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PHYTOCHEMICAL PROFILING AND QUANTIFICATION OF SECONDARY METABOLITES IN *SOYMIDA FEBRIFUGA* EXTRACT USING UV AND HPLC TECHNIQUES

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ABSTRACT

The present study was aimed at phytochemical profiling and quantification of secondary metabolites present in the hydroalcoholic extract of *Soymida febrifuga* using UV spectrophotometric and HPLC techniques. The extract was evaluated for physical characteristics, percentage yield, qualitative phytochemical screening, Thin Layer Chromatography (TLC), total phenolic content, total flavonoid content, and quantitative estimation of quercetin by HPLC analysis. The hydroalcoholic extract showed a black solid consistency with a percentage yield of 7.60%. Preliminary phytochemical screening revealed the presence of flavonoids, diterpenes, phenols, proteins, carbohydrates, and saponins, while alkaloids, glycosides, tannins, and sterols were absent. TLC analysis confirmed the presence of quercetin with an Rf value of 0.58 corresponding to the standard compound. Quantitative estimation showed total phenolic content and total flavonoid content of 0.574 mg/100 mg and 0.806 mg/100 mg respectively. HPLC analysis demonstrated the presence of quercetin in the extract with a retention time of 2.553 min, closely matching the standard quercetin peak at 2.556 min. The assay indicated 0.15% quercetin content in the extract. The findings suggest that *Soymida febrifuga* is a rich source of biologically active secondary metabolites and the developed analytical methods are suitable for phytochemical standardization and quality evaluation of the plant extract.

Keywords: *Soymida febrifuga*, Phytochemical profiling, Secondary metabolites, Quercetin, HPLC, UV spectroscopy, TLC, Flavonoids, Phenolic compounds, Hydroalcoholic extract.

INTRODUCTION

Medicinal plants have been widely utilized since ancient times as an important source of therapeutic agents for the prevention and treatment of various diseases. Plant-derived secondary metabolites such as alkaloids, flavonoids, tannins, phenolics, glycosides, saponins, and terpenoids possess diverse pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, anticancer, and antidiabetic properties. The growing interest in herbal medicine and natural products has increased the need for scientific validation,

phytochemical standardization, and quantitative analysis of bioactive constituents present in medicinal plants (Shakya *et al.*, 2016; Elshafie *et al.*, 2023).

Phytochemical profiling plays a significant role in the identification and characterization of biologically active compounds responsible for therapeutic efficacy. Qualitative and quantitative evaluation of secondary metabolites provides essential information regarding the chemical composition, purity, and standardization of herbal extracts (Riaz *et al.*, 2023).

Modern analytical techniques such as Ultraviolet-visible (UV-Visible) spectrophotometry and High Performance Liquid Chromatography (HPLC) are widely employed for the estimation and separation of phytoconstituents due to their sensitivity, accuracy, precision, and reproducibility (Mutha *et al.*, 2025). UV spectrophotometric methods are commonly used for preliminary quantitative estimation of phenolics, flavonoids, and other chromophoric compounds, whereas HPLC offers selective identification and quantification of individual phytochemicals in complex plant matrices (Harborne, 1998).

Soymida febrifuga, commonly known as Indian Redwood, belongs to the family Meliaceae and is distributed in various parts of India. Traditionally, different parts of the plant including bark, leaves, and stem have been used in indigenous systems of medicine for the treatment of fever, inflammation, diarrhea, dysentery, ulcers, and other disorders. The plant is reported to possess several pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and anticancer effects, which are mainly attributed to the presence of bioactive secondary metabolites (Sudhama *et al.*, 2024).

Previous phytochemical investigations on Soymida febrifuga revealed the presence of flavonoids, limonoids, tannins, phenolic compounds, steroids, alkaloids, and triterpenoids. However, comprehensive phytochemical profiling and quantitative estimation of secondary metabolites using advanced analytical techniques remain limited. Therefore, there is a need for systematic evaluation and standardization of the plant extract to establish its phytochemical composition and therapeutic potential.

The present study was undertaken to perform phytochemical profiling and quantification of secondary metabolites present in the extract of *Soymida febrifuga* using UV spectrophotometric and HPLC techniques. The study aims to identify major classes of phytoconstituents and quantify important bioactive compounds for future pharmacological and formulation studies.

MATERIALS AND METHODS

Materials

Leaves of *Soymida febrifuga* were collected, shade dried, and powdered for extraction. Hydroalcoholic solvent was used for the preparation of plant extract. Standard quercetin was used as a reference compound for TLC and HPLC studies. Analytical grade chemicals and reagents including methanol, ethanol, toluene, ethyl acetate, formic acid, ferric chloride, lead acetate, copper acetate, concentrated sulfuric acid, Fehling's reagent, Benedict's reagent, gelatin solution, Wagner's reagent, and Hager's reagent were used for phytochemical screening and chromatographic analysis.

UV spectrophotometer and HPLC instruments were employed for quantitative estimation of phenolic compounds, flavonoids, and quercetin content in the extract.

Methods

Extraction by maceration process

50 gram of dried leaves of *Soymida febrifuga* were coarsely powdered and subjected to extraction with petroleum ether by maceration (Handa *et al.,* 2008). The extraction was continued till the defatting of the material had taken place.

Defatted materials of *Soymida febrifuga* were extracted with hydroalcoholic solvent (Ethanol: Water; 70:30) using maceration process (48hrs). The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extract.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage (Arwande *et al.,* 2018). The percentage yields of each extract were calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powder taken}} \times 100$$

Qualitative evaluation

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (Chlorophyll, proteins, amino acids, etc.) and secondary bioactive constituents include (Alkaloids, phenols, flavonoids etc.). Phytochemical tests were done as per the methods given (Talukdar and Chaudhary, 2010).

Separation and Identification of phytoconstituents by TLC

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid (5:4:1) solvent system used. After pre-saturation with mobile phase for 20 min for development were used. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples. The developed thin layer chromatographic plates were visualized in normal light, short UV light (254nm), and long UV light (365nm) using TLC cabinet (Saxena *et al.,* 2005). The chromatogram was developed R_f Value of the spot was calculated using formula:

$$\mathbf{Rf} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Quantitative studies of bioactive constituents

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Javanmardi *et al.,* 2003).

10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Khan *et al.,* 2018).10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Identification of marker compound (Quercetin) by HPLC

The chromatographic separation was carried out using a reversed-phase HPLC system equipped with an octadecylsilane (C18) column having dimensions of 250 mm × 4.6 mm and a particle size of 5 µm. The mobile phase consisted of a mixture of acetonitrile and methanol in the ratio of 50:50 (v/v), which was delivered at a constant flow rate of 1.0 mL/min. The analysis was performed at room temperature. A sample volume of 20 µL was injected into the system, and detection was carried out using a UV detector set at a wavelength of 256 nm. Under these optimized chromatographic conditions, the analyte exhibited a retention time of 2.50 ± 0.5 minutes, indicating efficient and reproducible separation.

Preparation of standard solution

10mg of quercetin was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm. From stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Preparation of extract

10 mg extract was taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using 0.45µ membrane filter and then sonicated for 10 min.

RESULTS AND DISCUSSION

The present investigation was carried out to evaluate the phytochemical profile and quantify important secondary metabolites present in the hydroalcoholic extract of Soymida febrifuga using UV spectrophotometric and HPLC techniques. The extraction process yielded a black solid extract with a percentage yield of 7.60%, indicating satisfactory extraction efficiency of phytoconstituents from the plant material, as shown in Table 1. The physical appearance

and consistency of the extract suggested the presence of concentrated bioactive compounds.

Preliminary phytochemical screening revealed the presence of several important secondary metabolites in the hydroalcoholic extract (Table 2). The extract showed positive results for flavonoids, diterpenes, phenols, proteins, carbohydrates, and saponins, whereas alkaloids, glycosides, tannins, and sterols were absent. The presence of flavonoids and phenolic compounds indicates significant antioxidant potential of the plant extract. These phytoconstituents are known to contribute to various pharmacological activities including anti-inflammatory, antimicrobial, hepatoprotective, and antioxidant properties.

Thin layer chromatography (TLC) analysis confirmed the presence of quercetin in the hydroalcoholic extract of *Soymida febrifuga*. The chromatographic profile demonstrated that the extract exhibited spots with Rf values comparable to the standard quercetin under normal light, short UV, and long UV conditions (Table 3). The matching Rf value of 0.58 for both the standard and extract confirmed the occurrence of quercetin in the extract. Additionally, multiple spots observed in the extract indicated the presence of various phytoconstituents.

The quantitative estimation of total phenolic and flavonoid contents demonstrated appreciable concentrations of these secondary metabolites in the extract (Table 4). The total phenolic content was found to be 0.574 mg/100 mg, while the total flavonoid content was 0.806 mg/100 mg. The higher flavonoid content suggests that flavonoids may represent one of the major classes of phytochemicals present in the extract and may contribute significantly to its biological activity.

Further confirmation and quantification of quercetin were performed by HPLC analysis. The retention time of standard quercetin was observed at 2.556 min, whereas the hydroalcoholic extract showed a corresponding peak at 2.553 min (Table 5). The close similarity in retention times confirmed the presence of quercetin in the extract. Quantitative estimation revealed that the hydroalcoholic extract contained 0.15% quercetin, demonstrating the suitability of the developed HPLC method for identification and quantification of bioactive flavonoids in plant extracts.

The study confirmed that the hydroalcoholic extract of *Soymida febrifuga* contains valuable secondary metabolites, particularly flavonoids and phenolic compounds, which may be responsible for its therapeutic potential. The UV and HPLC analytical methods proved to be effective, reliable, and suitable for phytochemical profiling and standardization of the plant extract.

Table 1: Physical characteristics of leaves extract of *Soymida febrifuga*

Extract	Consistency	Colour	Weight of extract	% Yield
Hydroalcoholic	Solid	Black	3.80	7.60

Table 2: Qualitative chemical tests of extract of *Soymida febrifuga*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Hager's Test: Wagner's Test:	 -ve -ve
2.	**Glycosides** Conc. H_2SO_4 Test:	 -ve
3.	**Flavonoids** Lead acetate Test: Alkaline reagent test:	 +ve +ve
4.	**Diterpenes** Copper acetate Test:	 +ve
5.	**Phenol** Ferric Chloride Test:	 +ve
6.	**Proteins** Xanthoproteic Test:	 +ve
7.	**Carbohydrate** Fehling's Test: Benedict's Test:	 +ve +ve
8.	**Saponins** Froth Test:	 +ve
9.	**Tannins** Gelatin test:	 -ve
10.	**Sterols** Salkowski test	 -ve

+ ve – Present, - ve – Absent

Table 3: Identification of phytoconstituents (Quercetin) by TLC of *S. febrifuga*

Hydroalcoholic extract of *Soymida febrifuga*		
S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid (5:4:1)	***Rf* value**
1.	**(Quercetin)** Dis. travel by mobile phase= 6cm No. of spot at normal light= 1 No. of spot at short UV = 1 No. of spot at long UV= 1	 Normal- 0.58 Short- 0.58 Long- 0.58
2.	**(Hydroalcoholic extract)** Dis. travel by mobile phase= 6cm No. of spot at normal light= 2 No. of spot at short UV = 4 No. of spot at long UV= 6	 Normal- 0.58, 0.62 Short- 0.46, 0.58, 0.62, 0.70 Long- 0.46, 0.58, 0.62, 0.70, 0.76, 0.78
	Spot Sequence	
	Quercetin= 1st ; Hydroalcoholic extract = 2nd	

Table 4: Estimation of total phenol and flavonoids content of *Soymida febrifuga* extract

S. No.	Total phenol content	Total flavonoids content
1.	0.574 mg/100mg	0.806 mg/100mg

Table 5: Quantitative estimation of Quercetin in extract

S. No.	Standard/Extract	RT	% Assay
1.	Quercetin	2.556	-
2.	Hydroalcoholic extract	2.553	0.15%

CONCLUSION

The present study confirmed that the hydroalcoholic extract of Soymida febrifuga contains various bioactive secondary metabolites, particularly flavonoids and phenolic compounds. TLC and HPLC studies successfully identified and quantified quercetin in the extract. The results demonstrated that the developed UV and HPLC methods were simple, reliable, and suitable for phytochemical profiling and standardization of the plant extract. The presence of significant phytoconstituents suggests the potential therapeutic value of *Soymida febrifuga* for further pharmacological investigations.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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