EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 13
Issue: 5
Year: 2026

QUALITATIVE AND QUANTITATIVE STUDY OF SECONDARY METABOLITES OF *THEMEDA QUADRIVALVIS* USING UV AND HPLC

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DOI: https://doi.org/10.5281/zenodo.20132235

How to cite this Article: Ajay Gurjar*, B. K. Dubey, Deepak Kumar Basedia, Prabhat Kumar Jain, Anuj Kumar Asati, Sunil Shah. (2026). Qualitative and Quantitative Study of Secondary Metabolites of Themeda Quadrivalvis Using UV and Hplc. European Journal of Biomedical and Pharmaceutical Sciences, 13(5), 299–304.

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Article Received on 15/04/2026 **Article Revised on 05/05/2026** **Article Published on 10/05/2026**

ABSTRACT

The present study was undertaken to evaluate the qualitative and quantitative profile of secondary metabolites present in the hydroalcoholic extract of *Themeda quadrivalvis* using UV spectrophotometry and High-Performance Liquid Chromatography (HPLC). The extract was obtained as a dark brown solid with a percentage yield of 8.95%. Preliminary phytochemical screening revealed the presence of carbohydrates, glycosides, saponins, phenols, flavonoids, and proteins, while alkaloids and diterpenes were absent. Thin Layer Chromatography (TLC) analysis using Toluene: Ethyl acetate: Formic acid (5:4:1) confirmed the presence of quercetin with an Rf value of 0.60, comparable to the standard. Quantitative estimation by UV spectrophotometry showed total phenolic content of 0.865 mg/100 mg and total flavonoid content of 0.947 mg/100 mg of extract. HPLC analysis further confirmed the presence of quercetin with a retention time of 2.505 min in the extract, closely matching the standard quercetin (2.566 min), and the assay revealed 0.31% quercetin content. The findings demonstrate that *Themeda quadrivalvis* is a rich source of phenolic and flavonoid compounds and may serve as a potential candidate for further pharmacological investigations.

KEYWORDS: *Themeda quadrivalvis*, Secondary metabolites, Hydroalcoholic extract, Phytochemical screening, TLC, UV spectrophotometry, HPLC, Quercetin, Total phenolic content, Total flavonoid content.

INTRODUCTION

Secondary metabolites are biologically active organic compounds synthesized by plants that are not directly involved in primary metabolic processes such as growth and reproduction but play vital roles in plant defense, ecological adaptation, and environmental interactions (Bocso et al., 2022). These compounds include phenolics, flavonoids, alkaloids, tannins, terpenoids, saponins, and glycosides, many of which exhibit significant pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. The study of secondary metabolites has gained considerable importance in pharmaceutical and nutraceutical research due to their therapeutic potential and contribution to drug discovery (Kaushik et al., 2021).

Themeda quadrivalvis, a member of the Poaceae family, is a tropical grass species commonly found in subtropical and warm temperate regions. Although often regarded as a weed, several species within the genus *Themeda* have been reported to contain bioactive phytoconstituents with potential medicinal applications (Snyman et al., 2013).

However, comprehensive phytochemical profiling of *Themeda quadrivalvis* remains limited. A systematic qualitative and quantitative evaluation of its secondary metabolites is essential to understand its phytochemical composition and explore its potential biological activities.

Qualitative phytochemical screening provides preliminary identification of major classes of secondary metabolites present in plant extracts using standard chemical tests. Such screening helps determine the presence of compounds like flavonoids, phenolics, alkaloids, saponins, and tannins. Quantitative estimation further determines the concentration of these metabolites,

offering insight into the therapeutic relevance and comparative abundance of bioactive constituents in different solvent extracts (Maheshwaran et al., 2024).

Ultraviolet–Visible (UV–Vis) spectrophotometry is widely employed for the quantitative estimation of plant secondary metabolites due to its simplicity, rapidity, and cost-effectiveness. It is particularly useful for determining total phenolic and flavonoid content based on characteristic absorbance patterns of chromogenic reactions. High-Performance Liquid Chromatography (HPLC), on the other hand, is a highly sensitive and precise analytical technique used for the separation, identification, and quantification of individual phytoconstituents. HPLC enables detailed profiling of complex plant extracts, providing accurate quantification of specific secondary metabolites (Mutha et al., 2025).

Therefore, the present study aims to perform qualitative and quantitative analysis of secondary metabolites present in *Themeda quadrivalvis* using UV spectrophotometry and HPLC techniques. The study will contribute to the scientific understanding of its phytochemical composition and may provide a foundation for further pharmacological investigations and potential therapeutic applications.

MATERIAL AND METHODS

MATERIAL

The materials used in this investigation included the authenticated plant material of *Themeda quadrivalvis*, which was shade-dried and powdered for extraction. A hydroalcoholic solvent (ethanol and water) was used for extract preparation. Analytical reagents such as Wagner's reagent, Fehling's solution, ferric chloride, lead acetate, Legal's reagent, and aluminum chloride were employed for phytochemical screening and UV analysis. Silica gel TLC plates and a mobile phase of toluene: ethyl acetate: formic acid (5:4:1) were used for chromatographic studies, with standard quercetin as a reference compound. HPLC-grade methanol and water were used for quantitative HPLC analysis. All chemicals and reagents were of analytical or HPLC grade.

METHODS

Extraction procedure

Defatting of plant material

50 gram of dried aerial parts of *Themeda quadrivalvis* 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.

Extraction by maceration process

Defatted materials of *Themeda quadrivalvis* 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

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

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, sugar etc.) and secondary bioactive constituents include (Alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical tests were done as per the methods given (Talukdar and Chaudhary, 2010).

Separation and Identification of phytoconstituents by TLC

Thin layer chromatography is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase. 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 (Electronic India) (Saxena *et al.*, 2005).

1. Once the chromatogram was developed the R_f Value of the spot was calculated using the formula:

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

Quantitative studies of bioactive constituents

Estimation of total phenol content

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

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25µg/ml was prepared in methanol.

Preparation of Extract: 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.

Procedure: 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

Principle: Determination of total flavonoids content was based on aluminium chloride method (Khan *et al.*, 2018).

Preparation of Standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol.

Preparation of Extract: 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.

Procedure: 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

Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade water from Merck Ltd, ew Delhi, India. A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λ_{max}. The HPLC system (Waters) consisted of a pump, a U.V. Visible detector, a Thermo C_{18} (250 X 4.6 mm, 5µm) column, a Data Ace software.

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 mL min^{-1}. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm (Garg, 2021).

Table 1: Selection of Separation Variable.

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5 µm
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Acetonitrile	50
Methanol	50
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 µl
Detection wavelength	256 nm
Retention time	2.50± 0.5 min

Preparation of standard stock 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.

Preparation of working standard solution

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.

Analysis 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 focused on the qualitative and quantitative evaluation of secondary metabolites present in the hydroalcoholic extract of *Themeda quadrivalvis* using preliminary phytochemical screening, TLC, UV spectrophotometry, and HPLC analysis.

The physical characterization of the hydroalcoholic extract revealed a dark brown solid mass with a percentage yield of 8.95%. The moderate yield indicates efficient extraction of phytoconstituents using a hydroalcoholic solvent system, which is known to extract both polar and moderately non-polar compounds. The dark brown color further suggests the presence of phenolic and flavonoid compounds, which are commonly responsible for such coloration in plant extracts.

Qualitative phytochemical screening demonstrated the presence of carbohydrates, glycosides, saponins, phenols, flavonoids (positive in lead acetate test), and proteins,

whereas alkaloids and diterpenes were absent. The presence of phenols and flavonoids is particularly significant, as these compounds are widely associated with antioxidant and therapeutic activities. The positive ferric chloride test confirms phenolic constituents, while the alkaline reagent and lead acetate tests support the presence of flavonoids. The absence of alkaloids and diterpenes suggests that these classes may either be absent or present in negligible quantities in the hydroalcoholic extract.

Thin Layer Chromatography (TLC) analysis further supported the presence of flavonoid constituents. Using the mobile phase Toluene: Ethyl acetate: Formic acid (5:4:1), the standard quercetin exhibited an Rf value of 0.60 under long UV, short UV, and normal light. The hydroalcoholic extract showed a corresponding spot at Rf 0.60 along with an additional spot at Rf 0.72 under long UV. The matching Rf value between the standard quercetin and the extract indicates the probable presence of quercetin in *Themeda quadrivalvis*. The additional spot suggests the presence of other flavonoid or phenolic compounds in the extract.

Quantitative estimation revealed that the total phenolic content was 0.865 mg/100 mg and total flavonoid content was 0.947mg/100 mg. The slightly higher flavonoid content compared to total phenols indicates that flavonoids constitute a significant fraction of the phenolic compounds present in the extract. These findings correlate well with the positive qualitative tests and TLC results.

HPLC analysis provided further confirmation of quercetin in the extract. The standard quercetin showed a retention time (RT) of 2.566 minutes, while the hydroalcoholic extract exhibited a peak at 2.505 minutes, which is very close to the standard RT. This similarity in retention time confirms the presence of quercetin in the extract. The quantitative estimation indicated that the hydroalcoholic extract contains 0.31% quercetin. The slight difference in retention time may be attributed to matrix effects or minor variations in chromatographic conditions.

The results demonstrate that the hydroalcoholic extract of *Themeda quadrivalvis* contains significant secondary metabolites, particularly phenolic and flavonoid compounds, including quercetin. The combination of TLC and HPLC analysis provided reliable confirmation of phytoconstituents. The presence of these bioactive compounds suggests potential antioxidant and therapeutic properties, warranting further pharmacological evaluation of the plant extract.

Table 2: Physical characteristics of extract.

Extract	Consistency	Colour	% Yield
Hydroalcoholic	Solid	Dark Brown	8.95

Table 3: Qualitative chemical tests of extract of *Themeda quadrivalvis.*

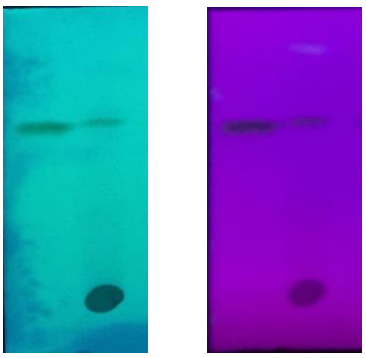
S. No.	Bioactive constituents	Hydroalcoholic extract
1.	**Alkaloids** Wagner's Test	-ve
2.	**Carbohydrates** Fehling's Test	+ve
3.	**Glycosides** Legal's Test	+ve
4.	**Saponins** Froth Test	+ve
5.	**Phenols** Ferric Chloride Test	+ve
6.	**Flavonoids** Alkaline Reagent Test Lead acetate Test	-ve +ve
7.	**Proteins** Xanthoproteic Test	+ve
8.	**Diterpenes** Copper acetate Test	-ve

+ ve – Present, - ve – Absent.

Table 4: Identification of phytoconstituents by TLC of *Themeda quadrivalvis.*

Hydroalcoholic extract of *Themeda quadrivalvis*		
S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid (5:4:1)	***Rf* value**
1.	**(Quercetin)**	Long- 0.60

	Dis. travel by mobile phase= 6cm No. of spot at long UV= 1 No. of spot at short UV = 1 No. of spot at normal light= 1	Short- 0.60 Normal- 0.60
2.	**(Hydroalcoholic extract)** Dis. travel by mobile phase= 6cm No. of spot at long UV = 2 No. of spot at short UV = 1 No. of spot at normal light= 1	Long- 0.60, 0.72 Short- 0.60 Normal- 0.60
	Spot Sequence	
	Quercetin	1st
	Hydroalcoholic extract	2nd



Short UV **Long UV**

Figure 1: TLC of *Themeda quadrivalvis* extract (Quercetin).

Table 5: Estimation of total phenol and flavonoids content.

S. No.	Total phenol content	Total flavonoids content
1.	0.865 mg/100mg	0.947 mg/100mg

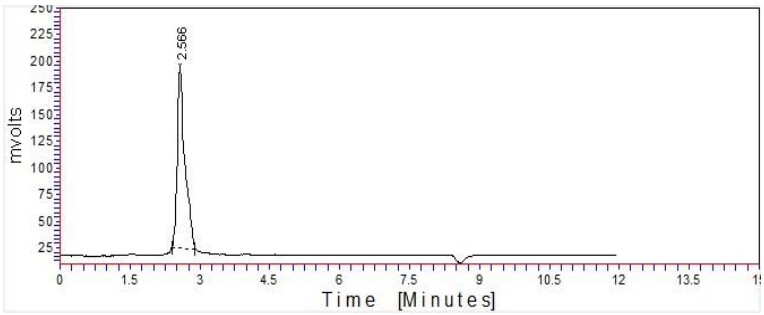


Figure 2: Chromatogram of standard Quercetin.

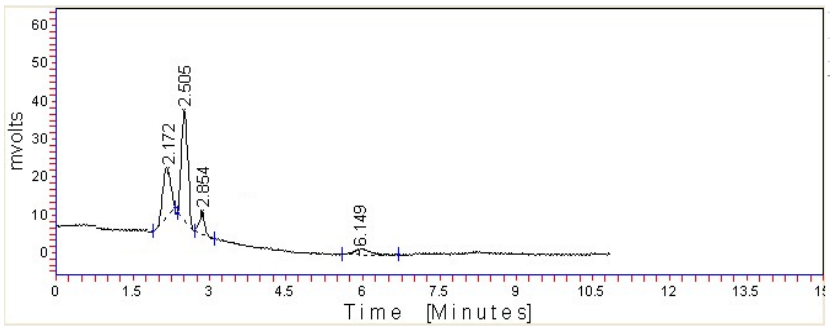


Figure 3: Chromatogram of hydroalcoholic extract of *Themeda quadrivalvis.*

Table 6: Quantitative estimation of Quercetin in extract.

S. No.	Standard/Extract	RT	% Assay
1.	Quercetin	2.566	-
2.	Hydroalcoholic extract	2.505	0.31%

CONCLUSION

The present study confirmed that the hydroalcoholic extract of *Themeda quadrivalvis* contains significant secondary metabolites, particularly phenolic and flavonoid compounds. Qualitative screening, TLC profiling, and HPLC analysis verified the presence of quercetin, with a quantified content of 0.31% in the extract. The appreciable total phenolic and flavonoid content further supports its potential antioxidant and therapeutic value. These findings provide a scientific basis for future pharmacological and bioactivity studies of *Themeda quadrivalvis*.

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