

Characterization of a Prepared Poly-Herbal Formulation and Comparison of its DPPH Scavenging Activity with Other Marketed Formulations

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Abstract: Present study involves preparation of a poly-herbal liquid formulation, its characterization and comparison of its antioxidant activity with some marketed formulations. Aqueous extract of the three plants namely, *Hippophae rhamnoides* (SBT), *Bacopa monerehii* (BM) and *Centella asiatica* (CA), were prepared using cold percolation method. These extracts were used further for preparation of poly-herbal syrup using simple syrup (66.67% w/v) as base. Characterization of this formulation was done by determination of total phenol content (Folin - Ciocalteu reagent (FCR) based assay) and evaluation of various physicochemical parameters like color, odor, taste, pH, specific gravity, refractive index etc. Total phenol content of the prepared poly-herbal syrup was found to be 11.410 mg/ml GAE. The antioxidant activity (DPPH scavenging activity) of the above prepared formulation was compared with other similar marketed formulations like Liv - 52 and New LIVFIT and it was found to be higher in case of Liv - 52 followed by prepared poly-herbal syrup and minimum in case of New LIVFIT. These results indicate that the prepared formulation appears to be a good candidate for use in the oxidative stress induced liver problems, however, its efficacy in living systems (*in vivo*) needs to be investigated.

Key Words: Poly-herbal formulation • Total phenol content • DPPH scavenging activity

INTRODUCTION

With the emerging interest in the world to adopt and study the traditional system and to exploit their potentials based on different health care systems, the evaluation of the rich heritage of the traditional medicine is essential. This has attracted a great deal of research interest in natural antioxidants and antioxidant based drugs/formulations for the prevention and treatment of complex diseases such as hepatic disorders.

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent, thus produces free radicals which start chain reactions. If free radicals reach high levels, oxidative stress in human body would be created, which leads to a variety of biochemical and physiological lesions and often results in metabolic impairment and cell death. On the other hand, high levels of active oxygen and free radicals could also cause lipid oxidation which led to a highly deteriorative process [1]. Antioxidants terminate these chain reactions by

removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. Most commonly known antioxidants are: Vitamin A, C, & E, polyphenols and carotenoids.

Aerobic organs such as the liver generate reactive oxygen species that induce oxidative tissue damage. These radicals react with cell membranes and thus induce lipid peroxidation or cause inflammation [2]. Liver diseases remain as one of the serious health problems. However we do not have satisfactory liver protective drugs in allopathic medical practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India [3].

Hippophae rhamnoides L. commonly known as Seabuckthorn (Family: Elaeagnaceae) growing in North-West Himalayas at high altitude (7000-15,000 feet), is a dwarf to tall (3-15 feet), branched and thorny nitrogen fixing deciduous shrub, native to Europe and Asia.

All parts of the plant are considered to be good source of a large number of bioactive substances [4]. SBT is a good source of antioxidant compounds [5-6] and has also been reported to possess hepatoprotective properties [7-8]. The medicinal effects of Seabuckthorn have been suggested to be due to the presence of high antioxidant contents. Seabuckthorn leaves are rich in flavonoides, tannins and triterpenes [4]. Literature review indicates that BM is having significant antioxidant activity [9] and has also been used in liver toxicity [10]. CA was also found to have antioxidant properties [11-13] and to be used as hepatoprotective [14].

For developing a satisfactory herbal formulation, there is a need to characterize the basic pharmaceutical properties of the dosage form developed and to evaluate the formulation for desired properties (In this case antioxidant activity) and compare it with other marketed formulations in order to evaluate its marketability. In view of the above, present study was undertaken to prepare a poly herbal formulation, characterize and compare its antioxidant activity with other marketed formulations.

MATERIALS AND METHODS

Collection of Plant Materials: Authenticated leaves of *Bacopa monerehii* and *Centella asiatica* were obtained from Dabur Research Foundation Ghaziabad (U.P.). *Hippophae rhamnoides* leaves were collected from Leh, India and authenticated by National Institute of Science Communication and Information Resources (NISCAIR).

Preparation of Extracts: The collected plant materials were dried under shade and size reduced into coarse powder. These powdered materials were soaked with distilled water separately (Raw material to solvent ratio 1:5) separately for 24 hrs. After 24 hrs, supernatants were decanted and fresh solvents were added. This process was repeated 4 times for complete extraction. After complete extraction supernatants were mixed separately for each plant. These mixed supernatants were filtered and concentrated under reduced pressure separately till a solid was obtained.

Preparation of Herbal Syrup: Simple syrup (66.67% w/v) was prepared as per Indian Pharmacopoeia. 4 gm of each of the extracts were dissolved in the prepared syrup and volume was made up to 100ml (Table 1).

Table 1: Formulation of syrup

Extract	Amount (mg) present in 10 ml of syrup
Bacopa monerehii	400
Centella asiatica	400
Hippophae rhamnoides	400
Syrup base	q.s.

Characterization of the Prepared Syrup: The above prepared formulation was characterized by determination of physicochemical parameters like interaction studies, specific gravity, refractive index etc. total phenol content and determination of DPPH scavenging activity.

Physicochemical Parameters: Physical parameters like color, odor, taste, specific gravity, pH and refractive index were analyzed as per the standard procedure mentioned in Indian Pharmacopoeia.

Total Phenol Content: The total phenol content was estimated by Folin - Ciocalteu reagent (FCR) based assay [15]. To the aliquot (50µl) taken from stock solution (10% v/v) of the prepared syrup, 3.5 ml distilled water and 250µl of FCR was added, the mixture was kept at room temperature for 1 - 8 min. and 750µl of 20% sodium carbonate solution was added. Mixture was kept at room temperature for 2 hrs and absorbance of the color developed was recorded at 765nm with the help of a UV-Visible spectrophotometer against blank. Total phenolic content was determined using Gallic acid standard curve ($R^2 = 0.986$) and expressed in mg/gm as Gallic Acid Equivalents.

DPPH Scavenging Activity: Different *in vitro* methods have been developed to measure the antioxidant activity of natural compounds. Among the *in vitro* assays, the ability of one compound to donate electrons to the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH \dot{y}) is one of the most widely used and reproducible assays. The DPPH method is described as a simple, rapid and convenient method independent of sample polarity for screening of samples for radical scavenging activity [16].

The DPPH scavenging activity was determined using the method described by Blois [17]. A 0.1mM solution of DPPH was prepared by using methanol. 2 ml of this solution was added to 2 ml of solution of each formulation (1% v/v) and the mixture was kept in dark for 20 min. After 20 min. absorbance of the color developed was recorded at 517nm with the help of a UV-Visible spectrophotometer against blank. Control was prepared by adding 2 ml of the DPPH solution to 2 ml methanol. Same procedure was repeated for estimation of DPPH scavenging activity in all the formulations.

Statistical Analysis: All measurements were performed at least in triplicate and values were averaged and reported along with the standard deviation. Values of $p < 0.05$ were considered as significantly different. The Statistical Package SPSS 12.0 for Windows was used to analyze the data.

RESULT AND DISCUSSION

Physicochemical Parameters: Results of physicochemical evaluation have been shown in Table 2.

Lack of standard quality control profile is one of the major impediments in the global acceptance of herbal drugs. Thus basic quality characteristics of the prepared formulation were evaluated through the determination of physicochemical properties. The results of physicochemical evaluation indicate that all the evaluated parameters of the prepared formulation are within the acceptable limits.

Total Phenol Content: Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. Phenolic compounds have been reported to have multiple biological effects, including antioxidant activity [18]. Since the prepared syrup was a poly herbal formulation, it was very difficult to identify biomarker (s) and quantify it. A number of articles have publicized that plants possess potent antioxidants which act as inhibitors of lipid peroxidation and scavengers of free radicals in the form of phenolic compounds, vitamins and flavonoides [19]. Hence, in order to characterize the formulation total phenol content was estimated. Total phenol content of the prepared syrup was estimated to be 11.41 ± 2.4 mg (GAE) per ml. Presence of such a high amount of total phenols may contribute to the antioxidant activity of the prepared formulation.

DPPH Scavenging Activity: Increased oxidative stress has been suggested to play a major role in liver damage due to cirrhosis of liver. Hence, when the normal levels of antioxidants are not enough for the eradication of free-radical-mediated injury, then administration of antioxidant compounds has a potential role to play in reducing liver damage [20]. All the three plants used in this study have been reported to exhibit antioxidant activity and also have hepatoprotective properties, hence, the formulation prepared by using extracts of the above three plants was evaluated for antioxidant activity

Table 2: Physicochemical evaluation

Parameter	Result
Color	Blackish-brown
Odor	Characteristic
Taste	Bitter
Viscosity	1.53 Cps (10% v/v)
Specific gravity	1.22 gm/ml
Refractive index	1.36
pH	4.8

and was compared with marketed preparations like Liv-52 and New LIVFIT which are being used as hepatoprotective.

DPPH scavenging based assay was used for estimation of antioxidant activity of the prepared syrup as well as marketed formulations. IC_{50} values were used to compare the antioxidant activity of all the above formulations. IC_{50} of the prepared syrup was found to be $87.55 \mu\text{g/ml}$. IC_{50} of Liv-52 and New LIVFIT were found $32.76 \mu\text{g/ml}$ and $300.03 \mu\text{g/ml}$ respectively. IC_{50} values of all these formulations indicate that antioxidant activity of the prepared formulation is more than New LIVFIT but less than that of Liv-52.

There are various reports which suggest that the antioxidant effect is mainly due to phenolic compounds, such as flavonoides, phenolic acids, tannins and phenolic diterpenes [21-24]. Phenolics have been considered powerful antioxidants *in vitro* and proved to be more potent antioxidants than Vitamin C and E and carotenoids [25-26]. Thus, it appears that the potent antioxidant activity expressed by the prepared formulation might be due to the presence of phenolic compounds.

All the three plants used in the present study have been reported to possess significant antioxidant activity. BM and CA, also, have the history of use in the cognitive disorders. It appears that antioxidant activity of BM and CA, at least in part, explains its role in facilitating cognitive functions [27, 28]. Therefore, the formulation prepared by using the extracts of these plants may be further investigated for its use in the cognitive disorders also.

CONCLUSION

On the basis of results obtained, it appears that the prepared formulation is having good antioxidant activity in comparison with the other marketed formulations and is likely to be a good formulation to be used in oxidative stress induced liver problems, however, its efficacy in living systems (*in vivo*) needs to be investigated.

RESULTS

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