International Journal of PharmTech Research

CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.13, No.04, pp 329-340, 2020

Seasonal Variations in Antioxidant Activity, Total Flavonoids Content, Total Phenolic Content, Antimicrobial Activity and Some Bioactive Components of *Ficus carica L.* in Palestine

Sireen Radwan[1], Ghassan Handal[2], Fuad Rimawi[3] and Michel Hanania[1,*]

[1]Department of Chemistry, Faculty of Science Bethlehem University, Bethlehem-Palestine
[2]Department of Biology, Faculty of Science, Bethlehem University, Bethlehem-Palestine
[3]Department of Chemistry, College of Science and Technology, Al-Quds University, Jerusalem, Palestine

Abstract: The genus Ficus is one of the largest genera of angiosperms with more than 800 species. This study aimed at investigating the pharmacological properties of *Ficus carica linn*. Leaves were collected in two seasons (May and October) and were extracted by Soxhlet extractor with different percentages of methanol-water, ethanol-water and pure water solvents. 100% methanol and 95% ethanol offered the highest yield (about 23%). Those extracts were further extracted/partitioned with n-butanol. May samples showed higher TPC, TFC and AA results than October samples. The ethanol-butanol extract had the highest TPC results (150.7±0.5 mg GA/g extract), while methanol-butanol extract showed the highest results for TFC and AA (350±4.3 mg Rutin/g extract and 368.1±1.9 mg $FeSO_4$/g extract, respectively). Ethanol and methanol extracts were weakly active against *E. coli*, while the extracts showed good inhibition activity against oral flora, where October samples gave higher activity than May samples. Some components of extracts were analyzed by a newly developed HPLC-PDA method. Eight compounds were identified: chlorogenic acid, caffeic acid, syringic acid, coumaric acid, rutin and *trans*-cinnamic acid. Quercetin was identified after acid hydrolysis of extracts in all sample extracts, while ferulic acid was present in water extract only.
Keywords : *Ficus carica linn*; pharmacological properties, antimicrobial activity, HPLC analyses.

Introduction

The genus Ficus constitutes one of the largest genera of angiosperms with more than 800 species of trees, epiphytes and shrubs in the tropical and sub-tropical regions worldwide. *Ficus carica linn* is the most popular member of the genus Ficus, and it is known by more than 135 names[1]. *F. carica* is an Asian species of flowering plant, commonly known as Fig tree[2,3]. The Mediterranean around which most of the fig growing

DOI: http://dx.doi.org/10.20902/IJPTR.2019.120404

countries are located has been the most important region of fig production from time immemorial[4]. Palestine, one of the original countries, is characterized by a wide range of environmental conditions and rich nature biodiversity. The fig trees are grown all over the country and mostly located in the marginal land. Moreover, fig names were mainly given based on skin ground color, internal color, country of origin and maturity date[5]. Twelve fig genotypes were identified in Palestine, specifically in the southern regions in West Bank, namely: Shhami, Ghzali, Bladi, Khdari, Swadi, Smari, Aswad, Ruzzi, Hmari, Moozi, Mouze and Mwazi[6].

This plant invites attention of researchers worldwide for its biological activities. The fruit of the tree is commonly eaten; it is used as a laxative to relieve constipation[7]. The health benefits of fig leaves are sourced from the availability of minerals such as iron, potassium, manganese and calcium, vitamins such as vitamin A, vitamin B1 and vitamin B2, and high fiber content[8-10]. Moreover, *F. carica* has remarkable pharmacological properties such as antioxidant, anticancer, cytotoxic and anti-inflammatory activities[11].

F. carica has been used in traditional medicine for a wild range of treatments. Its bark, fruit, leaves, roots and latex are medically used in different forms. In ethnomedicine, leaves are used as antidiabetic, vermifuge, contact dermatitis in humans, and photo toxicity in animals[12,13].

Researches showed that the leaves of *F. carica* contained alkaloids, tannins, phenols, organic acids, flavonoids, steroids and volatile compounds. Reports on the biological activities of the plant are mainly on its extract that have been proven to possess many biological activities[14]. Some of the most interesting therapeutic effects include anticancer, hepatoprotective, hypoglycemic and antimicrobial activities[15-16]. Over one hundred bioactive compounds have been identified in fig leaves including ferulic acid, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, psoralen, bergapten, organic acids (oxalic, citric, malic, quinic, shikimic, and fumaric acids) and coumarin[17].

This study aimed at comparing phytochemicals and pharmacological properties of fig leaves extract in Palestine in different seasons. Thus, total phenolic content (TPC), total flavonoids content (TFC), antioxidant activity (AA) and antimicrobial activity were measured. Additionally, the main chemical components of extracts were determined by a newly developed gradient-elution HPLC method.

Experimental

Fig leaves, solvents and chemicals

Mouazi fig leaves were collected from Tuqu village located southeast Bethlehem, Palestine in May and October 2019. Solvents used for HPLC were of HPLC grade, while all other solvents, reagents and standards used were of an analytical grade and were obtained from Sigma Aldrich Company. Bacterial species were obtained from The Holy Family Hospital-Bethlehem, Palestine. Deionized water was used for analysis and preparation of solutions.

Devices

Biochem Libra S22 UV-VIS spectrophotometer was used for the determination of flavonoids, total phenolics and antioxidant activity. HPLC Waters Alliance e2695 equipped with 2998 PDA detector was used for the analysis of bioactive compounds. BDS HYPERSIL C18 (Thermo Scientific) column was used for separation (Length: 250 mm, I.D.: 4.6 mm and particle size: 3µm). Data acquisition and control were carried out using Empower 3 chromatography data software.

Extraction of dry leaves (1st extract)

Leaves from May and October were dried at room temperature, ground and were directly extracted by Soxhlet extractor for two and a half hours, using 250 ml of different percentages of ethanol-water (95%, 75%, 50% and 25%), methanol-water (100%, 75%, 50% and 25%) and pure water solvents. Crude extracts were obtained by evaporating the solvent by rotary evaporator and were stored away from direct light at 4ºC.

Extraction of crude extract (2nd extract)

100% methanol and 95% ethanol crude extracts were further extracted (partitioned) with n-butanol. 70.0 mg of each extract was suspended in 10 mL water and extracted with two portions of 10 mL n-butanol. Butanol was then evaporated by rotary evaporator and the extract was stored away from direct light at 4ºC.

Stock solution preparation

For the 1st extract, 150 mg of the ethanol extracts were dissolved in 100.0 mL 50% ethanol, and 200 mg of the methanol extracts were dissolved in 100.0 mL 50% methanol. These solutions were reserved for the determination of total phenolic content, total flavonoid content and antioxidant activity. For the 2nd extract, 50 mg extracts were dissolved in 100.0 mL of 30% ethanol. The solutions were reserved for the determination of total phenolic content, total flavonoid content and antioxidant activity.

Total phenolic content (TPC)

TPC was determined by Folin-Ciocalteu method[18], which depends on the oxidation of phenolic compounds in the presence of Na_2CO_3. Briefly, the procedure is as the following: Gallic acid (GA) standard series was used for the construction of the quantification curve. Each sample was measured in triplicate. 40 µL of standard or 100 µL of sample was added to 1.2 mL of Folin-Ciocalteu reagent. After 5 minutes, 1.2 mL of 7.5% Na_2CO_3solution was added. The mixture was incubated for 1 hour at 27°C, then absorbance of the solutions was measured spectrophotometrically at 765 nm.

Total flavonoids content (TFC)

Aluminum chloride complex forming assay was used to determine the TFC of the extracts[19]. It relies on complexation reaction with Al (III) after nitration of the aromatic ring by $NaNO_2$ in alkaline medium. All analysis were carried out in triplicate. Rutin was used as a standard for the calibration curve with concentrations (5-100 ppm). 300 µL of 5% $NaNO_2$ was added to 1500 µL standard or 1000 µL sample. After 5 minutes 300 µL of 10% $AlCl_3$ solution was added and the reaction mixture was allowed to stand for 6 minutes, then 2000 µL of 1M NaOH solution was added to both standard and sample, while an extra 2000 µL of H_2O was added only to the sample solutions. The absorbance of this reaction mixture was recorded at 510 nm on an appropriate spectrophotometer.

Antioxidant Activity (AA)

Antioxidant capacity was determined by FRAP (ferric reducing antioxidant power)[20,21] method, which depends on the reduction of Fe^{+3} to Fe^{+2} by antioxidants producing blue colored solution. In this procedure, 1000 µL of FRAP solution and 1000 µL H_2O were added to 80 µL of standard or sample. The absorbance was recorded after 15 minutes at 593 nm spectrophotometrically.

HPLC-PDA Detection of Phytochemicals

Standards of gallic acid, chlorogenic acid, coumaric acid, syringic acid, ferulic acid, caffeic acid, rutin, quercetin, *trans*-cinnamic acid and luteolin were prepared at a concentration of 0.2 mg/ mL using 20% ethanol as a solvent. The concentration of samples of crude water, 100% methanol and 95% ethanol extracts of May and October samples was 200.0 mg/5.0 mL DMSO and ethanol (3:2). Water, methanol and ethanol solution extracts were hydrolyzed under acidic conditions. In the first step, solvents were evaporated by rotary evaporator to 50.0 mL, then the solution was refluxed with 50.0 mL 3M HCl for 30 minutes. After that, the mixture was evaporated to 50.0 mL.

HPLC analytical experiments of the crude water, methanol and ethanol extracts, acid hydrolyzed water, methanol and ethanol extracts were run under reversed phase condition. The mobile phase consisted of a mixture of 0.5% acetic acid (A) and acetonitrile (B) with a linear gradient mode as shown in table 1. All samples were filtered with 0.45 µm disposable filter. PDA range was set from 210 to 400 nm. Flow rate was 1.0 mL/minute, while injection volume was 20 µL and the column temperature was set to 25 ºC.

Table 1. Mobile phase composition.

Time (minutes)	A %	B %
0	98	2
35	80	20
55	65	35
60	40	60
65	10	90
67	98	2
70	98	2

Antimicrobial Activity

Escherichia coli, a Gram-negative bacteria (ATCC 29522) was used. Oral flora was collected from mouth cavity of three different persons with sterile cotton swab. The swabs were streaked on sheep blood agar under sterile conditions. The plates were incubated at 37℃ overnight. Two separate colonies were randomly sub-cultured overnight. Additional sub-culturing of the sub-cultured colonies was performed twice on two successive days. According to standard Gram Stain procedure[22], the test revealed that the isolated bacteria was Gram-positive. No further characterization of the isolated bacteria was made because the study aims at investigating the effect of *F. carica* leaves extract on the oral flora in general. Samples were prepared by dissolving 300.0 mg of each extract in 1.0 mL DMSO.

The antibacterial activity of the isolated bacteria was tested using Agar well diffusion method[23-25]. 24 h old cultures of test bacteria were aseptically swabbed on sterile Mueller Hinton agar plate[26]. Wells of 5 mm diameter were made aseptically in the inoculated plates. The volume of extract inoculum was 100µL in each well. Two plates were prepared per each trial with wells in each. One well contained DMSO as negative control; the second well contained amoxicillin (50mg/100ml) as positive control; the third well contained the sample. Concentration of bacterial samples were measured according to McFarland protocol[27]. Plates were incubated at 37℃ for 24 hours, after which inhibition zone was measured for each treatment.

Results and Discussion

Statistical analyses

All analyses were performed in triplicates. The averages and the standard deviations were calculated using Excel software version 11.5.1 (Microsoft, Redmond, USA). Statistical analyses were performed using JMP version 9.0 (SAS institute Inc.). The statistical analysis between different extraction solvents and between different collection dates of fig leaves was performed using analysis of variance (ANOVA), followed by Post hoc pairwise comparisons using the Tukey honestly significant difference test (HSD). Differences were considered significant if *p* values were lower than 0.05.

Extraction results showed that the highest yield was obtained when methanol (100%) and ethanol (95%) were used as extraction solvents with a yield of about 23%. The yield increases when the volume content of methanol or ethanol increases in the extraction solvent (from 25% to 50% and 75% to 100%). Extraction with water gave about 8% yield. Extraction yields are shown in table 2.

Table 2. Extraction yield for different solvents.

Extraction solvent	Percentage yield
100% methanol	23.7
75% methanol	13.0
50% methanol	12.2
25% methanol	7.3
95% ethanol	23.6
75% ethanol	19.4
50% ethanol	15.1
25% ethanol	7.7
Water	8.1

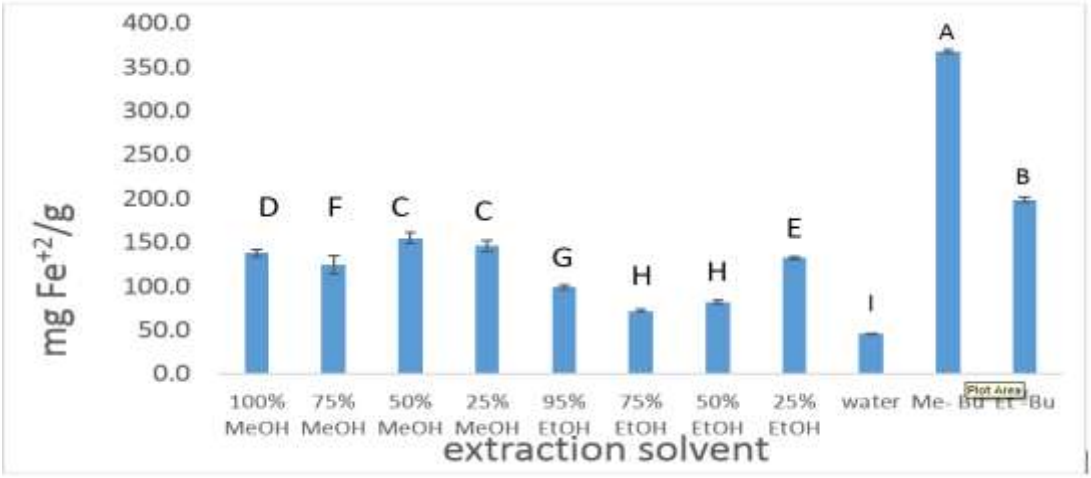
Effect of extraction solvent on AA, TFC and TPC of fig leaves

Statistically, the extraction solvent has an effect on the AA (mg Fe^{+2}/g). The significant differences ($p < 0.05$) between the AA and extraction solvent are indicated by different capital letters (A to I). It was found that highest AA was for MeOH-BuOH followed by EtOH-BuOH, methanol (25% and 50%) that statistically had no significant difference in their AA (indicated by letter C), followed by 100% methanol, 25% ethanol,75% methanol, 95% ethanol, and then 75% ethanol and 50% ethanol which has statistically not significant difference in their AA. The lowest AA was found for water that is significantly lower than all other extraction solvents (Figure 1 and Table 3).

Table 3: Effect of different extraction solvents on the AA, TPC and TFC of fig leaves collected on May 2019.

Extraction solvent	mg $FeSO_4$/1g extract*	mg Gallic acid/1 g extract*	mg Rutin/1g extract*
100% MeOH	138.5 ± 4.1 D**	69.4 ± 3.3 C**	123.06 ± 1.9 D**
75% MeOH	124.6 ± 10.0 F	37.7 ± 2.3 E	85.11 ± 0.97 H
50% MeOH	155.2± 6.1 C	41.4 ± 0.6 D	109.52 ± 1.2 E
25% MeOH	146.7± 6.3 C	37.8 ± 0.6 E	91.63 ± 0.96 G
95% EtOH	99.5± 2.4 G	35.4 ± 0.3 F	135.63 ± 1.0 C
75% EtOH	72.3 ± 1.8 H	30.1 ± 1.2 G	87.29 ± 2.0 H
50% EtOH	82.3 ± 2.5 H	24.5 ± 1.3 H	48.96 ± 0.60 I
25% EtOH	132.7 ± 1.4 E	34.2 ± 1.1 F	100.69 ± 2.0 F
100% H_2O	46.4 ± 0.52 I	14.9 ± 0.2 I	31.84 ± 0.54 J
MeOH-BuOH	368.1 ±1.9○ A	97.0 ± 1.3 B	350.42 ± 4.3 A
EtOH-BuOH	199.1 ± 3.3 B	150.7 ± 0.5 A	319.58 ± 3.5 B

* Results are expressed as average of three values ± SD.
**Different capital letters within column indicate significant difference ($p < 0.05$, $n = 3$).



* Results are expressed as average of three values ± SD. **Different capital letters within column indicate significant difference ($p < 0.05$, $n = 3$).

Figure 1: Effect of different extraction solvents on the AA.

Total phenolic content (TPC)

Statistical analysis showed that the extraction solvent has an effect on the Total phenolic content expressed as mg gallic acid per gram extract. The significant differences ($p < 0.05$) between the TPC and extraction solvent are indicated by different capital letters (A to I). It was found that highest TPC is for EtOH-

BuOH followed by MeOH-BuOH, followed by methanol (100% and 50%). Methanol 75% and 25% has not significantly different total phenolic content indicated by the letter E. Total phenolic content of ethanol extract has statistically lower TPC compared to methanol indicated by letters F, G, and H. The lowest TPC was found for water as extraction solvent, which is significantly lower than all other extraction solvents (See Table 3).

Total flavonoids content (TFC)

Results from statistical analysis showed that the extraction solvent has an effect on the total flavonoid content expressed as mg rutin per gram extract. The significant differences ($p < 0.05$) between the TFC and extraction solvent are indicated by different capital letters (A to J). It was found that the highest TFC is for MeOH-BuOH followed by EtOH-BuOH, followed by 95% ethanol and 100% methanol and 50% methanol, 25% ethanol, 25% methanol. Methanol and ethanol (both 75%) were found to have statistically similar TFC indicated by the capital letters H (about 85 mg rutin per g extract) followed by 50% ethanol solvent (48.96 ± 0.60). The lowest TFC was found when the fig leaves were extracted with water, which has 31.84 ± 0.54 mg rutin per g of extract (see Table 3).

Effect of collection season of fig leaves on TPC, TFC, and AA of fig leaves

To study the effect of date of collection of fig leaves on the antioxidant activity and total phenolic and flavonoids content, fig leaves were collected in May and October 2019. Results showed that there are significant differences in the AA of fig leaves extracted in May 2019 and October 2019, as indicated by capital letters (A and B). Same results were obtained for total phenolic content and total flavonoids content where significant differences were obtained for TPC and TFC when the leaves were collected between May and October.

Fig leaves extracts revealed a relatively high content of flavonoids and phenolic compounds and a high antioxidant activity during the two seasons May and October. The methanolic extract of May showed a higher activity than that of October. On the other hand, the ethanolic extract of October gave a higher activity than in May as shown in table 4.

The methanolic and ethanolic extracts of May showed a higher content of flavonoids and phenolic compounds than in October, and the highest results was obtained from butanol extract as shown in tables 5 and 6.

The principle of like dissolves like applies with fractionation with n-butanol. The compounds contained in the extract would be attracted by solvent with relatively similar polarity. The second extraction with n-butanol was carried out to separate compounds in the first extract according to their polarity so as concentrate the content of flavonoid and phenolic compounds[28]. Furthermore, studies showed that the solubility of flavonoid depends on the nature of flavonoids present in the extract either hydrophilic or hydrophobic[29]. The highest results obtained with n-butanol reveals the efficiency of butanol and the nature of flavonoid and phenolic compounds present that seems to be hydrophobic.

The high antioxidant activity is correlated to the presence of flavonoid and phenolic compounds[30]. Moreover, some phenolic compounds, with reported pharmacological properties have already been isolated from fig leaves, namely furanocoumarins like psoralen and bergapten, flavonoids like rutin, quercetin, and luteolin, phenolic acids like ferrulic acid, and phytosterols like taraxasterol[31,32].

Our results agree with previous studies in terms of AA. The results showed that methanolic extract gave higher antioxidant activity than aqueous extract[33]. However, our results were higher than other studies; TPC of Algerian fig leaf methanolic extract were found to be 42.8±0.35 mg GA/g extract[34-35].

Table 4. Effect of collection date of fig leaves on Antioxidant activity.

	mg $FeSO_4$/1g extract	
Extraction solvent	**May 2019***	**October 2019***
100% methanol	138.5 ± 4.1 A**	83.85 ± 7.03 B**
75% methanol	124.6 ± 10.0 A	94.63 ± 0.88 B
50% methanol	155.2± 6.1 A	108.59 ± 4.40 B
25% methanol	146.7± 6.3 A	134.35 ± 3.29 B
95% ethanol	99.5± 2.4 B	104.17 ± 3.90 A
75% ethanol	72.3 ± 1.8 B	99.86 ± 8.55 A
50% ethanol	82.3 ± 2.5 B	125.94 ± 1.36 A
25% ethanol	132.7 ± 1.4 B	140.60 ± 5.13 A
Water	46.4 ± 0.52 B	102.09 ± 7.75 A
Methanol-butanol	368.1 ±1.9 A	186.44 ± 20.20 B
Ethanol-butanol	199.1 ± 3.3 A	102.22 ± 7.38 B

* Results are expressed as average of three values ± SD.
**Different capital letters within row indicate significant difference ($p < 0.05$, $n = 3$).

Table 5. Effect of collection date of fig leaves on Total Phenolic Content.

	mg Gallic acid/1 g extract	
Extraction solvent	**May 2019***	**October 2019***
100% methanol	69.4 ± 3.3 A**	27.43 ± 1.90 B**
75% methanol	37.7 ± 2.3 A	24.87 ± 0.68 B
50% methanol	41.4 ± 0.6 A	26.18 ± 0.34 B
25% methanol	37.8 ± 0.6 A	30.11 ± 3.08 B
95% ethanol	35.4 ± 0.3 A	32.42 ± 0.20 B
75% ethanol	30.1 ± 1.2 A	23.87 ± 1.38 B
50% ethanol	24.5 ± 1.3 B	27.39 ± 0.61 A
25% ethanol	34.2 ± 1.1 A	27.37 ± 0.16 B
Water	14.9 ± 0.2 B	15.51 ± 1.54 A
Methanol-butanol	97.0 ± 1.3 A	58.86 ± 1.28 B
Ethanol-butanol	150.7 ± 0.5 B	37.91 ± 1.76 A

* Results are expressed as average of three values ± SD.
**Different capital letters within row indicate significant difference ($p < 0.05$, $n = 3$).

Table 6. Effect of collection date of fig leaves on Total Flavonoid Content.

	mg Rutin/1g extract	
Extraction solvent	**May 2019***	**October 2019***
100% methanol	123.06 ± 1.9 A**	85.47 ± 2.39 B**
75% methanol	85.11 ± 0.97 A	57.93 ± 0.69 B
50% methanol	109.52 ± 1.2 A	57.40 ± 1.66 B
25% methanol	91.63 ± 0.96 A	71.15 ± 1.88 B
95% ethanol	135.63 ± 1.0 A	123.97 ± 0.86 B
75% ethanol	87.29 ± 2.0 A	62.56 ± 1.46 B
50% ethanol	48.96 ± 0.60 A	45.04 ± 0.43 B
25% ethanol	100.69 ± 2.0 A	61.46 ± 0.68 B
Water	31.84 ± 0.54 A	9.77 ± 1.23 B
Methanol-butanol	350.42 ± 4.3 A	261.62 ± 5.10
Ethanol-butanol	319.58 ± 3.5 B	174.64 ± 6.73

* Results are expressed as average of three values ± SD.
**Different capital letters within row indicate significant difference ($p < 0.05$, $n = 3$).

HPLC-PDA detection of phytochemicals

Ten standards of flavonoids and phenolic compounds were separated by HPLC as shown in the below chromatogram (Figure 2).

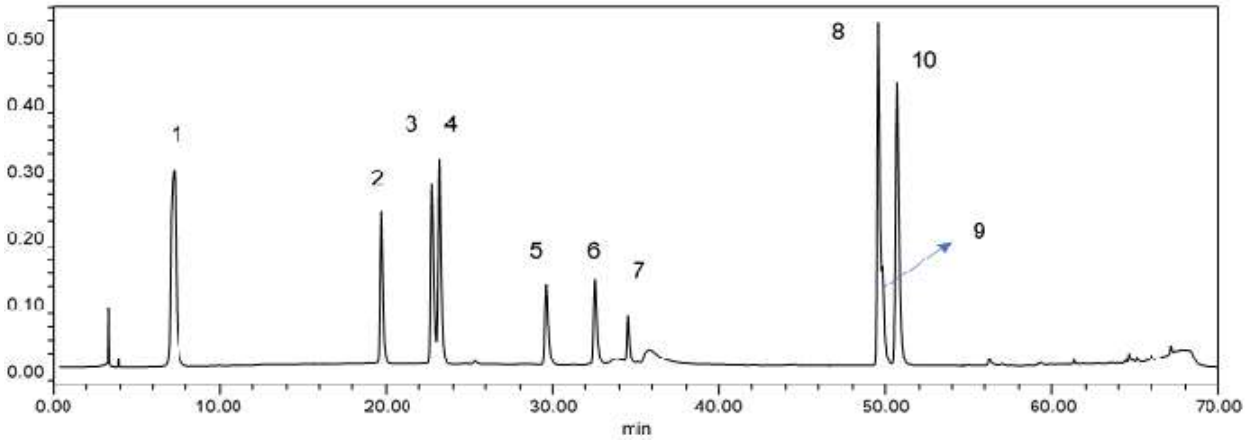


Figure 2. HPLC chromatogram for standards used: 1. Gallic acid, 2. Chlorogenic acid, 3. Caffeic acid, 4. Syringic acid, 5. Coumaric acid, 6. Ferulic acid, 7. Rutin, 8. Quercetin, 9. Luteolin, 10. *trans*-Cinnamic acid.

The following compounds were identified in sample extracts by HPLC: chlorogenic acid, caffeic acid, syringic acid, coumaric acid, rutin and *trans*-cinnamic acid as shown in figure 3. Quercetin was identified after acid hydrolysis of extracts in all sample extracts of May and October (100 methanol, 95% ethanol and water) as shown in figure 4. Acid hydrolysis and saponification are the most common means of releasing the acids. The acidic hydrolysis method involves treating the plant extract or the food sample itself with inorganic acid (HCl) at reflux or above reflux temperatures in aqueous or alcoholic solvents[36]. Phenolic acids such as, quercetin-3-O-glucoside and quercetin-3-O-rutinoside, have been isolated from the water extract of the leaves of *F. carica*[17]. On the other hand, ferulic acid was only identified in water extracts in May and October samples as shown in figure 5.

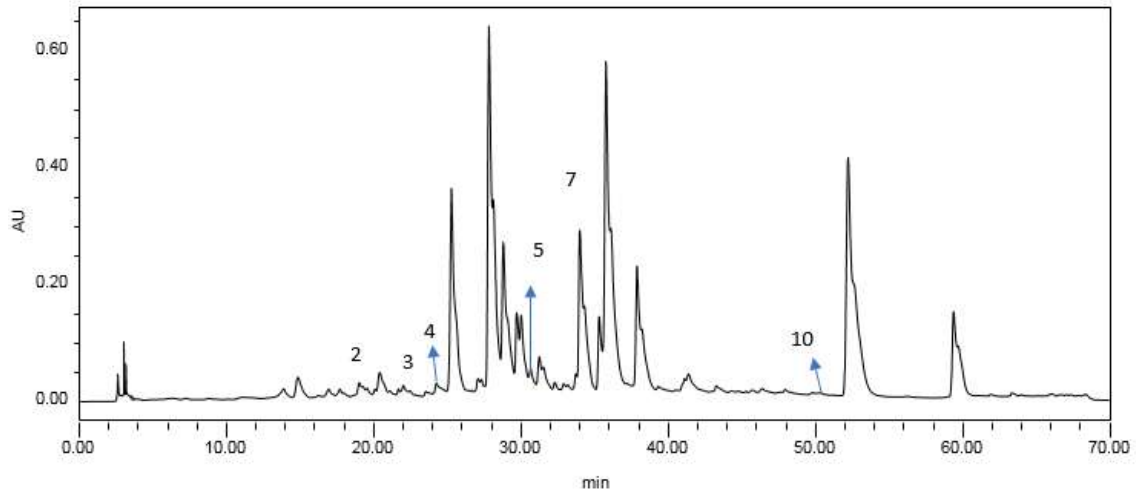


Figure 3. HPLC chromatogram for fig leaves collected in October 2019 extracted with 100% methanol at 300 nm.

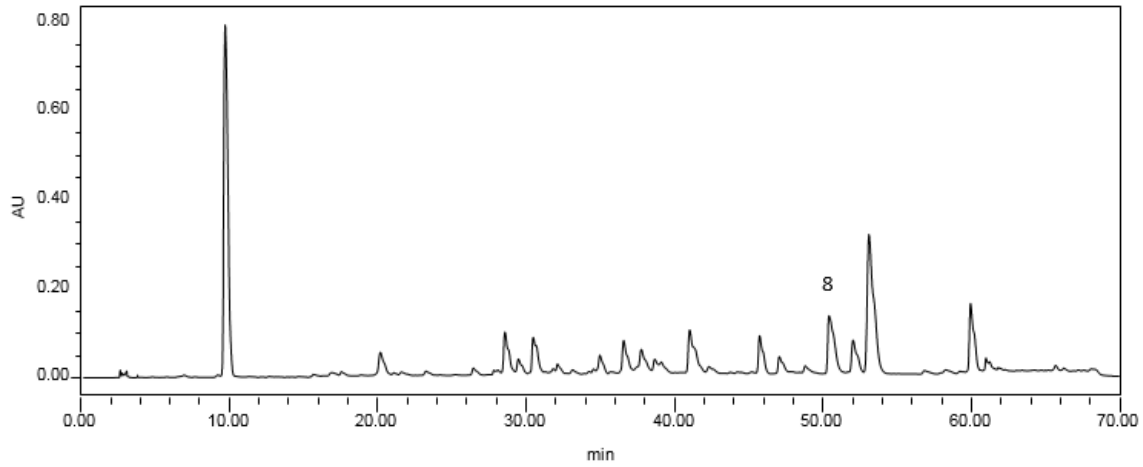


Figure 4. HPLC chromatogram for hydrolyzed methanol extract of October sample at 300 nm.

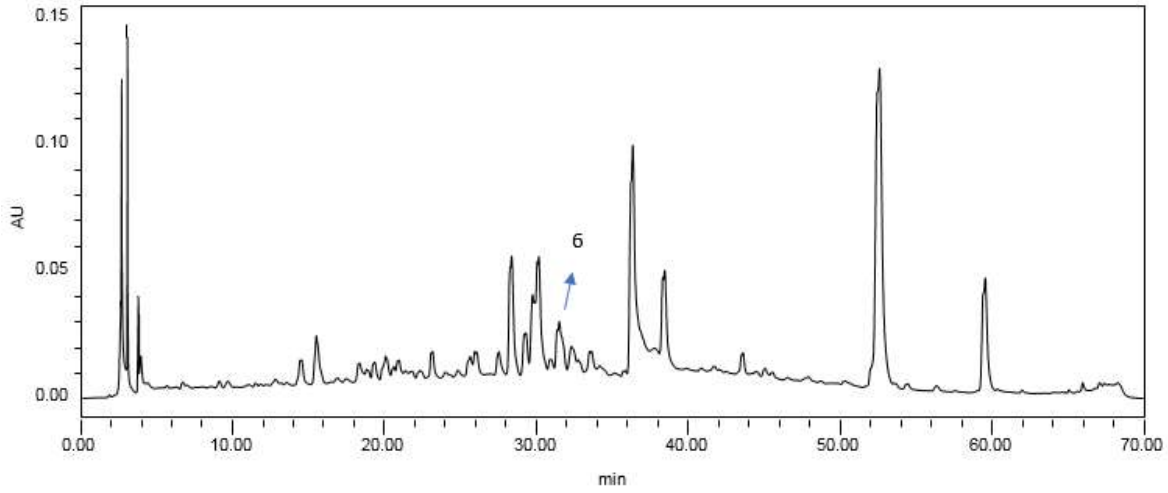


Figure 5. HPLC chromatogram for water extract of October sample at 300 nm.

Antimicrobial Activity

The oral cavity includes several sites that host several bacterial species. As a major entry to the human body, the oral cavity is considered as a chief site of human health concerns. The aim of this study was to investigate the antimicrobial effect of *F. carica* leaves extract on the oral flora, mainly Gram-positive bacteria, Additionally, *E. coli*, as Gram-negative bacteria, was investigated. Our results indicated the difference in antimicrobial activity between May and October samples. October samples showed no inhibition against *E. coli*, while 75% ethanol and 75% methanol extracts of May samples showed a relatively weak activity against *E. coli*.

October samples showed a higher activity than May samples against Gram-positive oral flora, and ethanol extracts of October samples revealed stronger activity than methanol extracts. 95% ethanol and 75% methanol extracts showed strong activity against Gram-positive bacteria oral flora, 75% of ethanol and methanol and 50% of methanol and ethanol extracts showed medium activity, 25% of ethanol and methanol extracts showed weak activity, while water extract showed no inhibition.

In general, May samples revealed a weak activity against Gram-positive oral flora, 95%, 75%, 50% ethanol extracts and 100% methanol extracts showed a medium activity, 75% methanol extract was weakly active, while all other extracts showed no activity against oral flora.

Our results are in agreement with previous studies on the activity of methanol extract of fig (*Ficus carica L.*) leaves in September against oral bacteria and *E. coli*[37]. However, further investigation should be made to explain the reason behind this. The extract content of late summer fig leaves samples must have contained some components that are not present in the early summer samples.

Our results showed that fig leaves contain a high content of phenolic and flavonoid compounds. Previous results showed that phenolic compounds possess diverse biological activities like antioxidant and antibacterial activity, as several reports have shown that some flavonoid compounds have antibacterial activity against oral bacteria[38]. Flavonoid compounds have their own mechanism against bacteria. Flavonoids inhibit nucleic acid synthesis, inhibit energy metabolism and disrupt cytoplasmic membrane function[39]. Flavonoids, as antibacterial agents, have multiple cellular targets. They are able to form complexes with proteins through secondary forces, such as hydrogen bond and hydrophobic effects, as well as by forming covalent bonds[40].

Conclusion

In this work, optimization of extraction method using Soxhlet extractor was performed applying ethanol, methanol and water solvents in different percentages. Seasonal variation showed that October plant consisted of high nutrients and polyphenolic compounds, thus showing high TPC and TFC values. This is attributed to the fact that this time of the year is the harvest time of fig fruits, and thus fruits and leaves are totally ripened and full of diverse constituents of compounds, mainly polyphenols and flavonoids. Utilizing a newly developed gradient elution HPLC-PDA method showed some main components of the extract. Fig leaves extract showed weak antimicrobial activity against Gram-negative bacteria, but was very active against oral flora. Our results propose fig leaves extracts as a good candidate for commercial oral hygiene product.

Acknowledgments

Authors are grateful to the Deanship of Research-Bethlehem University for financial support through the Palestinian Ministry of Higher Education and Research.

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