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**Research Paper**

# FTIR Spectral Analysis and Bio-functional Characterization of *Ficus carica* and *F. racemosa* Leaves for Therapeutic Applications

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## Abstract

*Ficus carica*, a common fig and *Ficus racemosa*, a cluster fig which are well-known plants of the genus *Ficus* (Moraceae family) that demand comparative therapeutic assessment. The study was designed to bio-fabricate a therapeutic application and Fourier transform infrared spectral analysis of both *Ficus* species. Microwave-assisted extraction method was used to prepare aqueous leaf extracts. Antioxidant profile was determined through total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical scavenging assays. Antibacterial activity was assessed using the agar well diffusion method. Alpha-amylase inhibition and hemolytic assays were used to determine antidiabetic and cytotoxic potentials, respectively, in aqueous leaf extracts of *F. carica* and *F. racemosa*. The *F. carica* and *F. racemosa* exhibited 113.74 ± 3.61 mg GAE/100g and 89.51 ± 5.65 mg GAE/100g TPC ($p > 0.05$), respectively. TFC in *F. carica* was 55.13 ± 4.04 mg CE/100g and 45.89 ± 1.29 mg GAE/100g in *F. racemosa* ($p > 0.05$). The percentage of DPPH radical scavenging activity in *F. carica* was 43.61 ±2.01% while in *F. racemosa*, it was 53.41 ± 2.23% ($p > 0.05$). Both samples exhibited mild antimicrobial activities (8 to 20 mm growth inhibition zones). Moderate alpha-amylase inhibition was observed (*F. carica*: 25.54 ± 0.88% and *F. racemosa*: 21.29 ± 15.81%), while hemolytic activity was higher in *F. racemosa* (13.2 ± 1.22%) than in *F. carica* (12.47 ± 2.79 %). FTIR analysis confirmed the presence of alcohols, carboxylic acids, aldehydes, phenols, alkenes, alkynes, esters, ethers, fluoride, amines and aromatics. It is concluded that both medicinal plants exhibited antioxidant, antimicrobial and antidiabetic activities. The *F. racemosa* and *F. carica* extracts exhibited low hemolytic activity, indicating good biocompatibility, with FRA being marginally more hemolytic than *F. carica* but also having lower cytotoxic effects. These findings support their future application in pharmacological formulations. Future research involving different extraction techniques and animal trials could improve therapeutic understanding and clinical applications.

## Introduction

A wide variety of plants found throughout the world have been used since ancient times for therapeutic purposes (Chaachouay & Zidane, 2024). Plants from certain taxonomic groups, also known as medicinal plants, produce compounds called secondary metabolites through various metabolic pathways in response to environmental stress or pathogens (Castelli and Lopez, 2022). Due to their effectiveness against a range of inflammatory and pathogenic diseases, these bioactive compounds are essential in both traditional remedies and modern pharmacotherapy (Riaz et al., 2023)

*Ficus carica* and *F. racemosa*, two medicinal plants, belong to the genus *Ficus* (Family Moraceae). *F. carica* is generally identified as "common fig or wild fig," whose leaves fall seasonally. It is the oldest cultivated tree, has historical value in many cultures, and is widely cultivated due to its edible and nutritious fruit. It possesses several phytochemicals that are potent for therapeutic purposes (Fazel et al., 2024). In addition, its potent therapeutic consequence has also been mentioned in the hadith (Spagnoli & Yavari, 2022).

Different parts of *F. carica* have chemicals like phenolic compounds, amino acids, vitamins and minerals. Phytochemicals include phenolic acids, flavonoids, ceramides, steroids, cerebrosides and triterpenoids (Hajam & Saleem, 2022). Leaves, fruits, roots and bark of this plant possess significant antimicrobial, antioxidant, antidiabetic, cytotoxic, hepatoprotective, anticholinesterase, anti-inflammatory, antipyretic and anti-angiogenesis activities. These activities are useful to treat a number of diseases like diabetes, asthma, ulcers, vomiting, gonorrhea, skin and heart diseases (Rasool et al., 2023).

*F. racemosa* is generally identified as "cluster fig." It is characterized by its cluster fruit, which grows directly on the trunk and branches. Different sections of the *F. racemosa* tree, such as fruits, leaves, roots and bark, possess proteins, lipids, carbohydrates and minerals. Phytochemicals in this plant include alkaloids, flavonoids, glucosides, sterols, furanocoumarins and terpenoids (Pahari et al., 2022). Research studies showed that this plant also exhibits antimicrobial, antioxidant, anti-inflammatory, antidiabetic, cytotoxic, antipyretic, anti-analgesic, and antidiarrheal properties. Various parts of this plant can treat many diseases like diarrhea, mumps, tonsillitis, menorrhagia and other liver, urinary and inflammatory diseases (Kannan et al., 2024)

Limited comparative assessment of *F. carica* and *F. racemosa* leaves, particularly in terms of evaluating a broad range of bioactivities, is available. Additionally, as per our knowledge, none of the previous studies have reported the hemolytic potential of these two plants. The objective of this research was to conduct a relative bio-fabrication of the therapeutic (antioxidant, antidiabetic, antimicrobial, and cytotoxic properties) application, along with Fourier transform infrared (FTIR) spectral analysis of aqueous leaf extracts from these two well-known species of the *Moraceae* family.

## Materials and Methods

### Sample preparation

Leaves of *F. carica* and *F. racemosa* collected from the Institutional Botanical Garden were shade-dried and ground into a powder form. The sample and water (1:5 ratio) mixture was placed in the microwave. The subsequent heating, cooling and heating were done three times till the separation of extracts using the filtration method. The samples were dried in the water bath at 52-55°C. The *F. carica* and *F. racemosa* leaves aqueous extracts were used for analysis of their bio-fabrication and FTIR spectral analysis (Abubakar & Haque, 2020).

### Antioxidant contents and activity

For the estimation of total phenolic content (TPC) as milligrams of gallic acid equivalents (GAE/100 g), 50 µL test sample (S), 40 µL sodium carbonate ($Na_2CO_3$) and 10 µL (10%) FC reagent were mixed, incubated (2 hours) at room temperature and absorbance was measured (765 nm). For total flavonoid content (TFC) assessment as milligrams of catechin equivalent (mg CE) per 100 grams, 10 µL S, 10.5 µL sodium nitrate and 67 µL of distilled water were mixed. Then 19 µL (10%) $AlCl_3$ was added and the absorbance was evaluated (510 nm). In the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay for antioxidant activity, a mixture of 250 µL DPPH solution and 2.5 µL S was incubated at ambient temperature for 35 minutes. Ascorbic acid was the positive control (C). At 517 nm, absorbance (Abs) was calculated (Ali et al., 2022). Percent Antioxidant activity: [(Abs. of C – Abs. of S) / Abs. of C] ×100.

### Antimicrobial activity

In the well diffusion assay, plant extracts and agar medium were prepared in Eppendorf tubes and flasks, respectively. The flask containing agar and empty petri plates was autoclaved. Then, inoculum (100 µL), *Staphylococcus aureus (ATCC 25923)* and *Escherichia coli (ATCC 25922)* were poured into the separate flasks. Plant extracts (80 µL) were added to the wells along with the bacterial strains. Ciprofloxacine served as the positive control and in the negative control sample, distilled water was added instead of the plant extract. After overnight incubation (37°C), the zones of growth inhibition (mm) were calculated (Ahmed et al., 2020).

### Antidiabetic activity (Alpha amylase inhibition assay)

For assay, 30 µL of sample (s) and acarbose (c) were mixed with the enzyme solution (10 µL). After pre-incubation at room temperature for 10 minutes, 1 percent starch (40 µL) was added. The reaction was halted by adding 20 µL of 1 M HCl, followed by a 30-minute incubation. For the detection of substrate unhydrolyzed by the enzyme, iodine (75 µL) was added and absorbance (A) was recorded at 580 nm (Ali et al., 2022). % inhibition = [1-Ac / As] × 100).

### Cytotoxic activity (Hemolytic assay)

Briefly, 3 mL of human blood and 5 mL of chilled phosphate-buffered saline (PBS) were mixed and centrifuged for 5 minutes. This washing step was repeated three times to isolate

red blood cells (RBCs). Subsequently, 20 µL of plant sample (s) was mixed with 180 µL of the RBC suspension and centrifuged again for 5 minutes. The supernatant was diluted with 900 µL of chilled PBS, and absorbance (a) was assessed at 570 nm (Kauser et al., 2018). Triton-X 100 was the positive control (pc) and PBS was the negative control (nc). % hemolysis: [(a.s – a.nc)] /a.pc × 100. Where, a.nc: absorbance of negative control, a.s: absorbance of test samples, a.pc: absorbance of positive control.

**FTIR spectral analysis**

For Fourier transform Infrared (FTIR) spectroscopy, plant extracts (1 mg) were finely ground with 100 mg potassium bromide (KBr) in a ratio of 1:100. After mixing, it was pressed under high pressure to form a pellet and subsequently investigated in the spectral range of 400 - 4000 $cm^{-1}$ (Kamran et al., 2019).

**Statistical analysis**

For triplicate measurements, results are reported as Mean ± Standard Deviation. ANOVA was used to determine the significance of the results using Minitab statistical software version 17.

**Results**

The percentage yield of extracts obtained by the microwave-assisted method was 45.98% for *F. carica* and 34.35% for *F. racemosa.*

**Antioxidant contents and activity**

The *F. carica* and *F. racemosa* exhibited 113.74 ± 3.61 mg GAE/100g and 89.51 ± 5.65 mg GAE/100g TPC, respectively. TFC in *F. carica* was 55.13 ± 4.04 mg CE/100g and 45.89 ± 1.29 mg GAE/100g in *F. racemosa*. The percentage of DPPH radical scavenging activity in *F. carica* was 43.61 ± 2.01% while in *F. racemosa*, it was 53.41 ± 2.23% (Figure 1). The difference between TPC, TFC and antioxidant activities of *F. carica* and *F. racemosa* was non-significant ($p>0.05$). Although the mean values differ, the differences are not statistically significant, which means they could be attributable to random variation.

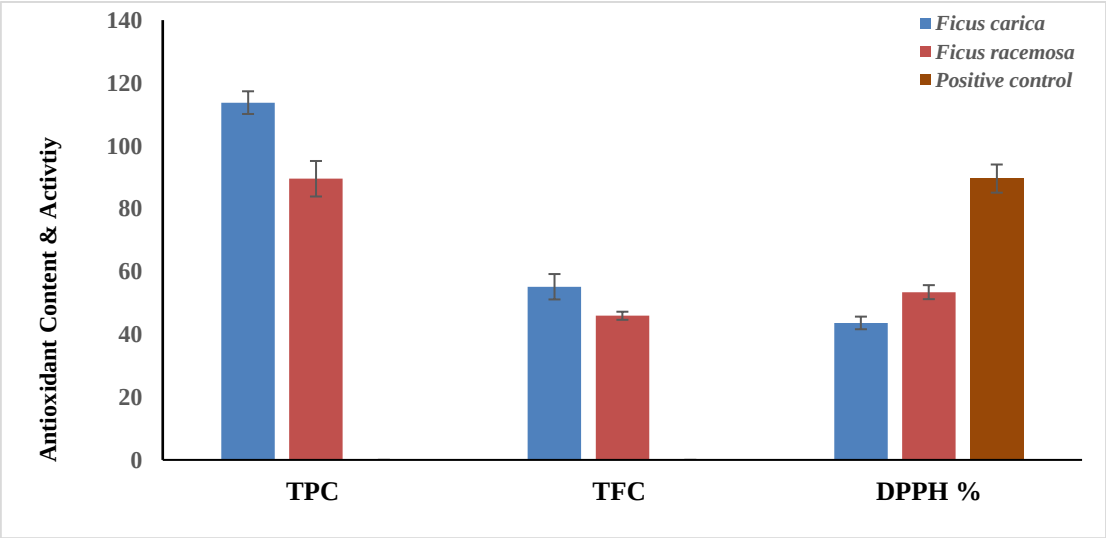


Figure 2. Antioxidant Profile

## Antimicrobial activity

Figure 2 represents the antimicrobial activity of *F. carica* extract against *E. coli*, with an 8 mm zone of growth inhibition (ZGI) and 8 mm ZGI against *S. aureus*. Meanwhile, the *F. racemosa* showed a ZGI of 20 mm against *E. coli* and an 8 mm ZGI against *S. aureus*. Both samples exhibited mild antimicrobial activities. The positive control (Ciprofloxacin) showed 27 mm ZGI for *E. coli*, while *Streptococcus aureus* showed a 28 mm ZGI. The negative control presented 0 mm zone of inhibition against both strains.

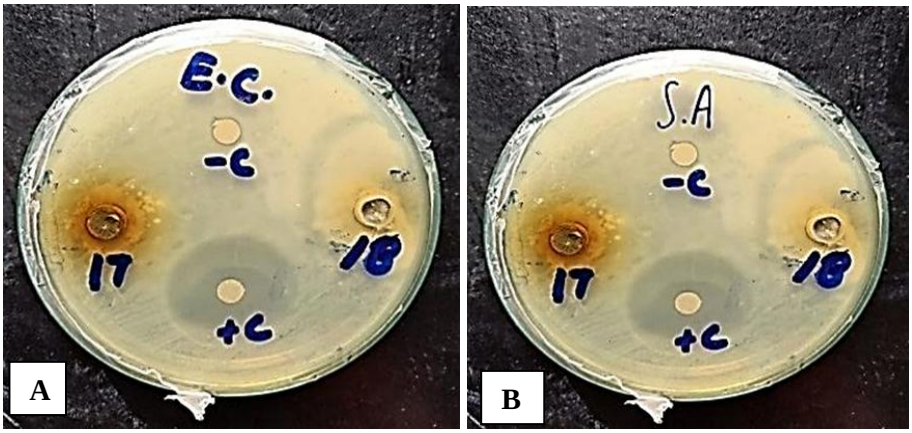


Figure 2. Antimicrobial activity of *F. carica* (17) and *F. racemosa* (18) against *E. coli* (A), and against *S. aureus* (B)

## Antidiabetic activity

Almost analogous ($p > 0.05$) antidiabetic activity was exhibited by both samples. The aqueous extract of *F. carica* showed 25.54 ± 0.88 % inhibition of alpha amylase; whereas the aqueous extract of FRA indicated 21.29 ± 15.81 % enzyme inhibitory potential and the percentage enzyme inhibition by the positive control was 81.47 ± 5.34 %. Both samples showed moderate antidiabetic potential (Figure 3).

## Cytotoxicity

The aqueous extracts of *F. carica* and *F. racemosa* leaves showed 12.47 ± 2.79% and 13.2 ± 1.22% hemolytic activities ($p > 0.05$), respectively, indicating the safe effect of plant samples being less toxic towards RBCs. However, 94.9% cell lysis was observed by the positive control (Figure 3).

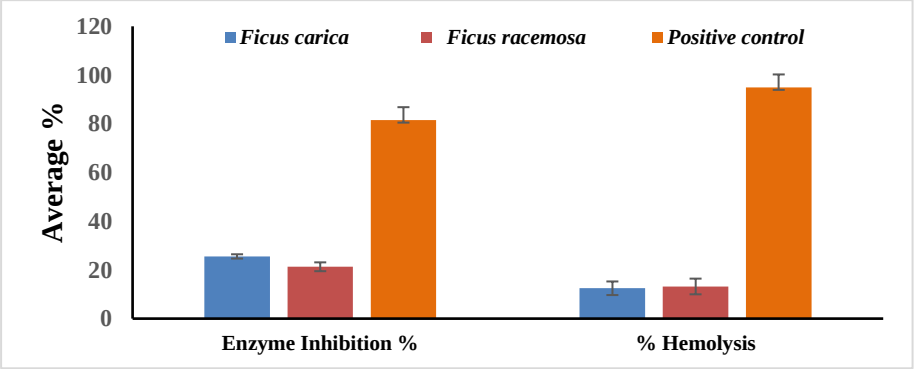


Figure 3. Antidiabetic and Cytotoxic Potential

## FTIR spectral analysis

Different functional groups were identified through FTIR in the *F. carica* (Table 1, and Figure 4). Alcoholic compounds were spotted by peaks at 3900.7 $cm^{-1}$, 3880.2 $cm^{-1}$, 3863.4 $cm^{-1}$, 3814 $cm^{-1}$, 3744.1 $cm^{-1}$ and 3668.6 $cm^{-1}$. The presence of alcohols and alkyne was found at strong, broad and sharp peaks of 3270.7 $cm^{-1}$. Amine salt, alkane, alcohol, and carboxylic acid compounds were detected as a strong band measured at 2918 $cm^{-1}$. Carboxylic acid, amine salt, alkane, and alcohol were all observed at a peak of 2849 $cm^{-1}$. A peak at 2353.8 $cm^{-1}$ showed the presence of carboxylic acid, amine salt and alcohol. Aldehyde was present at a strong peak at 1733.2 $cm^{-1}$. A solid band at 1716.4 $cm^{-1}$ showed the presence of α, β-unsaturated ester. Conjugated acid and conjugated aldehyde were detected at 1695.9 $cm^{-1}$. Conjugated alkene, cyclic alkene and amine were present at 1634.4 $cm^{-1}$. A band at 1539.4 $cm^{-1}$ showed the presence of a nitro group. A peak at 1455.5 $cm^{-1}$ showed the presence of an aromatic group. A band at 1418.3 $cm^{-1}$ showed the presence of the alcohol group. Phenol and sulphone were detected at a peak of 1317.6 $cm^{-1}$. A solid band at 1235.6 $cm^{-1}$ showed the presence of alkyl, aryl, ether and amine. Amine and sulfoxide were detected at 1010 $cm^{-1}$.

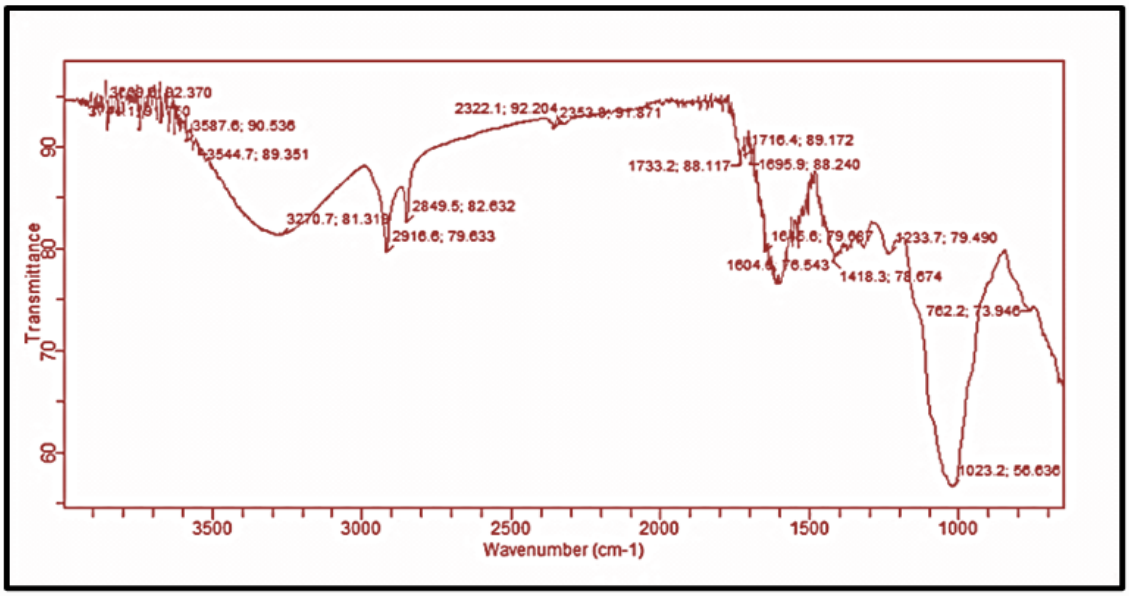


**Figure 4. FTIR spectra of *F. carica* leaves**

The values of absorption that were anticipated by the FTIR for *F. racemosa* are shown in Table 1 and Figure 5. Alcohols were present as indicated by peaks at 3744.1 $cm^{-1}$, 3669.6 $cm^{-1}$, 3587.6 $cm^{-1}$ and 3544.7 $cm^{-1}$. Alcohols, alkynes and carboxylic acid were present as shown by the band measured at 3270.7 $cm^{-1}$. Alkanes were detected at a peak of 2916.6 $cm^{-1}$. Alkanes and aldehydes were present at 2849.5 $cm^{-1}$. Amino groups were detected at medium and strong peaks of 2322.1 $cm^{-1}$. Aromatic compounds and aldehydes were detected at a peak of 1733.2 $cm^{-1}$. A solid band at 1716.4 $cm^{-1}$ showed the presence of αβ-unsaturated ether, aliphatic ketones and carboxylic acid. Peak at 1695.9 $cm^{-1}$, showed the presence of aromatic compounds. Imine was detected at a peak of 1645.6 $cm^{-1}$. Conjugated alkenes, amine and cyclic alkene were detected at a peak of 1604.6 $cm^{-1}$. Carboxylic acid and alcohols were present at a medium peak of 1418.3 $cm^{-1}$. Alkyl, aryl, amine and ether were detected at 1233.7 $cm^{-1}$.

Amines were detected at a peak of 1032 $cm^{-1}$. At a peak of 762.2 $cm^{-1}$, the presence of a medium peak of halo compounds was shown.

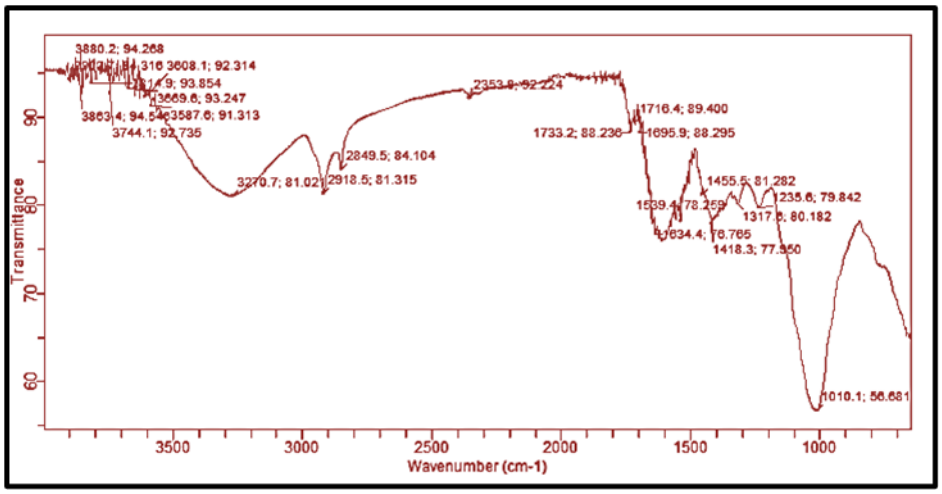


Figure 5. FTIR spectra of *F. racemosa* leaves

Table 1: FTIR Spectral analysis

| ***F. carica*** | | | ***F. racemosa*** | | |
|---|---|---|---|---|---|
| **Peak (Wave number $cm^{-1}$)** | **Bond** | **Functional group** | **Peak (Wave number $cm^{-1}$)** | **Bond** | **Functional group** |
| 3900.7, 3880.2, 3744.1, 3669.6 | O-H | Alcohols | 3744.1, 3669.6 3587.6, 3544.7 | O-H | Alcohol |
| 3270.7 | O-H C-H | Alcohol Alkyne | 3270.7 | O-H, C-H | Alcohol Alkyne |
| 2918.5 | O-H N-H C-H | Alkane, Alcohol, carboxylic Acid | 2916.6, 2849.5 | C-H | Alkane Aldehyde |
| 2849.5, 2353.8 | O-H N-H | Carboxylic acid, Alcohol, Amino salts | 2353.8, 2322.1 | N-H C-H | Amino group |
| 1733.2 | C=O | Aldehyde | 1733.2 | C=O | Aldehyde |
| 1716.4 | C=O | α, β-unsaturated ester | 1716.4 | C=O | α,β unsaturated ester |
| 1695.9 | C=O | Aromatic compound | 1695.9 | C-H | Aromatic compound |
| 1634.4 | C=C, N-H | Alkene, Amines aromatic Alkene | 1645.6 | C=N | Imine/oxime |
| 1539.4 | N-O | Nitro group | 1604.6 | C=C, N-H | Alkene, Amine aromatic Alkene |
| 1455.5 | C-H | Aromatic group | 1418.3 | O-H | Alcohol |
| 1418.3 | O-H | Alcohol | 1233.7 | C-O, C-N | Amine group, ether linkage with alkyl & aryl group |
| 1317.6 | O-H S=O | Phenolic group | 1023.2 | C- N | Amines |
| 1235.6 | C-O, C-N | Amine group, ether linkage with alkyl & aryl group | 762.2 | C- C | Halogenated groups |
| 1010.1 | C-N, S=O | Amino & sulfinyl groups | - | - | - |

## Discussion

The current study evaluated the phytochemical content, antioxidant, antimicrobial, antidiabetic, cytotoxic activities, and functional group composition of aqueous leaf extracts of *F. carica* and *F. racemosa*. In this investigation, both *F. carica* and *F. racemosa* aqueous extracts exhibited moderate levels of TPC and TFC. *F. carica* recorded 113.74 ± 3.61 mg GAE/100g TPC and 55.13 ± 4.04 mg CE/100g TFC, while FRA showed 89.51 ± 5.65 mg GAE/100g TPC and 45.89 ± 1.29 mg CE/100g TFC. These TPC values in our research were higher than those reported by Reveny et al. (2023) (33.93 - 40.76 mg GAE/g). These TFC values in our research were lower than those documented by El Ghouizi et al. (2023) (74.58 - 148.17 mg CE/g). For *F. racemosa*, the TPC observed in this study exceeded the 40.68 ± 9.17 mg GAE/g reported by Sharma and Kumar (2021). Regarding TFC, the values obtained in this work for *F. carica* were lower than those reported by El Ghouizi et al. (2023) (148.17 ± 8.54 mg CE/g) and Pawar et al. (2023) (65.76 ± 0.29 mg CE/g), emphasizing the influence of solvent choice on flavonoid extraction.

In terms of antioxidant activity, our findings showed that *F. racemosa* exhibited higher DPPH radical scavenging activity (53.41 ± 2.23%) compared to *F. carica* (43.61 ± 2.01%), though both values were lower than the 75.5% inhibition reported by Abdel-Rahman et al. (2021) for ethanolic *F. carica* extracts. The $IC_{50}$ values reported in previous studies for *F. racemosa* methanol extracts (31.87 and 334.95 μg/ml) suggest greater potency than the crude percentage inhibition measured in the current analysis. Although it is counterintuitive that extracts with lower Tops and TFC exhibit higher antioxidant activity but some possible reasons for such results are given. Presence of other active compounds like terpenes (that behave as antioxidants) and synergistic effects of different compounds may increase overall antioxidant capacity. Moreover, high TFC and TPC may exhibit pro-oxidant activity that ultimately reduces antioxidant potential.

Assessment of antimicrobial potential was limited as it was tested against two strains only, which refers to the general screening of plant extracts. The results revealed that *F. racemosa* showed a significantly larger zone of inhibition (20 mm) against *E. coli* than *F. carica* (8 mm), while both extracts had equal activity (8 mm) against *S. aureus*. Compared with the positive control, Ciprofloxacin (27–28 mm), the plant extracts showed moderate but noteworthy inhibition.

These findings partially agree with prior studies; Tikent et al. (2024) reported higher ZGIs for *F. carica* hydroethanolic extracts - 12.6 mm for *E. coli* and 10.5 mm for *S. aureus* - than those observed in the current study, possibly due to differences in solvent polarity or extract concentration. Similarly, Al-Ogaili et al. (2020) reported modest antimicrobial activity for a 20% aqueous *F. carica* extract, showing 10 mm and 9 mm zones against *E. coli* and *S. aureus*, respectively, which are also slightly higher than the 8 mm ZGIs observed in the present work. Pant et al. (2025) reported smaller zones (13 mm and 12 mm) for *F. racemosa* fruit extracts compared to the larger inhibition observed in our leaf extract assays, suggesting variability depending on plant part and extraction conditions. The current investigation demonstrated moderate α-amylase inhibitory activity for *F. carica* (25.54 ± 0.88%) and *F. racemosa* (21.29 ± 15.81%), substantially lower than the positive control (81.47 ± 5.34%).

These results indicate the limited enzyme inhibitory potential of the aqueous extracts. This contrasts with stronger antidiabetic effects observed in previous studies: Lin and Zhang (2023) reported a 35.75% glucose reduction with a dichloromethane *F. carica* extract, while Pahari et al. (2022) observed blood glucose reductions of 18.4% and 17.0% at 5 and 24 hours, respectively. The discrepancy highlights differences in solvent polarity and assay models, as the current *in vitro* evaluation may underestimate complex *in vivo* effects.

In this research, hemolytic activity was low for both *F. carica* (12.47 ± 2.79%) and *F. racemosa* (13.2 ± 1.22%), compared to the positive control, which induced 94.9% cell lysis, indicating that both extracts are largely non-cytotoxic to normal human red blood cells. These results concur with Nirwana et al. (2018), who reported high cell viability (>77%) even at elevated concentrations of *F. carica* ethanol extracts. However, previous research indicates selective cytotoxicity against cancer cell lines; Purnamasari et al. (2019) observed 82.78% inhibition of liver cancer cells by methanolic *F. carica* extract, while Khan et al. (2017) reported 57.37% DLA cell death at an $IC_{50}$ of 175 µg/mL using ethanol extract of *F. racemosa* leaves, and Gorla et al. (2016) observed 47.83% cytotoxicity against the A-549 lung cancer cell line using a hexane extract at 200 µg/mL; both reported significant cytotoxicity in other cancer cell models. This suggests the aqueous extracts tested in this analysis have biocompatibility with normal cells, while *Ficus* species may selectively target cancer cells.

FTIR analysis in the present investigation revealed multiple key functional groups in both *F. carica* and *F. racemosa*. *F. carica* showed strong O–H stretches at 3900.7, 3744.1, and 3668.6 $cm^{-1}$, and aldehyde groups at 1733.2 $cm^{-1}$. *F. racemosa* exhibited O–H peaks at 3744.1, 3587.6, 3544.7, and 3669.6 $cm^{-1}$, with additional imine (1645.6 $cm^{-1}$) and halo group signals (762.2 $cm^{-1}$). Both extracts shared peaks related to alcohols/alkynes (3270.7 $cm^{-1}$), alkanes/carboxylic acids (2918–2849 $cm^{-1}$), and ethers/amines (1235–1010 $cm^{-1}$).

These results align with those reported by Abu-Seraj et al. (2021) who observed O-H and N-H stretching at 3471.87- 3329.14 $cm^{-1}$, respectively; alkane at 2881.65 $cm^{-1}$ and 2850.79 $cm^{-1}$, nitro group (N-O) Symmetric stretching at 1334.67 $cm^{-1}$ and 1313.52 $cm^{-1}$, aliphatic amines at 1232.51- 1028.06 $cm^{-1}$, alkenes (=C-H) bending at 989.48 -719.45 $cm^{-1}$ and alkyl halides stretching at 667.37 - 657.73 $cm^{-1}$. FTIR spectra provided limited information about phytochemicals, as it identified only the functional groups present. A more comprehensive structural characterization with high-performance liquid chromatography and gas chromatography mass spectrometry is warranted to correlate the phyto-constituents with therapeutic efficacy.

## Conclusion

The current research study observed that aqueous extracts of *F. carica* and *F. racemosa* are rich in a few phenolic compounds. Both medicinal plants exhibited antioxidant, antimicrobial and antidiabetic activities. The *F. racemosa* and *F. carica* extracts exhibited low hemolytic activity, indicating good biocompatibility, with *F. racemosa* being marginally more hemolytic than *F. carica* but also having lower cytotoxic effects. FTIR of both plants characterized the structure and identified the functional groups of both *F. carica* and *F. racemosa*. In conclusion, this research indicated that aqueous extracts of both have extraordinary pharmacological

activities and in-depth studies in other polar and non-polar solvents are required to further evaluate the remarkable properties and therapeutic potential of these medicinal plants.

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