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Research Article

PHYTOCHEMICAL STANDARDIZATION AND ANTIOXIDANT POTENTIAL OF AYURVEDA FORMULATION *DARVYADI RASKRIYA*

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ABSTRACT

The study represents phytochemical standardization and antioxidant potential of ayurveda formulation *Darvyadi Raskriya*. The study involves development of high-performance thin-layer chromatographic (HPTLC) method for analysis of formulation. The study utilizes analysis of glycyrrhizin in formulation which is the phytoconstituent of *Glycyrrhiza glabra* one of the component of formulation. The sample in ethanol was applied on aluminium TLC plates using Linomat 5 spray (CAMAG). Linear ascending development was performed in twin trough glass chamber saturated with mobile phase. The mobile phase consisted of ethyl acetate-methanol-formic acid (10:5:1 v/v/v). The spectrodensitometric detection was performed at the wavelength of 254 nm. The regression analysis was found to be linear with $r^2=0.997$ in the concentration range 5-25 ppm. The antioxidant activity of formulation was also found to be significant as compared to control.

Keywords: Standardization, *Glycyrrhiza glabra*, Glycyrrhizin, HPTLC, Antioxidant.

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INTRODUCTION

The standardization of drugs is very important aspect to ensure quality and integrity of a specific drug. The standardization also play vital role towards the quality concern of herbal drugs however the standardization of herbal drugs are difficult as compared to modern allopathic medicine due to the presence of large number of phytoconstituents. Recently many researchers emphasized need of herbal drug standardization for enhancing global acceptance of herbs and herbal

formulation. The traditional as well as modern instrumental methods were used extensively for the standardization of natural drug in last two decades¹⁻³. High Performance Thin Layer Chromatography (HPTLC) is one of the important instrumental methods used for this purpose. This method enables researchers to identify or quantify marker compounds in herbal formulation and play vital role in standardization of natural remedies. HPTLC is not only accurate and precise technique but it also having other advantages

such as; cost effectiveness and selectivity^{4, 5}. HPTLC applied for the qualitative and quantitative estimation of herbs, fermentation mixtures, drugs obtained from animal sources and ayurveda formulation⁶.

Darvyadi Raskriya is an ayurveda formulation which is prepared as juice or decoction till to become semisolid⁷. *Darvyadi Raskriya* offer many therapeutic properties and used in the treatment of dry eye and infected eyes. It consisted of *Berberis aristata*, *Trichosanthes dioica*, *Glycyrrhiza glabra*, *Nelumbo nucifera*, *Prunus puddum*, *Nelumbo nucifera* and *Azadirachta indica*^{8, 9}. *Glycyrrhiza glabra* is one of the chief components of formulation; considering this aspect present study involves standardization of *Darvyadi Raskriya* using analytical study of chemical constituent of *Glycyrrhiza glabra* in formulation.

MATERIALS

The *Darvyadi Raskriya* was ordered and purchase from local market. CAMAG TLC system comprising of a Linomat-5 applicator and CAMAG TLC III scanner was used with stationary phase silica gel G60F254. The plates were developed in a CAMAG twin trough glass chamber by ascending method. The solvents and other reagent were of high purity grade.

METHODS¹⁰

Preparation of standard solutions

The standard solution was prepared by dissolving 10 mg Glycyrrhizinate in 10 ml methanol. The aliquots of varying concentration were prepared by further dilution i.e; 5, 10, 15, 20 and 25 µg/ml.

Chromatographic Conditions

The chromatographic analysis was performed on 20 cm × 10 cm HPTLC silica gel G60F254 plates with fluorescent indicator. The sample and standard solutions were applied as bands using CAMAG Linomat automated spray equipped with a 100 µl syringe, other settings were as follow:

- Band length 6 mm
- Application rate 3 µl/sec
- Distance between bands 4 mm
- Distance from the plate side edge 7 mm
- Distance from the bottom of the plate 2 cm.

Calibration Curve

Different concentration of standard solution was applied onto TLC plate to obtain linear relationship using calibration curve. The chromatograms were developed in specified chromatographic conditions. The plate was air dried followed by drying in hot air oven at 105°C for 5 min. The spots were measure by linear scanning at 254 nm CAMAG TLC Scanner.

Analysis of glycyrrhizin in formulation

The *Darvyadi Raskriya* was taken in conical flask and extracted several times with ethanol then sonicated. The final extract of formulation was filtered through Whatman filter paper and filtrate was used for further analysis. The sample solution was run in identical

chromatographic conditions and spot of biomarker identified as mentioned in **figure 1**.

Method Validation

Repeatability: Repeatability was carried out using the three replicates of same spot 1000 ng/spot.

Intermediate precision: The intra-day and inter-day variations for determination of standard were carried out at different concentration levels.

Recovery Studies: Recovery study was performed by spiking samples with standard at three different concentration level. The recovery study was performed in triplicate; the study ensures recovery of drugs at different levels of formulations.

Antioxidant Activity (DPPH Radical Scavenging Assay)

The antioxidant property of plant was measured using DPPH radical scavenging method¹¹. The antioxidant activity was compared with ascorbic acid. The antioxidant activity of formulation was performed in varying concentration. 1.5 ml of 0.1 mM DPPH solution was mixed with 1.5 ml of various concentrations of formulation. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 on spectrophotometer. The solution without any sample along with DPPH and methanol was used as control. Inhibition of DPPH free radical in percentage was calculated by the following formula:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}] \times 100$$

Where; A_{control} is the absorbance of the control (L-Ascorbic acid)

A_{test} is the absorbance of reaction mixture sample (in the presence of sample).

RESULT AND DISCUSSION

Mobile Phase Development

The solvent system composed of ethyl acetate-methanol-formic acid (10:5:1 v/v/v) was used for estimation of glycyrrhizin. Separate chromatogram was observed with R_f value 0.35 ± 0.03 as mentioned in **figure 2 & 3**.

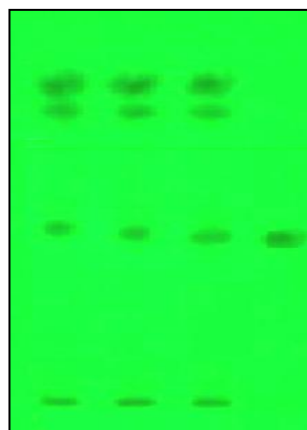


Figure 1: TLC profile of Test solutions and Standard solutions

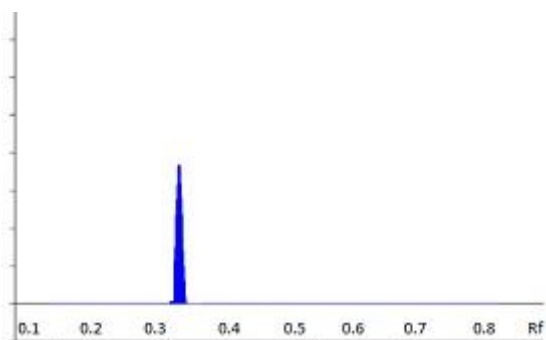


Figure 2: HPTLC chromatogram of standard.

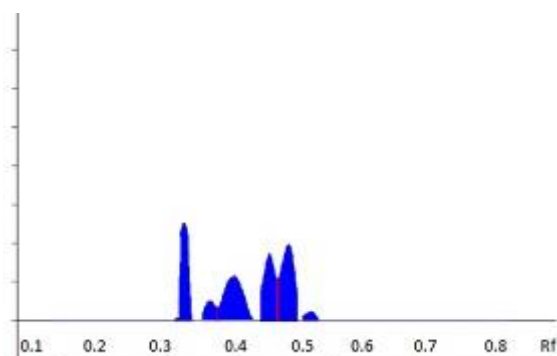


Figure 3: Chromatogram of sample.

Linearity

Linear regression data showed good linear relationship with in specified concentration range. The correlation coefficient r^2 was found to be $r^2=0.997$ as shown in figure 4.

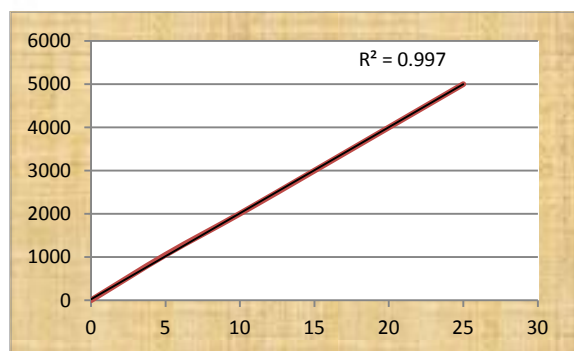


Figure 4: Calibration graph shows linear relationship between concentration and peak area.

Validation parameters

Repeatability showed very low % RSD of peak area of active component of formulation. Intermediate precision and recovery studies were also found to be within range as mentioned in table 1.

DPPH Radical Scavenging Assay

The selected ayurveda formulation was also evaluated for its antioxidant potential using DPPH radical scavenging activity. The result of study observed dose dependent response for ayurveda formulation as shown

in figure 5. Potent antioxidant activity was observed with high concentration of formulation extract. The antioxidant potential of formulation attributed to its phytoconstituents¹².

Table 1: The results of validation parameters

Repeatability study		
Sample applied ng	% RSD	
1000	0.055	
1000	0.058	
1000	0.053	
Intermediate precision		
Sample applied ng	Intraday % RSD	Interday % RSD
200	1.96	1.6
400	1.98	1.89
1000	1.95	1.92
Recovery studies		
% Amount added	% RSD	
20	0.169	
40	0.298	
60	0.275	

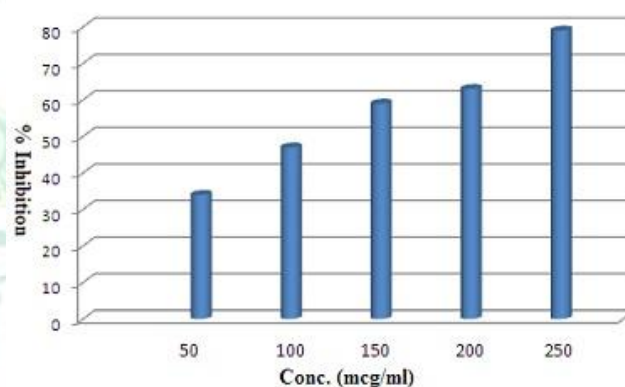


Figure 5: Antioxidant activity of sample

CONCLUSION

The present study involves phytochemical standardization and antioxidant potential of ayurveda formulation *Darvyadi Raskriya*. The standardization of formulation was performed using high-performance thin-layer chromatographic (HPTLC) method using glycyrrhizin (*Glycyrrhiza glabra*) as marker which is one of the important components of formulation. The linear relationship between concentration and area was observed with $r^2=0.997$ for regression analysis. The validation parameters for HPTLC method were also found within range and identical chromatogram was detected in formulation with $R_f 0.35 \pm 0.03$. The formulation also evaluated for antioxidant potential using DPPH assay. The antioxidant activity of formulation was also found to be significant as compared to control. The results of study may be used as trail for the further development of ayurveda formulation.

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