is shown in Table 2.

Table 2: Composition of hepatoprotective polyherbal formulation

Sr. No.	Ingredients (in % w/w)	Formulations Code		
		F1	F2	F3
1	*Berberis aristata* extract	250mg	250mg	250mg
2	*Boerhaavia diffusa* extract	250mg	250mg	250mg
3	*Piper longum* extract	250mg	250mg	250mg
4	Sucrose	66.7%	66.7%	66.7%
5	Sodium benzoate	0.3%	0.2%	0.1%
6	Xanthan gum	0.2%	0.1%	0.05%
7	Tween 80	0.5%	0.5%	0.5%
8	Flavour (Mixed fruit)	0.5%	0.5%	0.5%
9	Purified water q.s.	100ml	100ml	100ml

Procedure for hepatoprotective polyherbal formulation

The required quantities of solubilizers (Tween 80) was transferred to a volumetric flask (100ml capacity) containing 100ml of distilled water and heated until complete dissolved with occasional shaking. Then required amount of extracts of drugs were added and heated until complete dissolved with occasional shaking to dissolve the drug completely. Then required amount of hydrotropic agent (sodium benzoate) and suspending agent (xanthan gum) were added and heated until complete dissolved with occasional shaking. Then 66.7% w/w sucrose was added and again the flask was shaken and continuous heating until it dissolve with occasional shaking. Then the volume was made up to the mark with distilled water. Now cool the formulation for some time & then add flavouring agent (mixed fruit flavour) to it. The final developed Polyherbal formulation was preserved in airtight container.

Evaluation of formulated polyherbal formulation

Physicochemical parameters [5-6]: The herbal formulation was evaluated for various physicochemical parameters such as organoleptic characters (colour, odour, and taste), pH, viscosity, density and specific gravity.

a) **Colour:** Five ml final polyherbal formulation was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.
b) **Odour:** Two ml of final polyherbal formulation was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.
c) **Taste:** A pinch of final polyherbal formulation was taken and examined for its taste on taste buds of the tongue.
d) **Determination of pH:** The pH of polyherbal formulation was determined by using pH meter. The pH meter was calibrated using distilled water, buffer (at pH 4 and 9) till constant readings were obtained. Measurement of pH of polyherbal formulation was carried out at different time intervals to evaluate the physical stability and palatability, using digital pH meter.
e) **Determination of Viscosity:** Viscosity is another parameter of quality control of polyherbal formulation physical stability and portability. Viscosity of formulated polyherbal formulation was determined by Ostwald viscometer [7-8]
f) **Determination of Specific gravity:** Pycnometer was used to determine the specific gravity at 25 ^{0}C. The tare weight of the pycnometer was subtracted from the filled weight. The weight per millilitre was determined by dividing the weight in air, expressed in g, of the quantity of polyherbal formulation which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature. Specific gravity of the final polyherbal formulation was obtained by dividing the weight of the polyherbal formulation (expressed in gm.) contained in the pycnometer by the weight of water (in ml), contained both determined at 25 ^{o}C.
g) **Determination of Density:** Taking 10ml of formulation and weighing then calculated density of polyherbal formulation at room temperature. Density was determined for polyherbal formulation with the help of density bottles. Density of polyherbal formulation was determined dividing mass of polyherbal formulation (expressed in gm.) by the volume of polyherbal formulation (expressed in ml).

Stability studies of hepatoprotective polyherbal formulation [9-10].

Stability testing: Stability testing of the prepared poly herbal formulation was performed on keeping the samples at

accelerated temperature conditions. Three portions of the final formulation (A, B and C) were taken in amber coloured glass bottles and were kept at accelerated temperature at 4 °C Room temperature and 47° respectively. The samples were tested for all the physicochemical parameters, turbidity and homogeneity at the interval of 24hr., 48hr. and 72hr. to observe any change.

2. Results

Percentage yield of various plants extracts by continuous hot extraction (soxhlet)

Table 3: Percentage yield of various plants extracts by continuous hot extraction (soxhlet extraction)

Sr. No.	Drug	Solvent	Nature of extract	Colour	Weight (gm.)	Percentage yield (w/w)
1.	*Berberis aristata*	Methanol	Solid	Greenish black	1.24	4.96%
2.	*Boerhaavia diffusa*	Methanol	Solid	Greenish black	1.27	5.11%
3.	*Piper longum*	Methanol	Semi solid and sticky	Green	1.22	4.88%

The percentage yield is calculated in term of air dried weight of plant material. In case of Boerhaavia *diffusa* the percentage yield obtained was 5.11% w/w, in case of *Berberis aristata* it was 4.96% w/w and in case of *Piper longum* percentage yield obtained was 4.88% w/w. The percentage yield of various plants extracts shown in Table 3

Percentage yield of various plants extracts by cold extraction (maceration)

Table 4: Percentage yield of various plants extracts by cold extraction (maceration extraction)

Sr. No.	Drug	Solvent	Nature of extract	Colour	Weight (gm)	Percentage yield(w/w)
1.	*Berberis aristata*	Methanol	Solid	Greenish Black	1.19	4.7%
2.	*Boerhaavia diffusa*	Methanol	Solid	Greenish Black	1.20	4.8%
3.	*Piper longum*	Methanol	Semi solid and sticky	Green	1.11	4.4%

The percentage yield is calculated in term of air dried weight of plant material. In case of *Boerhaavia diffusa* the percentage yield obtained was 4.8% w/w, in case of *Berberis aristata* it was 4.7% w/w and in case of Piper *longum* the percentage yield obtained was 4.4% w/w. The percentage yield of various plants extracts shown in Table 4

By comparing percentage yield of all extracts developed by maceration extraction and soxhlet extraction (from Table No.: 1.3, 1.4). The Percentage yield of all methanolic extracts by Soxhlet extraction (continuous hot extraction) was more than Maceration extraction (cold extraction). Hence it may conclude that extracts developed from soxhlet extraction is further used for Hepatoprotective Polyherbal Formulation.

Preliminary phytochemical investigation

Table 5: Preliminary Phytochemical Investigations of various extracts

Sr. No.	Name of the test	*Berberis aristata*	*Boerhaavia diffusa*	*Piper longum*
A.	**Carbohydrates**	**Methanolic extract**	**Methanolic extract**	**Methanolic extract**
	Molisch`s test	+	+	+
	Fehling`s test	+	+	+
B	**Proteins**			
	Biuret`s test	+	+	+
	Million`s test	+	+	+
C	**Alkaloids**			
	Dragendorff `s test	+	+	+
	Hager`s test	+	+	+
D	**Steroids**			
	Salkowski reaction	+	+	+
E	**Flavonoids**			
	Lead acetate test	+	+	+
	Ferric chloride test	+	+	+
F	**Glycosides**			
	Legal test	+	+	+
	Kellar Killani test	+	+	+
	Borntrager`s test	+	+	+

+Present,-Absent

The extracts obtained by soxhlet extraction were subjected to preliminary phytochemical analysis Phytochemical investigation of extracts of *Berberis aristata*, *Boerhaavia diffusa* & *Piper longum* show the presence of alkaloids, glycosides, flavonoids, steroids, Proteins & carbohydrates. Hence it may concluded that berberine in *Berberis aristaa* extract, piperine in *Piper longum* extract and boeravinones in *Boerhaavia diffusa* extract is present. Preliminary Phytochemical Investigations of Various Extracts shown in Table 5

Development of polyherbal formulation

In the present study, the use of mixed- solvency has been explored to develop liquid Polyherbal Formulation of water soluble herbal drugs to give quick onset of action and better bioavailability. Optimized formulae for development of formulation with herbal extracts shown in Table 1. Formulation Code A shows that the herbal test extracts were not properly soluble in the simple syrup containing 66.7%w/w. In Formulation Code B & C the solution formed with PEG 400 & PEG 800 (Solubilzer) was turbid and test extracts were not properly soluble. Formulation Code D shows the different concentration of sodium citrate (hydrotropic agents). The solution thus formed was not clear & was hazy. Test extracts were not properly soluble. The formulations thus formulated gave negative results. In Formulation Code E the solution formulated with Tween 80 (wetting agent) was clear and test extracts were properly soluble. Formulation was further developed by using Tween 80.

Composition of Hepatoprotective Polyherbal Formulation is shown in Table 2. Formulations (F1 & F2) contain more concentration of suspending agent (xanthan gum) and other additives so that it enhances viscosity of Formulation (F1 & F2). The flow of formulations are not uniform. Formulation F3 has adequate quantity of solubilzers, hydrotopic agent (sodium benzoate) and suspending agent (xanthan gum). It has optimum viscosity. Hence Formulation F3 was selected for the development of Hepatoprotective Polyherbal Formulation.

Physicochemical parameters of developed polyherbal formulation f3

Table 6: Physicochemical parameters of developed polyherbal formulation f3

Sr. No.	Physicochemical Parameters	Observed Values
1	Colour	Reddish brown
2	Odour	Characteristics
3	Taste	Sweet
4	Viscosity(centipoise)	18.19 centipoise
5	pH	6.7
6	Density (g/cm^3)	1.1708 g/cm^3
7.	Specific gravity(g/ml)	1.234g/ml

Prepared Polyherbal Formulation F3 was evaluated for physiochemical parameters as mentioned already and results were summarized in Table 6. Organoleptic studies of the Polyherbal Formulation was performed by subjective evaluation. The formulation had pleasant appearance and acceptable colour, odour and taste. There was no change in the organoleptic properties in the respect to colour, odour and taste.

Results of viscosity studies indicate prepared formulation F3 possess optimum viscosity for physical stability. The pH values of formulation were almost constant from 24hr to 72hr indicating physical stability and palatability. Densities were found to be satisfactory and consistent to provide maximum possible stability.

Stability studies of hepatoprotective polyherbal formulation f3

Table 7: Stability testing of developed polyherbal formulation F3

Sample No.	Time (hour)	Temp (ºC)	Physicochemical parameters					
			Colour	Odour	Taste	pH	Specific gravity (g/ml)	Turbidity/ Homogeneity
A1	24hr	4 ºC	NC	NC	NC	6.7	1.234	No turbidity
B1		R.T.	NC	NC	NC	6.7	1.234	X
C1		47 ºC	NC	NC	NC	6.7	1.234	Homogeneity
A2	48hr	4 ºC	NC	NC	NC	6.7	1.234	No turbidity
B2		R.T.	NC	NC	NC	6.7	1.234	X
C3		47 ºC	NC	NC	NC	6.7	1.234	Homogeneity
A1	72hr	4 ºC	NC	NC	NC	6.7	1.234	No turbidity
B2		R.T.	NC	NC	NC	6.7	1.234	X
C3		47 ºC	NC	NC	NC	6.7	1.234	Homogeneity

R.T: Room Temperature
NC: No Change

The Polyherbal Formulation with Hepatoprotective activity was subjected for stability testing to assess the effect of storage at different temperature and humidity. Polyherbal Formulation was found to be stable when subjected to accelerated stability studies at variable temperature and humidity condition. There was no significant change in the physicochemical and organoleptic properties. In the present study, stability studies were carried out during 24hr, 48hr and 72hr of storage formulation was evaluated for physicochemical parameters pharmacological activity to rule out the significant changes. After 72hr, stored formulation was subjected for thorough physicochemical evaluation along with hepatoprotective activity studies following results were obtained as shown in Table 7

Conclusion

Every year 18,000 people are reported to die due to liver cirrhosis caused by hepatitis. Traditional system of medicine, especially Ayurveda advocates number of preparations for treating liver & GIT disorders. Allopathic medicine provides only symptomatic relief with added side effects in the treatment of liver disease. Herbal drugs, used in Indian System of Medicine are however claimed to be effective and safe in such ailments. Polyherbal preparations are considered safe and effective products consisting of multiple extracts/active principles from medicinal plants. By considering the above aspects, the present proposal of study is designed for the development of Hepatoprotective Polyherbal Formulation for effective management of liver diseases using some of the indigenous medicinal plants.

In the present study, coarsely powdered shade dried stem of *Berberis aristata* of family berberidaceae, roots of *Boerhaavia diffusa* of family nyctaginaceae and fruits of *Piper longum* of family piperaceae were subjected to continuous hot extraction (soxhlet extraction) and cold extraction(Maceration extraction) with methanol.

The results obtained from the pharmacological screening are methanolic extracts of stem of *Berberis aristata* DC, roots of Boerhaavia *diffusa* Linn and fruits of Piper *longum* Linn shown more prominent hepatoprotective activity.

In the present work an attempt has been made to formulate the Hepatoprotective Polyherbal Formulation for the significant extracts and evaluated the same for various parameters.

Polyherbal Formulation was formulated, using the required quantities of solubilizer in a volumetric flask (100ml capacity) containing 100ml of distilled water and heated until complete dissolved with occasional shaking. Then required amount of extracts of drugs were added and heated until complete dissolved with occasional shaking to dissolve the drug completely. Then required amount of sodium citrate and xanthan gum were added and heated until complete dissolved with occasional shaking. Then sucrose was added and again the flask was shaken and continuous heating until it dissolve with occasional shaking. Then the volume was made up to the mark with distilled water. After some time add flavouring agent to the formulation.

Formulated Polyherbal Formulation was evaluated for organoleptic characters (colour, odour and taste), density, viscosity, pH, specific gravity, turbidity and homogeneity. Organoleptic properties of the formulations were checked after formulation and stability studies. There was no change in the organoleptic properties in respect to colour, odour and taste.

Viscosity and pH of all the prepared Polyherbal Formulations were evaluated 24hr, 48hr and 72hr. Data revealed that there is no much change in viscosity and pH of formulation, indicating the poor ability and palatability of the Polyherbal Formulation.

Homogeneity and turbidity was evaluated for formulated

polyherbal formulation at room temperature.
Density was evaluated for formulated Polyherbal Formulation at room temperature. Density of formulation was found to 1.234g/cm^3 hence the density is slightly more than that of water.

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