

Comparative Phytochemical Profiling and Total Phenolic Content of Aqueous and Phenolic-Rich Ethanolic Extracts of *Ocimum tenuiflorum*

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ABSTRACT

Ocimum tenuiflorum L. is a medicinally important plant widely recognised for its diverse phytochemical constituents and therapeutic potential. The extraction solvent plays an important role in determining the qualitative and quantitative composition of plant extracts. The present study aimed to comparatively evaluate the phytochemical profiles and total phenolic contents of aqueous and ethanolic extracts of *O. tenuiflorum* leaves. Fresh leaves were collected, authenticated, shade-dried and powdered before extraction. The aqueous extract was prepared by hot-water maceration, whereas the ethanolic extract was prepared using Soxhlet extraction with ethanol. Both extracts were subjected to preliminary phytochemical screening for alkaloids, terpenoids, flavonoids, tannins, steroids, anthraquinones, saponins, resins, glycosides and phenolic compounds. Total phenolic content was determined by the Folin-Ciocalteu method and expressed as mg gallic acid equivalent per gram of extract. Both extracts showed the presence of several classes of phytoconstituents, including alkaloids, terpenoids, tannins, steroids, saponins, resins and phenols. Flavonoids and glycosides were detected in the ethanolic extract but not in the aqueous extract. The ethanolic extract exhibited a higher total phenolic content of 52.27 mg GAE/g compared with 20.52 mg GAE/g for the aqueous extract. The findings demonstrate that extraction solvent influences the phytochemical profile and phenolic content of *O. tenuiflorum*, with ethanol providing a phenolic-rich extract under the conditions employed in the present study.

Keywords: *Ocimum tenuiflorum*; Tulsi; phytochemical screening; total phenolic content; aqueous extract; ethanolic extract

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1. INTRODUCTION

Medicinal plants represent an important source of biologically active compounds and have been extensively investigated for their potential applications in pharmaceutical and healthcare research. The therapeutic properties of medicinal plants are generally attributed to the presence of secondary metabolites such as phenolics, flavonoids, alkaloids, terpenoids, tannins, saponins and glycosides.[1]

Ocimum tenuiflorum L., belonging to the family Lamiaceae and commonly known as Tulsi, is an important medicinal plant traditionally used in the Indian system of medicine.[2] The plant contains diverse phytochemical constituents, including phenolic acids, flavonoids and other secondary metabolites. Literature reports have identified compounds such as rosmarinic acid, caffeic acid, luteolin, apigenin, kaempferol and related polyphenolic constituents in *O. tenuiflorum*. [3]

Phenolic compounds are an important group of plant secondary metabolites because of their structural diversity and antioxidant properties.[4] Previous investigations have demonstrated considerable phenolic and flavonoid contents in *O. tenuiflorum* leaves, although the reported values vary depending on the plant material, extraction solvent and analytical method used.[5]

The extraction procedure is an important factor affecting the recovery of phytoconstituents from plant material. Different solvents possess different polarities and therefore vary in their ability to extract specific classes of compounds. Comparative studies of *Ocimum* species have demonstrated that solvent selection can substantially influence the recovery of phenolics, flavonoids and other secondary metabolites.[6]

Therefore, the present study was undertaken to comparatively evaluate the qualitative phytochemical profiles and total phenolic contents of aqueous and ethanolic extracts of *O. tenuiflorum* leaves prepared using the extraction procedures described in the present study.

Objective

The objective of the study was to compare the phytochemical constituents and total phenolic content of aqueous and phenolic-rich ethanolic extracts of *O. tenuiflorum* leaves.

2. MATERIALS AND METHODS

2.1 Collection and authentication of plant material

Fresh, healthy and mature leaves of *Ocimum tenuiflorum* L. were collected from the surroundings and medicinal plant garden of Nitte College of Pharmaceutical Sciences, Bengaluru, Karnataka, India, during January 2026. The collected leaves were washed thoroughly with distilled water to remove adhering dirt and foreign matter. The leaves were shade-dried at room temperature and subsequently pulverised into coarse powder using a mechanical grinder. The powdered material was stored in an airtight container until further use.



Fig No: 01 Shade drying of *Ocimum tenuiflorum*

The plant material was authenticated by **Dr. P. Santhan, PhD (Bot), Taxonomist, NABET (QCI) Accredited Functional Area Expert–Ecology and Biodiversity**, and a voucher specimen was deposited for future reference.

2.2 Preparation of aqueous extract

Approximately **100 g** of dried powdered leaves were subjected to aqueous extraction using **1000 mL of distilled water**, maintaining a 1:10 w/v ratio.

The water was preheated to approximately 70–80°C and the powdered plant material was immersed in the hot solvent. The extraction vessel was covered with aluminium foil and maintained for 24 h with intermittent stirring. Following extraction, the mixture was filtered through muslin cloth and subsequently through Whatman No. 1 filter paper.

The resulting filtrate was concentrated on a water bath maintained at approximately 50–60°C to obtain the concentrated aqueous extract.[7]

2.3 Preparation of ethanolic extract

Approximately **100 g** of dried powdered leaves were packed into a cellulose extraction thimble and subjected to Soxhlet extraction using approximately **800–1000 mL of 95% ethanol**.

Extraction was carried out at approximately 78°C for 6–8 h. After completion of extraction, the extract was cooled, filtered through Whatman No. 1 filter paper and concentrated on a water bath maintained at approximately 40–50°C.

The concentrated extract was stored appropriately until further analysis.[8]



Soxhlet extraction of *Ocimum tenuiflorum*

2.4 Preliminary phytochemical screening

The aqueous and ethanolic extracts were subjected to qualitative phytochemical screening using standard chemical tests.

Phytoconstituent	Test Adopted	Procedure	Positive Observation
Alkaloids	Mayer's Test	To 2 mL of the extract, add a few drops of dilute HCl and filter. Add 2–3 drops of Mayer's reagent to the filtrate.	Cream or pale yellow precipitate indicates the presence of alkaloids.
Terpenoids	Salkowski Test	Mix 2 mL of extract with 2 mL chloroform. Carefully add 1–2 mL concentrated H ₂ SO ₄ along the side of the test tube.	Formation of a reddish-brown ring at the interface indicates terpenoids.
Flavonoids	Sodium Hydroxide Test	Add 2 mL of 10% NaOH solution to 2 mL of extract. Then add dilute HCl.	Yellow colour that disappears on adding acid indicates flavonoids.
Tannins	Lead Acetate Test	Add 2–3 drops of 10% lead acetate solution to 2 mL of extract.	Yellowish-white precipitate indicates tannins.
Steroids	Chloroform Test	Dissolve 2 mL extract in 2 mL chloroform and carefully add concentrated H ₂ SO ₄ .	Red upper layer with yellow-green fluorescence indicates steroids.
Anthraquinones	Free Anthraquinone Test	Shake 2 mL extract with 5 mL benzene, filter, and add an equal volume of 10% ammonia solution.	Pink or red colour in the ammoniacal layer indicates anthraquinones.
Saponins	Foam Test	Shake 2 mL extract vigorously with 5 mL distilled water for 2 minutes.	Stable froth persisting for 10–15 minutes indicates saponins.
Resins	Sodium Hydroxide Test	Add 2 mL sodium hydroxide solution to 2 mL extract and mix well.	Turbidity or precipitate after acidification indicates resins.
Glycosides	Keller–Killiani Test	Mix 2 mL extract with 2 mL glacial acetic acid containing one drop of 5% FeCl ₃ . Carefully add concentrated H ₂ SO ₄ along the side of the test tube.	Brown ring at the interface indicates cardiac glycosides.
Phenols	Ferric Chloride Test	Add 2–3 drops of 5% ferric chloride solution to 2 mL extract.	Blue, green, or violet colour indicates phenolic compounds.

The respective chemical tests were performed according to standard phytochemical procedures described in the study.[9]

2.5 FTIR Analysis

The Fourier-transform infrared (FTIR) analysis of the **aqueous and ethanolic extracts of *Ocimum tenuiflorum*** was performed using an **Alpha II FTIR spectrometer** in **attenuated total reflectance (ATR) mode**. The extract samples were placed directly on the ATR crystal, and spectra were recorded over **4000–600 cm⁻¹** at a spectral resolution of **4 cm⁻¹**. We collected 32 scans for each sample. The resulting spectra were analysed for characteristic absorption bands corresponding to the functional groups present in the extracts.[10]

2.6 Determination of total phenolic content

The total phenolic content (TPC) of the aqueous and ethanolic extracts was determined using the **Folin-Ciocalteu colourimetric method** with gallic acid as the reference standard.

A calibration curve was prepared using different concentrations of gallic acid. The absorbance was measured spectrophotometrically, and the calibration curve obtained in the study showed the equation:

$$y = 0.034x + 0.048$$

with an **R² value of 0.9985**.

The total phenolic content was expressed as:

mg gallic acid equivalent per gram of extract (mg GAE/g).[11]

2.7 Statistical analysis

The results of quantitative analysis were expressed as appropriate for the experimental determinations. The comparison of total phenolic content between the aqueous and ethanolic extracts was based on the measured TPC values.

3. RESULTS AND DISCUSSION

3.1 Preliminary phytochemical screening

Preliminary phytochemical screening revealed differences in the qualitative phytochemical profiles of the two extracts.

The aqueous extract showed the presence of **alkaloids, terpenoids, tannins, steroids, saponins, resins and phenolic compounds**. Flavonoids, glycosides and anthraquinones were not detected in the aqueous extract.

The ethanolic extract showed the presence of **alkaloids, terpenoids, flavonoids, tannins, steroids, saponins, resins, glycosides and phenolic compounds**, while anthraquinones were not detected.

Thus, the ethanolic extract demonstrated a broader qualitative phytochemical profile, particularly with respect to flavonoids and glycosides.

Table 1. Preliminary phytochemical screening of aqueous and ethanolic extracts of *O. tenuiflorum*

Phytoconstituent	Aqueous extract	Ethanolic extract
Alkaloids	+	+
Terpenoids	+	+
Flavonoids	–	+
Tannins	+	+
Steroids	+	+
Anthraquinones	–	–
Saponins	+	+
Resins	+	+
Glycosides	–	+
Phenolic compounds	+	+

+ = Present;

– = Absent

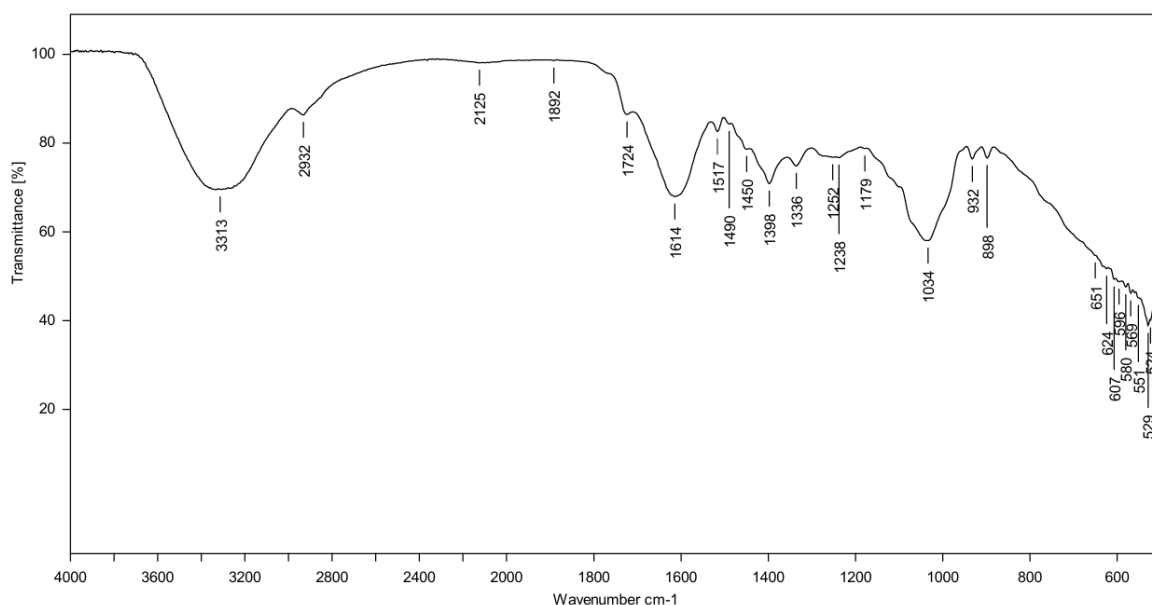
Discussion

The presence of multiple classes of phytoconstituents in both extracts demonstrates the chemical complexity of *O. tenuiflorum* leaves. The detection of flavonoids and glycosides in the ethanolic extract but not in the aqueous extract indicates that extraction conditions influenced the qualitative recovery of secondary metabolites. Such solvent-dependent variation has also been reported in published phytochemical investigations of *O. tenuiflorum*. For example, previous studies have demonstrated differences in the detection of tannins, flavonoids, glycosides, steroids, terpenoids and saponins among extracts prepared using different solvents.

3.2 FTIR Results

Aqueous Extract

The FT-IR spectrum of the **aqueous extract of *Ocimum tenuiflorum*** exhibited characteristic absorption bands at **3313 cm⁻¹**, corresponding predominantly to O–H stretching, indicating the presence of hydroxyl-containing constituents such as phenolic compounds, flavonoids and tannins. The absorption band at **2932 cm⁻¹** was attributed to aliphatic C–H stretching, suggesting the presence of aliphatic and terpenoid constituents. A distinct band at **1724 cm⁻¹** was associated with C=O stretching of carbonyl-containing compounds. The bands observed at **1614 and 1517 cm⁻¹** corresponded mainly to aromatic skeletal C=C vibrations, suggesting the presence of aromatic phenolic and flavonoid constituents. Bands at **1490, 1450 and 1398 cm⁻¹** were associated with aromatic and aliphatic C–H bending vibrations. The absorption bands at **1336, 1252 and 1238 cm⁻¹** were attributed mainly to C–O-related vibrations, while the bands at **1179 and 1034 cm⁻¹** were associated with C–O/C–O–C stretching, which may indicate oxygenated constituents including phenolic compounds and glycosidic structures. Overall, the FT-IR spectrum demonstrated the presence of functional groups characteristic of **phenolic, flavonoid, terpenoid and other oxygenated phytochemical constituents** in the aqueous extract of *O. tenuiflorum*.

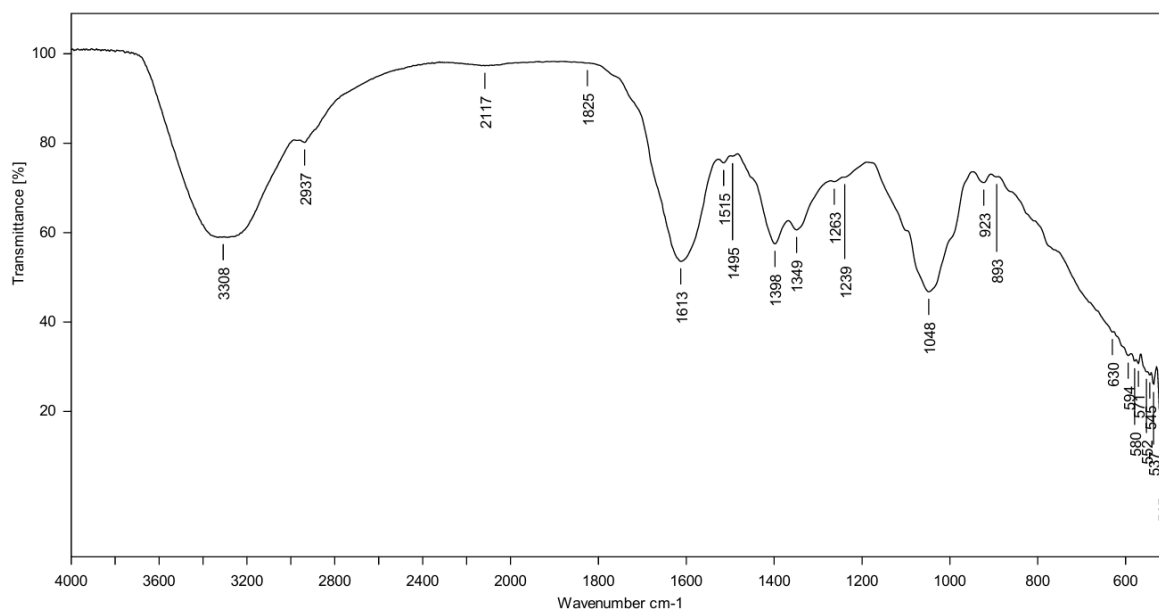


Aqueous extract

Ethanolic Extract

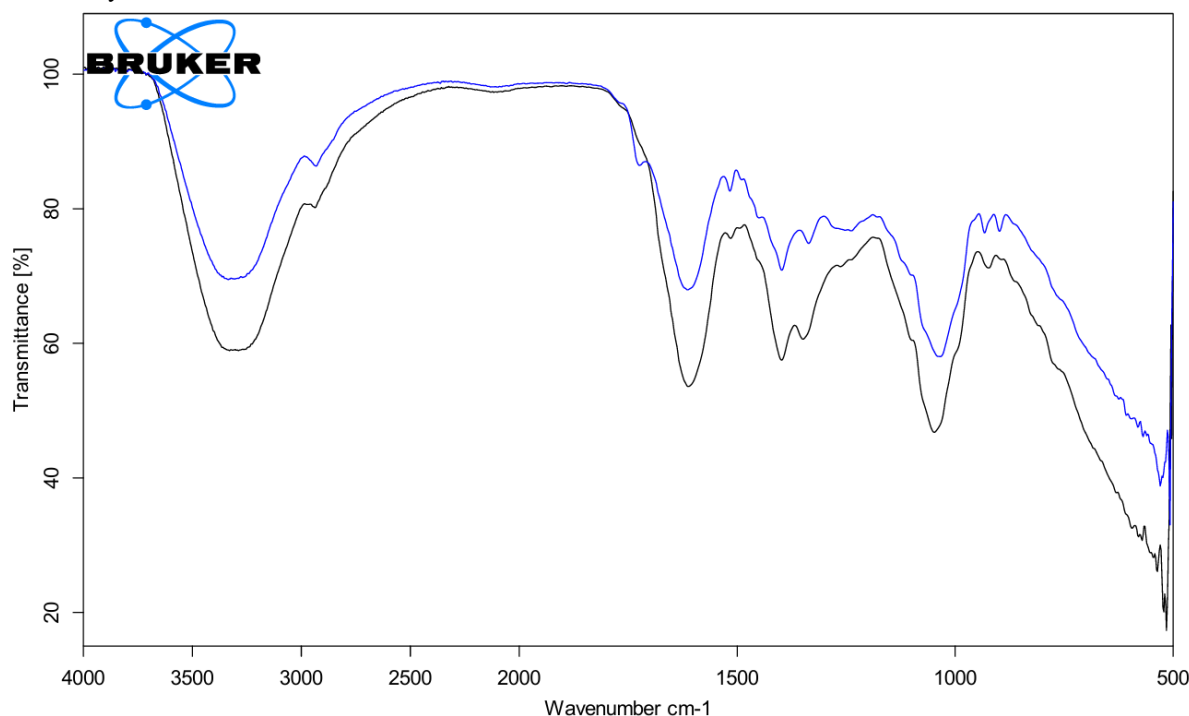
The FT-IR spectrum of the **ethanolic extract of *Ocimum tenuiflorum*** exhibited characteristic absorption bands at **3308, 2937, 1613, 1515, 1495, 1398, 1349, 1263, 1239 and 1048 cm⁻¹**. The broad absorption band at **3308 cm⁻¹** was attributed to **O–H stretching**, indicating hydroxyl-containing constituents such as phenolic compounds, flavonoids and tannins. The band at **2937 cm⁻¹** corresponded to **aliphatic C–H stretching**, suggesting the presence of aliphatic and terpenoid constituents. The absorption bands at **1613 and 1515 cm⁻¹** were associated with **aromatic C=C skeletal vibrations**, supporting the presence of aromatic phenolic and flavonoid constituents. Bands at **1495 and 1398 cm⁻¹** were related to aromatic and aliphatic C–H bending vibrations. The bands observed at **1349, 1263 and 1239 cm⁻¹** were mainly associated with **C–O-related vibrations**, while the band at **1048 cm⁻¹** corresponded predominantly to **C–O/C–O–C stretching**, which may be associated with oxygenated compounds and glycosidic structures. Overall, the FT-IR profile of the ethanolic extract demonstrated functional groups characteristic of **phenolic, flavonoid, terpenoid and other oxygenated phytoconstituents**.

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Ethanol extract

The FT-IR spectra of both extracts showed common bands around **3300, 2930, 1615, 1515, 1400, 1240 and 1040 cm^{-1}** , indicating similar functional groups associated with phenolic, flavonoid, terpenoid and other oxygenated constituents. The ethanolic extract showed slight differences in band positions and additional characteristic absorptions compared with the aqueous extract, suggesting differences in the relative phytochemical composition extracted by the two solvents.



3.3 Total phenolic content

The total phenolic content of the aqueous extract was **18.52 mg GAE/g**, whereas the ethanolic extract demonstrated a substantially higher value of **50.27 mg GAE/g**.

Table 2. Total phenolic content of *O. tenuiflorum* extracts

Extract	Total phenolic content (mg GAE/g)
Aqueous extract	18.52

Phenolic-rich ethanolic extract	50.27
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The ethanolic extract therefore contained approximately **2.55-fold higher total phenolic content** than the aqueous extract under the extraction and analytical conditions used in this study.

Discussion

The higher TPC observed in the ethanolic extract indicates that ethanol was more effective than the aqueous extraction procedure used in the present study for recovering phenolic constituents from *O. tenuiflorum* leaves.

This observation is consistent with the general finding that extraction solvent strongly influences the recovery of plant polyphenols. However, reported TPC values for *O. tenuiflorum* vary considerably between studies because of differences in geographical origin, plant part, drying conditions, solvent composition, extraction method, extraction duration and analytical methodology. Published studies have reported substantial phenolic contents in *O. tenuiflorum* extracts prepared using different solvents, supporting the importance of extraction conditions when comparing phytochemical results.

The higher phenolic content of the ethanolic extract also corresponds with the broader qualitative phytochemical profile observed in the present study. Nevertheless, TPC represents the combined response of phenolic substances to the Folin-Ciocalteu assay and should not be interpreted as identification or quantification of individual phenolic compounds. Confirmation of specific constituents would require chromatographic or spectrometric techniques such as HPLC, LC-MS or LC-MS/MS. Recent *O. tenuiflorum* research has used advanced analytical approaches to identify individual flavonoid and phenolic constituents.

3.3 Comparative phytochemical profile

The comparative findings demonstrate that both extraction procedures recovered several common phytochemical classes, whereas the ethanolic extraction additionally detected flavonoids and glycosides.

Table 3. Comparative summary of phytochemical findings

Parameter	Aqueous extract	Ethanolic extract
Number of detected phytochemical classes	7	9
Alkaloids	Present	Present
Terpenoids	Present	Present
Tannins	Present	Present
Steroids	Present	Present
Saponins	Present	Present
Resins	Present	Present
Phenolics	Present	Present
Flavonoids	Not detected	Detected
Glycosides	Not detected	Detected
Anthraquinones	Not detected	Not detected
TPC	20.52 mg GAE/g	52.27 mg GAE/g

The comparative results indicate that the ethanolic extraction procedure used in the present study yielded an extract with both a **broader qualitative phytochemical profile and higher total phenolic content**.

4. CONCLUSION

The present study demonstrated that extraction solvent influenced the phytochemical profile and total phenolic content of *Ocimum tenuiflorum* leaf extracts. Both aqueous and ethanolic extracts contained several important classes of phytoconstituents, including alkaloids, terpenoids, tannins, steroids, saponins, resins and phenolic compounds. Flavonoids and glycosides were detected specifically in the ethanolic extract.

The total phenolic content was **20.52 mg GAE/g** in the aqueous extract and **52.27 mg GAE/g** in the ethanolic extract, demonstrating a substantially higher phenolic content in the latter. Under the conditions employed in the present investigation, the ethanolic extraction procedure therefore produced a **phenolic-rich extract with a broader qualitative phytochemical profile**.

These findings provide a phytochemical basis for selecting the ethanolic extract for further pharmacological investigation. However, identification and quantification of individual phenolic constituents using advanced chromatographic techniques are required to establish the detailed chemical composition of the extract.

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