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ORIGINAL ARTICLE

Marker Based Standardization of Polyherbal Formulation Bonjigar by Spectrophotometric Method

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ABSTRACT

Background: Bonjigar is an herbal oral preparation having multiple ingredients with the effect of liver protection including *Glycyrrhiza glabra*, *Eclipta elba*, *Tamrix gallica*, *Sliymum marianum*, *Sphaeranthus indicus*, *Boerhaavia diffuse* and *Cichorium intybus*. They have hepatoprotective, anti-inflammatory, antioxidant, antihyperlipidemic and antipyreticetc activities which could be used as a potential source of bioactive substances.

Objectives: The objective of the study is to standardize Bonjigar using flavones, tannins and sapogenin as chemical markers with the aid of spectroscopy.

Methodology: Quantitative determination of flavones, sapogenins and phenolic compounds was evaluated at 360nm, 430nm and 277nm. Sample was prepared for the quantitative determination of each marker and was determined with a spectrophotometer.

Result: Evidently found that Bonjigar individually consist of flavonoids, tannins and sapogenins. Total flavonoids as rutin, tannins as gallic acid and total sapogenins as diosgenin were found to be 0.564 mg/gm, 14.30 mg/gm and 2.839 mg/gm respectively.

INTRODUCTION

Bonjigar is an oral preparation manufactured by Herbion Naturals. It is a combination of *Eclipta alba, Cichorium intybus, Glycyrrhiza glabra, Boerhavia diffusa, Sphaeranthaus indicus, Tamarix gallica* and *Silybum marianum* (Table **1**).

The preparation is indicated in liver disorders. It is used to treat liver disorders, hepatitis whether chronic or acute , infectious hepatitis , hepatocellular jaundice, fatty degeneration, impaired function of liver as a consequence of industrial chemicals, systemic infections, and hepatotoxic medicines [1].

The liver protecting effect of Bonjigar is attributed by a number of herbal constituents present in it. Different analytical tests have been carried out on the individual components of Bonjigar. It has been cited by Christian M that kaempferol and quercetin have been identified, along with 13 unidentified free and bound flavonoids in quantitative and qualitative analysis of *Boerhavia diffusa* using TLC and HPLC [2]. The phytochemical, antioxidant and biochemical composition of *Cichorium intybus* roots, leaves, stem and seeds have revealed that leaves possess a higher content of water soluble proteins, total sugars, phenolic acids, flavonoids and anti-oxidants as compared to seeds that contains higher value of reducing sugars,

saponins and salt soluble proteins. The presence of saponins, terpenoids, anthocyanins, tannins, flavonoids and cardiac glycosides has also been confirmed its phytochemical screening [3].

It has been cited by Badr et al. that aliphatic acids, including tartaric acid, acetic acid and butyric acid were present in *Glycyrrhiza glabra* when HPLC analysis of the organic acids in licorice was carried out. The effect on liver enzymes, total cholesterol, triglycerides, red blood cells, white blood cells, serum minerals and body weight was also determined whereas, cytotoxic activity was also found in the methanolic extract [4]. Dharmender et al. reported the chemical constituents of *Eclipta alba* responsible for its pharmacological action [5].

A number of studies have also been carried out on *Sphaeranthaus indicus.* Chakraborty has reviewed the therapeutic activity of the whole plant as well as different parts of the drug which includes its leaves, seeds, flowers and concluded that therapeutic potential of the drug i.e. anti inflammatory, hepatoprotective, antioxidant, anti convulsion, antihyperlipidemic, anxiolytic, antipyretic etc activity is due to its active constituents and the plant could be used as a potential source of bioactive substance [6]. Another study cited that the drug possesses anti-microbial activity. In the study various extracts of the whole plant were prepared and the plant was found to have comparable anti microbial activity to Nystatin and Gentamicin [7]. Mishra et.al isolated a novel flavonoid *C*-glycoside, 5-hydroxy-7-methoxy-6-*C*-glycosylflavone from the aerial parts of the plant [8].

The genetic variation of *Silybum marianum* has been reported by Ottai and associates [9]. The phenolic and flavonoid content of *Tamarix Gallica* have been analyzed by Chaturvedy and et.al. anti-oxidant activity was also determined. The results showed that the total content of phenol was found to be 6.99498mg/100g, whereas the total flavonoid content was 47.61905mg/100g and IC50of 0.5mg/ml was obtained for the antioxidant activity [10]. Another study on *Tamarix gallica* reviewed its phytochemical constituents and anti-malarial, expectorant, antihaemorrhoid, anthelmintic, astringent, hepatoprotective, anti-hyperlipidemic, antioxidant, antidiarrhoeal, antinociceptive, antimicrobial, anticancer and liver protecting activity of the plant [11].

Standardization helps to ensure the quality of the constituents present in the formulation [12]. This study aims to standardize Bonjigar using flavones, tannins and sapogenin as chemical markers with the aid of spectroscopy.

Table 1. Unit composition of Bonjigar capsule.

S. No.	Ingredients	Quantity
1	Glycyrrhiza glabra - Mulethi	150 mg
2	Eclipta elba - Bhangra	350 mg
3	Tamrix gallica - Maeen kalan	200 mg
4	Sliymum marianum - Oant karara	250 mg
5	Sphaeranthaus indicus - Mundee	100 mg
6	Boerhaavia diffusa - Biskhapra	150 mg
7	Cichorium intybus - Kasni	100 mg
8	**Product Extract**	225 mg
9	Talcum powder	250 mg
10	Dicalcium Phosphate	110 mg
11	Citric Acid	20 mg
12	Methyl paraben	1.52 mg
13	Propyl paraben	0.3 mg
14	Potassium Sorbate	0.5 mg

METHODOLOGY

Reagents and Chemicals

All the reagents and chemicals used in the experiment were of analytical grade.

Instrumentation

UV/Vis spectrophotometer had been used for the experiment.

Quantitative Determination of Flavones as Rutin

Sample preparation

Weigh approximately 1 gram of the powdered extract in a 50 ml conical flask. Sonicate it for 15 minutes after adding 30ml of acetone. After sonication transfer it to a separating funnel and separate out the bottom layer into a conical flask and filter out the top acetone layer. The same procedure was repeated 3 times. The extract was then completely dried using water bath. The dried extract was transferred into a 50 ml conical flask and then dissolved in 40ml of methanol. The remaining volume was made up with the same solvent. The resultant solution was then analyzed for spectrophotometric determination at 360nm.

Quantitative Determination of Total Sapogenins

Preparation of solution A & B

Solution A is 0.5 ml *p*-anisaldehyde and 99.5 ml ethyl acetate, while solution B is 50 ml concentrated sulfuric acid and 50 ml ethyl acetate.

Sample preparation

Take 1 gm of powdered extract in 100 mL conical flask. Add 20 mL acetone and sonicate it for15 minutes. Filter the solution. Dry the filtrate under water bath. Add 5 mL ethyl acetate, 2.5 mL solution A and 2.5 mL solution B in the residue. Stir for about 10 minutes. Place it in water bath maintained at 60^{o}C for 10 minutes to develop color. Allow it to cool at 25^{o}C water bath. Pipette out 5 mL of this solution and transfer in 50 mL volumetric flask. Make up the volume by ethyl acetate. Filter the solution and use the filtrate as sample. Determine the optical density of the solution at 430 nm against blank preparation prepared by mixing 5 mL ethyl acetate, 2.5 mL solution A and 2.5 mL solution B.

Quantitative Determination of Total Phenolic Compounds

- **Sample preparation**

For the determination of total phenolic compounds as gallic acid two solutions are prepared:

Test solution 1: weigh 1 gram of powdered extract and transfer it to 50 ml retort and add 10ml of water. Connect the retort to a reverse refrigerator and heat it in boiling water, steam bath for 10 minutes. Cool it to the room temperature and filter through an ash less filter paper in a 10 ml volumetric flask and make up the volume with water.

Test solution 2: Transfer 1 ml of the test solution 1 in a 100 ml measuring flask and add buffer solution pH 9.0 to bring the volume to the mark. Measure the optical density of the solution using spectrophotometer at the wavelength 277 nm Use buffer solution with pH 9.0 as a comparison solution.

RESULTS AND DISCUSSION

The potency of a formulation depends on the quantity and level of the constituents. Standardization helps to ensure it. Bonjigar is a poly herbal formulation used for the management and treatment of liver ailments. The constituents in Bonjigar individually consist of flavonoids, tannins and sapogenins (Table **2**). In this experiment marker-based standardization of Bonjigar has been carried out using spectrophotometry. Sample was prepared for the quantitative determination of each marker and was determined with a spectrophotometer. Total flavonoids, sapogenins and gallic acid were the markers. Total flavonoids as rutin, tannins as gallic acid and total sapogenins as diosgenin were found to be 0.564 mg/gm, 14.30 mg/gm and 2.839 mg/gm respectively (Table **3**).

Table 2. Individual extraction of Bonigar capsule.

S. No.	Extract	Common Name	Total Flavones as Rutin	Tannins as Gallic Acid	Total Sapongenin as Disogenin
			Result: mg/gm		
1	Boerhaavia diffusa	biskhapra	0.58	16.72	6.308
2	Eclipta elba extract	bhangra	2.9	35.504	8.890
3	Tamrix gallica	maeen kalan	1.7	30.65	4.346
4	Glycyrrhiza glabra	mulethi	12.1	41.309	42.83
5	Silymbum marianum	oant karara	0.55	26.717	8.703
6	Cichorium intybus	Kansi	1.517	14.29	7.796
7	Sphaeranthaus indicus	mundee	6.678	22.25	22.64

Table 3. Result of Bonjigar extract B# Ex-C-015/17.

S. No.	Extract Name	Total Flavones as Rutin	Tannins as Gallic Acid	Total Sapongenin as Disogenin
		Result: mg/gm		
1	Bonjigar Extract	0.564	14.30	2.839

CONCLUSION

The experiment confirms the presence of rutins, gallic acid and diosgenin in the Bonjigar formulation. Hence, concluded that the formulation is efficacious is treating liver ailments.

REFERENCES

1. Naturals H. Bonjigar syrup. Available from: http://www.herbion.com/OnlineStore/ProductDetails.aspx?ProductID=3&ProductPriceID=76.
2. Christian M. Flavonoids-chemical constituents of Boerhaavia diffusa L. through TLC and HPLC. Int Res J Chem. 2013:27-36.
3. Shad MA, Nawaz H, Rehman T, Ikram N. Determination of some biochemicals, phytochemicals and antioxidant properties of different parts of Cichorium intybus L.: A comparative study. J Anim Plant Sci. 2013;23(4):1060-6.
4. Badr SE, Sakr DM, Mahfouz SA, Abdelfattah MS. Licorice (Glycyrrhiza glabra L.): chemical composition and biological impacts. Res J Pharm Biol Chem Sci. 2013; 4:606-21.
5. Jaglan D, Brar AS, Gill R. Pharmacological activity and chemical constituents of Eclipta alba. Global Journal of Medical Research. 2014.
6. Chakraborty AK, Behera M, Roy H, Rambhade S. Sphaeranthus indicus Linn- A Phytopharmacological review. International Journal of Drug Formulation & Research. 2010; 1(3);113-33.
7. Duraipandiyan V, Kannan P, Ignacimuthu S. Antimicrobial Activity of Sphaeranthus indicus L. Ethnobotanical Leaflets. 2009;2009(2):5.
8. Mishra BB, Yadav SB, Singh RK, Tripathi V. A novel flavonoid C-glycoside from Sphaeranthus indicus L. (family compositae). Molecules. 2007; 12(10):2288-91.
9. Ottai ME, Abdel-Moniem AS. Genetic parameter variations among milk thistle, Silybum marianum varieties and varietal sensitivity to infestation with seed-head weevil, Larinus latus Herbst.

International Journal of Agriculture and Biology. 2006; 6:862-6.
10. Drabu S, Chaturvedi S, Sharma M. Antioxidant Activity, Total Phenolic, And Flavonoid Content of Aerial Parts of Tamarix Gallica. Phytomedicine. 2012; 4(2):174-80.
11. Urfi MK, Mujahid Md, Badruddeen, Akhtar J, Khalid M, Khan MI. et al. Tamarix gallica: For traditional uses, phytochemical and pharmacological potentials. J Chem Pharm Res. 2016; 8(1):809-14.
12. Ladva BJ, Mahida VM, Kantaria UD, Gokani RH. Marker based standardization of polyherbal formulation (SJT-DI-02) by high performance thin layer chromatography method. J Pharm Bioallied Sci. 2014; 6(3):213.