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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *ZIZYPHUS JUJUBA* L. LEAF EXTRACTS

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Abstract: The aim of the present study was to investigate the biological activities of the extracts obtained from leaf of *Ziziphus jujuba*. To reach this purpose, total phenolic content (TPC), total flavonoid content (TFC), DPPH and ABTS radical scavenging activity, as well as antibacterial activity extracts were evaluated. The content of total phenols in leaf ranged from 22.71 to 28.69 mg GAE/g, the total content of flavonoids ranged from 5.15 mg RE/g to 8.25 mg RE/g depending on the applied extraction method. All extracts showed very high antioxidant activity. The ethanol extracts of *Ziziphus jujuba* leaf were screened for antibacterial activities against *Salmonella typhimurium, Salmonella enteritidis, Listeria ivanovii, Listeria monocytogenes, Staphylococcus aureus* and *Pseudomonas aerogenes* using microdilution method. Furthermore, the highest antibacterial activity was observed against *Salmonella typhimurium* and *Salmonella enteritidis*. The present results suggested the promising antioxidant and antibacterial properties of *Ziziphus jujuba* leaf extracts, which can be used in the fabrication of functional bioactive ingredients for different purposes.

Keywords: phytochemicals, antibacterial evaluation, *Ziziphus jujuba*

Introduction

Chinese jujube (*Zizyphus jujuba* Miller) with over 700 subspecies has been used during thousands of years of cultivation in the temperate and subtropical areas of the Northern Hemisphere (Wang et al., 2016) and as a crude drug in traditional medicine for the purpose of analeptic, palliative, antibechic actions (Li et al., 2007).

Different parts of jujube are rich in medicinal properties such as analgesic, antiseptic, and antidiabetic. Jujube fruit is rich in vitamins A, B, and C, as well

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as minerals such as calcium, phosphorus, and iron, it contains protein, fat, carbohydrates and various compounds such as alkaloids, flavonoids, tannins, saponins, sterols and fatty acids (Ozturk et al., 2021). Jujube also has significant antioxidant properties that can neutralize the activity of free radicals, so its fruit is involved in traditional medicine (Tatari et al., 2016). This antioxidant activity has been attributed to the high level of phenolic compounds.

The jujube leaf, which is the main byproduct of jujube, has also been used for thousands of years (i.e., to improve sleep, to nourish the heart and soothe the nerves, and to reduce hemorrhaging and diarrhea). Jujube leaves are rich in bioactive components and have various physiological and pharmacological functions (the aqueous ethanol extracts of the jujube leaf were used as energetic constituent for hepatosis and wound healing in animal trials) (Zhang et al., 2019). In addition, natural antioxidants have the capacity to improve food quality and stability, and can also act as nutraceuticals to terminate free radical chain reactions in biological systems, and thus may provide additional health benefits to consumers (Sampath Kumara et al., 2012). Phenolic and flavonoids were believed to be the major bioactive components in jujube leaves, which have been shown to be responsible for cardioprotective, anticancer, antidiabetic, anti-aging and neuroprotective effects.

Most studies focused on the bioactivity and chemical constituents of jujube seeds and fruits, and less attention was devoted to the jujube leaf.

The number of bioactive compounds recovered from plant materials depends on the extraction methods used. Therefore, the objective of this study is to explore the phenolic composition and antioxidants of the ethanol–water and water extracts from *Chinese jujube*, to provide sufficient experimental evidence for antioxidant activity and potential for further development and utilization of jujube.

Materials and methods

The extraction process

5 g of leaf sample (ground) was extracted with 50 mL of solvent (50% ethanol or water). The extraction methods are given below.

1. Infusion - water 80 °C;
2. Infusion - water 100 °C;
3. Maceration water - 22 h;
4. Maceration 50% ethanol - 22 h;
5. Ultrasonic extraction with water, 30 min.;
6. Ultrasonic extraction with 50% ethanol, 30 min.

The samples were filtered and total phenols, flavonoids and antioxidant activity were determined from the filtrate. UV/Vis spectrophotometry was used to determine the concentration of total phenols, flavonoids and antioxidant capacity. Spectrophotometric measurements were performed on a UV-VIS spectrophotometer (Cary Series 300 Agilent Technologies).

For determining antibacterial activity of leaf extract - the extracts were obtained by maceration (24h) with 50% ethanol. After decanting, the extracts were evaporated in a rotary vacuum evaporator at 45℃ to a gelatinous mass. The concentrated extract was kept in a screw cap bottle at 4℃. The extracts were dissolved in 10% dimethylsulfoxide at an initial concentration of 200 mg/mL.

Determination of total phenolic acids (TPC)

The TP contents were determined by the Folin–Ciocalteau method (Singleton et al., 1965). The extract samples (40 μL) were mixed with 3.16 water, after that 200 μL Folin–Ciocalteu was added. After reaction for 8 min, 600 μL of sodium carbonate was added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. The TP concentration was expressed as the equivalent to milligrams of gallic acid per g of dried weight (mg GAE/g DW).

Determination of total flavonoids (TFC)

The total flavonoid content was measured by a colorimetric assay. The extracts (1 mL) were added to a test tube containing 4 mL of water. Sodium nitrite solution (5%, 0.3 mL) was added to the mixture and reacted for 5 min, followed by the addition of 0.3 mL of 10% aluminum chloride. After 5 min, 2 mL of 1 M sodium hydroxide was added and supplemented with 2.4 mL of distilled water and mixed well. The absorbance of mixture was measured at 510 nm. The results were expressed as the equivalent to milligrams of rutin per g of dried weight (mgRE/ g DW).

Antioxidant capacity

Antioxidant properties were determined by the ABTS assay (Re et al., 1999). The ABTS stock solution in distilled water was prepared from 7 mM ABTS and 2.45 mM potassium persulphate, and then incubated in the dark for 12 - 16 h at room temperature. The ABTS working solution was prepared by diluting the stock solution with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. A 10 μL of

extracts was mixed with 1 mL of prepared ABTS solution and mixed for 6 min. Absorbance was measured at 734 nm, with distilled water as a reference. Results were expressed as percentage of inhibition of the ABTS radical.

The antioxidant capacity of the extracts was also studied through the evaluation of the free radical-scavenging effect on the 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical. An aliquot 0.1 mL of extract was mixed with 3.9 mL DPPH solution ($6 \cdot 10^5$M). The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. After that, the absorbance was measured at 515 nm, with distilled water as a reference. Results were expressed as percentage of inhibition of the DPPH radical.

Antibacterial activity - minimum inhibitory concentrations

The antibacterial (AB) activity was tested against the *Listeria ivanovii, Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aerogenes, Salmonellla enteritidis*. Bacterial colonies from the plates incubated overnight at 37 °C were resuspended in sterile NaCl and adjusted to the 0.5 McFarland standard. The inoculum prepared above is diluted to 1×10^6 CFU/mL. Determination of minimum inhibitory concentrations (MIC) was performed following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines using the broth dilution method in a 96-well microtiter plate. 100 μL of the initial concentration extract was added to the first row of the microtiter plate. 50 μL of MHB was added to all wells in a row. After that, 50 μL of the extract was transferred from the first well to the second well and so on. In this way, the extract was diluted twice. 50 μL of bacterial suspension was added to all wells except the last (negative control). The next day, the activity of the extracts was read. The last well in which turbidity did not occur was read as the minimum inhibitory concentration of the extract.

Results and discussion

The content of total phenols, flavonoids and antioxidant activity of the leaf extracts are given in the Table 1. The content of extracted polyphenols and flavonoids as well as the antioxidant activity of leaf extracts depends on the applied solvent and method of extraction.

The content of total phenols in leaf of *Zizyphus jujuba* ranged from 22.71 to 28.69 mg GAE/g. The highest content of total phenols was in the extract obtained by maceration with 50% ethanol as a solvent (28.69 mg GAE/g) and

infusion 100 ℃ (26.80 mg GAE/g). The total content of flavonoids ranged from 5.15 mg RE/g (ultrasonic extraction with 50% ethanol) to 8.25 mg RE/g (maceration with water). Phenolic compounds and flavonoids are well known for their free radical scavenging (antioxidant) activities (Đurović et al., 2023).

Table 1. Phytochemical content and antioxidant activity of leaf of *Zizyphus jujuba*

Leaf	Total phenols mg GAE/g	Total flavonoids mg RE/g	DPPH % inhibition	ABTS % inhibition
Infusion 80 ℃	25.26±1.30	8.23±0.33	76.80±2.28	99.52
Infusion 100 ℃	26.80±1.32	7.70 ±0.33	74.75±0.09	99.13
Maceration water	24.05±0.38	8.25±0.25	80.36±0.63	99.18
Maceration 50% etanol	28.69±0.36	7.70±0.20	86.34±0.15	99.98
Ultrasonic extraction - water	22.97±0.36	7.55±0.34	82.50±1.15	99.62
Ultrasonic extraction - 50% ethanol	22.71±0.19	5.15±0.21	87.48±0.46	99.66

All extracts showed a high level of inhibition and had strong free radical scavenging activities. The reason may be attributed to the differences of their phenolic contents and flavonoids contents. Various antioxidant activity assays such as the (DPPH) scavenging activity and ABTS assays were used to evaluate the effect of extraction method and solvents on the antioxidant activity in the leaf. These findings agree well with previous reports indicating that leaves of the *Ziziphus genus* showed a high antioxidant activity due to their high bioactive compounds content such as polyphenols including tannins and flavonoids (Esteki et al., 2012; Hossain et al., 2016).

Many studies have reported the impact of different solvents on the content of secondary metabolites and/or their antioxidant activity (Khaou et al., 2013; Ngo et al., 2017). In addition, the extraction techniques also have noticeable effect on the recovery of phytochemical content.

Antibacterial activity

Plants are a rich source of compounds that do not appear essential for primary metabolism, including thousands of secondary metabolites. In addition to their function in physiology or in structural maintenance, some of the

secondary metabolites (e.g. flavonoids, anthocyanins, monoterpenes and tetraterpenes) serve for defense against microbes (Wink, 2008).

As shown in Table 2, the antibacterial activity of *Ziziphus jujuba* leaf extract was tested against three Gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes* and *Listeria ivanovii*) and three Gram-negative (*Salmonella typhimurium*, *Salmonella enteritidis* and *Pseudomonas aerogenes*) bacteria**.**

The results of the antibacterial activity of 50% ethanol *Ziziphus jujuba* leaf extracts show that the tested extracts have an antibacterial effect. The leaf extract acted in concentrations of 25 to 50 mg/mL (*Salmonella typhimurium* and *Salmonella enteritidis* – 25 mg/mL) and on other tested bacteria (*Listeria ivanovii, Listeria monocytogenes, Staphylococcus aureus, Pseudomonas aerogenes*) in concentrations of 50 mg/mL.

Table 2. Minimum inhibitory concentrations (MIC) of ethanolic extract of *Ziziphus jujuba* leaf

Bacteral Spp.	MIC of leaf extract (mg/mL)
Listeria ivanovii	50
Listeria monocytogenes	50
Salmonella typhimurium	25
Staphylococcus aureus	50
Pseudomonas aerogenes	50
Salmonellla enteritidis	25

The results of this work were in agreement with Ghazghazi et al. (2014), who evaluated the antibacterial potential of jujube extract on several Gram-negative and Gram-positive bacteria (S. *typhi, E. coli, S. aureus*, and *B. cereus*). The antimicrobial effect is related to the ability of the compounds based on shape and size to penetrate into the outer membrane and reach their site of action (Kavak et al., 2010). The bactericidal effect depends on the concentration of active components in extracts and type of bacterial strains.

Conclusion

The results of the present investigation demonstrated the effects of extraction solvent and method on phenolic and flavonoid content and antioxidant activities of *Ziziphus jujuba* leaf. The results revealed that the extraction method greatly influences the phytochemical content and bioactivity and strongly recommends that any plant samples, intended to study, must undergo several extraction processes to reveal the actual phytochemical

content. In this study, important phytochemical constituents were detected in both ethanol and aqueous leaf extracts of *Z. jujuba*. The phytochemicals detected in the plant extracts could be responsible for the observed antibacterial activities. The extracts exhibited antibacterial activities. Thus, further investigations should be carried out for individual phenolic compounds for potential medicinal and industrial uses. The toxicity profile of this plant should be carried out so as to establish its safety/therapeutic index.

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