



Phytochemical Profile and Biological Activities of *Ficus carica* Latex from the West Bank, Palestine

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Ficus carica, commonly known as the fig tree, is widely cultivated across many regions of the world, particularly in the Middle East, due to its nutritional, medicinal and economic importance. The latex of this tree is a milky white sap that can be found in all tissues, particularly the unripen leaves and fruits. In this study, fig latex collected from the West Bank of Palestine was extracted using 100% ethanol, 70% ethanol, 80% methanol and ethyl acetate. Antioxidant activity assessed by the DPPH assay showed high inhibition for 80% methanol ($79.5 \pm 1.4\%$) and 70% ethanol ($71.1 \pm 1.5\%$), moderate inhibition for 100% ethanol ($66.3 \pm 1.6\%$) and low inhibition for ethyl acetate ($20.2 \pm 1.3\%$), compared with vitamin C ($94.0 \pm 1.5\%$). Antibacterial testing revealed activity of the 70% ethanol extract against *Escherichia coli* only, with no activity against *Staphylococcus aureus* or *Staphylococcus epidermidis*. HPLC analysis identified gallic acid, *p*-coumaric acid, ellagic acid, rutin, quercetin and trace levels of sinapic acid and chrysin.

Keywords: Fig tree latex, Antioxidant activity, Antibacterial activity, HPLC analysis.

INTRODUCTION

Ficus carica L., commonly referred to as the fig tree, is a member of the *Moraceae* family and is widely distributed throughout the Mediterranean region [1-3]. The tree has been cultivated since ancient times for its fruit, which is rich in sugars, fibers and bioactive compounds [4,5]. In addition to its edible fruits, the fig tree exudes a white milky latex when its branches, leaves or immature fruits are cut [6,7]. All *Ficus* species possess latices within their vasculatures, affording protection and self-healing from physical assaults [8]. Traditionally, the latex has been used in folk medicine across various cultures to treat skin infections, warts, gastrointestinal disorders and other ailments [8-11].

Latex from *Ficus carica* contains a diverse mixture of bioactive compounds including phenolics, flavonoids, enzymes (such as ficin) and terpenoids [12]. These compounds are thought to be responsible for the various therapeutic effects attributed to fig latex including antioxidant [13], antimicrobial [14], anti-inflammatory [15] and anticancer activities [16,17]. The antibacterial potential of *F. carica* latex has also gained attention in recent years. Studies demonstrated that fig

latex exhibits inhibitory effects against a variety of bacterial strains, including *Staphylococcus aureus* and *Escherichia coli*. These effects are believed to be due to the synergistic action of phenolic compounds and proteolytic enzymes, which may disrupt bacterial cell membranes and interfere with vital metabolic processes [18,19].

In the West Bank of Palestine, fig trees are abundant and often grow in non-cultivated areas, making them a readily accessible resource for natural product research [20]. However, the chemical composition and biological activity of *F. carica* latex collected specifically from this region remain poorly studied. Environmental factors such as soil type, climate and altitude can significantly influence the phytochemical makeup of plant extracts, thus underlining the importance of region-specific investigations [21,22]. Moreover, the rise in antibiotic-resistant bacterial infections has renewed interest in discovering novel antimicrobial agents from plant sources, including fig latex [23].

Despite its extensive traditional use, the biological properties and phytochemical constituents of fig latex have not been comprehensively explored, particularly in the Palestinian context, a region with unique climatic and environmental condi-

tions that may influence phytochemical composition. Hence, characterizing the phenolic profile of fig latex and assessing its antioxidant properties is an essential step in validating and expanding its medicinal applications. The current study was designed to fill this knowledge gap by investigating the antioxidant capacity, antibacterial activity of *F. carica* latex collected from the West Bank. Furthermore, high-performance liquid chromatography (HPLC) was employed to identify main phenolic compounds. This integrative approach aims to provide a scientific basis for the traditional use of fig latex and to explore its potential for development into therapeutic or nutraceutical products.

EXPERIMENTAL

All standards, solvents and reagents used were provided by Sigma-Aldrich and were used without additional purification. Fresh *Ficus carica* latex was collected in mid-October 2024 from fruits harvested in As-Sawiya village (32°05'05" N, 35° 15'28"E), located south of Nablus, Palestine. Latex exudate was obtained by gently squeezing each fruit and allowing the secretion to drip directly into sterile 15 mL glass vials, without applying mechanical pressing to the pulp. Collected samples were immediately refrigerated and stored until subsequent analyses.

For sample preparation, 0.5-1.0 mL of *F. carica* latex was added to 10 mL of each solvent *viz.* 100% ethanol, 70% ethanol, 80% methanol and ethyl acetate. The resulting mixtures were sonicated for 2 h to ensure complete dispersion of the latex. The extraction yield was approximately 6% for 100% ethanol, 70% ethanol and 80% methanol, whereas a lower yield of about 2% was obtained with ethyl acetate.

HPLC analysis: For the analysis of bioactive compounds in *Ficus carica* latex, a Waters Corporation HPLC Waters Alliance e2695 system equipped with a 2998 PDA detector was employed. The following analytical standards (purity > 98%) were individually dissolved in 20% ethanol to obtain stock solutions with a concentration of 25 mg/100 mL: gallic acid, 3,4-dihydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, chlorogenic acid, 4-hydroxyphenylacetic acid, vanillic acid, caffeic acid, syringic acid, isovanillic acid, p-coumaric acid, ferulic acid, sinapic acid, rutin, verbascoside, quercetin, *trans*-cinnamic acid, apigenin, hesperetin, naringenin, hesperidin, naringin, chrysin, ellagic acid, luteolin, galangin, daidzein and kaempferol. Subsequently, 1.0 mL aliquots of each standard solution were transferred into separate 50 mL volumetric flasks and diluted to volume with the same solvent.

The mobile-phase composition and gradient elution conditions used for the analysis of the major compounds are summarized in Table-1. Solvent A consisted of 1.0% acetic acid in water, while solvent B was acetonitrile. Chromatographic separation was carried out using a Restek Roc C₁₈ column (3 μm, 150 × 4.6 mm) at a flow rate of 0.6 mL/min. The PDA detector was operated over a wavelength range of 210-400 nm. The column temperature was maintained at 25 °C and the injection volume was set at 20 μL. Prior to injection, all samples were filtered through a 0.45 μm disposable membrane filter.

TABLE-1
CONDITIONS OF GRADIENT ELUTION
FOR THE HPLC METHOD USED

Time (min)	A (%)	B (%)
0	93	7
40	80	20
50	65	35
70	40	60
75	10	90
78	93	7
80	93	7

Antioxidant activity using DPPH solution: Each dried extract was dissolved in an appropriate solvent (methanol or ethanol) to obtain a 100 mg/mL stock solution. An aliquot of 1 mL of each extract was mixed with 1 mL of freshly prepared DPPH solution in a test tube, vortexed gently and incubated in the dark at room temperature for 30 min. The absorbance was then recorded at 517 nm. A mixture of 1 mL solvent and 1 mL DPPH solution served as the control and methanol was used as blank. Ascorbic acid (vitamin C) was included as the positive control. The percentage of DPPH inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of DPPH without sample and A_{sample} is the absorbance of DPPH with extract.

Antimicrobial analysis: The antibacterial activity of the extracts was evaluated using the disc diffusion method [24]. Two Gram-positive bacterial strains, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228 along with one Gram-negative strain, *Escherichia coli* ATCC 25922, were selected for the study. Sterile Mueller-Hinton agar medium (Sigma-Aldrich) was poured into sterile Petri dishes and allowed to solidify for 2 h before use. All extracts were prepared using dimethyl sulfoxide (DMSO) (Sigma-Aldrich) as the solvent. Bacterial suspensions were prepared and evenly spread onto the surface of the agar plates. Sterile filter paper discs (6 mm diameter, 1 mm thickness) were then placed on the inoculated agar surface and 50 μL of each extract was carefully loaded onto the discs using a micropipette. DMSO served as the negative control. For the positive controls, gentamycin (0.01 mg/mL) was used against *E. coli*, while penicillin (0.01 mg/mL) was used against *S. aureus* and *S. epidermidis*. Bacterial suspensions (100 μL, 10⁶ CFU/mL) were spread uniformly on the agar plates prior to disc placement. The plates were subsequently incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the clear inhibition zone formed around the discs, which indicated the ability of the extracts to inhibit bacterial growth.

Statistical analysis: All experiments were performed in triplicate (n = 3) and results are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

HPLC analysis: Fig. 1 shows the 26 standards used for fingerprinting the components in the samples, shown at 290 nm, which represents the best fit for all components. HPLC results

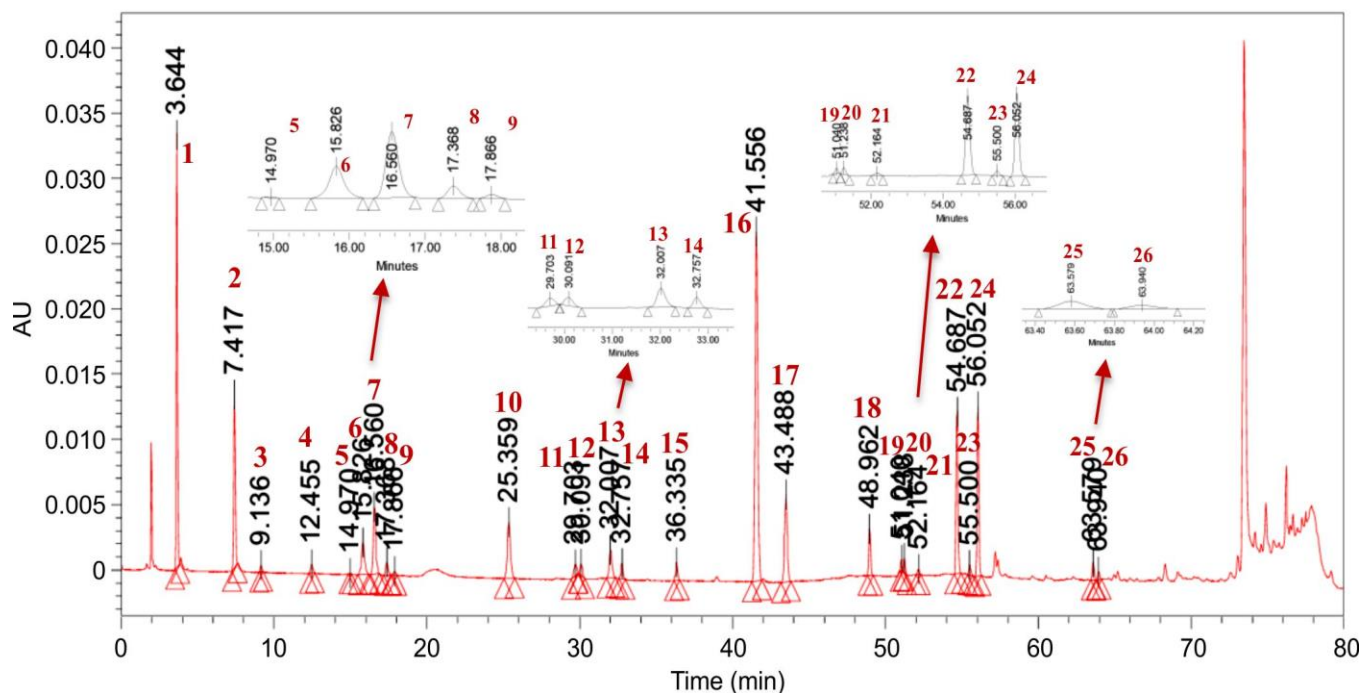


Fig. 1. HPLC chromatogram for standards used at 290 nm: (1) gallic acid, (2) 3,4-dihydroxybenzoic acid, (3) 3,4-dihydroxyphenylacetic acid, (4) chlorogenic acid, (5) 4-hydroxyphenylacetic acid, (6) isovanillic acid, (7) caffeic acid, (8) syringic acid, (9) vanillic acid, (10) *p*-coumaric acid, (11) ferulic acid, (12) sinapic acid, (13) ellagic acid, (14) rutin, (15) verbascoside, (16) naringin, (17) hesperidin, (18) diadzein, (19) luteolin, (20) quercetin, (21) *trans*-cinnamic acid, (22) naringenin, (23) kaempferol, (24) hesperetin, (25) chrysin and (26) galangin

for extracted samples were obtained at different wavelengths (245, 290 and 330 nm) since some compounds have no or different absorption values at various wavelengths. Detection of main components of the fig latex was accomplished by comparing the retention times and UV scans for standards and samples from the PDA device. The detected compounds were tentatively identified based on their HPLC-PDA chromatographic profiles. The HPLC analysis of the 80% methanol extract revealed the presence of several phenolic and flavonoid compounds. At 245 nm (Fig. 2a), gallic acid was clearly identified along with traces of ellagic acid. When the chromatogram was monitored at 290 nm (Fig. 2b), *p*-coumaric acid and traces of sinapic acid were detected. Further analysis at 330 nm (Fig. 2c), which is suitable for flavonoid detection, showed the presence of rutin and quercetin, along with traces of chrysin.

In contrast, the 70% ethanol extract exhibited only two unidentified peaks at retention times of 39.54 and 51.29 min, respectively (Fig. 3). These peaks did not correspond to any of the reference standards used in the study. Moreover, no detectable peaks were observed at 290 nm or 330 nm for this extract. Similarly, the ethyl acetate extract (Fig. 4) did not show any significant or unidentified peaks when analyzed at 245, 290 and 330 nm, indicating the absence or very low concentration of detectable phenolic and flavonoid compounds under the applied chromatographic conditions.

Only a limited number of compounds were detected in the ethyl acetate extract, suggesting the presence of less polar constituents or phytochemicals occurring at relatively low concentrations. This difference in compound recovery can mainly be attributed to variations in solvent polarity, which plays a crucial role in the extraction efficiency of plant secondary

metabolites. Phenolic acids and flavonoids generally contain multiple hydroxyl groups, making them more polar in nature and therefore more soluble in polar or semi-polar solvents such as aqueous methanol and ethanol. In comparison, ethyl acetate possesses intermediate polarity and is comparatively less effective in extracting highly polar phenolic compounds from plant matrices. These observations emphasize the importance of selecting an appropriate solvent system for the efficient extraction and maximum recovery of bioactive phytochemicals.

Antimicrobial activity: The antibacterial results showed that the effectiveness of *F. carica* latex against some strains of bacteria depends on the type of extract solvent used (Table-2). Of the tested extracts, only 70% ethanol extract produced an inhibition zone against *E. coli* (15 mm). No inhibition was observed for *S. aureus* or *S. epidermidis* with any other extract. Gentamicin (positive control) produced inhibition zones of 20 mm, 17 mm and 20 mm against *S. aureus*, *S. epidermidis* and *E. coli*, respectively, confirming assay validity. The present results agree with recent research on the inhibition of *E. coli* [25], while disagree with another research in which alcoholic extract showed inhibition activity against *S. aureus* bacteria [26].

TABLE-2
INHIBITION ZONES (mm) FOR VARIOUS
SAMPLES AGAINST GENTAMYCIN

Sample/bacteria	<i>S. aureus</i>	<i>S. epidermis</i>	<i>E. coli</i>
80% Methanol	0	0	0
100% Ethanol	0	0	0
70% Ethanol	0	0	15
Gentamycin	20	17	20

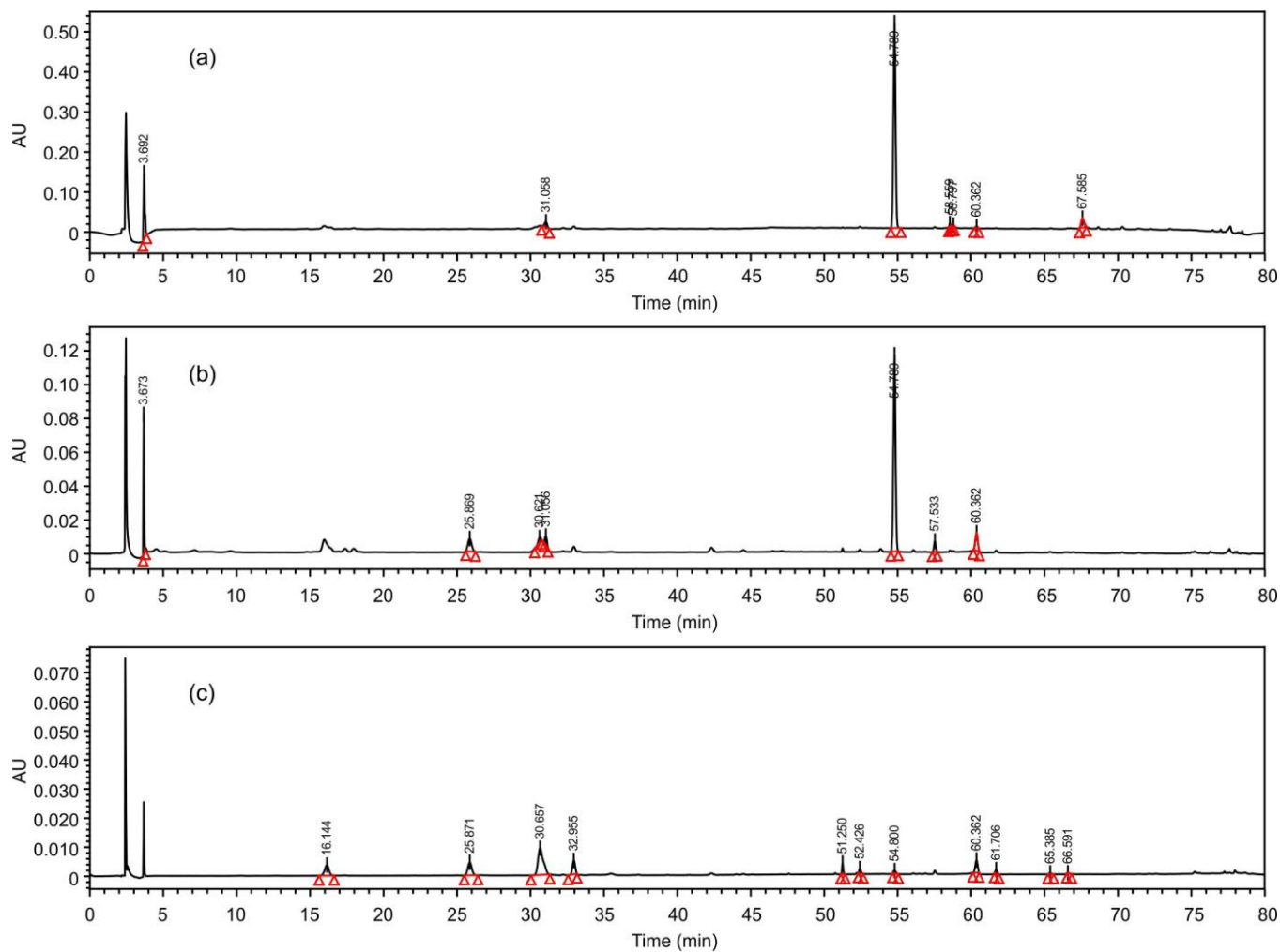


Fig. 2. Chromatograms for the 80% methanol sample run at 245 nm (a), 290 nm (b) and 330 nm (c)

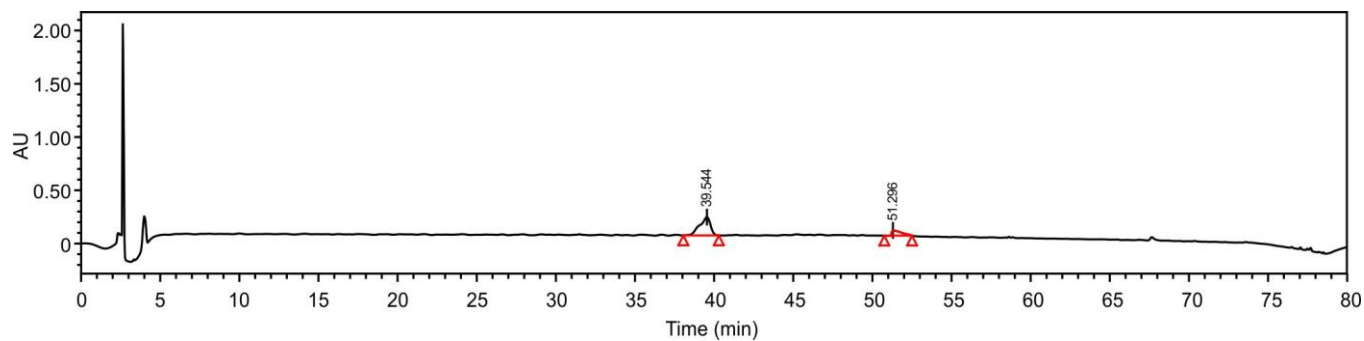


Fig. 3. Chromatogram for the 70% ethanol sample run at 245 nm

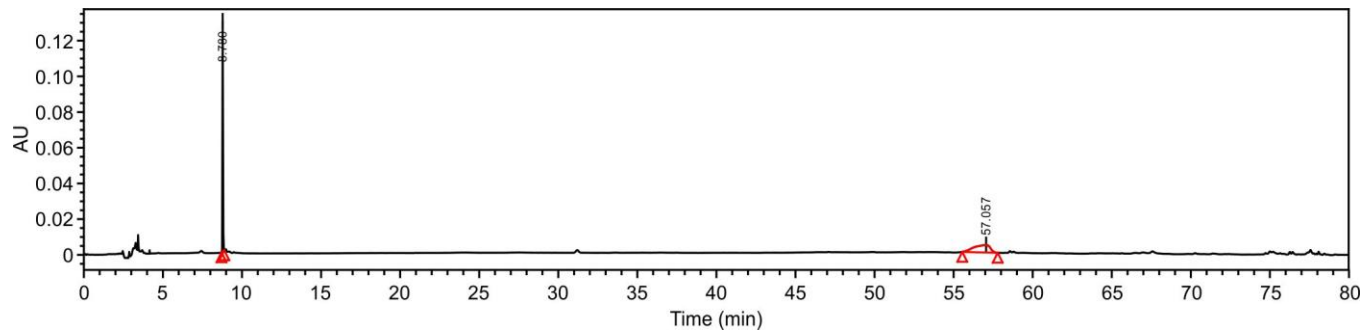


Fig. 4. Ethyl acetate sample showing unknown peaks

Antioxidant activity: Table-3 presents the percentage inhibition values of the different extracts in comparison with the ascorbic acid standard. Among the tested samples, the 80% methanol extract exhibited the highest antioxidant activity, with an inhibition percentage of $79.15 \pm 1.4\%$, whereas the ethyl acetate extract showed the lowest activity, with an inhibition percentage of $20.2 \pm 1.3\%$. The observed antioxidant activity can be attributed to the presence of polyphenolic and flavonoid compounds, which are generally polar in nature. Since methanol is more polar than ethanol and ethyl acetate, it is expected to extract a higher amount of these bioactive secondary metabolites. Consequently, the methanolic extract demonstrated superior antioxidant potential compared to the less polar solvent extracts [27].

TABLE-3
PERCENTAGE OF DPPH INHIBITION
OF THE SELECTED EXTRACTS

Extract solvent	% Inhibition at 100 µg/mL
80% Methanol	79.5 ± 1.4
100% Ethanol	66.3 ± 1.6
70% Ethanol	71.1 ± 1.5
Ethyl acetate	20.2 ± 1.3
Ascorbic acid (standard)	94.0 ± 1.5

In addition to solvent polarity, the water content of the extraction solvent also plays an important role in enhancing the solubility and recovery of phenolic compounds. The presence of water promotes swelling of the plant matrix through the adsorption of solvent molecules onto the hydroxyl and carboxyl groups of cellulose fibers [28]. This swelling increases the distance between fibers, leading to matrix expansion and a larger surface area, which facilitates solvent penetration and improves the release of soluble phytochemicals into the extraction medium [29]. The antioxidant activity observed in this study is consistent with previously reported DPPH radical scavenging activity of Malaysian fig latex extracts [13].

Conclusion

The present study explored the phytochemical composition and biological activities of *Ficus carica* latex collected from the northern region of the West Bank, Palestine. Different extraction solvents with varying polarities were employed to evaluate their influence on the recovery of bioactive compounds and biological efficacy. Among the tested solvents, the 80% methanol extract demonstrated the highest antioxidant activity in the DPPH radical scavenging assay, showing strong activity when compared with vitamin C used as the reference standard. In terms of antibacterial activity, the 70% ethanol extract exhibited moderate inhibitory effects against *E. coli*, whereas no significant inhibition was observed against the other tested bacterial strains or with extracts prepared using the remaining solvents. The developed HPLC method enabled the identification of seven secondary metabolites including both polyphenolic and flavonoid compounds. However, further comprehensive studies are recommended to isolate additional bioactive constituents and to further investigate their pharmacological and therapeutic applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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