



King Saud University
Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com



ORIGINAL ARTICLE

Appraisal of wild fig fruits for nutra-pharmaceutical and biological attributes



Naveed Ahmad ^{a,*}, Farooq Anwar ^{b,c}, Ali Abbas ^d, Muhammad Shahid ^e,
Munawar Iqbal ^a, Samiah H. Al-Mijalli ^f, Shahid Iqbal ^a, Mengfei Tian ^g,
Chunjian Zhao ^g, Arif Nazir ^h

^a Department of Chemistry, Division of Science and Technology, University of Education, Lahore, Pakistan

^b Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

^c Institute of Chemistry, University of Sargodha, Sargodha 40100, Pakistan

^d Department of Chemistry, Govt. Islamia Graduate College, Chiniot, Pakistan

^e Department of Bioinformatics and Biotechnology, Government College University, Faisalabad 38000, Pakistan

^f Department of Biology, College of Sciences, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia

^g Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, China

^h Department of Chemistry, The University of Lahore, Lahore Pakistan

Received 8 September 2022; accepted 26 January 2023

Available online 1 February 2023

KEYWORDS

Wild fig;
Phenolic bioactive profiling;
Biological attributes;
Nutra-pharmaceuticals

Abstract The fruits of wild fig, native to Soon valley of Pakistan, were appraised for phenolic compounds and high-value nutrients. These fruits were shade-dried and extracted with different solvents of varying polarity to recover a wide range of antioxidant components, where hydroxyethanol exhibited highest extraction yield of antioxidant compounds. Among others, hydroxyethanol-derived crude concentration extracts (CCEs) and phenolic rich fractions (PRFs) showed greater amount of total phenolic, flavonoids and superior biological properties. The fruits of tested fruits were found to possess potassium (11.34 g/kg) and calcium (4.19 g/kg) as major elements, glucose (5.63g/100g DW) as dominant natural sugar and acetic acid (2.40 mg/100 g of dry matter) as principal organic acid. There was a considerable ($p < 0.05$) variation in phenolic content and biological properties of various extracts recovered with different solvents. Furthermore, the biological properties of tested fruits were found to be significantly correlated with their intrinsic total phenolic content (TPC) and total flavonoid content (TFC). Based upon the findings of present study, the fruits

* Corresponding author.

E-mail address: mnavedahmad@yahoo.com (N. Ahmad).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

of wild fig could be treated as promising source of beneficial nutrients and potent phenolic components for their applications in the development of nutraceuticals.

© 2023 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Reactive oxygen species (ROS) and free radicals can initiate oxidation in lipid molecules which is associated with the loss of organoleptic and nutritive properties of various food stuffs (Manian et al., 2008). The prevalence of different disorders including aging, metabolic syndromes, inflammations and cardiovascular disorders is directly linked to these radicals (Anwar et al., 2013). Antioxidant molecules have been documented to control the process of lipid oxidation. Due to the perceived toxic effects of synthetic antioxidants, scientists have been searching for safer plant-based-natural antioxidants with multiple medicinal properties.

Different plants and plant-derived foods are propitious source of folk medicine in different civilizations due to availability of potent secondary metabolites including phenolic and flavonoid compounds (Irfan et al., 2021). These plant bioactives are used to regulate and maintain different bodily functions (Manzoor et al., 2013) by representing their antagonistic effect against different ailments including aging, metabolic disorders, and inflammations (Noori et al., 2022). The issues of food security and public health with rapidly expanding population of the world, prompt us to explore vast resources of under-utilized parts (leaves, bark, fruits, roots and stem) of wild plants for important phytonutrients and bioactives which are essential to maintain the physiological functions in living organisms (Shahidi, 2009).

Pakistan has a variety of plant species for biological prospecting. Various types of medicinal flora have been found to be wildly grown in different areas of Pakistan which are extensively employed in traditional folk medicine due to their intrinsic phytonutrients and potent medicinal compounds (Ramadan et al., 2012). In this context, the fruits of wild fig are potential candidates for the exploration of valuable phytonutrients and bioactives with multiple therapeutic applications. Fig (*Ficus carica* L.), member of *Moraceae* family, is native to Syria and Iran. This fruit crop spread to other regions by humans. It grows in dry and warm climates. Its fruits can be used in fresh and dried form; however, its dried form is very popular in the world as the process of drying can enhance its stability by preserving its nutrients. Fig fruits have widely been utilized as food and medicine due to its nutrients including organic acids, individual sugars, different vitamins, important minerals and amino acids. There is no fat and cholesterol in the fruits of fig tree (Lazreg Aref et al., 2011). The natural potent antioxidants in figs have been believed to offer shielding effect to plasma proteins against oxidative stress and consequently the antioxidant attributes of plasma is enhanced (Vinson et al., 2005). The diet containing fig fruits has been published as one the healthiest food which is directly linked with longevity (Trichopoulos et al., 2006).

Due to the medicinal value of phenolic components and existing gap of scientific information motivated us to evaluate wild fruits for phytonutrients and phenolic compounds. The main objective of this project was to study the fruits of wild fig for beneficial nutrients, phenolic compounds and their biological characteristics.

2. Material and methods

2.1. Pretreatment, extraction and fractionation

The ripened fruits of wild fig were collected from Soon valley, district Khushab, Punjab, Pakistan (geographical coordi-

nates: 32°58'N 72°15'E, lowest temperature: 1 °C, highest temperature: 36 °C and annual rainfall: 50 cm). Prior to experimental procedure, collected fruit samples were authenticated by a taxonomist, then washed with tap water and shade-dried. These dried fruits (10.0 g) were milled and immersed in different solvents (100 mL) of varying polarity including absolute methanol, aqueous methanol (80:20, methanol:water), absolute ethanol, aqueous ethanol (80:20, ethanol:water), absolute acetone and aqueous acetone (80:20, acetone:water) for five days to extract bioactives (Ahmad et al., 2011).

After separating insoluble residual biomass through filtration (Whatman, 8μ), recovered extracts were freed from solvents under vacuum using rotovap (EYELA, Tokyo, Japan) to have CCEs which were further fractionated to produce PRFs (Maheshwari et al., 2011). Briefly, each CCE (5 g) was reconstituted in distilled water (100 mL) and processed for sequential extraction with *n*-hexane (100 mL) to eliminate non-polar components while ethyl acetate (100 mL) was used to purify phenolic bioactives. The additional solvent from each ethyl acetate extract was detached under vacuum using rotavapor to yield phenolic rich fraction (PRF). The recovered CCEs/PRFs were stored at −20 °C.

2.2. Total phenolic content (TPC)

The CCEs/PRFs of tested fruits were analyzed for TPC using Folin reagent method (Singleton and Rossi, 1965). TPC in different CCEs/PRFs were presented as gallic acid equivalents (GAE g per 100 g dry matter). Standards and samples were run in triplicate under same experimental protocols.

2.3. Total flavonoids content (TFC)

The CCEs/PRFs of tested fruits were analyzed for presence of TFC (Zhishen et al., 1999, Zhuang et al., 1992). TFC was given as catechin equivalents (CE g/100 g of dry matter). Standards and samples were run in triplicate under same experimental protocols.

2.4. Antioxidant properties

Antioxidant properties of different CCEs/PRFs, derived from the tested fruits, were assessed by employing various in-vitro antioxidant assays such as radical scavenging ability, reducing capacity and inhibition of peroxidation in lipid. Tepe et al. (2005) was used for DPPH radical scavenging activity (as IC₅₀ value). Reducing capacity was calculated using a protocol described by Yen et al. (2000). Tested extracts were appraised to retard the formation of peroxide in lipids following a method described by Ahmad et al. (2011). This inhibition potential directly correlated with antioxidant capacity of various CCEs/PRFs. All treatments and positive control were run under same experimental protocols.

2.5. Antimicrobial activity

Antimicrobial attributes of tested CCEs/PRFs were performed out against different infective microbes (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum*) using micro dilution broth and disc diffusion methods (NCCLS, 1999, 1997). Bacterial and fungal strains were cultured on nutrient agar (37 °C) and potato dextrose agar (29 °C), respectively. Standard drugs and negative control were also run under same experimental protocols. The growth inhibition zones (mm) of each tested microbe was measured.

Microbial (fungus and bacteria) cultures were grown on nutrient broth (37 °C) and sabouraud dextrose broth (29 °C), respectively, to assess minimal inhibitory concentration (MIC). Sterility control (CCE/PRF + NB + Tween 80), positive control (microbial culture only) and growth control (NB + Tween 80) were also executed under same experimental protocols. MIC value of CCE/PRF can be calculated by finding its lowest concentration that inhibited microbial growth completely.

2.6. Quantification of minerals

The evaluation of valuable minerals in tested fruits was done using inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Link et al., 1998). USEPA method (3051A) was employed for the digestion of sample with a digestion mixture (concentrated HCl: concentrated HNO₃, 1:3) in microwave oven at 182 °C for 9.5 mins followed by cooling, filtration, centrifugation and dilution of final reaction mixture with water to required volume for ICP-OES analysis. High-purity water (18 MΩcm) was used to prepare standard and sample's solutions.

2.7. Analysis of organic acids and individual sugars

According to Mahmood et al. (2012), tested fruits were appraised for the presence of organic acid and individual sugars. Briefly, the fruit juice was obtained by blending fresh aliquot (10 g) of fruits with distilled water (20 mL) in electrical blender (Deuron, GL-109) which was spun (3000 g) and then filtered (0.25 µm) to remove the suspended debris. Organic acid profiling of tested fruits juice was carried out by analyzing on HPLC (Varian Pro star, USA) equipped with UV-vis detector and RP-C₁₈ column (250 × 2.0 mm × 1/4") while individual sugars were estimated by analyzing on HPLC (Shimadzu, Japan) where amino packing column and refractive index detector (10A) were employed. Water was used as diluent in sugar analysis.

2.8. Other biological activities

The tested CCEs/PRFs were evaluated for inhibition of bio-film produced by different bacterial strains (*S. aureus* and *E. coli*) (Stepanović et al., 2000). Haemolytic and antithrombotic capacity of tested extracts/fractions were estimated by spectrophotometer method (Yang et al., 2005) and Prasad et al., (2006), respectively.

2.9. Statistical analysis

All the treatments were conducted in three replicates unless stated otherwise and data thus produced was statistically analyzed by analysis of variance (ANOVA) using STATISTICA 5.5 (Stat SoftInc, Tulsa, Oklahoma, USA) software. $P \geq 0.05$ was taken as probability value to indicate statistically significant difference.

3. Results and discussion

3.1. Extraction yield

Fruits of wild fig were extracted with a range of extracting solvents to recover extractable bioactives of varying polarity. The yield of CCEs (g/100 g) were determined to vary considerably in the range of 3.88–53.25 %. Hydroxyethanol recovered the highest yield (53.25 %) and the lowest yield (3.88 %) was extracted with absolute acetone. The tested fruit's CCE are in crude form and thus contain some moieties of interfering components that offer resistance to detect and consequently alter their antioxidant properties. Therefore, these interfering impurities were removed from CCEs by sequential liquid-liquid extraction (LLE) to get PRFs whose yield was found to range from 2.70 g/100 g to 19.00 g/100 g of CCE. The maximum yield (19.00 %) was recovered from ethanolic CCE while hydroxymethanolic CCE produced the lowest yield (2.70 %) of PRF (Table 1).

Hydroxyethanol recovered higher amount of bioactives from tested fruits exhibiting its superior efficacy as compared to other extracting solvents. The yield of bioactives using different solvents was varied significantly ($p < 0.05$). Manzoor et al., (2013) and Shabir et al., (2011) showed higher efficiency of hydroxymethanol and hydroxyethanol to extract bioactives from different plant matrices. In this analysis, the efficiency of hydroxymethanol and hydroxyethanol was found to be better than other extracting solvents for optimum recovery of antioxidant molecule from tested fruits.

Soni et al. (2014) recovered the yield (11.47 %) of crude extract from the fruits of fig using methanol which is in lesser quantity compared to methanolic extract of current analysis. This difference in extraction yield has been attributed to various factors including agroclimatic conditions, type of soil and content of bioactive components (Mahmood et al., 2012).

3.2. Total phenolic content (TPC) and total flavonoids content (TFC)

The antioxidant activity of various plants is attributed to the natural occurrence of physiologically active components including flavonoid and phenolic components in them (Katalinic et al., 2006). Due to antioxidant activity, plants have extensive application in food industry. TPC and TFC in tested CCEs/PRFs were calculated to be varied from 0.07 to 1.87 % GAE and 0.01 to 0.13 % CE, respectively. Hydroxyethanol-derived CCE exhibited maximum TPC (1.87 % GAE) and TFC (0.13 % CE) and acetone-derived CCE displayed the lowest of TPC (0.07 % GAE) and TFC (0.01 % CE). (Table 1).

Table 1 Yield, TPC and TFC.

Solvents	Yield (g/100 g)		TPC (GAE g/100 g)		TFC (CE g/100 g)	
	CCE	PRF	CCE	PRF	CCE	PRF
Methanol	36.10 ± 1.16 ^d	7.40 ± 0.67 ^b	1.33 ± 0.10 ^b	2.49 ± 0.28 ^b	0.13 ± 0.02 ^a	0.35 ± 0.03 ^b
Hydroxymethanol	53.25 ± 2.14 ^a	2.70 ± 0.35 ^c	1.22 ± 0.07 ^{ab}	2.24 ± 0.19 ^b	0.11 ± 0.04 ^{ab}	0.36 ± 0.07 ^b
Ethanol	39.12 ± 1.03 ^c	19.00 ± 1.36 ^a	1.34 ± 0.08 ^b	2.74 ± 0.21 ^b	0.07 ± 0.03 ^b	0.40 ± 0.11 ^{ab}
Hydroxyethanol	49.63 ± 2.13 ^b	8.90 ± 0.86 ^b	1.87 ± 0.07 ^a	3.80 ± 0.35 ^a	0.13 ± 0.06 ^a	0.53 ± 0.04 ^a
Acetone	3.88 ± 0.41 ^f	—	0.07 ± 0.01 ^d	—	0.01 ± 0.01 ^c	—
Hydroxyacetone	7.63 ± 0.52 ^e	—	0.12 ± 0.03 ^c	—	0.02 ± 0.01 ^c	—

Values (mean ± SD) are average of three replicates, analyzed individually. Different superscripts in a column denotes significant differences ($p < 0.05$) of mean among various solvents.

As a result of fractionation of tested fruit's crude extracts, impurities were removed to produce PRFs in which TPC and TFC varied in the range of 2.24–3.80 GAE (%) and 0.35–0.53 CE (%), respectively. The maximum TPC (3.80 % GAE) and TFC (0.53 % CE) was exhibited by PRF derived from hydroxyethanolic CCE, while the lowest TPC (2.24 % GAE) and TFC (0.35 % CE) was displayed by PRF derive from hydroxymethanolic CCE, respectively. TPC was considerably ($p < 0.05$) varied in CCEs/PRFs, extracted with different solvents from tested fruits. In this analysis, phenolic components in PRFs were found to be significantly enhanced (3–4 times) than their corresponding CCEs which is ascribed to the successful fractionation CCEs through LLE.

The determination of TPC and TFC from tested fruits was scarce. The results of TPC of wild fig fruit was higher when compared to those of various cultivars of fig fruits by, (Nakilcioğlu and Hışıl, 2013), (Çalışkan and Aytekin Polat, 2011), (Bey et al., 2013) and (Soni et al., 2014), nevertheless, lesser to results of Pourghayoumi et al. (2012). TFC in fig fruits was in lesser quantity than the results reported by (Nakilcioğlu and Hışıl, 2013). These differences in concentration of TPC and TFC in various plant matrices can be associated to different factors including genetics, harvesting season, maturity stage and agro-climatic regions.

3.3. Reducing capacity

The CCEs/PRFs, recovered with different extracting solvents from fig fruits, were explored for their ability to reduce iron (III) into iron (II) by phenolic compounds in them which have been documented to behave like reducing agents to convert ferric into ferrous (Joshi et al., 2010). Therefore, reducing ability (antioxidant attributes) of plant matrices might be correlated to phenolic identities in them (Zou et al., 2004). By enhancing the concentration of CCEs/PRFs, a regular increase in its reducing capacity was recorded (Table 2). There was a notable ($p < 0.05$) difference in the reducing capacities of tested CCEs/PRFs. Similar trends of enhanced reducing capacity with increasing concentration of extracts was also recorded by Ahmad et al., (2011).

3.4. Free radical scavenging ability

DPPH is stable free radical whose $\lambda^{\max}$ falls in visible region (515–528 nm) that turns to yellow by accepting hydrogen from phenolic component present in plant extract. Therefore, free radical scavenging capacity has been documented to be enhanced with the increase of bioactives in the extract (Larrauri et al., 1999). Wild fig's CCEs/PRFs were explored for their ability to scavenge free radical and the results thus obtained were presented as IC₅₀ values that ranged from 0.15 to 1.96 mg/mL and 0.04 to 0.23 mg/mL, respectively (Table 2).

The hydroxyethanol-derived CCE/PRF showed lowest IC₅₀ value (0.15 mg/mL, 0.04 mg/mL) representing their maximum radical scavenging capacity compared to other extracts/fractions. BHT was also run along with other treatments and its radical scavenging ability was found to be higher than those of investigated CCEs/PRFs. PRFs of tested fruits exhibited higher ($p < 0.05$) free radical capacity (0.04–0.23 mg/mL) compared to CCEs (0.15–1.96 mg/mL) showing their higher antioxidant attributes which is ascribed to greater amount of phenolics in them as a result of successful fractionation of CCEs. The capacity of plant extract to scavenge free radicals has been found to be directly associated with their phenolic content (Siddhuraju et al., 2002).

Wild fig fruit's CCEs/PRFs were evaluated for the first time to scavenge free radical. The antioxidant properties (IC₅₀ value) of tested fruit was lower compared to the results of Uddin, (2013), Faleh et al. (2012), Nakilcioğlu and Hışıl, (2013) and Pourghayoumi et al. (2012). These differences in DPPH radical scavenging ability of various plant matrices can be associated to different factors including genetics, harvesting season, maturity stage and agro-climatic regions.

3.5. Inhibition of lipid peroxidation

The oxidation of lipids produces peroxides which combine with thiocyanate to give a complex ($\lambda^{\max} = 500$ nm). The tested CCEs/PRFs were appraised to hinder the formation of peroxide in lipids which were found to vary over the range

Table 2 Reducing capacity, DPPH radical scavenging activity and Inhibition of peroxidation in linoleic acid.

Solvents		Reducing Power ($\lambda = 700$ nm)				IC ₅₀ value (mg/mL)	Inhibition of peroxidation (%)
		5 mg	10 mg	15 mg	20 mg		
Methanol	CCE	0.25 ± 0.04	0.77 ± 0.06	0.95 ± 0.02	1.10 ± 0.07	0.51 ± 0.07 ^b	59.16 ± 3.81 ^b
	PRF	0.55 ± 0.04	0.64 ± 0.07	0.71 ± 0.02	0.93 ± 0.04	0.23 ± 0.04 ^a	72.20 ± 2.39 ^{ab}
Hydroxymethanol	CCE	0.22 ± 0.03	0.39 ± 0.04	0.52 ± 0.04	0.64 ± 0.05	0.34 ± 0.04 ^c	63.35 ± 3.57 ^a
	PRF	0.55 ± 0.07	0.59 ± 0.02	0.86 ± 0.05	0.93 ± 0.07	0.16 ± 0.03 ^b	74.11 ± 2.58 ^{ab}
Ethanol	CCE	0.21 ± 0.01	0.36 ± 0.05	0.51 ± 0.03	0.64 ± 0.04	0.28 ± 0.05 ^c	58.37 ± 2.72 ^b
	PRF	0.70 ± 0.03	0.84 ± 0.04	0.86 ± 0.07	0.89 ± 0.03	0.08 ± 0.03 ^c	70.46 ± 2.16 ^b
Hydroxyethanol	CCE	0.20 ± 0.05	0.43 ± 0.02	0.53 ± 0.04	0.70 ± 0.02	0.15 ± 0.03 ^d	65.88 ± 3.37 ^a
	PRF	0.54 ± 0.05	0.58 ± 0.06	0.82 ± 0.06	0.95 ± 0.02	0.04 ± 0.01 ^d	82.22 ± 1.96 ^a
Acetone	CCE	0.09 ± 0.02	0.21 ± 0.01	0.31 ± 0.02	0.40 ± 0.01	1.96 ± 1.41 ^a	37.05 ± 1.87 ^d
	PRF	—	—	—	—	—	—
Hydroxyacetone	CCE	0.09 ± 0.01	0.14 ± 0.02	0.21 ± 0.01	0.27 ± 0.03	0.61 ± 0.09 ^b	43.63 ± 2.49 ^c
	PRF	—	—	—	—	—	—

Explanations as given in Table 1.

of 37.05–65.88 % and 70.46–82.22 %, respectively. Hydroxyethanol-derived CCE/PRF inhibited the formation of peroxides to the highest levels 65.88 % and 82.22 %, respectively, while the minimum potential was observed for acetone-derived CCE (37.05 %) and ethanolic PRF (70.46 %). Hydroxyethanolic CCE/PRF of tested fruits were found to hinder the formation of peroxides in linoleic acid representing their higher antioxidant properties which is positively correlated with occurrence of higher amount of phenolics in them. Positive control (BHT) was also processed with other treatments to retard the formation of peroxides in linoleic acid which was found to be higher (92.01 %) than other tested CCEs/PRFs of tested fruits. PRFs exhibited superior inhibition potential than those of CCEs, which is an indication of successful fractionation of CCEs to purify phenolic identities. The findings of this study could not be compared with others due to unavailability of reports in the literature.

3.6. Antimicrobial attributes

The occurrence of potent antimicrobial compounds in plants makes them applicable in folk medicinal system to cure different ailments (Ahmad et al., 2011; Hussain et al., 2011, 2008). In order to detect these potential medicinal compounds, antimicrobial properties of CCEs/PRFs were determined by performing disc diffusion plate assay and measurement of MIC values (Table 3). The lowest concentration of bioactive component with minimum microbial growth is referred as MIC (Walsh et al., 2003).

The tested CCEs/PRFs were assessed to be most effective against *S. aureus* with bigger inhibition zone (7–17 mm and 15–18 mm) and corresponding lower MIC values (182–247 µg/

mL and 168–193 µg/mL), respectively. Among others, hydroxymethanolic CCE/PRF showed lowest MIC value (182 µg/mL and 172 µg/mL) representing their maximum antibacterial properties against *S. aureus*, while acetone derived extracts showed minimum antibacterial attributes with higher MIC value (223–258 µg/mL). Positive control was also processed under similar set of experimental conditions of treatments and exhibited higher ($p < 0.05$) antibacterial potential compared to tested CCEs/PRFs.

The tested CCEs/PRFs were most effective against *F. oxysporum* with bigger zone of inhibition (6–17 mm and 11–18 mm) and corresponding lower MIC values (187–273 µg/mL and 169–209 µg/mL), respectively. CCEs/PRFs showed lowest antifungal properties against *A. flavus* with smaller zone of inhibition (6–12 mm; 11–14 mm) with greater MIC (206–280 µg/mL; 201–245 µg/mL), respectively (Table 4). In comparison to others, hydroxyethanolic CCE/PRF exhibited maximum antifungal attributes indicating higher concentration of intrinsic bioactive compounds. Various biological properties including antioxidant and antimicrobial potential of different plants have been linked with the natural occurrence of bioactives in them (Ahmad et al., 2011).

3.7. Inhibition of biofilm formation

Different bacterial strains have been known to exhibit biofilm formation for their improved resistance against a variety of antibiotics which is serious environmental and health concerns. Isolation of different medicines from plant matrices have gained the attention of scientists to control various infectious diseases caused by multiple-drug resistant bacterial strains (Prabu et al., 2006).

Table 3 Antibacterial activity.

Solvents	Zone of inhibition (mm)						MIC (µg/mL)					
	CCE			PRF			CCE			PRF		
	<i>S. aureus</i>	<i>E.coli</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>E.coli</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>E.coli</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>E.coli</i>	<i>B. cereus</i>
Methanol	12.0 ± 0.4 ^{c_{ab}}	10.0 ± 0.6 ^{d_c}	9.0 ± 0.4 ^{d_a}	15.0 ± 0.9 ^{c_b}	11.0 ± 0.8 ^{c_b}	10.0 ± 0.5 ^{d_a}	217 ± 9 ^{b_c}	241 ± 9 ^{a_a}	225 ± 7 ^{b_c}	193 ± 12 ^{a_{ab}}	214 ± 9 ^{a_a}	229 ± 11 ^{a_a}
Hydroxymethanol	15.0 ± 0.3 ^{b_c}	14.0 ± 0.7 ^{b_c}	11.0 ± 0.6 ^{c_b}	16.0 ± 0.8 ^{c_d}	16.0 ± 0.7 ^{c_a}	12.0 ± 0.7 ^{c_b}	182 ± 10 ^{d_b}	211 ± 9 ^{b_c}	264 ± 9 ^{a_a}	172 ± 8 ^{b_a}	184 ± 7 ^{b_a}	210 ± 9 ^{a_{ab}}
Ethanol	15.0 ± 0.4 ^{a_{bc}}	13.0 ± 0.8 ^{c_b}	9.0 ± 0.5 ^{d_a}	17.0 ± 0.6 ^{c_d}	14.0 ± 0.6 ^{d_a}	10.0 ± 0.8 ^{d_b}	195 ± 10 ^{c_d}	207 ± 9 ^{c_a}	231 ± 7 ^{b_c}	177 ± 5 ^{b_{ab}}	197 ± 9 ^{a_{ab}}	225 ± 8 ^{b_b}
Hydroxyethanol	17.0 ± 0.7 ^{b_a}	16.0 ± 0.9 ^{b_b}	13.0 ± 0.7 ^{b_a}	18.0 ± 0.9 ^{b_{ab}}	20.0 ± 1.1 ^{b_a}	14.0 ± 0.6 ^{b_a}	190 ± 8 ^{c_d}	194 ± 8 ^{d_b}	211 ± 5 ^{c_b}	168 ± 8 ^{b_b}	152 ± 6 ^{c_{ab}}	194 ± 9 ^{c_b}
Acetone	7.0 ± 0.2 ^{d_b}	9.0 ± 0.5 ^{d_a}	6.0 ± 0.3 ^{c_b}	—	—	—	247 ± 9 ^{a_{ab}}	223 ± 8 ^{c_b}	258 ± 8 ^{a_b}	—	—	—
Hydroxyacetone	7.0 ± 0.3 ^{b_d}	10.0 ± 0.4 ^{d_{ab}}	7.0 ± 0.3 ^{c_b}	—	—	—	231 ± 9 ^{b_{ab}}	216 ± 9 ^{b_c}	237 ± 7 ^{b_b}	—	—	—
Rifamycin	24 ^a	26 ^a	18 ^a	24 ^a	26 ^a	18 ^a	124 ^c	96 ^c	146 ^c	124 ^c	96 ^d	146 ^c

Explanations as given in Table 1.

Table 4 Antifungal activity.

Solvents	Zone of inhibition (mm)						MIC(µg/mL)					
	CCE			PRF			CCE			PRF		
	<i>A. niger</i>	<i>A. flavus</i>	<i>F. oxysporum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>F. oxysporum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>F. oxysporum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>F. oxysporum</i>
Methanol	8.0 ± 0.6 ^{d_c}	10.0 ± 0.8 ^{c_a}	10.0 ± 0.8 ^{c_a}	10.0 ± 0.8 ^{d_b}	11.0 ± 0.8 ^{c_b}	11.0 ± 0.6 ^{c_a}	268 ± 8 ^{b_a}	257 ± 10 ^{a_b}	243 ± 10 ^{b_b}	229 ± 8 ^{b_a}	218 ± 9 ^{b_a}	209 ± 8 ^{a_c}
Hydoxymethanol	14.0 ± 0.8 ^{b_c}	11.0 ± 0.8 ^{b_c}	15.0 ± 0.9 ^{c_a}	11.0 ± 0.7 ^{d_b}	14.0 ± 0.9 ^{b_b}	16.0 ± 0.9 ^{c_a}	217 ± 11 ^{c_a}	229 ± 8 ^{c_a}	209 ± 8 ^{c_b}	256 ± 10 ^{a_a}	201 ± 7 ^{c_a}	188 ± 6 ^{b_b}
Ethanol	9.0 ± 0.7 ^{d_c}	9.0 ± 0.7 ^{c_b}	12.0 ± 0.9 ^{d_a}	13.0 ± 0.6 ^{c_b}	10.0 ± 0.8 ^{c_b}	14.0 ± 0.5 ^{d_a}	251 ± 12 ^{b_c}	234 ± 11 ^{c_a}	205 ± 9 ^{c_b}	208 ± 11 ^{c_a}	245 ± 6 ^{a_a}	194 ± 5 ^{b_{ab}}
Hydroxyethanol	16.0 ± 0.8 ^{b_a}	12.0 ± 0.9 ^{b_b}	17.0 ± 1.1 ^{b_a}	17.0 ± 0.8 ^{a_b}	13.0 ± 0.8 ^{b_a}	18.0 ± 0.7 ^{b_a}	198 ± 7 ^{d_{ab}}	206 ± 13 ^{b_d}	187 ± 6 ^{d_b}	183 ± 7 ^{d_b}	221 ± 8 ^{b_{ab}}	169 ± 5 ^{c_c}
Acetone	3.0 ± 0.4 ^{f_c}	9.0 ± 0.5 ^{a_c}	6.0 ± 0.4 ^{e_a}	—	—	—	327 ± 11 ^{a_a}	251 ± 9 ^{b_b}	273 ± 8 ^{a_c}	—	—	—
Hydroxyacetone	7.0 ± 0.8 ^{b_b}	6.0 ± 0.3 ^{d_b}	8.0 ± 0.5 ^{f_a}	—	—	—	269 ± 9 ^{a_b}	280 ± 10 ^{a_a}	248 ± 9 ^{c_b}	—	—	—
Fluconazole	23 ^a	24 ^a	20 ^a	23 ^a	24 ^a	20 ^a	134 ^c	126 ^f	160 ^c	134 ^d	126 ^d	160 ^c

Explanations as given in Table 1.

In this experiment, tested CCEs/PRFs were applied against *E. coli* and *S. aureus* to retard biofilm formation and their efficacy was found to vary from 26.15 to 78.34 % and 63.29 to 80.71 %, respectively (Table 5). The maximum biofilm inhibition potential (76.16–78.34 % and 72.67–80.71 %) was expressed by CCE/PRF, recovered with hydroxyethanol, and acetone-derived CCE (26.15–45.93 %)/methanolic PRF (63.29–66.13 %) showed the lowest potential to inhibit biofilm formation compared to other extracts/fractions. Hydroxyethanolic CCE/PRF showed better biofilm inhibition potential which might be associated with the occurrence of higher phenolic contents. Due to the pre-concentration, PRFs exhibited superior biofilm inhibition potential than CCEs. The rifamycin (reference drug) showed higher inhibition potential (87.91–89.43 %) compared to tested CCEs/PRFs. The findings of this study could not be compared with others due to unavailability of reports in the literature.

3.8. Thrombolytic efficiency

The use of synthetics drugs to cure the problem of thrombus in living organisms has some severe health concerns. Therefore, a variety of medicinal plants have been explored to exhibit their potential to treat thrombus which might be associated to the occurrence of potent biological active components (McLaughlin et al., 1998, Maheshwari et al., 2011).

Tested CCEs/PRFs were evaluated for thrombolytic potential which ranged from 38.74 to 55.21 % and 43.51 to 66.52 %, respectively (Table 5), where hydroxyethanolic CCE (55.21 %) and PRF (66.52 %) showed highest thrombolytic efficiency and the lowest thrombolytic activity was exhibited by acetone-derived CCE (38.74 %) and methanolic PRF (43.51 %). Due to its enhanced phenolic contents, the thrombolytic ability of hydroxyethanolic CCE/PRF was also higher when compared to other extracts/fractions. Furthermore, thrombolytic activity of PRFs has been noted to be higher in comparison to their crossponding CCEs which might be attributed to purification/fractionation of CCEs. Standard drug (streptokinase) was also processed along with other treatments and its thrombolytic potential (87.91–89.43 %) was higher than tested CCEs/PRFs of wild fig fruits. The findings of this study could not be compared as there is no report available on thrombolytic activity of wild fig fruits.

3.9. Haemolytic capacity

Erythrocytes are one of the most important types of blood cell which are used to deliver oxygen and drug molecules to different body tissues (Hamidi and Tajerzadeh, 2003). Oxidative stress has been found to produce hemolysis by damaging the erythrocyte (Ko et al., 1997). Tested CCEs/PRFs were examined for hemolysis which was found to be in the range of 2.17 to 3.32 % and 2.51 to 2.72 %, respectively, where lowest hemolysis (2.17 %) was exhibited by ethanolic CCE and the highest (3.32 %) was displayed by acetone-derived CCE, compared to other tested CCEs. Among other PRFs, the minimum hemolysis (2.51 %) was recorded for methanolic PRF and highest hemolysis (2.72 %) was showed by hydroxymethanolic PRF (Table 5).

The damaging effect of tested PRFs on erythrocytes was found to be 1–2.50 times lower when compared to those of

Table 5 Biofilm inhibition, hemolytic and thrombolytic activity.

Solvents	Biofilm inhibition (%)				Hemolytic potential (%)		Thrombolytic capacity (%)	
	CCEs		PRFs		CCEs	PRFs	CCEs	PRFs
	<i>S.aureus</i>	<i>E.coli</i>	<i>S.aureus</i>	<i>E.coli</i>				
Methanol	64.13 ± 1.45 ^{cd}	61.09 ± 1.73 ^d	66.13 ± 1.74 ^d	63.29 ± 1.56 ^d	2.83 ± 0.10 ^b	2.51 ± 0.08 ^b	40.31 ± 1.75 ^{cd}	43.51 ± 1.51 ^c
Hydroxymethanol	68.71 ± 1.88 ^b	70.31 ± 1.91 ^c	72.10 ± 1.92 ^b	71.32 ± 1.70 ^b	2.22 ± 0.13 ^{cd}	2.72 ± 0.03 ^a	42.51 ± 1.01 ^c	51.81 ± 1.61 ^b
Ethanol	74.16 ± 1.52 ^{bc}	67.31 ± 1.95 ^{cd}	80.43 ± 2.02 ^a	68.52 ± 1.56 ^c	2.39 ± 0.12 ^c	2.56 ± 0.05 ^b	47.32 ± 1.40 ^b	49.32 ± 1.57 ^b
Hydroxyethanol	78.34 ± 1.60 ^a	76.16 ± 2.25 ^a	80.71 ± 1.88 ^a	72.67 ± 1.21 ^{ab}	2.17 ± 0.10 ^d	2.56 ± 0.06 ^b	55.21 ± 1.65 ^a	66.52 ± 1.72 ^a
Acetone	26.15 ± 1.19 ^e	45.93 ± 2.04 ^b	—	—	3.32 ± 0.11 ^a	—	38.74 ± 0.79 ^d	—
Hydroxyacetone	41.09 ± 1.70 ^b	62.45 ± 1.69 ^d	—	—	2.94 ± 0.15 ^b	—	39.09 ± 1.05 ^d	—
Control (Rifamycin)	87.91 ± 4.12 ^a	89.43 ± 3.94 ^a	87.91 ± 4.12 ^a	89.43 ± 3.94 ^a	—	—	—	—
PBS	—	—	—	—	1.54	—	—	—
Triton X-100	—	—	—	—	100	—	—	—
Water	—	—	—	—	—	—	3.84	—
Streptokinase	—	—	—	—	—	—	92.34	—

Explanations as given in Table 1.

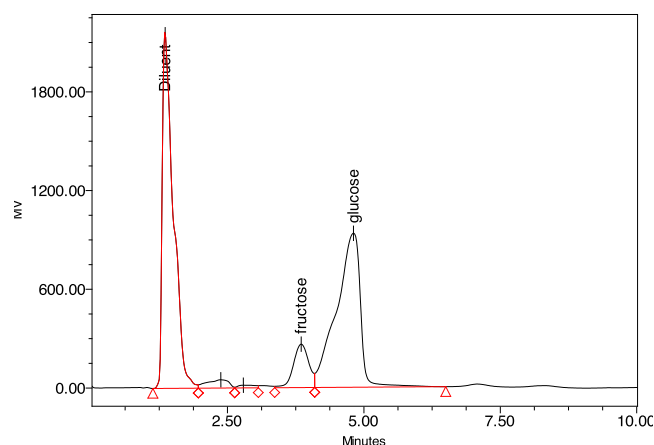


Fig. 1 Estimation of individual sugars in Fig fruit.

CCEs which might be linked with improved phenolic content. The hemolytic potential of PBS (1.54 %) was observed to be lower than CCEs/PRFs of tested fruits.

3.10. Individual sugars and organic acid profiling

Organoleptic characteristics of different plant-derived food is attributed to its individual sugars and organic acids. The concentration of organic acids and individual sugars were found

to fluctuate in different phases of maturation (Zhang et al., 2021).

The fruits of wild fig were evaluated for individual sugars and organic acids. Glucose (5.63 %) was detected as the most dominant individual sugar followed by fructose (0.98 %) (Fig. 1). According to the results of present analysis, acetic acid was recorded as major organic acid (2.40 mg/100 g DW) with relatively lower concentration of citric, malic, gluconic and oxalic acids (Tables 6). The varying concentration of organic acids has been documented in different plant based foods due to varietal and difference in weather conditions of experimental sites (Poyrazoğlu et al., 2002). The findings of current assay recommended the consumption of tested fruits as a excellent source of organic acids and natural sugars.

Slatnar et al. (2011) analyzed fruits of fig cultivars from Slovenia to contain malic (0.52–9.07 g/kg) and citric acids (1.36–10.54 g/kg), respectively. Malic acid (9.89–32.64 %), oxalic acid (1.11–8.54 %) and citric acid (53.06–84.97 %) were found to be present as principal organic acids in Japanese fig cultivars fruit's juice (Shiraishi et al., 1996).

3.11. Estimation of minerals

The tested fruits were appraised for the presence of useful minerals. Potassium (11339.22 mg/kg) and calcium (4193.69 mg/kg) were detected as major minerals and substantial concentration of other high-value minerals including sodium, magnesium, phosphorus and iron were also present

Table 6 Estimation of individual sugar and organic acid.

Sugar content (g/100 g DW)				Organic acid (mg/100 g)					
Glucose	Sucrose	Galactose	Fructose	Succinic acid	Gluconic acid	Malic acid	Oxalic acid	Citric acid	Acetic acid
5.63 ± 0.32	ND	ND	0.98 ± 0.19	ND	0.12 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.02	2.40 ± 0.07

Explanations as given in Table 1.

ND: not determined.

Table 7 Mineral profiling.

Elements	Concentration (mg/kg)	Elements	Concentration (mg/kg)
Al	109.50 ± 6.54	Se	2.47 ± 0.04
Ca	4193.69 ± 64.02	Cu	6.52 ± 0.07
Ba	5.44 ± 0.09	Cr	1.11 ± 0.02
Mg	2084.74 ± 24.15	Na	217.72 ± 11.25
La	0.31 ± 0.02	Fe	115.43 ± 9.85
Mn	10.76 ± 1.12	B	20.52 ± 5.64
Ni	1.75 ± 0.03	Be	ND
Sr	42.78 ± 4.6	Co	0.05 ± 0.01
Zn	11.53 ± 2.21	P	655.17 ± 19.64
K	11339.22 ± 129	Sb	0.77 ± 0.02
Ti	ND	Si	133.10 ± 7.98
Cd	0.06 ± 0.01	V	ND
Pb	2.57 ± 0.02		

Explanations as given in Table 1.

ND: Not Detected.

Table 8 Correlation analysis between TPC and biological attributes of tested wild fruits.

	TPC	IC ₅₀ Value	Inhibition Potential	Antibacterial activity			Antifungal activity			Biofilm inhibition		Hemolytic activity	Thrombolytic activity
				S.aureus	E.coli	B. cereus	A. niger	A. flavus	F. oxysporum	S. aureus	E.coli		
TPC	1												
IC ₅₀ Value	-0.762**	1											
Inhibition Potential	0.804**	-0.807**	1										
Antibacterial (S.aureus)	0.890**	-0.829**	0.841**	1									
Antibacterial (E.coli)	0.759**	-0.816**	0.852**	0.792**	1								
Antibacterial (B. cereus)	0.702**	-0.717**	0.547**	0.703**	0.621**	1							
Antifungal (A. niger)	0.816**	-0.813**	0.864**	0.859**	0.845**	0.749**	1						
Antifungal (A. flavus)	0.526*	-0.641**	0.810**	0.614**	0.695**	0.618**	0.719**	1					
Antifungal (F. oxysporum)	0.754**	-0.841**	0.787**	0.802**	0.826**	0.749**	0.867**	0.687**	1				
Biofilm inhibition (S. aureus)	0.787**	-0.749**	0.820**	0.817**	0.896**	0.658**	0.895**	0.644**	0.814**	1			
Biofilm inhibition (E.coli)	0.324	-0.201	0.182	0.112	0.144	0.178	0.060	-0.145	0.215	0.167	1		
Hemolytic activity	-0.523*	0.758**	-0.689**	-0.582**	-0.781**	-0.458	-0.695**	-0.632**	-0.687**	-0.682**	-0.198	1	
Thrombolytic activity	0.785**	-0.751**	0.627**	0.773**	0.657**	0.805**	0.689**	0.690**	0.759**	0.652**	0.120	-0.436*	1

**Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level.

Table 9 Correlation analysis between TFC and biological attributes of tested wild fruits.

	TFC	IC ₅₀ Value	Inhibition Potential	Antibacterial activity			Antifungal activity			Biofilm inhibition		Hemolytic activity	Thrombolytic activity
				S.aureus	E.coli	B. cereus	A. niger	A. flavus	F. oxysporum	S. aureus	E.coli		
TFC	1												
IC ₅₀ Value	-0.674**	1											
Inhibition Potential	0.710**	-0.741**	1										
Antibacterial (S.aureus)	0.769**	-0.765**	0.877**	1									
Antibacterial (E.coli)	0.701**	-0.875**	0.891**	0.815**	1								
Antibacterial (B. cereus)	0.725**	-0.715**	0.586**	0.670**	0.667**	1							
Antifungal (A. niger)	0.733**	-0.870**	0.890**	0.878**	0.879**	0.718**	1						
Antifungal (A. flavus)	0.409*	-0.659**	0.776**	0.649**	0.732**	0.597**	0.715**	1					
Antifungal (F. oxysporum)	0.697**	-0.875**	0.795**	0.781**	0.847**	0.791**	0.887**	0.782**	1				
Biofilm inhibition (S. aureus)	0.685**	-0.897**	0.864**	0.851**	0.879**	0.686**	0.901**	0.680**	0.879**	1			
Biofilm inhibition (E.coli)	0.245	-0.141	0.124	0.101	0.131	0.158	0.081	-0.214	0.218	0.179	1		
Hemolytic activity	-0.386	0.782**	-0.685**	-0.589**	-0.752**	-0.478	-0.643**	-0.587**	-0.694**	-0.740**	-0.180	1	
Thrombolytic activity	0.782**	-0.659**	0.594**	0.761**	0.684**	0.847**	0.708**	0.688**	0.747**	0.718**	0.124	-0.454*	1

**Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level.

in tested fruits (Tables 7). Sodium and potassium have been reported to be present in the body at suitable concentration for optimum blood pressure (NRC, 1989). The findings of this analysis support the optimum use of tested fruits as a potential source of nutrients for smooth functioning of different physiological processes. Valvi and Rathod, (2011) reported the occurrence of potassium (1922 mg/100 g) as principal mineral along with significant amount of other important minerals including calcium, sodium, iron and magnesium in Indian fig fruit cultivars.

3.12. Correlation analysis

According to Pearson method ($P = 0.001$), a strong positive correlation was observed between bioactive components (TPC and TFC) and its biological potential including antimicrobial, inhibition capacity, thrombolytic and biofilm activities ($P = 0.614\text{--}0.867$, 0.804 , 0.785 and $0.324\text{--}0.787$), ($P = 0.586\text{--}0.891$, 0.710 , 0.782 , $0.245\text{--}0.685$), however, strong negative correlation was also recorded for haemolysis and IC_{50} value ($P = -0.523$, -0.762), ($P = -0.386$, -0.674), respectively (Tables 8, 9). Our research group (Ahmad et al., 2016) reported the presence of eight phenolic acid and flavonoids in tested fruits, therefore the tested biological attributes are directly associated with its TPC and TFC.

4. Conclusions

The fruits of wild fig have been evaluated for high-value nutrients and potent bioactives that contributed significantly to their biological potential (determined rarely before). Potassium and calcium along with considerable amount of other essential nutrients, gluconic acid and glucose were also detected as major organic acid and individual sugar in tested fruits. Among other extracting solvents, aqueous-ethanolic CCEs/PRFs showed higher TPC, TFC and biological attributes. These biological properties of tested fruits are significantly correlated with their TPC and TFC. The findings of this study suggest the consumption of the fruits of wild fig as a potential source of valuable phytonutrients and potent bioactives in the development of nutraceuticals.

Funding

This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R158), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors express their gratitude to Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R158), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

References

- Ahmad, N., Anwar, F., Hameed, S., Boyce, M.C., 2011. Antioxidant and antimicrobial attributes of different solvent extracts from leaves and flowers of *akk* [*Calotropis procera* (Ait.) Ait. F.]. *J. Med. Plant Res.* 5, 4879–4887.
- Ahmad, N., Zuo, Y., Lu, X., Anwar, F., Hameed, S., 2016. Characterization of free and conjugated phenolic compounds in fruits of selected wild plants. *Food Chem.* 190, 80–89. <https://doi.org/10.1016/j.foodchem.2015.05.077>.
- Anwar, F., Zreen, Z., Sultana, B., Jamil, A., 2013. Enzyme-aided cold pressing of flaxseed (*Linum usitatissimum* L.): Enhancement in yield, quality and phenolics of the oil. *Grasas y Aceites* 64, 463–471. <https://doi.org/10.3989/gya.132212>.
- Bey, M.B., Louaileche, H., Zemouri, S., 2013. Optimization of phenolic compound recovery and antioxidant activity of light and dark dried fig (*Ficus carica* L.) varieties. *Food Sci. Biotechnol.* 22, 1613–1619. <https://doi.org/10.1007/s10068-013-0258-7>.
- Çalışkan, O., Aytekin Polat, A., 2011. Phytochemical and antioxidant properties of selected fig (*Ficus carica* L.) accessions from the eastern Mediterranean region of Turkey. *Sci. Hortic. (Amsterdam)* 128, 473–478. <https://doi.org/10.1016/j.scienta.2011.02.023>.
- Faleh, E., Oliveira, A.P., Valentão, P., Ferchichi, A., Silva, B.M., Andrade, P.B., 2012. Influence of Tunisian *Ficus carica* fruit variability in phenolic profiles and in vitro radical scavenging potential. *Rev. Bras. Farmacogn.* 22, 1282–1289. <https://doi.org/10.1590/S0102-695X2012005000132>.
- Hamidi, M., Tajerzadeh, H., 2003. Carrier erythrocytes: An overview. *Drug Deliv. J. Deliv. Target. Ther. Agents* 10, 9–20. <https://doi.org/10.1080/713840329>.
- Hussain, A.I., Anwar, F., Hussain Sherazi, S.T., Przybylski, R., 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chem.* 108, 986–995. <https://doi.org/10.1016/j.foodchem.2007.12.010>.
- Hussain, A.I., Anwar, F., Iqbal, T., Bhatti, I.A., 2011. Antioxidant attributes of four Lamiaceae essential oils. *Pakistan J. Bot.* 43, 1315–1321.
- Irfan, A., Imran, M., Khalid, M., Sami Ullah, M., Khalid, N., Assiri, M.A., Thomas, R., Muthu, S., Raza Basra, M.A., Hussein, M., Al-Sehemi, A.G., Shahzad, M., 2021. Phenolic and flavonoid contents in *Malva sylvestris* and exploration of active drugs as antioxidant and anti-COVID19 by quantum chemical and molecular docking studies. *J. Saudi Chem. Soc.* 25, <https://doi.org/10.1016/j.jscs.2021.101277>.
- Joshi, S.C., Verma, A.R., Mathela, C.S., 2010. Antioxidant and antibacterial activities of the leaf essential oils of Himalayan Lauraceae species. *Food Chem. Toxicol.* 48, 37–40. <https://doi.org/10.1016/j.fct.2009.09.011>.
- Katalinic, V., Milos, M., Kulisic, T., Jukic, M., 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem.* 94, 550–557. <https://doi.org/10.1016/j.foodchem.2004.12.004>.
- Ko, F.N., Hsiao, G., Kuo, Y.H., 1997. Protection of oxidative hemolysis by demethylisoeugenol in normal and β -thalassemic red blood cells. *Free Radic. Biol. Med.* 22, 215–222. [https://doi.org/10.1016/S0891-5849\(96\)00295-X](https://doi.org/10.1016/S0891-5849(96)00295-X).
- Larrauri, J.A., Sánchez-Moreno, C., Rupérez, P., Saura-Calixto, F., 1999. Free radical scavenging capacity in the aging of selected red spanish wines. *J. Agric. Food Chem.* 47, 1603–1606. <https://doi.org/10.1021/jf980607n>.
- Lazreg Aref, H., Gaaliche, B., Fekih, A., Mars, M., Aouni, M., Chaumon, J.P., Said, K., 2011. In vitro cytotoxic and antiviral activities of *Ficus carica* latex extracts. *Nat. Prod. Res.* 25, 310–319. <https://doi.org/10.1080/14786419.2010.528758>.

- Link, D.D., Walter, P.J., Kingston, H.M., 1998. Development and validation of the new EPA microwave-assisted leach method 3051A. *Environ. Sci. Technol.* 32, 3628–3632. <https://doi.org/10.1021/es980559n>.
- Maheshwari, D.T., Yogendra Kumar, M.S., Verma, S.K., Singh, V.K., Singh, S.N., 2011. Antioxidant and hepatoprotective activities of phenolic rich fraction of Seabuckthorn (*Hippophae rhamnoides* L.) leaves. *Food Chem. Toxicol.* 49, 2422–2428. <https://doi.org/10.1016/j.fct.2011.06.061>.
- Mahmood, T., Anwar, F., Abbas, M., Saari, N., 2012. Effect of maturity on phenolics (Phenolic acids and flavonoids) profile of strawberry cultivars and mulberry species from Pakistan. *Int. J. Mol. Sci.* 13, 4591–4607. <https://doi.org/10.3390/ijms13044591>.
- Manian, R., Anusuya, N., Siddhuraju, P., Manian, S., 2008. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chem.* 107, 1000–1007. <https://doi.org/10.1016/j.foodchem.2007.09.008>.
- Manzoor, M., Anwar, F., Ahmed Bhatti, I., Jamil, A., 2013. Variation of phenolics and antioxidant activity between peel and pulp parts of pear (*Pyrus communis* L.) fruit. *Pakistan J. Bot.* 45, 1521–1525.
- McLaughlin, J.L., Rogers, L.L., Anderson, J.E., 1998. The use of biological assays to evaluate botanicals. *Ther. Innov. Regul. Sci.* 32, 513–524. <https://doi.org/10.1177/009286159803200223>.
- Nakilcioğlu, E., Hışıl, Y., 2013. Research on the Phenolic Compounds in Sarilop (*Ficus Carica* L.) Fig Variety (in English). *Gıda* 38, 267–274. <https://doi.org/10.5505/gida.2013.08208>.
- NCCLS, 1997. National Committee for Clinical Laboratory Standards (NCCLS). Approved Standard M2 A6, 1997, fifth ed. NCCLS: Wayne, PA.
- NCCLS, 1999. National Committee for Clinical Laboratory Standards (NCCLS). M100-S9., 1999, NCCLS: Wayne, PA.
- Noori, S., Kiasat, A.R., Kolahi, M., Mirzajani, R., Seyyed Nejad, S. M., 2022. Determination of secondary metabolites including curcumin in *Rheum ribes* L. and surveying of its antioxidant and anticancer activity. *J. Saudi Chem. Soc.* 26., <https://doi.org/10.1016/j.jscs.2022.101479> 101479.
- NRC, 1989. National Research Council. Recommended dietary allowances., 1989, (10th ed.). Washington: National Academy Press. 1989.
- Pourghayoumi, M., Bakhshi, D., Rahemi, M., Jafari, M., 2012. Effect of pollen source on quantitative and qualitative characteristics of dried figs (*Ficus carica* L.) cvs “Payves” and “Sabz” in Kazerun - Iran. *Sci. Hortic. (Amsterdam)* 147, 98–104. <https://doi.org/10.1016/j.scienta.2012.08.026>.
- Poyrazoğlu, E., Gökmen, V., Artık, N., 2002. Organic Acids and Phenolic Compounds in Pomegranates (*Punica granatum* L.) Grown in Turkey. *J. Food Compos. Anal.* 15, 567–575. <https://doi.org/10.1006/jfca.2002.1071>.
- Prabu, G.R., Gnanamani, A., Sadulla, S., 2006. Guaijaverin - A plant flavonoid as potential antiplaque agent against *Streptococcus mutans*. *J. Appl. Microbiol.* 101, 487–495. <https://doi.org/10.1111/j.1365-2672.2006.02912.x>.
- Prasad, S., Kashyap, R.S., Deopujari, J.Y., Purohit, H.J., Taori, G. M., Dagainawala, H.F., 2006. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. *Thromb. J.* 4, 9–12. <https://doi.org/10.1186/1477-9560-4-14>.
- Ramadan, A., Soliman, G., Mahmoud, S.S., Nofal, S.M., Abdel-Rahman, R.F., 2012. Evaluation of the safety and antioxidant activities of *Crocus sativus* and Propolis ethanolic extracts. *J. Saudi Chem. Soc.* 16, 13–21. <https://doi.org/10.1016/j.jscs.2010.10.012>.
- Shabir, G., Anwar, F., Sultana, B., Khalid, Z.M., Afzal, M., Khan, Q. M., Ashrafuzzaman, M., 2011. Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of gold mohar [*Delonix regia* (Bojer ex Hook.) Raf.]. *Molecules* 16, 7302–7319. <https://doi.org/10.3390/molecules16097302>.
- Shahidi, F., 2009. Nutraceuticals and functional foods: Whole versus processed foods. *Trends Food Sci. Technol.* 20, 376–387. <https://doi.org/10.1016/j.tifs.2008.08.004>.
- Shiraishi, S., Kawakami, K., Widodo, S.E., Shiraishi, M., Kitazaki, M., John, S., 1996. Organic Acid Profiles in the Juice of Fig Fruits Literature of fig (83 ~ s carica L.) has been voluminously documented since a long time ago (Condit and Enderud, 1956), and almost all well-known fig varieties have been well described (Condit, 1955 40, 29–33).
- Siddhuraju, P., Mohan, P.S., Becker, K., 2002. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chem.* 79, 61–67. [https://doi.org/10.1016/S0308-8146\(02\)00179-6](https://doi.org/10.1016/S0308-8146(02)00179-6).
- Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 16, 144–158.
- Slatnar, A., Klancar, U., Stampar, F., Veberic, R., 2011. Effect of Drying of Figs (*Ficus carica* L.) on the Contents of Sugars, Organic Acids, and Phenolic Compounds. *J. Agric. Food Chem.* 59, 11696–11702.
- Soni, N., Mehta, S., Satpathy, G., Gupta, R.K., 2014. “Estimation of nutritional, phytochemical, antioxidant and antibacterial activity of dried fig (*Ficus carica*)”. ~ 158 ~. *J. Pharmacogn. Phytochem.* 3, 158–165.
- Stepanović, S., Vuković, D., Dakić, I., Savić, B., Švabić-Vlahović, M., 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J. Microbiol. Methods* 40, 175–179. [https://doi.org/10.1016/S0167-7012\(00\)00122-6](https://doi.org/10.1016/S0167-7012(00)00122-6).
- Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., Polissiou, M., 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chem.* 90, 333–340. <https://doi.org/10.1016/j.foodchem.2003.09.013>.
- Trichopoulou, A., Vasilopoulou, E., Georga, K., Soukara, S., Dilis, V., 2006. Traditional foods: Why and how to sustain them. *Trends Food Sci. Technol.* 17, 498–504. <https://doi.org/10.1016/j.tifs.2006.03.005>.
- Uddin, N., 2013. DPPH Scavenging Assay of Eighty Four Bangladeshi Medicinal Plants. *IOSR J. Pharm. Biol. Sci.* 6, 66–73. <https://doi.org/10.9790/3008-0656673>.
- Valvi, S.R., Rathod, V.S., 2011. Mineral composition of some wild edible fruits MINERAL COMPOSITION OF SOME WILD EDIBLE FRUITS FROM. *Biol. J Appl Tech, Phar* 2, 392–396.
- Vinson, J.A., Zubik, L., Bose, P., Samman, N., Proch, J., 2005. Dried Fruits: Excellent in Vitro and in Vivo Antioxidants. *J. Am. Coll. Nutr.* 24, 44–50. <https://doi.org/10.1080/07315724.2005.10719442>.
- Walsh, S.E., Maillard, J.Y., Russell, A.D., Catrenich, C.E., Charbonneau, D.L., Bartolo, R.G., 2003. Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. *J. Appl. Microbiol.* 94, 240–247. <https://doi.org/10.1046/j.1365-2672.2003.01825.x>.
- Yang, Z.G., Sun, H.X., Fang, W.H., 2005. Haemolytic activities and adjuvant effect of *Astragalus membranaceus* saponins (AMS) on the immune responses to ovalbumin in mice. *Vaccine* 23, 5196–5203. <https://doi.org/10.1016/j.vaccine.2005.06.016>.
- Yen, G.C., Duh, P.D., Chuang, D.Y., 2000. Antioxidant activity of anthraquinones and anthrone. *Food Chem.* 70, 437–441. [https://doi.org/10.1016/S0308-8146\(00\)00108-4](https://doi.org/10.1016/S0308-8146(00)00108-4).
- Zhang, X., Wei, X., Ali, M.M., Rizwan, H.M., Li, B., Li, H., Jia, K., Yang, X., Ma, S., Li, S., Chen, F., 2021. Changes in the content of organic acids and expression analysis of citric acid accumulation-related genes during fruit development of yellow (*Passiflora edulis* f. *flavicarpa*) and purple (*passiflora edulis* f. *edulis*) passion fruits. *Int. J. Mol. Sci.* 22. <https://doi.org/10.3390/ijms22115765>.
- Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64, 555–559.

Zhuang, X.P., Lu, Y.Y., Yang, G.S., 1992. Extraction and determination of flavonoid in ginkgo. *Chinese Herbal Medicine* 23, 122–124.

Zou, Y., Lu, Y., Wei, D., 2004. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. in vitro. *J. Agric. Food Chem.* 52, 5032–5039. <https://doi.org/10.1021/jf049571r>.