IJP (2025), Vol. 12, Issue 9 **(Research Article)**

Received on 12 September 2025; received in revised form, 28 September 2025; accepted, 29 September 2025; published 30 September 2025

DEVELOPMENT OF QUALITY PARAMETERS AND DETERMINATION OF GLYCYRRHETINIC ACID AND PIPERINE CONTENT IN YASTIMADHUVATI

Vipul Chaudhary, Mamta Shah and Karuna Modi *

Department of Pharmacognosy and Phytochemistry, L. M. College of Pharmacy, Ahmedabad - 380009, Gujarat, India.

Keywords:

Colorimetry, Glycyrrhetinic acid, HPTLC, Piperine, Yastimadhuvati

Correspondence to Author:
Dr. Karuna Modi

Department of Pharmacognosy,
L. M. College of Pharmacy,
Ahmedabad - 380009, Gujarat, India.

E-mail: karuna.modi@lmcp.ac.in

ABSTRACT: `Yastimadhuvati' is a popular Ayurvedic formulation prescribed in cough, cold, respiratory diseases and chronic fever. The proposed study is aimed at developing physico-chemical parameters and estimating the contents of glycyrrhetinic acid and piperine in the formulation. Pills were prepared according to the Vaidyaka chikitsasar. The pills were subjected to pharmacognostical and phytochemical screening and evaluated for the standards visually, weight variation, friability and hardness tests. Further glycyrrhetinic acid was quantified by colorimetric method. Also a simultaneous HPTLC method was developed for quantification of glycyrrhetinic acid and piperine. The pills showed microscopical features like sclereids, crystal fibres, vittae, epidermis with subepidermis and oil glands. The acid insoluble ash was found to be 0.5 ± 0.61, Water soluble extractive value was higher than the alcohol soluble extractive value. The pills contained higher amounts of phenolics. The content of glycyrrhetinic acid was found to be almost same (0.4% w/w) when estimated by colorimetric and HPTLC methods. Piperine content was found to be 3.5%w/w using the same HPTLC method. The quality parameters developed for the Yastimadhuvati would serve as useful tool for gauging the quality and also the simultaneous HPTLC method developed for the estimation of glycyrrhetinic acid and piperine is simple and reproducible.

INTRODUCTION: Medicines prepared in the form of pills or tablets are known as `Vati' or `Gutika' which contains one or more drugs obtained from plants, animals or mineral origin. Yastimadhuvati, a well known Ayurvedic formulation, is commonly used in cough and respiratory diseases [1]. The major ingredients of the vati (pills) include glycyrrhiza, black pepper, cardamom, beheda, fennel, black catechu, clove and peppermint.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.12(9).762-65

Article can be accessed online on: www.ijpjournal.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.IJP.12(9).762-65

A standard formulation was preparedin laboratory as mentioned in Vaidyakachikitsasar and evaluated for quality parameters and content of glycyrrhetinic acid by both colorimetric [2] and HPTLC methods and piperine content by HPTLC method.

MATERIALS AND METHODS:

Plant Materials and Preparation of Formulation: Crude drugs for formulation were collected from local market of Ahmedabad and were identified by morphological characters. The raw materials were powdered separately to 60 mesh size and Yastimadhuvati was prepared according to the formulation of Vaidyakachikitsasar **Table 1.** Pills of 5mm were prepared mixing the powdered material using distilledwater and dried at room temperature till they attained constant weight.

TABLE 1: YASTIMADHUVATI FORMULATION

Sr. no.	Common Name	Latin Name	Part used	Quantity (Tola)
1	Baheda	*Terminalia belerica* Linn.	Fruit	4
2	Variyali	*Foeniculum vulgare* Miller.	Fruit	4
3	Elaichi	*Elettaria cardamom* Maton.	Seed	4
4	Jethimadha	*Glycyrrhiza glabra* Linn.	Root or stolon	4
5	Jethimadha no shiro	*Glycyrrhiza glabra* Linn.	Aqueous extract	16
6	Kali mirch	*Piper nigrum* Linn.	Fruit	4
7	Laving	*Eugenia caryophyallus* Thunb.	Flower bud	4
8	Katha	*Acacia catechu* Wild.	Heart wood	4
9	Pippermint	Various species of *Mentha*	-	1

Evaluation of Parameters of Yastimadhuvati: Yastimadhuvati was subjected to evaluation for weight variation, friability, hardness and disintegrationtests [3].

Pharmacognostical and Phytochemical Analysis: The pills were powdered and studied for the microscopical characters, ash values, extractive values [4] and presence of various secondary metabolites [5-8]. The content of volatile oil [4], alkaloids [9], flavanoids [10], phenolics [10] and tannins [11] were also estimated.

Estimation of Glycyrrhetinic Acid by Colorimetric Method [2]: The content of glycyrrhetinic acid in the pills was estimated by the method of Srivastava *et al*. The powdered vati after suspending in dioxane was subjected to hydrolysis using H_2SO_4. The hydrosylate was neutralized and shaken with $CHCl_3$. The $CHCl_3$ extract after drying was treated with H_2SO_4 followed by alcoholic solution of vanillin. The mixture was kept in boiling water bath for five minutes and optical density was measured at 545 nm (Schimadzu UV 1700, Japan).

Estimation of Glycyrrhetinic Acid and Piperine by HPTLC: A simultaneous HPTLC method was developed to quantify glycyrrhetinic acid and piperine in yastimadhuvati. Reagents like 1,4-dioxane, toluene, ethylacetate, methanol and ammonia used were of chromatography grade. Reference standard of glycyrrhetinic acid and piperine were purchased from Sigma Aldrich, India. The HPTLC was done on precoated TLC plates of silica gel 60 F_{254} (Merck)as stationary phase and 1,4-Dioxane: toluene: ethyl acetate: methanol: ammonia (1.5:2:1: 1:0.3) as mobile phase using Camag Linomat V equipped with Camag TLC Scanner and Win CATS integration software(version 1.4.3.6336).

Calibration curves of reference standards were prepared using stock solution (1mg/ml) of piperine and glycyrrhetinic acid in methanol. A fixed volume of standard solution (1, 2, 3, 4, 5 µl) was spotted. Calibration curves of peak area *vs*. concentration of standard were plotted. The test solution was prepared extracting 10 g of yastimadhuvati powder with 50ml distilled water exhaustively. It was hydrolyzed with 10 ml 2N H_2SO_4, neutralized and extracted with 50 ml of chloroform twice. The residue after removing $CHCl_3$ was weighed and 5mg/ml solution was prepared and; 10 and 20µl of this was spotted. The plates were visualized and scanned at 254 nm. Derivatization was done using anisaldehyde sulphuric acid for glycyrrhetinic acid and dragendorff's reagent for piperine.

RESULT AND DISCUSSION: Yastimadhuvati was round shaped and dark brown to black coloured with aromatic odour and astringent taste. Powdered yastimadhuvati can be characterized by presence of crystal fibres, vittae, epidermis with subepidermis, group of stone cells, sclereides and oil glands **Fig. 1.**

Total ash, water-soluble ash and acid insoluble ash values were found to be 7.8 ± 0.76, 3.80 ± 0.27 and 0.5±0.61%w/w, respectively indicating very less extent of earthy matter in the formulation. Water soluble extractive was found to be appreciably higher than alcohol soluble extractive values.

The value of loss on drying and volatile oil content were 26.41±0.40 % w/w and 10.6±0.11% v/w respectively revealing significance for aromatic drugs used in formulation **Table 2.** Yastimadhuvati complied with IP limits for weight variation test, friability test, hardness and disintegration tests **Table 3.**

Phytochemical screening revealed the presence of saponins, alkaloids, flavanoids, sterol and triterpenoids, phenolics, tannins, and carbohydrates. Among the phytoconstituents estimated, total phenolic content was the highest **Table 4.**

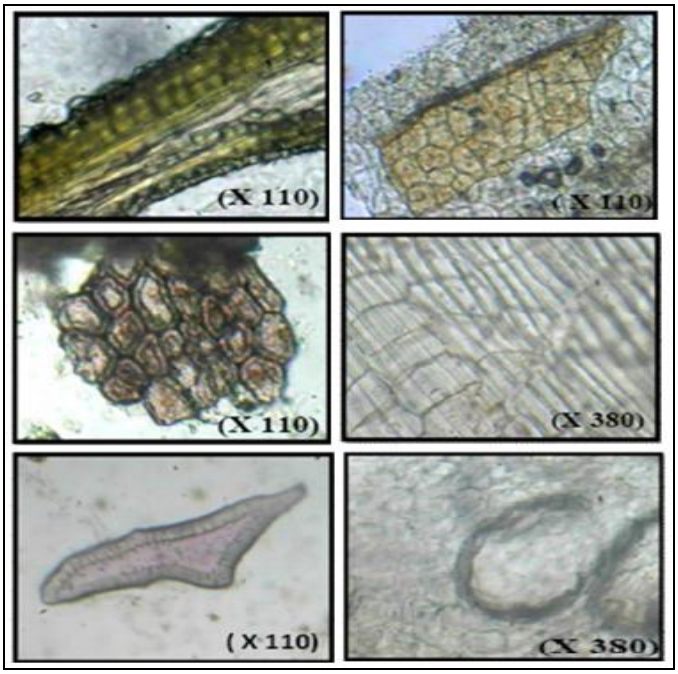

FIG. 1: MICROSCOPICAL CHARACTERS OF YASTIMADHUVATI

TABLE 2: PHYSICO-CHEMICAL PARAMETERS OF YASTIMADHUVATI

Quality parameter	Percentage
Water soluble extractive	35.3 ± 0.98 % w/w
Alcohol soluble extractive	8.6 ± 0.41 % w/w
Loss on drying	26.41 ± 0.40 % w/w
Volatile oil content	10.6 ± 0.11 % v/w

Standard deviation (±SD), Number of reading (n) = 3.

TABLE 3: STANDARD PARAMETERS OF YASTIMADHUVATI

Parameters	Value
Weight variation test	
No. of vat is showing deviation from average weight by more than 7.5%	15
No. of vat is showing average weight ± 7. 5%	5
Disintegration test (min)	34.5
Friability (%)	0.1
Hardness test (kg/cm^2)	6.8

TABLE 4: PHYSICO-CHEMICAL PARAMETERS OF YASTIMADHUVATI

Phytoconstituents	%w/w
Alkaloids	0.542 ± 0.00
Phenolics	14.1 ± 0.26
Flavanoids	1.324 ± 0.42
Tannins	1.23 ± 0.02

Standard deviation (±SD), Number of reading (n) = 3.

Glycyrrhetinic acid was estimated by colorimetric assay method 0.42±0.12% w/w in yastimadhuvati. Simultaneous HPTLC method for quantification of glycyrrhetinic and piperine was found to be sensitive, reproducible and suitable for routine analysis. A simple sample preparation method was generated, which was economic for the purpose of quality control. Co-chromatography with reference standards glycyrrhetinic acid and piperine revealed resolution of the same in yastimadhuvati, at R_f 0.31 and 0.82 **Fig. 2**.

The content of glycyrrhetinic acid and piperine indicated 0.40±0.005% w/w and 3.5±0.01% w/w respectively. Also glycyrrhetinic acid content was found to be almost similar when estimated by colorimetric method. The results of the present study would serve as a valuable gauge for evaluation of quality of yastimadhuvati.

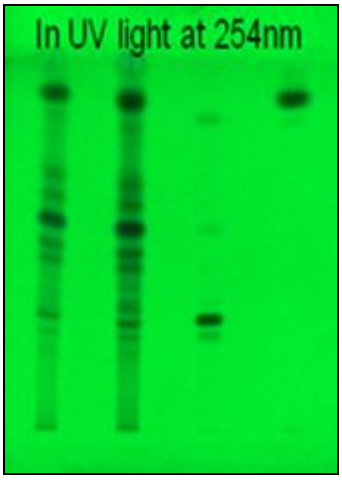

FIG. 2: TLC OF YASTIMADHUVATI

CONCLUSION: The present study successfully established quality control parameters for *Yastimadhuvati* and developed reliable methods for quantification of its key phytoconstituents, glycyrrhetinic acid and piperine. Pharmacognostical, phytochemical, and physicochemical evaluations confirmed the presence of characteristic features and revealed significant phenolic content, thereby validating the formulation's traditional use. The content of glycyrrhetinic acid determined by both colorimetric (0.42% w/w) and HPTLC (0.40% w/w) methods showed close agreement, while piperine was quantified as 3.5% w/w using HPTLC. The developed HPTLC method proved simple, sensitive, reproducible, and cost-effective, making

it suitable for routine quality assessment. These findings provide a scientific basis for standardization of *Yastimadhuvati*, ensuring its quality, safety, and efficacy. The study thus contributes to strengthening quality assurance practices in Ayurvedic formulations and may serve as a reference for future pharmacopoeial standards.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Thakkur GK: Vaidyakachikitsasar. Sindh Ayurvedic Pharmacy, First Edition 1936; 1.
2. Srivastava VK, Mukerjee SK and Maheshwari ML: Estimation of glycyrrhitinic acid in glycyrrhiza roots. Indian Drugs 1977; 14(4): 80-82.
3. Anonymous: Indian Pharmacopoeia. Ministry of Health and Family Welfare 2007; 1.
4. Anonymous: Ayurvedic Pharmacopoeia of India. Ministry of Health and Family welfare, First Edition 1999; 2.
5. Sim SK: Medicinal Plant Alkaloids. University of Toronto Press 1969.
6. Geissman A: Modern Methods of Plant Analysis. Springer Verlag 1955; 3: 471.
7. Evans WC and Evans D: Pharmacognosy. WB Saunders Company Ltd., Fifteenth Edition 2002.
8. Robinson T: The organic constituents of higher plants, their chemistry and interrelationships. Burgers Publishing Company 1964.
9. Sreevidya N and Mehrotra S: Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. Journal of AOAC International 2003; 86(6): 1124-1127.
10. Kalola J and Shah M: Free radical scavenging activity of *Inula cappa*. Ars Pharmaceutica 2006; 47(4): 387-388.
11. Anonymous: State Pharmacopoeia of the Union of Soviet Socialist Republics IX. Ministry of health of USSR, Ninth Edition 1961.

How to cite this article:
Chaudhary V, Shah M and Modi K: Development of quality parameters and determination of glycyrrhetinic acid and piperine content in yastimadhuvati. Int J Pharmacognosy 2025; 12(9): 762-65. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.12(9).762-65.