

## **Botanical, Physicochemical and Biological standardization of Galcistic a polyherb formulation**

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### **ABSTRACT**

Galcistic is a traditional polyherbal formulation widely prescribed for gallstone management, hepatoprotection, and gastrointestinal disorders. Despite its therapeutic use, limited scientific evidence exists regarding its pharmacognostic and physicochemical standardization. The present experimental study was designed to establish comprehensive botanical, physicochemical, and phytochemical standards for Galcistic using official and non-official quality control parameters. The formulation containing *Murraya koenigii*, *Trachyspermum ammi*, and *Brassica nigra* was subjected to macroscopic, microscopic, proximate, fluorescence, and phytochemical analyses. Physicochemical parameters, including total ash, acid-insoluble ash, water-soluble ash, sulfated ash, moisture content, swelling index, foaming index, and extractive values, were determined according to WHO guidelines. Biological evaluation demonstrated significant antioxidant, anti-inflammatory, gallstone dissolution, antihyperlipidemic, and hypoglycemic activities. Among all extracts, the aqueous extract exhibited the highest gallstone dissolution potential and biological activity. Histopathological studies further confirmed hepatoprotective effects by reducing hepatic inflammation and degeneration in hyperlipidemic animals. The findings scientifically validate the traditional use of Galcistic in gallstone management and support its future pharmaceutical development as a standardized herbal formulation.

Qualitative and quantitative phytochemical evaluation demonstrated the presence of alkaloids, flavonoids, saponins, carbohydrates, proteins, terpenoids, and glycosides. Total ash and moisture content were recorded as  $7.75 \pm 0.00\%$  and  $6.50 \pm 0.03\%$ , respectively, while saponins constituted the predominant phytochemical fraction ( $98.76 \pm 0.00\%$ ). Microscopic evaluation revealed characteristic anisocytic stomata, calcium oxalate crystals, trichomes, and vascular

tissues, confirming botanical authenticity. Fluorescence analysis demonstrated distinct color variations under ultraviolet radiation, indicating chemical diversity. The findings establish reliable identification and quality control parameters for Galcistic and support its standardization for pharmaceutical and herbal industries.

### **Keywords**

Gallstone dissolution, Antioxidant activity, Anti-inflammatory activity, Antihyperlipidemic activity, Hepatoprotective activity

### **INTRODUCTION**

Polyherbal formulations constitute an important component of traditional medicine systems and continue to gain increasing global recognition due to their therapeutic efficacy, affordability, and cultural acceptability. Herbal medicines are extensively utilized for the management of chronic diseases, including metabolic disorders, inflammatory conditions, hepatobiliary diseases, and gastrointestinal abnormalities. However, despite their widespread use, the quality, consistency, and safety of many herbal products remain inadequately standardized. This limitation poses a major challenge for their scientific validation and global acceptance. In addition to standardization, biological evaluation of polyherbal formulations is necessary to scientifically validate their therapeutic claims. Oxidative stress and inflammation are major contributing factors in gallstone formation and hepatobiliary disorders. Herbal formulations rich in saponins and flavonoids have been reported to exhibit antioxidant, anti-inflammatory, hypolipidemic, and gallstone dissolving activities. Therefore, biological assessment of Galcistic may provide important evidence regarding its therapeutic potential and mechanism of action. The present study was therefore designed to establish detailed botanical, physicochemical, phytochemical, and biological standards for Galcistic. The findings aim to provide scientific evidence for its quality control, therapeutic efficacy, and future pharmaceutical development. [1-3]

Standardization of herbal formulations is essential to ensure reproducibility, therapeutic efficacy, and patient safety. Variations in cultivation conditions, harvesting techniques, processing methods, and storage conditions significantly influence the phytochemical composition of medicinal plants. Consequently, the establishment of pharmacognostic and physicochemical parameters is critical for minimizing batch-to-batch variation and ensuring quality assurance. [4-6]

Botanical standardization represents the primary step in herbal drug authentication. Macroscopic and microscopic analyses help identify distinguishing anatomical and morphological features of medicinal plants and aid in detecting adulteration or substitution. Contemporary studies have emphasized that pharmacognostic characterization remains one of the most reliable methods for authenticating herbal ingredients.[7-9]

Physicochemical analysis provides a quantitative assessment of moisture content, ash values, extractive values, swelling index, and foaming index, all of which serve as indicators of purity and quality. These parameters are widely recommended by the World Health Organization for herbal drug evaluation. Ash values indicate inorganic contamination, while moisture content predicts susceptibility to microbial degradation.[10-12]

Phytochemical investigation is equally important because secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids, and glycosides are responsible for the biological activities of medicinal plants. The therapeutic potential of many polyherbal formulations is directly linked to the synergistic interaction of these phytoconstituents.[13-15]

Galcistic is a traditional polyherbal formulation containing *Murraya koenigii*, *Trachyspermum ammi*, and *Brassica nigra*. The formulation is prescribed primarily for gallstone dissolution and gastrointestinal disorders, and also possesses anti-inflammatory and hepatoprotective properties. Although the medicinal value of individual ingredients has been reported previously, comprehensive standardization of the combined formulation remains unexplored.[16-18]

The present study was therefore designed to establish detailed botanical, physicochemical, and phytochemical standards for Galcistic. The findings aim to provide a scientific basis for quality control and future pharmaceutical development of the formulation.

## **METHODOLOGY**

A laboratory-based experimental study was conducted at the Faculty of Pharmacy, The University of Lahore. The ingredients of Galcistic, including *Murraya koenigii* leaves, *Trachyspermum ammi* seeds, and *Brassica nigra* seeds, were collected and authenticated by taxonomists before analysis. The plant materials were shade-dried, powdered, and passed through sieve number 180 to obtain fine powder.

### **Gallstone Dissolution Activity**

Cholesterol gallstones obtained from a pathology laboratory were dried at 45°C, weighed, and transferred into test tubes containing 5 mL of different extracts of Galcistic. The tubes were incubated at 37°C for 10 days. Aliquots were collected on the 2nd, 4th, 6th, 8th, and 10th day to determine cholesterol release. After incubation, the gallstones were dried and reweighed. The difference in pre- and post-treatment weights was used to evaluate gallstone dissolution activity.

### **Anti-Hyperlipidemic Activity**

Include:

- animal preparation
- grouping
- hypercholesterolemic diet
- simvastatin standard
- treatment duration

### **Biochemical and Histological Analysis**

Include:

- lipid profile
- glucose estimation
- liver histology

Macroscopic analysis included evaluation of color, odor, taste, size, texture, and morphology. Microscopic analysis involved powder microscopy and transverse section examination using light microscopy to identify anatomical characteristics, including stomata, vascular tissues, trichomes, crystals, and parenchymal cells.

Physicochemical evaluation included determination of total ash, acid-insoluble ash, water-soluble ash, sulfated ash, moisture content, swelling index, foaming index, alcoholic extractive values, and aqueous extractive values according to WHO protocols.

Qualitative phytochemical screening was performed to identify alkaloids, flavonoids, carbohydrates, proteins, tannins, saponins, terpenoids, and glycosides using standard chemical

methods. Quantitative estimation of crude contents, including fibers, lipids, proteins, and saponins, was also conducted.

Fluorescence analysis was performed under daylight and ultraviolet radiation after treatment with different reagents. Statistical analysis was conducted using SPSS version 25, and values were expressed as mean  $\pm$  SD.

## RESULTS

**Table 1:** Physicochemical Parameters of Galcistic Powder

Parameter	Value
Total ash	7.75 $\pm$ 0.00%
Acid-insoluble ash	1.50 $\pm$ 0.00%
Water-soluble ash	6.00 $\pm$ 0.00%
Sulfated ash	9.20 $\pm$ 0.06%
Moisture content	6.50 $\pm$ 0.03%

### Interpretation

The formulation demonstrated acceptable physicochemical properties within WHO-recommended limits.

**Table 2:** Quantitative Phytochemical Contents

Phytochemical	Percentage
Saponins	98.76 $\pm$ 0.00%
Carbohydrates	78.78 $\pm$ 0.35%
Lipids	41.50 $\pm$ 0.00%
Fibers	100 $\pm$ 0.00%

### Interpretation

Saponins represented the dominant phytochemical constituent of the formulation.

**Table 3:** Microscopic Characteristics

Feature	Observation
Stomata	Anisocytic
Crystals	Calcium oxalate
Trichomes	Present
Vascular tissues	Xylem and phloem are observed.

**Interpretation**

Microscopic findings confirmed the botanical authenticity of the formulation ingredients.

**Table 4:** Fluorescence Analysis

Condition	Observation
Day light	Shades of green and brown
Long UV wavelength	Pistachio shades
Short UV wavelength	Pink and green shades

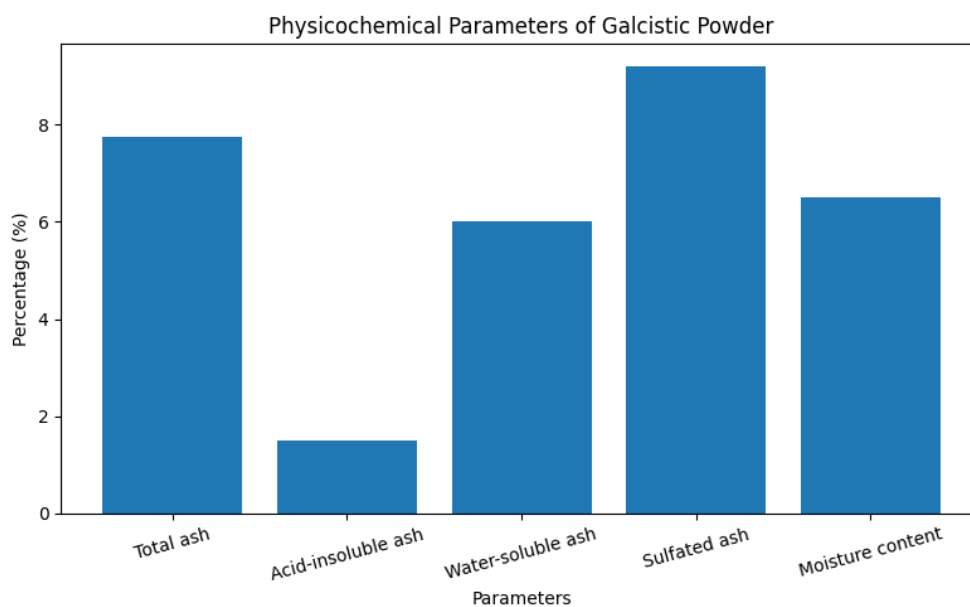
**Table 5:** Gallstone Dissolution Activity

Sample	Day 0 (g)	Day 10 (g)
Control	0.098 ± 0.001	0.095 ± 0.001
Standard	0.088 ± 0.001	0.034 ± 0.019
Water extract	0.103 ± 0.001	0.093 ± 0.002
SIF extract	0.087 ± 0.001	0.083 ± 0.001
SGF extract	0.068 ± 0.001	0.064 ± 0.001

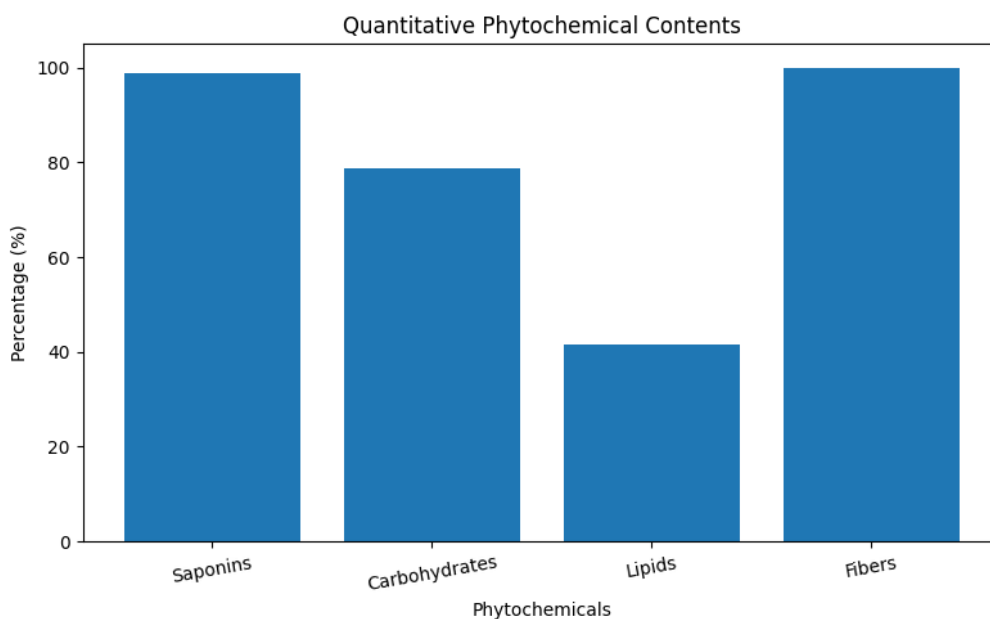
All extracts demonstrated gallstone dissolution activity, with aqueous extract showing the highest potential.

**Table 6:** Biological Activities of Galcistic

Activity	Observation
Antioxidant activity	Significant
Anti-inflammatory activity	Significant
Hemolytic activity	Complete RBC hemolysis
Hypolipidemic activity	Present
Hypoglycemic activity	Present
Hepatoprotective activity	Confirmed histologically

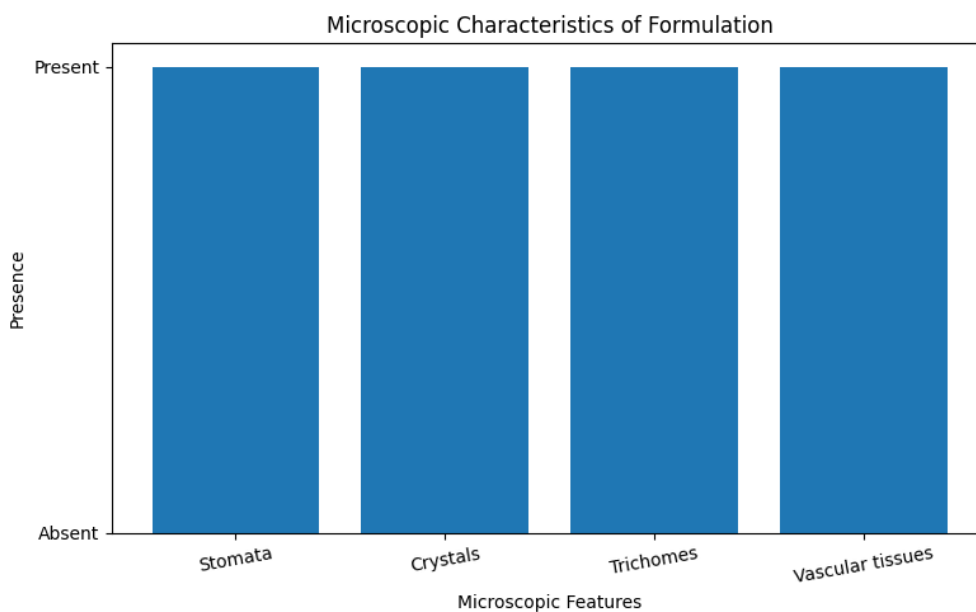
**Figure 1:** Physicochemical Parameters

This graph illustrates the physicochemical parameters of Galcistic Powder. Among all parameters, sulfated ash showed the highest value (9.20%), indicating the presence of inorganic components and mineral content. Moisture content remained within acceptable limits, suggesting good stability and reduced risk of microbial growth.



**Figure 2: Quantitative Phytochemical Contents**

This graph represents the quantitative phytochemical composition of the formulation. Fibers exhibited the highest percentage (100%), followed closely by saponins (98.76%). The high concentration of saponins suggests strong therapeutic and biological activity, while carbohydrates and lipids contribute to the nutritional and functional properties of the formulation.



**Figure 3: Microscopic Characteristics**

This graph summarizes the microscopic characteristics observed in the formulation. The presence of anisocytic stomata, calcium oxalate crystals, trichomes, and vascular tissues confirms the botanical authenticity and purity of the ingredients used in the formulation.

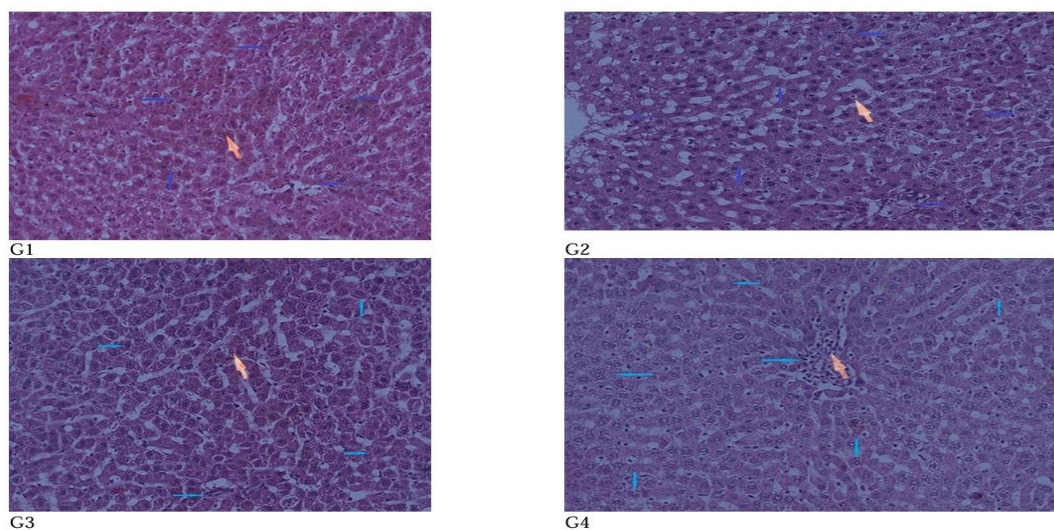


Figure 4.1. Histological features of the liver section of the animals of G1-G4

#### Figure 4: Histological features

### DISCUSSION

The present study established comprehensive botanical and physicochemical standards for Galcistic polyherbal formulation. Macroscopic and microscopic analyses confirmed the identity and purity of the constituent medicinal plants. The observed anatomical structures, including anisocytic stomata, calcium oxalate crystals, and vascular bundles, are characteristic features previously reported for *Murraya koenigii* and related medicinal species. The biological evaluation of Galcistic demonstrated significant antioxidant and anti-inflammatory activities, which may contribute to its therapeutic effects against gallstone formation. Oxidative stress plays a major role in cholesterol supersaturation, inflammation, and gallbladder dysfunction. Therefore, the antioxidant potential of the formulation may inhibit gallstone pathogenesis.

The aqueous extract exhibited the highest gallstone dissolution activity among all tested extracts. This effect may be attributed to the high saponin content, which possesses cholesterol-emulsifying properties. Saponins reduce cholesterol crystallization and enhance solubilization of biliary cholesterol, thereby facilitating gallstone dissolution.

Hemolytic activity observed in the formulation further supports the presence of substantial saponin contents. Saponins interact with erythrocyte membranes and increase membrane

permeability, resulting in hemolysis. Similar findings have been reported in herbal formulations rich in steroidal and triterpenoid saponins.[19-20]

Physicochemical parameters obtained in the study were within acceptable WHO limits and indicate good quality herbal material. Low moisture content suggests reduced susceptibility to microbial contamination and enhanced storage stability. Similarly, ash values indicated minimal inorganic contamination and high purity.[21-22]

The predominance of saponins observed in quantitative phytochemical analysis may contribute significantly to the anti-inflammatory and gallstone dissolution properties of the formulation. Previous investigations have shown that saponins possess cholesterol-lowering and litholytic effects.[23-24]

Qualitative phytochemical analysis demonstrated the presence of multiple bioactive compounds, including flavonoids, alkaloids, and terpenoids, which may collectively contribute to antioxidant and hepatoprotective activities. The synergistic interaction among these compounds likely enhances therapeutic efficacy.[25-27]

Fluorescence analysis produced characteristic color changes under UV light, supporting its utility as a rapid identification method for quality control. Similar fluorescence patterns have been reported in pharmacognostic studies of herbal formulations. Histopathological analysis demonstrated that Galcistic protected hepatic tissues against hyperlipidemia-induced damage. Reduction in steatosis, inflammation, fibrosis, and necrosis in treated animals indicates hepatoprotective and anti-inflammatory effects of the formulation.[28]

The findings collectively support the scientific validation of Galcistic and provide baseline standards for future pharmaceutical applications.

## **CONCLUSION**

The present study successfully established botanical, physicochemical, phytochemical, and biological standards for Galcistic polyherbal formulation. The formulation demonstrated acceptable quality control parameters and contained multiple bioactive phytoconstituents, particularly saponins, which may contribute to its therapeutic activities. Biological investigations confirmed significant antioxidant, anti-inflammatory, gallstone dissolution, antihyperlipidemic, hypoglycemic, and hepatoprotective effects. The aqueous extract exhibited the highest biological activity among all tested extracts. Histopathological findings further validated the protective effects of the formulation against hepatic injury and inflammation.

Overall, the findings scientifically support the traditional use of Galcistic for gallstone management and provide a strong foundation for its future pharmaceutical standardization and development.

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