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Phytochemistry of *Ziziphus Mauritiana*; its Antioxidant and Antimicrobial Potential

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Abstract

Background: *Z. mauritiana* is a very common and easily available plant all over the world, including Pakistan. It finds a lot of importance due to its nutritional and medicinal value.

Methods: Current studies were performed to investigate the phytochemical nature and antioxidant and antimicrobial potential of *Z. mauritiana* (Lahore, Pakistan). Total phenolic contents in leaves and pulp extracts were identified using a modified Folin-Ciocalteu's method while total sugars were determined by Lane and Eynon method. Antioxidant activity was determined by DPPH radical-scavenging activity assay using a UV-Visible spectrophotometer at 517 nm. The antimicrobial potential was tested by agar well diffusion method using imipenem as standard drug.

Results: The methanolic extracts of leaves and pulp contained tannins, saponins, phlobatannins, alkaloids and flavonoids while steroids were absent in both the samples. The pulp and leave extracts have shown the presence of 57.3% and 37.89% sugar contents, respectively. The concertation of total phenolic contents (TPC) was found higher in pulp extracts as compared to that observed in leaves. The methanolic pulp extract has shown higher antioxidant potential as compared to the leaves extract. However, methanolic leaves extract exhibited higher antibacterial potential as compared to the methanolic pulp extract.

Conclusion: The methanolic extracts of *Z. Mauritiana* pulp and leaves contain many biologically active ingredients and are biologically active against most of the tested bacterial strains. Its pulp and leaves are richer in numerous antioxidants and secondary metabolites. The pulp extract exhibited higher antioxidant potential as compared to the leaves extract.

Introduction

Plants find a tremendous significance due to their nutritional and medicinal potential [1-3].The treatment of diseases by using plant extracts is a field of great interest for researchers throughout the world [4-6] and a large number of drugs of plant origin have been adopted in modern pharmacotherapy [7-9]. There are numerous reports regarding the traditional use of various parts of the plants for the treatment of several diseases, like asthma, fever, stomach pain, allergies, mental depression and ulcers etc. [10].

Ziziphus Mauritiana (Figure 1) is a common nutritional and medicinal plant [11, 12] which belongs to the family Rhamnaceae [10]. The genus Ziziphus is comprised of 40 species that are distributed in warm temperate and subtropical regions [13]. *Z. mauritiana* is commonly known as ber (Pakistan), berra (Afghanistan), bozoi, kool, kul (Bangladesh), badari, ber, beri (India), Chinee apple, Chinese apple (Australia), bidara, dara, widara (Indonesia) and lang tsao, tsaotsao, hongtsao (China), dattechinoise (French), azufaifo, yuyuba (Spanish), Chinese date and Chinese fig (English). Its origin is Central Asia; however, it is commonly availabe worldwide [14, 15] including Zimbabwe, Malaysia, England and Thailand [16] and in warmer parts of Europe, Asia and Australia [17]. The average fruit production of this plant is 80 kg to 200 kg depending upon age and climatic conditions [18, 19]. *Z. mauritiana* has been mentioned in Sura Saba, Sura An-najm and and Sura Waqiya of Holy Quran [20, 21]. According to a Hadith narrated by Muhammad Ahmad Zahbi (Rahmat ullah Alayhi), "The first fruit eaten by Hazrat Adam (Alayhi Sallam) after His descent on the earth, was the fruit of the Zizyphus" [22, 23]. The fruits of *Z. mauritiana* are nutritionally highly rich in carotene, protein, vitamin C, phosphorus and calcium contents as compared to the apple [24, 25]. In *Z. mauritiana* species, carbohydrates, proteins, starch, sugar, mucilage, and vitamins are abundantly present [26]. The leaves of the plant are used by cattle, camels, goats etc. as a food and provide useful minerals for cattle health [27]. The fruits of *Z. mauritiana* are also rich in nutritional contents and are very useful for human health [15]. There are many applications of this plant in traditional medicines e.g., its ripe fruit in dried form is used as a mild laxative and also useful for treatment of ulcer and cuts. The leaves of *Z. mauritiana* are very helpful in fever, asthma and liver issues whereas root powder of this plant is dusted on wounds [28]. *Z. mauritiana* exhibits neuroprotective, anti-biotic, anti-spermatogenic and anti-oxidant activity due to the presence of numerous bioactive compounds. The plant also contains total cholesterol regulatory and carbohydrate metabolism compounds [29].

Keeping in view the nutritional and medicinal importance of *Z. mauritiana*, current studies were performed to investigate the (a) phytochemistry of methanolic extracts of pulp and leaves of *Z. mauritiana* (Lahore, Pakistan) (b) Measurement of total phenolic contents by using Folic-cioealteu reagent (c) Determination of DPPH scavenging activity (d) Comparison of antioxidant potential of leaves and pulp with the standard materials (e) Investigation of antimicrobial activity against different bacterial strains.

Figure 1: *Ziziphus Mauritiana*, Gol Ber

Methods

a. Collection of Plant Material

Ripped fresh fruits and mature leaves of *Z. mauritiana* were collected from a village Theter, District Lahore (Punjab, Pakistan) and washed to remove dust and debris. They were then dried under shade for 25 days, crushed into powder and stored carefully.

The pyrex origin glassware was used in experimental work. All the chemicals and the solvents were of analytical grade. Antioxidant activity of leaves and pulp extracts was determined by using DPPH radical-scavenging activity assay and measured by UV-Visible spectrophotometer at 517 nm.

b. Extract Preparation

100 g of crushed dried pulp and leave powder of *Z. mauritiana* were taken in two separate flasks. The materials were then extracted by maceration in methanol solvent by adding 500 mL of methanol in each flask; they were kept in well-tighten containers with frequent shaking (2-3 times a day) for up to a week. The macerate from each flask was then separately filtered using Whatman filter paper No. 41. Rotary Evaporator was used to concentrate the crude methanolic extracts. The extracts were then allowed to stand followed by evaporation of remaining methanol. Finally, dark brown

gummy solids were produced which were kept in clean petri plates in dark and a cool place for further studies (Figure 2).

Figure 2: (a) Leaves and (b) pulp extract of *Z. mauritiana*

c. Phytochemical Analysis of Phytochemicals

The methanolic extracts of leaves and pulp of *Z. mauritiana* were qualitatively screened through various tests (tests for alkaloids, steroids, flavonoids, tannins, saponins and phlobatannins) by a reported procedure [30]. Total phenolic contents in leaves and pulp extracts were identified using a modified Folin-Ciocalteu's method [31] while total sugars were determined by Lane and Eynon method [32].

d. Antioxidant Studies

Antioxidant activity of leaves and pulp extract of *Z. mauritiana* was investigated by DPPH radical scavenging activity with UV-Vis spectrophotometer at 517 nm [33]. It is a commonly used assay for antioxidant evaluation of natural products [34, 35] and employs the fact that an antioxidant is a hydrogen donor. The hydrogen is accepted by DPPH which is used for measuring radical scavenging activity of compound. This process is chosen because DPPH acts as a stable nitrogen centered free radical and is available on commercial scale [36, 37].

e. Antimicrobial Activity of *Z. mauritiana* Leaves and Pulp

The leaves and pulp extracts of *Z. mauritiana* were investigated for their antimicrobial potential against five bacterial strains. For bacterial growth, nutrient agar medium was used. The medium was prepared, autoclaved for 15 minutes at 121°C and then poured on to the petri plates and allowed to solidify. Five isolated strains of bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi* were used for assessment of antimicrobial activity of test solutions (20 µL, 40 µL and 60 µL) in DMSO using agar well diffusion method [38].

Results

The leaves and pulp of *Z. mauritiana* were subjected to phytochemical characterization (phytochemical analysis, total phenolic content, sugar contents) and biological evaluation (antioxidant and antimicrobial activities).

a. Phytochemical analysis

The methanolic extract of *Z. mauritiana* pulp revealed the presence of saponins, phlobatannins, alkaloids, flavonoids and tannins; its leaves (methanolic extract) also exhibited the presence of same contents. The steroids were absent in both the pulp and leaves of *Z. mauritiana*.

b. Determination of total phenolic content (TPC)

For total phenolic content (TPC) determination, quantitative phytochemical analysis of methanolic extracts of pulp and leaves of *Z. mauritiana* was done by using UV-Vis spectrophotometer at 765 nm. Gallic acid was used as a standard. By using calibration curve of standard gallic acid, the TPC present in pulp and leaves extracts of *Z. mauritiana* were determined; the results are revealed in terms of mg gallic acid equivalent (mg GAE/g) at 765 nm. Figure 3 shows the concentration of total phenolic compounds in methanolic extract of *Z. mauritiana* (leaves and pulp).

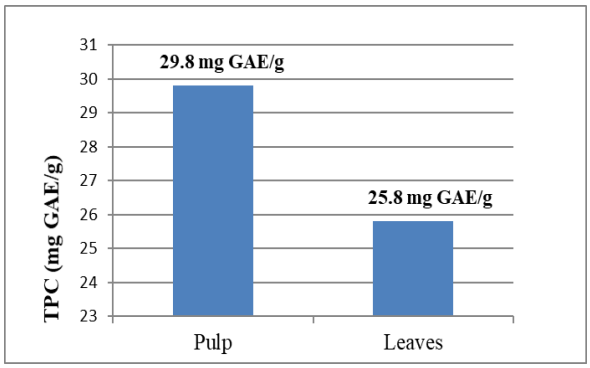

Figure 3: TPC (mg GAE/g) in pulp and leaves of *Z. mauritiana*

c. Quantitative investigation of sugar contents

Sugar contents were determined qualitatively in the methanolic extract of leaves and pulp of *Z. mauritiana* and the results are summarized in Table 1 and Figure 4.

	Total sugar%age	Reducing sugar%age	Non-reducing sugar%age
Pulp	57.3	23	34.3
Leaves	37.89	19.16	18.73

Table 1: Sugar contents in methanolic extract of leaves and pulp of *Z. mauritiana.*

d. Antioxidant activity

Plants are commonly investigated for their antioxidant potential [39-41]. The presence of antioxidants was investigated in methanolic extracts of leaves and pulp of *Z. mauritiana* and the results are displayed in Table 2 and Figures 5 and 6.

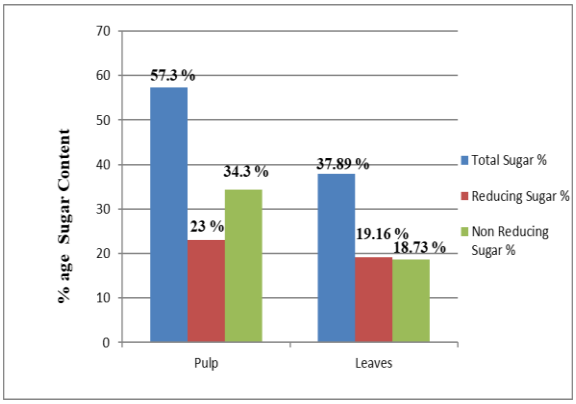

Figure 4: Sugar contents in methanolic extract of pulp and leaves of *Z. mauritiana.*

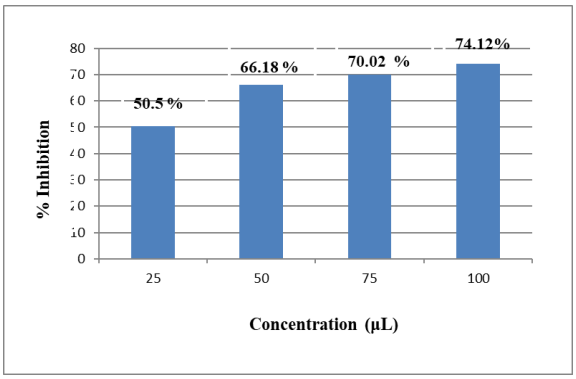

Figure 5: Antioxidant activity of methanolic extract of leaves of *Z. mauritiana.*

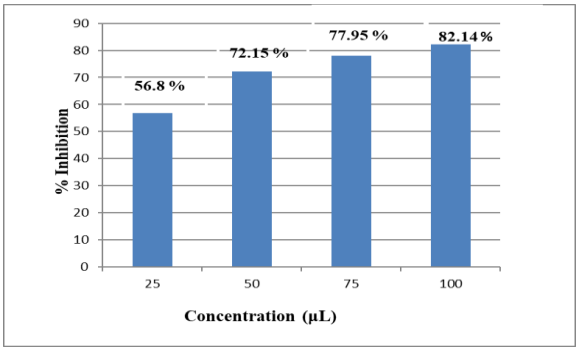

Figure 6: Antioxidant activity of methanolic extract of pulp of *Z. mauritiana*.

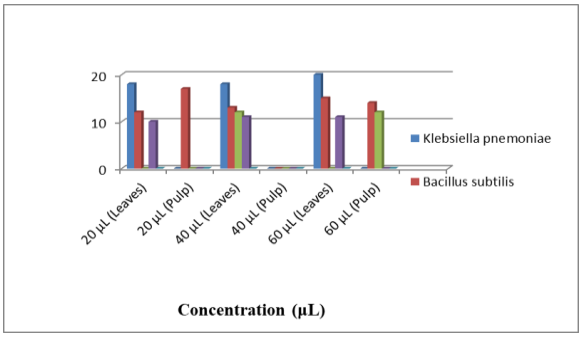

Figure 7: Comparison of antibacterial activities of different dilutions of *Z. mauritiana* leaves and pulp extracts.

e. Antimicrobial activities

The antimicrobial activities of leaves and pulp extracts of *Z. mauritiana* against five microbial species were evaluated by well diffusion method. Imipenem (10%) was used as a standard antibacterial drug. For each bacterial species, three dilutions with concentrations 20 µl/ml, 40 µl/ml and 60 µl/ml were made from pulp and leave extracts of *Z. mauritiana*. The diameter of inhibition zones of plant extracts and standards are given in Table 3. The comparison of antimicrobial activities of methanolic extract of *Z. mauritiana* leaves and pulp against different bacterial strains are shown in Figure 7.

Discussion

The results of current study demonstrate significant antioxidant potential and some antimicrobial activity of its pulp and leaves. Due to presence of numerous active compounds in pulp of *Z. mauritiana*, it exhibits antimicrobial and antioxidant potential and may find many pharmacological applications. The pulp and leaves of *Z. mauritiana* have shown the presence of 29.8 and 25.8 mg gallic acid equivalent (mg GAE/g) at 765 nm. It was demonstrated that methanol, due to its polar nature, has the ability to dissolve total phenolic compounds from both leaves and pulp of *Z. mauritiana*. TPC in methanolic extract of *Z. mauritiana* pulp are in higher concentration as compared to those present in methanolic extract of its leaves. The pulp (*Z. mauritiana*) has shown comparatively higher concentration of total sugar contents, reducing sugar and non-reducing sugar as compared to its leaves (Figure 4). The results of DDPH radical scavenging evaluations (Table 2, Figures 5 & 6) indicate that methanolic extract of *Z. mauritiana* leaves and pulp have excellent antioxidant potential. By comparing the results, it can easily be concluded that pulp extract of *Z. mauritiana* possesses higher antioxidant potential as compared to its leaves extract. Thus, both the pulp and leaves extracts possess the ability to inhibit the production of oxidative species produced in body. The antioxidant activity of was decreased with lowering the concentration of methanolic extracts of leaves as well as that of pulp. In case of leaves *Z. mauritiana* (Figure 5), the highest antioxidant activity (74.12%) was observed with a concentration of 100 µL of methanolic extract whereas antioxidant potential was reduced to 50.5% by lowering the concentration of leaves extract to 25 µL. In case of pulp of *Z. mauritiana* (Figure 6), the highest (82.14%) and lowest (56.80%) antioxidant activities were observed with the methanolic pulp extracts of 100 µL and 25 µL concentrations, respectively.

Test Organisms	Zone of inhibition (mm)						
	20 µl/ml		40 µl/ml		60 µl/ml		Std. (Imipenem) 10%
	Leaves	Pulp	Leaves	Pulp	Leaves	Pulp	
K. pneumonia	18	Nill	18	Nill	20	Nill	25
B. subtilis	12	17	13	Nill	15	14	37
S. aureus	Nill	Nill	12	Nill	Nill	12	18
P. aeruginosa	10	Nill	11	Nill	11	Nill	23
S. typhi	Nill	Nill	Nill	Nill	Nill	Nill	4.0

Table 2: Antimicrobial activities of *Z. mauritiana* leaves and pulp

Sr. No.	Leaves extract				Pulp extract			
	Conc. (µL)	Absorbance	%age inhibition	%age inhibition (Ascorbic acid)	Conc. (µL)	Absorbance	%age inhibition	%age inhibition (ascorbic acid)
1	25	0.4462	50.50	72.02	25	0.3895	56.80	72.02
2	50	0.3049	66.18	88.40	50	0.2439	72.15	88.40
3	75	0.2703	70.02	92.03	75	0.1984	77.95	92.03
4	100	0.2333	74.12	96.05	100	0.1614	82.14	96.05

Table 3: Antioxidant activity of methanolic extracts of leaves and stem of *Z. mauritiana*

The antimicrobial potential of leaves and pulp extracts of *Z. mauritiana* was tested against five bacterial strains using imipenem as a standard drug. The results (Table 3, Figure 7) have shown that antibacterial activity of the leaves extract against various microbial strains was decreased in the following order: *Klebsiella pnemonae* > *Bacillus subtilis* > *Pseudomonas aeroginosa* > *Staphylococcus aureus*. The pulp extract of *Z. mauritiana* has shown antimicrobial activity only against Bacillus subtili and *Staphylococcus aureus* whereas no antibacterial activity was observed by the pulp extract against all other bacterial species. The leaves and pulp extracts, both have shown no activity against Salmonella typhia. It can easily be concluded that higher antimicrobial activity is generally displayed by leaf extract of *Z. mauritiana* as compared to the pulp extract against all tested bacterial species except Salmonella typhi.

It was concluded that phytochemicals like alkaloids, flavanoids, tannins, saponins and phlobatanins were present whereas steroids were not present in both extracts of pulp and leaves of *Z. mauritiana*. The contents of TPC in pulp and leaves were 29.8 mg GAE/g and 25.8 mg GAE/g in methanolic extracts of pulp and leaves, respectively. Pulp and leaves extract showed maximum percentage inhibition 82% and 74%, respectively at maximum concentration of 100 µL. Sugar content is also the part of chemical constituents of both the pulp and leaves of *Z. mauritiana*. The plant contains many biologically active constituents which display good antimicrobial and antioxidant activities. The methanolic pulp extract of *Z. mauritiana* has shown high antioxidant potential as compared to the leaves extract.

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author Contributions

Mohsin Javed (Supervision of MS research), Shabbir Hussain (Writing of manuscript and submission), Rabiya Bibi (Experimental work for her MS degree), Muhammad Riaz (Analysis), Adnan amjad (Interpretation of results), Hamid Raza (Antioxidant potential), Shah muhammad Haroon (Antibacterial activities)

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