CHEMICAL FINGERPRINT AND PHYTOCHEMICAL STUDIES OF *OCIMUM BASILICUM* AND *OCIMUM TENUIFLORUM*

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ABSTRACT: The present study investigated the antidiabetic, antioxidant, and antibacterial properties, along with the chemical composition of *Ocimum basilicum* and *Ocimum tenuiflorum* leaves in different solvents. Antioxidant activity was evaluated using phenolic, flavonoid contents and DPPH free radical scavenging assays that revealed ethyl acetate as the most effective solvent with a DPPH scavenging rate of 60.07% and 77.84% respectively for both plants. Both basil species exhibited moderate antioxidant effects, with significant variations ($P < 0.05$) in flavonoid and phenolic contents. Antibacterial activity against *S. aureus* and *E. coli* was demonstrated using the agar well diffusion method, resulting in zones of 12 mm and 10 mm inhibition for *S. aureus*, and 14mm and 7mm for *E. coli* respectively for *O. basilicum* and *O. tenuiflorum*. Furthermore, aqueous extracts exhibited promising antidiabetic activity, with maximum percentages of 97.31% ± 0.03, 91.03% ± 0.01 glycation inhibitions and 51.13% ± 0.03, 55.13% ±0.03 alpha amylase inhibitions respectively. High-Performance Liquid Chromatography analysis identified quercetin and gallic acid as principal components, while Fourier Transformed Infrared Spectroscopy showed the presence of phenols, carboxylic acids, and amines groups. This comprehensive investigation underscores the potential pharmacological significance of these plants in the context of antioxidant, antibacterial and antidiabetic therapy.

Keywords: Antioxidant, Antibacterial, Antidiabetic, HPLC, FTIR.

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INTRODUCTION

Medicinal plants have been served as a main basis of healing practices in local communities throughout the world for centuries. Approximately 85% population depends on natural compounds because they are considered as a principal source of healthcare (Fitzgerald *et al*., 2019). This reliance highlights the profound significance of medicinal plants in treating the health issues. Furthermore, drug formulations rely on natural products, with an 80% effect originating from plant-derived compounds (Chaachouay & Zidane, 2024). Despite notable advancements in synthetic drug chemistry and antibiotic therapies, plants serve as a valuable source of bioactive compounds for combating diverse human ailments (Rasker, 2023).

Ocimum, a genus within the Lamiaceae family, consist of approximately 150 diverse species well-known for their medicinal properties, notably exemplified by *Ocimum basilicum* and *Ocimum tenuiflorum*. Widely cultivated across various regions, Ocimum species, herbaceous perennials, have garnered universal recognition for their therapeutic potential (Saini *et al*., 2022). These plants have a diverse range of metabolites that contribute to their pharmacological activity. These metabolites give the plant a wide range of powerful properties such as antibacterial, antioxidant and antidiabetic benefits (Dhama *et al*., 2023).

Among secondary metabolites, phenols emerge as a prominent class for their diverse biological functions (Oliveira *et al*., 2023). Because of their diverse range of pharmacological characteristics including their anti-inflammatory, analgesic, anti-tumor, antibacterial, antioxidant and immunostimulant actions, flavonoids a subset of phenolic compounds have gained a lot of attention (Nazir *et al*., 2020). Studies have demonstrated the therapeutic potential of *O. basilicum* and *O. tenuiflorum* leaves extracts in managing hyperglycemia, oxidative stress, lipid peroxidation and enzyme activities related to glucose metabolism. Notably, these extracts exhibit high levels of antioxidant, antibacterial and anti-diabetic activities (Almatroodi *et al*., 2020). The current study was planned to investigate the antioxidant, antibacterial and antidiabetic (antiglycation, enzyme inhibitory) properties of solvent fractions made from the leaves of these medicinal plants.

Experimental:

Collection and identification of plant material: The leaves of *O. tenuiflorum* and *O. basilicum* were collected randomly from a local market in Faisalabad, Pakistan. Subsequently, the leaves underwent thorough examination and certification at the Department of Botany, University of Agriculture, Faisalabad. This certification process ensured the authenticity and quality of the plant material utilized for the experimental analyses.

Preparation of Extract: The leaves were ground to form powdered sample and kept in sealed, transparent bags to preserve their integrity. The extraction procedure was then completed at room temperature. The maceration method was applied to samples of both *Ocimum* species using three different solvents: methanol, ethyl acetate and water. The plant material was mixed in the appropriate ratios with the solvents and shaken for six hours to facilitate the maceration process. The resulting combinations underwent evaporation in a water bath after being thoroughly filtered to remove any residual plant material during maceration. The concentrated extracts were lastly placed in low-temperature storage to maintain efficacy and stability (Safdar *et al*., 2017).

Total Phenolic Content (TPC): For TPC estimation, the Folin-Ciocalteu reagent assay was employed. In a 96-well plate, a mixture comprising Na2CO3 (200 µL), the test sample (250 µL) and a diluted Folin-Ciocalteu reagent (10%: 50 µL) was accurately prepared. The plate was then incubated for a period of two hours to facilitate the reaction. The wavelength used to test the absorbance was 710 nm. The findings were shown as mg GAE/100g (Ali *et al*., 2022).

Total Flavonoid Content (TFC): The AlCl3 colorimetric technique was employed for TFC assessment. In a 96-well plate, plant extract (38 µL each) was combined with 9.5 µL of NaNO2 and 156 µL of distilled water. After incubation for a period of 10 minutes, addition of 19 µL of 10% AlCl3 solution was done and again incubated (5 minutes). Finally, the absorbance was taken (510 nm wave length). The findings were displayed as mg CE/100g (Hussain *et al*., 2021).

DPPH Radical Scavenging Assay: The antioxidant activity was assessed via Hussain *et al*. (2021) methodology. To create a 250µL solution of DPPH, 0.004 mg of DPPH was added to 100 mL of methanol. 2.5 µL of plant extract and the prepared stock solution were put to an ELISA plate. For 35 minutes, the mixture was incubated. The absorbance was determined using a spectrophotometer set at characteristics absorbance of 517 nm. The prepared DPPH solution served as control. The calculations were made in triplicates (Hussain *et al*., 2021).

$$\% \text{ DPPH scavenging: } \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs Control}} \times 100$$

Antibacterial Assay: Using the agar well diffusion technique, the antibacterial properties of these both plants were assessed against the bacterial strains *Escherichia coli* and *Staphylococcus aureus.* Sample was prepared in Eppendorf tube by mixing 5mg ethyl acetate extract in 1mL DMSO solution. Agar solution was prepared by adding 2.66g agar in 70mL distilled water in two separate flasks. Agar solution and petri plates were autoclaved. Temperature of petri plates and agar solution was cool down to 40^{o} C and add 100µL bacterial strains, *E. coli* (gram negative) in one flask and *S. aureus* (gram positive) in another flask. Bacteria containing agar solution was then poured onto two petri plates in laminar air flow and was allowed to solidify. After that, three wells were created in gel by using tip (1mL) of micropipette and 100µL of both samples were poured onto the two wells in both plates and third well was filled with ciprofloxacin as positive control. The widths of the inhibitory zones were measured in mm (millimeters) after incubation of 24 hours at 37 °C (Ahmed *et al*., 2020).

Antidiabetic Evaluation

Antiglycation Potential: A 67 mM phosphate buffer having a pH 7.2 have been applied to create a solution having bovine serum albumin (BSA) and D-glucose with a concentration of 10mg. Subsequently, 1 mL of this prepared solution mixed with 150 µL of the plant sample in an Eppendorf tube, and the mixture was incubated for a period of 2 days. 200 µL of the incubated sample was transferred to ELISA plate, and each well received an equal addition of 200 µL of distilled water. A spectrophotometer has been used to compute the absorbance of the resultant solution at two distinct wavelengths 440 nm for emission and 370 nm for excitation (Ali *et al*., 2022).

Calculation of % inhibition:

$$\% \text{ inhibition} = \frac{\text{Absorbance at 440nm}}{\text{Absorbance at 370nm - Absorbance at 440nm}} \times 100$$

Alpha-Amylase Inhibition Assay: Combined 30 µL of the sample and 10 µL of a phosphate buffer solution containing 1 gramme of bacterial alpha-amylase, then incubate for 10 minutes in a 96-well plate. After that, an additional half-hour was spent incubating 40 µL of starch solution. After injecting a 1M HCl solution to stop the enzymatic reaction, iodine solution was added to see how much starch was still present. At 580 nm, absorbance was measured in comparison to a control. (Ali *et al*., 2022)

Absorbance at 440nm

$$\% \text{ inhibition} = \frac{\text{A (control)}}{\text{A (sample)}} \times 100$$

Where, A (control) represented the absorbance of control and A (sample) represented absorbance of test samples.

Structural Analysis

High-Performance Liquid Chromatography (HPLC): Hydrolyzed dried leaves of *O. basilicum* and *O. tenuiflorum* were used in this study. 0.5 g of dry mass of plant sample and 20 mL of ethanol with 1 g/L of BHT (butylated hydroxytoluene) were combined to create a mixture.10mL of 1 mol/L HCl were then added to the mixture. After thorough mixing, sonication was carried out for 15 minutes. After injecting sample, a 280-nm measurement was taken. Several compounds were identified using their retention period (Teofilović *et al*., 2021).

Fourier Transform Infrared Spectroscopy (FTIR): For FTIR analysis, Bruker Tensor 27 Fourier transform infrared (FTIR) spectrometer was used. With the help of Potassium bromide (KBr) plant leaves were grind into fine powder, which was subsequently compressed using compression dye at high pressure until a pellet formed and analyzed in the range of 400–4000 cm^{-1} (Kustiati *et al*., 2022).

Statistical analysis: All experiments were carried out in triplicate. Results of all activities were expressed as mean ± S.D. Significance of results was measured by t-test with Minitab statistical software version 17. One-way ANOVA was employed to analyze the data, enabling the simultaneous examination of multiple population means (Montgomery, 2017).

RESULTS AND DISCUSSION

Antioxidant Activity: Table 1 presents the antioxidant potential of *O. basilicum* and *O. tenuiflorum* extracts using different solvents. Significant variations were observed in the Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and DPPH free radical scavenging activity across the solvents used for extraction. In *O. basilicum*, the maximum TPC was observed in the aqueous extract (429.93 ± 16.77 mg GAE/100g), while the highest TFC was observed in the methanol extract (998.37 ± 0.40 mg CE/100g). Ethyl acetate exhibited the highest DPPH scavenging activity in both species, with *O. tenuiflorum* showing a higher percentage inhibition compared to *O. basilicum* across all solvents. These findings highlight the diverse antioxidant potential of the two *Ocimum* species, with solvent choice significantly influencing the extraction efficiency and resultant bioactivity. This result was in accordance with a previous study by Hussain *et al*. (2017), which showed solvent-dependent variations in Ocimum sanctum's metabolite content. The methanol extract had the greatest levels of total flavonoids (0.67 g/100 g dry plant material) and total phenolics (1.36 g/100 g dry plant material), with ethanol and *n*-hexane extracts following. According to a study by Mousavi *et al*. (2018), methanol extraction produced the highest number of polyphenolics from *O. tenuiflorum* leaves when compared to butanol and ethanol solvents. An additional investigation by Sharma *et al*. (2022) concerning the DPPH radical scavenging of different extracts revealed that the greatest scavenging activity was exhibited by ethanolic extracts of all Ocimum plants including, *O. tenuiflorum* and *O. basilicum*. These extracts were followed by methanol extracts (68–71%), acetone extracts (66–67%), and aqueous extracts (20–36%).

Table 1: Antioxidant potential.

Solvents	*O. basilicum*			*O. tenuiflorum*		
	TPC	**TFC**	**DPPH**	**TPC**	**TFC**	**DPPH**
Ethyl Acetate	248.72±14.32	82.93±0.66	60.07±1.21	170.05±27.96	67.23±0.52	77.84±1.48
Methanol	330.41±19.60	998.37±0.40	42.11±0.91	243.02±7.03	743.64±0.66	70.75±0.49
Aqueous	429.93±16.77	843.28±0.26	16.96±1.07	337.20±29.20	209.60±0.526	63.37±1.23

Data is presented as mean ± standard error for the triplicate measurements. TPC: total phenolic contents expressed as mg gallic acid equivalents (GAE) /100 g dry weight; TFC: total flavonoid contents expressed as mg catechin equivalents (CE)/100 g dry weight; percentage DPPH: 2, 2-diphenyl l-picrylhydrazyl.

Antidiabetic Evaluation: A comparative analysis of antidiabetic properties between *Ocimum basilicum* and *Ocimum tenuiflorum* extracts using various solvents is presented in Table 2. Notable differences were observed in antiglycation and alpha-amylase inhibition activities. In *Ocimum basilicum*, ethyl acetate showed the highest antiglycation activity (97.31%), while methanol exhibited the highest alpha-amylase inhibition (51.13%). Conversely, in *Ocimum tenuiflorum*, ethyl acetate demonstrated superior antiglycation activity (91.03%), with methanol displaying the highest alpha-amylase inhibition (55.13%). The three species' aqueous extracts had the lowest anti-diabetic effectiveness, with inhibition levels between 20 and 27%.Parasuraman *et al*. (2015) discovered that the methanolic extract of *O. tenuiflorum* has antidiabetic effects exceeding 50%. These results are

in line with other research by Parasuraman *et al*. (2015) that revealed among all three *Ocimum* species, the acetone extract showed the strongest antidiabetic efficacy, with α-amylase inhibition ranging from 61% to 69%, followed by methanol extracts with inhibition ranging from 59% to 60%, and ethanol extracts with inhibition ranging from 51% to 59%. Similarly, the hexane extract of *O. sanctum* exhibited 40–50% antidiabetic action, according to Suanarunsawat *et al*. (2016). This is in line with other research showing that medicinal plant extracts suppress α-amylase activity.

Table 2: Antidiabetic Evaluation.

Solvents	*O. basilicum*		*O. tenuiflorum*	
	Antiglycation activity	Alpha amylase inhibition	Antiglycation activity	Alpha amylase inhibition
Ethyl Acetate	97.31±0.03	29.02±0.03	91.03±0.01	26.02±0.02
Methanol	57.67±0.51	51.13±0.03	65.83±0.02	55.13±0.03
Aqueous	8.51±0.38	31.98±0.04	17.70±0.002	33.11±0.05
Control	76.04± 0.004	81.47±0.00	76.04± 0.004	81.47±0.00

Data is mean percentage ± standard error. Means with similar letter combinations in either a row or a column is not statistically significant (P>0.05).

Antimicrobial Assay" According to the presence or lack of inhibitory zones and zone width, *O. basilicum* extracts' potency was determined by their antimicrobial activities against the bacterial strains that were the subject of the current investigation, as shown in Table 3. In this study, the zone diameters against *E. coli* and *S. aureus* are 14 mm and 12 mm, respectively. Similarly, a study carried out by Rubab *et al*. (2021) reported that the methanolic extract of *Ocimum basilicum* stem exhibited significant antimicrobial activity against a range of gram-positive, gram-negative bacteria including *E. coli* and *S. aureus* strains. A different study by Srichok *et al*. (2022) found that *O. tenuiflorum's* ethanolic extract exhibited antibacterial activity against *S. aureus* and *S. agalactiae*, with minimum bactericidal concentrations (MBC) ranging from 15.6 to 500 µg/mL and minimum inhibitory concentrations (MIC) ranging from 3.9 to 31.2 µg/mL. This study too confirmed our results. The research on microbicidal activity of synthesized ZnO nanoparticles using *O. basilicum* at a concentration of 100 mg/mL was assessed against pathogenic strains by Irshad *et al*. (2020), showing zones of inhibition of 1.05 mm ± 0.137 against *Staphylococcus aureus*, 36.15 mm ± 0.304 against *Escherichia coli*, and 24.10 mm ± 0.05 against *Aspergillus niger*.

Table 3: Antibacterial potential.

Bacterial Strains	Plant Extract - Inhibition zone	Positive Control - Inhibition zone
Escherichia coli	*Ocimum basilicum* - 14 mm *Ocimum tenuiflorum* - 7 mm	39mm
Staphylococcus aureus	*Ocimum basilicum* - 12 mm *Ocimum tenuiflorum* - 10 mm	39mm

FTIR Analysis: Table 4 shows the FTIR analysis of both plants *O. basilicum* and *O. tenuiflorum*. Each plant had specific characteristics, such as various peak sizes, positions, and intensities, which suggested the existence of several functional groupings. FTIR analysis revealed distinct functional groups in *O. basilicum* and *O. tenuiflorum*. In *O. basilicum*, peaks at 3270.7 cm^-1 (O-H stretching), 2918.5 cm^-1 (C-H stretching), and 1604.6 cm^-1 (C=C, -H stretching) were observed, indicating the presence of alcohols, alkanes, and amines/amides, respectively (Fig 1, Fig 2).

O. tenuiflorum exhibited similar O-H stretching (3276.3 cm^-1) and C-H stretching (2849 cm^-1), along with peaks at 1735.1 cm^-1 (C=O stretching) and 1015.7 cm^-1 (C-O, C-N stretching), suggesting the presence of aldehydes/esters and various organic compounds. The FT-IR profile of tulsi extracts revealed a wide band between 3400 and 3200 cm-1 and this band was assigned to the OH- group, confirming that the extracts included phenolic chemicals as evidenced by Sharma *et al*. (2022). Another investigation by Osei Akoto *et al*. (2020) observed a band at 2849.5 cm-1 indicating the C-H group which confirms the presence of carboxylic acid and aldehyde in *O. tenuiflorum* and is in line with our findings. In a study by Oliveira *et al*. (2016) was found that peak positions within the range of 3300–3500 cm−1 were associated with hydroxyl group O-H stretching, while those within the range of 2800–3000 cm−1 were attributed to the alkanes group. Additionally, the peak observed at 1600 cm−1 was assigned to amines and amides stretching. FTIR spectra for both plants are given below:

Table 4: FTIR analysis of *Ocimum basilicum* and *Ocimum tenuiflorum*.

Ocimum basilicum			*Ocimum tenuiflorum*		
Absorption cm^{-1}	**Functional group**	**Compound**	**Absorption cm^{-1}**	**Functional group**	**Compound**
3270.7	O-H	Alcohols, phenols carboxylic acid	3276.3	O-H	Alcohols, phenols
2918.5	C-H	Alkanes	2849	C-H, O-H	Aldehyde, carboxylic acid
2849.5	C-H	Aldehyde, carboxylic acid	1735.1	C=O	Aldehyde, Ester
1604.6	C=C, -H	Amines and amides	1015.7	C-O, C-N	Alcohol, ethers, esters, carboxylic acid, anhydrides. Amines
1541.3	C=O	Phenyl	-	-	-
1375.4	C-H, S=O	CH3-bend Sulfones, sulfonyl	-	-	-
1317.6	S=O	Sulfones, sulfonyl, sulfates	-	-	-

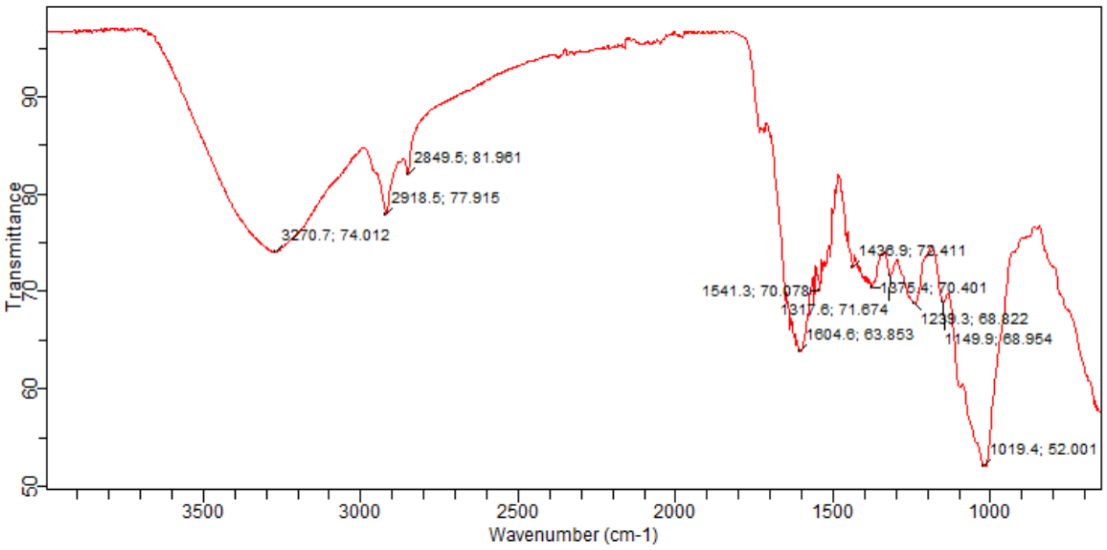

Fig 1 FTIR Spectra of *Ocimum basilicum*

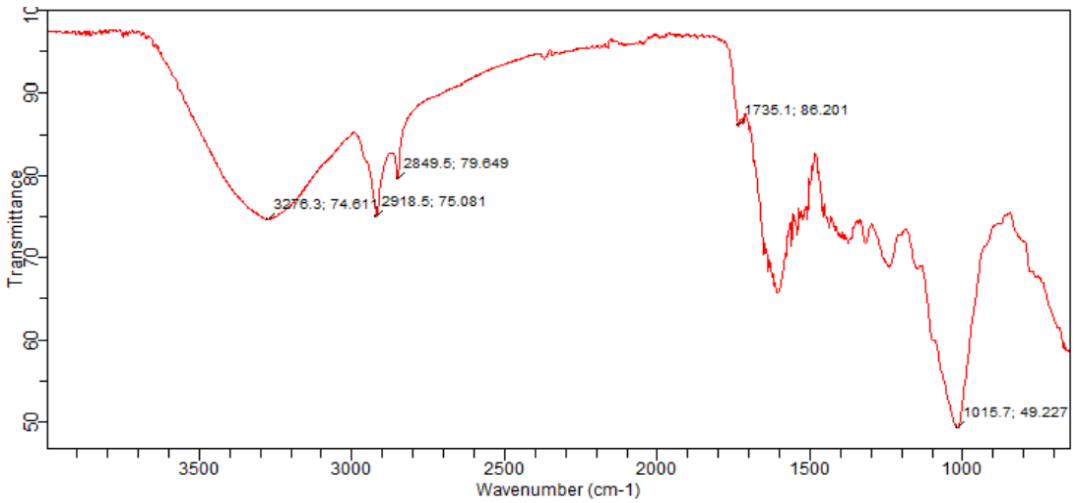

Fig 2 FTIR Spectra of *Ocimum tenuiflorum*

HPLC Analysis: In our investigation, a total of eight compounds were identified in *Ocimum basilicum* leaves using High Performance Liquid Chromatography (HPLC). The findings are explained in Table 5 and showed graphically (Fig 3, Fig 4). Quercetin was detected at a retention time of 2.700 minutes, with a concentration of 17.25 ppm. Gallic acid and Caffeic acid eluted at retention times of 4.280 minutes and 12.540 minutes, respectively, with concentrations of 28.0439 ppm and 12.9184 ppm. Similarly, vanillic acid and chlorogenic acid were identified at retention times of 13.573 minutes and 15.00 minutes, accompanied by peak areas and concentrations of 897.881 mV.s, 606.881 mV.s, and 55.6687 ppm, 47.3368 ppm, respectively.

Table 5 HPLC analysis of *Ocimum basilicum* and *Ocimum tenuiflorum*.

Ocimum basilicum				*Ocimum tenuiflorum*			
Compound name	**Retention time (min)**	**Peak area (mV.s)**	**Amount (ppm)**	**Compound name**	**Retention time (min)**	**Peak area (mV.s)**	**Amount (ppm)**
Quercetin	2.700	325.54	17.25	Quercetin	2.687	589.215	31.2284
Gallic Acid	4.280	778.996	28.0439	Gallic Acid	4.240	401.226	14.442
Caffeic Acid	12.540	280.833	12.9184	Caffeic Acid	12.633	378.598	17.4155
Vanillic Acid	13.573	897.881	55.6687	Vanillic Acid	13.347	141.631	8.7812
Chlorogenic Acid	15.000	606.881	47.3368	Benzoic Acid	14.507	417.423	44.2469
Syringic Acid	16.960	186.309	4.6578	Chlorogenic Acid	15.440	563.225	43.9316
P-Coumaric Acid	17.547	329.378	4.2820	P-Coumaric Acid	17.947	131.577	1.7105
Cinnamic Acid	25.140	800.317	28.0111	M-Coumaric Acid	20.100	469.126	5.6296

The retention times for syringic acid and P-Coumaric acid were determined as 16.960 minutes and 17.547 minutes, respectively, with peak areas and concentrations of 186.309 mV.s, 329.378 mV.s, and 4.6578 ppm, 4.2820 ppm. Cinnamic acid was discerned at a retention time of 25.140 minutes, with a concentration of 28.0111 ppm. Our findings of various compounds via HPLC are in line with the previous research. Vlase *et al*. (2014) stated that HPLC has been developed for the identification of phenolic compound present in *O. basilicum* and reported that quercetin was observed with RT value of 23.64min with an amount of 0.85ppm. Ullah *et al*. (2022) described that gallic acid and caffeic acid have RT values of 6.21min and 9.61min. In the current study, ten compounds were identified in *Ocimum tenuiflorum* by HPLC. Beltrán Noboa *et al*. (2022) stated that ferulic acid has RT value of 11.84min. HPLC spectra are given below:

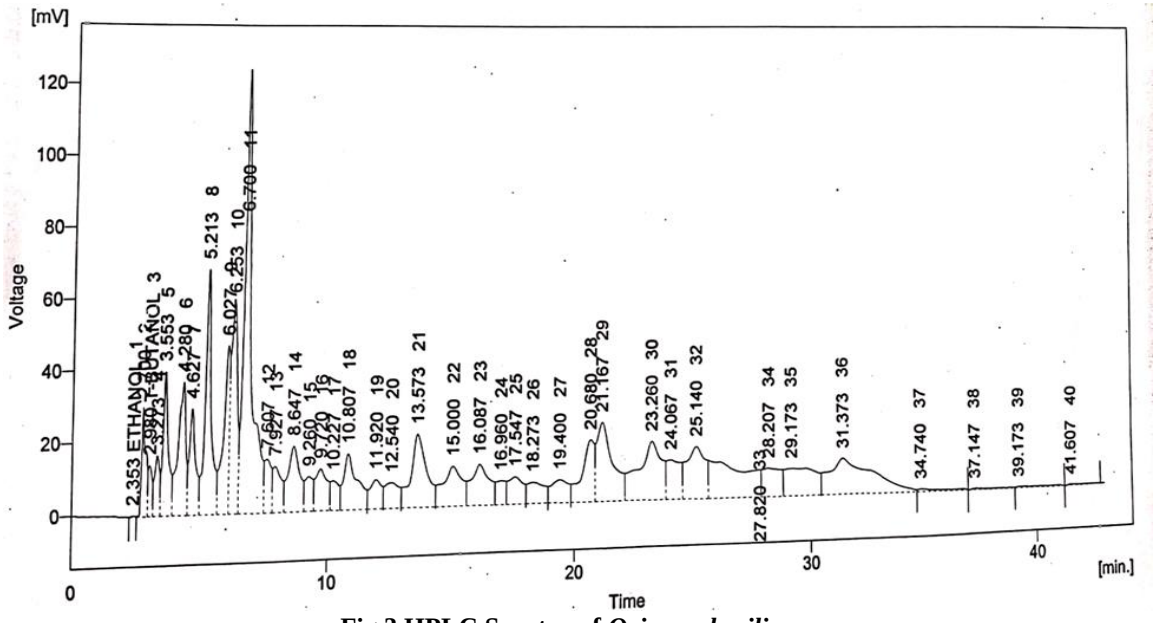

Fig 3 HPLC Spectra of *Ocimum basilicum*

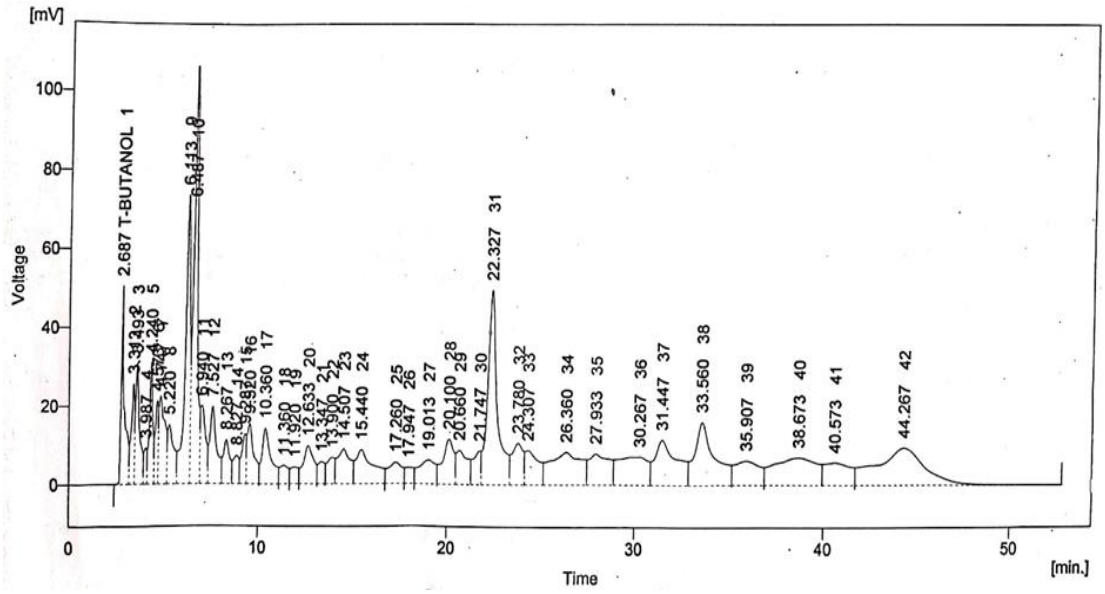

Fig 4 HPLC Spectra of *Ocimum tenuiflorum*

Conclusion: The present study investigated the antioxidant, antibacterial, and antidiabetic activities, as well as the chemical composition of *O. basilicum* and *O. tenuiflorum* extracts. Utilizing various solvents for extraction, the extracts were evaluated for their phenolic and flavonoid contents, DPPH free radical scavenging activity, inhibition of alpha-amylase, and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Notably, *O. basilicum* extract emerged as the most potent in terms of antioxidant and anti-diabetic properties. Significantly, the extracts exhibited pronounced efficacy against *Staphylococcus aureus*, suggesting their potential as natural antimicrobial agents. Moreover, the methanolic extracts of both plants demonstrated superior inhibition of alpha-amylase, indicating their potential in diabetes management. The investigation extensively employed FTIR and HPLC analyses to delineate the composition of *O. tenuiflorum* and *O. basilicum* extracts and found compounds of significant importance including quercetin gallic acid, caffeic acid, vanillic acid, etc. These findings highlight the necessity for further research to unravel the biochemical effects and interactions of these extracts. Such activities hold promise for the development of novel therapeutic interventions aimed at addressing various health challenges.

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