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RESEARCH ARTICLE

Phenolic contents, antioxidant activity and proximate analysis of *Ziziphus oxyphylla* Edgew. (Angrezi ber) from Soon Valley, Salt Range, Pakistan

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Abstract

The purpose of this research was to investigate the phenolic composition, antioxidant activity and proximate composition of *Ziziphus oxyphylla* Edgew. (Angrezi ber) through the use of ethanol and aqueous extracts. The flavonoid concentration of the extracts exhibited significant variation as a result of employing two distinct extraction methods, specifically sun-drying and shade-drying. Shade-dried aqueous extracts of leaves yielded the highest TPC of 599 ± 1.00 mg/100 g GAE (where GAE stands for Gallic acid equivalent), while shade-dried ethanol extracts of leaves exhibited a minimum value of TPC 281 ± 1.00 mg/100 g GAE. In leaves, the highest flavonoid content was observed in sun-dried ethanol extracts at 496.296 ± 3.70 mg/100 g CE (where CE stands for catechin equivalent), while the minimum was observed in shade-dried aqueous extracts at 159.259 ± 48.15 mg/100 g CE. Sun-dried ethanol extract 3711.35 ± 2.70 µg/100 g AAE (where AAE stands for ascorbic acid equivalent) exhibits a higher level of antioxidants as compared to shade-dried aqueous extracts valued at 462.70 ± 2.70 µg/100 g AAE. Aqueous and ethanol extracts of fruit yielded 758.000 mg/100 g GAE and 739.333 mg/100 g GAE respectively. At the same time, aqueous and ethanol extracts of fruit yielded 405.045 µg/100 g AAE and 329.369 µg/100 g AAE antioxidants respectively. Proximate analysis of *Z. oxyphylla* fruit yielded the following results: Moisture 11.9333 ± 0.0208 %, fat 8.2466 ± 0.02081 % fiber 5.03 ± 0.02 %, ash 5.6666 ± 0.1527 %, protein 2.0366 ± 0.0152 % and NFE (Nitrogen-Free Extract) 79.233 ± 0.02081 %. The results obtained from this study, which focused on the total phenolic contents and proximate compositions of *Z. oxyphylla*, can serve as a foundation for further investigations into its antioxidant capabilities also.

Keywords

Ziziphus oxyphylla; Soon Valley; nutritional analysis; Folin-Ciocalteu reagent; food source

Introduction

Food serves as a vital source of energy and essential building blocks for the restoration of damaged tissues as well as the production of crucial physiological components. Hence, it is imperative to ensure the provision of appropriate quality and amount of food to an individual. The assessment of food quality can be based on its nutritive values, which are determined by analyzing the composition of food in terms of its carbohydrate, protein, fat, mineral and vitamin content. The caloric content of food is contingent upon

the quantity of these constituents. The presence of insufficient quantities of one or more essential components can lead to the manifestation of signs and symptoms associated with a range of disorders. These several components collaborate in order to maintain the proper functioning of the human body. Nevertheless, the nutritional benefit of a diverse dietary regimen is solely advantageous if the essential components are sufficiently accessible in appropriate amounts and compositions (1-3).

Antioxidants serve the purpose of mitigating cellular damage caused by free radicals, which are molecules produced as a result of the regular metabolic process of oxidation. Several types of free radicals are present in biological systems, such as reactive hydroxyl radicals, reactive oxygen free radicals species, the superoxide anion radical, peroxyl and hydrogen peroxides. These molecules are capable of generating metabolic products that can initiate attacks on DNA or lipids in cell membranes. These factors are implicated in several forms of biological harm, including the development of cancer, cellular deterioration associated with aging, DNA damage and their contribution to heart disease and arthritis (4).

Currently, there exists a global liking for the utilization of aromatic and medicinal plants as sources of antioxidants in the context of food (5). The primary source of antioxidative properties in plants can be traced to phenolic compounds, including phenolic diterpenes, flavonoids and phenolic acids. These compounds possess the capacity to effectively neutralize free radicals, either by scavenging them, donating hydrogen atoms or electrons, or chelating metal cations (6). A strong positive connection was observed between the antioxidant capability and total phenol levels of several plant species (7-9). In addition to their antioxidant capacity, phenolic compounds demonstrate a diverse array of physiological activities, encompassing antiallergenic, anti-inflammatory, antiartherogenic, antibacterial, antithrombotic, vasodilatory and cardioprotective actions (10). Hence, the addition of antioxidant plant phenols to a food product may potentially confer a positive impact on human health.

Ziziphus oxyphylla, also known as Indian jujube or Ber, belongs to the family Rhamnaceae (known as the buckthorn family) and is native to Southeast Asia and South Asia. The tree grows in scrublands, wastelands and dry deciduous woods. In Southeast and South Asia, it is found in India, Pakistan, Bangladesh and Sri Lanka. The plant has been utilized in traditional medicine for the treatment of many ailments i.e., diabetes (11), jaundice (12, 37), hypertension (13) as well as gas troubles (14) for a long.

Materials and Methods

Plant Material: *Ziziphus oxyphylla* was collected from Soon Valley, Khushab District, Punjab, Pakistan (Fig. 1). Our collection site was precisely located at N 32°30’05.7” and S 072°09’30.2”, situated at an elevation of 856 m above sea level.

Fig. 1. Collection site (Soon Valley).

Furthermore, the plant was collected during fruiting season; the identified and scientific name of the plant specimen was cross-referenced using the World Flora Online (WFO) (https://worldfloraonline.org) that was deposited in the Herbarium of the Department of Botany, University of Sargodha, Pakistan under voucher number GA-640 for future reference.

Sample Preparation: Sun and shade-dried leaves were crushed into a fine powder, and this powder was used to make solutions for different assays. Using 5 g of plant materials (leaves and fruits), 2 types of solutions were made (100 % aqueous and 20 % ethanol and 80 % aqueous) for quantification of total phenolic content and antioxidants.

Plant material (5 g) from sun and shade dried leaves and fruits was added in 100 mL of solvent using different beakers. Solutions were thoroughly mixed with a vortex mixer and then left to rest for a period of 24 h. Then, this solution was filtered using Whatman filter paper no. 42, these solutions were used for further analysis.

For the proximate analysis, the fruit sample was dried in the shade for a period of 3 weeks; then, this sample was further dried using the hot air-drying method.

Determination of Total Phenolic Content: TPC was calculated using a slightly adjusted version of the Folin-Ciocalteu reagent technique (15). Total phenols were measured using ethanol with Gallic acid as a reference and were represented as (GAE) Gallic acid equivalents per 100 g of fresh weight sample. All experiments were carried out 3 times, whereas the experiments were conducted out twice.

Determination of total Flavonoid content: The approach given was used to compute FC (16). The Catechin was used as the standard; results were represented as mg of (+) -catechin equivalent per 100 g of fresh weight. All experiments were conducted in triplicate as were all determinations.

Determination of Total Antioxidants: The technique was employed to assess the overall antioxidant activity in water-solubilized samples (17). The results are presented both as micrograms of Ascorbic acid equivalent per mL of pulp and as mol TE (Trolox equivalent) per mL of pulp, utilizing AA (Ascorbic acid) for generating standard calibration curves. The process was repeated 3 times (in triplicate) to ensure accuracy in all computations and experiments.

Proximate analysis: Proximate analysis was conducted in IFSN (Institute of Food Science and Nutrition) at the University of Sargodha. This analysis encompassed proximate factors, including crude protein, crude fat, moisture, ash and NFE, crude fiber, ash and NFE, by following the procedure outlined by (18). All the values obtained were converted into %.

Results and Discussion

Phytochemical screening of *Z. oxyphylla* yielded the following results. Shade-dried aqueous extracts of leaves yielded the highest TPC, 599 ± 1.00 mg/100 g GAE (where GAE stands for Gallic acid equivalent), followed by sun-dried aqueous extracts 589 ± 1.00 mg/100 g GAE, sun-dried and shade dried ethanol extracts showed minimum amount of TPC 367 ± 1.00 mg/100 g GAE and 281 ± 1.00 mg/100 g GAE respectively (Table 1).

Table 1. Mean values of Total phenolic contents (leaves)

Solvent	Drying method		Mean
	Sun dry	Shade	
Ethanol	367 ± 1.00^{C}	281 ± 1.00^{D}	324 ± 47.11^{B}
Aqueous	589 ± 1.00^{B}	599 ± 1.00^{A}	594 ± 5.55^{A}
Mean	478 ± 121.60^{A}	440 ± 174.18^{B}	

Units= mg/100 g GAE (GAE stands for Gallic acid equivalent). Significantly different mean values do not share a letter.

The inherent characteristics of phenolic compounds present challenges in devising a comprehensive extraction process (19). The polarity and characteristics of the solvent employed have an impact on the solubility of phenolic compounds (20).

The highest flavonoid content was observed in sun-dried ethanol extracts, 496.296 ± 3.70 mg/100 g CE (where CE stands for catechin equivalent), followed by shade-dried ethanol extracts at 451.852 ± 3.70 mg/100 g CE. Sun and shade-dried aqueous extracts showed minimum flavonoid content of 177.778 ± 3.70 mg/100 g CE and 159.259 ± 48.15 mg/100 g CE (Table 2). The flavonoid compositions of *Z. oxyphylla* exhibit similarities to those found in certain plants, such as *Artemisia campestris* and *A. arvensis* belonging to the same family as reported (21).

Table 2. Mean of flavonoids (leaves)

Solvent	Drying method		Mean
	Sun dry	Shade	
Ethanol	496.296 ± 3.70^{A}	451.852 ± 3.70^{A}	474.074 ± 24.57^{A}
Aqueous	177.778 ± 3.70^{B}	159.259 ± 48.15^{B}	168.519 ± 32.18^{B}
Mean	337.037 ± 174.49^{A}	305.556 ± 163.14^{A}	

Units= mg/100 g CE (CE stands for catechin equivalent). Significantly different mean values do not share a letter.

Sun-dried ethanol extract 3711.35 ± 2.70 µg/100 g AAE (where AAE stands for ascorbic acid equivalent) exhibits a higher level of antioxidants compared to the shade-dried extract 1443.78 ± 2.70 µg/100 g AAE. Likewise, the sun-dried aqueous extract 597.84 ± 7.15 µg/100 g AAE demonstrates a significantly higher antioxidant value compared to the shade-dried aqueous extract 462.70 ± 2.70 µg/100 g AAE (Table 3).

Ethanol extracts, whether from shade or sun-dried samples, display greater antioxidant activity when contrasted with both sun and shade-dried aqueous extracts.

Previous research conducted by (22, 23) demonstrated that the choice of solvent used for extraction significantly affects the antioxidant capacity of

Table 3. Mean of antioxidants (leaves)

Solvent	Drying method		Mean
	Sun dry	shade	
Ethanol	3711.35 ± 2.70^{A}	1443.78 ± 2.70^{B}	2577.57 ± 1242.00^{A}
Aqueous	597.84 ± 7.15^{C}	462.70 ± 2.70^{D}	530.27 ± 74.17^{B}
Mean	2154 ± 1705.35^{A}	953.24 ± 537.37^{B}	

Units= µg/100 g AA (AAE stand for ascorbic acid equivalent). Significantly different mean values do not share a letter.

the extracts. These findings align with the results obtained in the present study. The observed differences in antioxidant activity may be attributed to the varying levels of polyphenols in each solvent, as suggested by (24, 25). However, it is important to note that the quality of polyphenols, flavonoids and tannins also contributes to this variation. The utilization of solvents possessing varying polarity enables the extraction of a specific subset of antioxidants, hence influencing the estimation of antioxidant capacity (26).

Total phenolic contents in the fruit of *Z. oxyphylla* quantified as gallic acid equivalent are presented in Table 4. The total phenolic content of the fruit sample was measured to be 758.000 mg/100 g GAE for aqueous extracts and 739.333 mg/100 g GAE for ethanol extracts. Antioxidant content for fruit was measured to be 405.045 µg/100 g AAE for aqueous extracts and 329.369 µg/100 g AAE. The inherent characteristics of phenolic compounds present challenges in devising a comprehensive extraction process (19). Similar reports are on many bioactive compounds in the methanolic extract of *Artocarpus hirsutus* leaves (36). The polarity and characteristics of the solvent employed have an impact on the solubility of phenolic compounds (20).

Table 4. Means for TPC and antioxidants (Fruit)

Treatment	TPC	Total Antioxidants
Aqueous	758.000^{A}	405.045^{A}
Ethanol	739.333^{B}	329.369^{B}

Units= mg/100g GAE (GAE stands for Gallic acid equivalent) for TPC and µg/100g AA (AAE stand for ascorbic acid equivalent) for antioxidants. Means that do not share a letter are significantly different

Proximate analysis of fruit: The moisture content of *Z. oxyphylla* was measured to be 11.9333 ± 0.0208 %. This moisture content found in *Z. oxyphylla* (11.9333 ± 0.0208 %) close to some common leafy vegetables like *Xanthosoma sagittifolum* and *Adansonia digitata* with 13.2 % and 9.50 % respectively (27). The fruit sample consisted of 8.2466 ± 0.0208 % fat; the findings of this study closely align with the fat content (28), who examined "nutrient value of North East Indian plant foods" and (29) with the topic "nutrient composition of less familiar leaves consumed by the tribals of Udaipur region." Fat plays a vital role in facilitating the absorption of essential vitamins like A, B, E and K, making it a valuable energy source (30). Crude fiber % was found to be 5.03 ± 0.02 %. The findings from this study are corroborated by the results obtained in the research conducted earliest (31). Ash content was 5.6666 ± 0.1527 %, (32) considered the ash content, which ranged from 1.91 % to 8.73 %, to be rich. Results revealed that crude protein content was 2.0366 ± 0.0152 %. The present study benefits from the support of prior research conducted by (33-35), which validated the current outcomes. The NFE (Nitrogen-Free Extract) content of *Z. oxyphylla* was measured at 79.233 ± 0.02081% (Table 5) (Fig. 2), which is similar and can be compared to the carbohydrate content found in other fruits like *Morus alba, Mangifera indica, Prunus nepalensis, P. cerasoides* and *Terminalia chebula*, which posses carbohydrate contents of 87.5 %, 84.0 %, 82.6 %, 84.1 % and 80.6 % respectively (32).

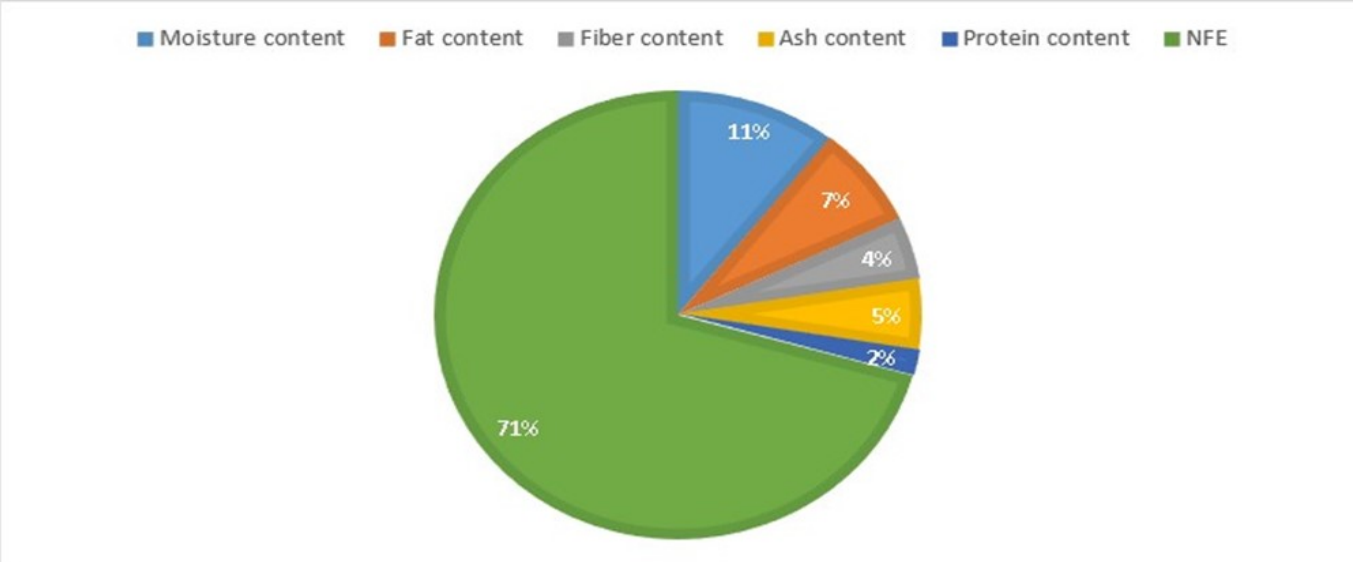

Fig. 2. Results of proximate analysis shown as %.

Table 5. Proximate analysis of *Z. oxyphylla* fruit

SAMPLE	Moisture %	Fat %	Fiber %	Ash %	Protein %	NFE %
Ziziphus oxyphylla	11.9333 ± 0.0208	8.2466 ± 0.02081	5.03 ± 0.02	5.6666 ± 0.1527	2.0366 ± 0.0152	79.233 ± 0.02081

Conclusion

In this study, total phenolic content, antioxidant activity of fruit and leaves, and proximate composition of fruit of *Ziziphus oxyphylla* were determined using aqueous and ethanol extracts. The leaves and fruits of this plant contain a high amount of total phenolic content, considerable total flavonoid content, antioxidant activity and high nutritional value. The results obtained from this study, which focused on the total phenolic contents and proximate compositions of *Z. oxyphylla*, can serve as a foundation for further investigations into its antioxidant capabilities. These research endeavours will help improve the National Food Composition Database in the country.

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Authors' contributions

The manuscript is written by JN as M.Phil scholar. SR (as M.Phil supervisor) and MN (as M.Phil co-supervisor) helped in the tabulation of data. The rest of the authors AS,MK, GS, TT, UZ, ABS and NR helped in the finalization of the paper. All the authors approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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