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INVESTIGATION OF THE ANTIRADICAL ACTIVITY OF THE ZIZIPHORA PLANT (ZIZIPHORA SPP.)

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Abstract
In this study, the antiradical activity of ethanol extracts obtained from *Ziziphora clinopodioides* subsp. *rigida* was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The influence of different volumes of the extract (25, 50, 75, and 100 µL) on the DPPH radical solution was examined spectrophotometrically at 517 nm. The absorbance reduction was used as a measure of free radical neutralization. The experimental data confirmed the extract's significant antiradical potential, with an IC_{50} value calculated at 99.62 µL, indicating moderate antioxidant efficiency. These findings suggest that *Ziziphora clinopodioides* may serve as a natural source of bioactive compounds with antioxidant properties, potentially useful in pharmaceutical or nutraceutical applications.

Keywords: Ziziphora, antiradical activity, DPPH, IC_{50}, spectrophotometry, ethanol extract, free radicals, natural antioxidants, phytochemicals, bioactive compounds.

Introduction

In recent years, the detrimental effects of free radicals on living organisms have garnered significant scientific attention, particularly due to their role in accelerating the development of cancer, cardiovascular diseases, diabetes, and the aging process. Free radicals can damage cellular DNA, proteins, and lipid structures, leading to oxidative stress and cellular dysfunction [1].

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As a result, the search for natural antioxidants—especially bioactive compounds derived from medicinal plants such as phenolics, flavonoids, and essential oils—has become increasingly important. These compounds play a crucial role in protecting biological systems against oxidative damage. Among the widely used methods for evaluating the antioxidant capacity of plant extracts, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is one of the most reliable and reproducible techniques. In this method, antioxidant activity is assessed based on the discoloration of the DPPH radical solution, measured spectrophotometrically at 517 nm [2].

Ziziphora clinopodioides subsp. *rigida*, a medicinal plant belonging to the family Lamiaceae, is traditionally used for its anti-inflammatory, antiseptic, and sedative properties. The plant is rich in essential oils, flavonoids, and other phenolic constituents known for their strong biological activity. Hence, it is of significant interest to investigate the antioxidant potential of this species using a standardized scientific approach [3, 4].

The DPPH radical scavenging assay provides a convenient method to determine the hydrogen-donating or electron-transferring ability of potential antioxidants. The purple-colored DPPH• radical becomes reduced and discolored in the presence of antioxidant molecules. In this study, a slightly modified version of the Blois [2] method was applied to assess the free radical inhibition capacity of *Ziziphora* extracts [4].

Materials and Methods

Preparation of DPPH• Working Solution. A 7.92 mM solution of DPPH• was prepared by dissolving DPPH in ethanol in a 100 mL volumetric flask. The solution was wrapped in aluminum foil and kept in the dark at room temperature for 30 minutes before use.

Preparation of Plant Extract. The plant sample of *Ziziphora clinopodioides subsp. rigida* was used for extraction. A total of 0.75 g of dried aerial parts were extracted in 25 mL of 96% ethanol using an ultrasonic bath (60 °C, 20 minutes). The obtained extract was filtered through a 0.45 µm syringe filter and stored for further analysis [1]. Before analysis, the extract was diluted fivefold with ethanol.

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Determination of Antiradical Activity. A quartz cuvette (4 mL volume) was filled with 3 mL of DPPH solution and 100 µL of ethanol as the control. The cuvette was then placed into a spectrophotometer (Model K7000, YOKE, China), and absorbance at 517 nm (D_1) was recorded every 5 minutes for 30 minutes. To assess the antiradical activity of the samples, 25, 50, 75, and 100 µL of the plant extract were added to 3 mL of DPPH solution in separate cuvettes. The remaining volume was adjusted to 3.1 mL with ethanol. Absorbance at 517 nm (D_2) was measured in the same manner. The percentage of antiradical activity (ARA%) was calculated using the following equation:

$$ARF\% = \frac{D_1 - D_2}{D_1} \cdot 100\%$$

Results and Discussion

The results are presented in Table 1, which includes the measured absorbance values for the control and treated samples along with the calculated antiradical activity percentages for various extract volumes.

Table 1. Absorbance values and calculated antiradical activity (%) of ethanol extract of *Ziziphora clinopodioides* at different volumes

Volume, µl	Time, min.	Sample					
		Abs, D	ARF%		Time, min.	Abs, D	ARF%
25	0	1,096	0,00	75	0	1,096	0,0
	5	0,986	10,04		5	0,848	22,6
	10	0,974	11,13		10	0,763	30,4
	15	0,965	11,95		15	0,74	32,5
	20	0,956	12,77		20	0,723	34,0
	25	0,952	13,14		25	0,711	35,1
	30	0,948	13,50		30	0,702	35,9
50	0	1,096	0,00	100	0	1,096	0,00
	5	0,946	13,69		5	0,752	31,39
	10	0,895	18,34		10	0,613	44,07
	15	0,876	20,07		15	0,583	46,81
	20	0,862	21,35		20	0,561	48,81
	25	0,846	22,81		25	0,545	50,27
	30	0,836	23,72		30	0,533	51,37

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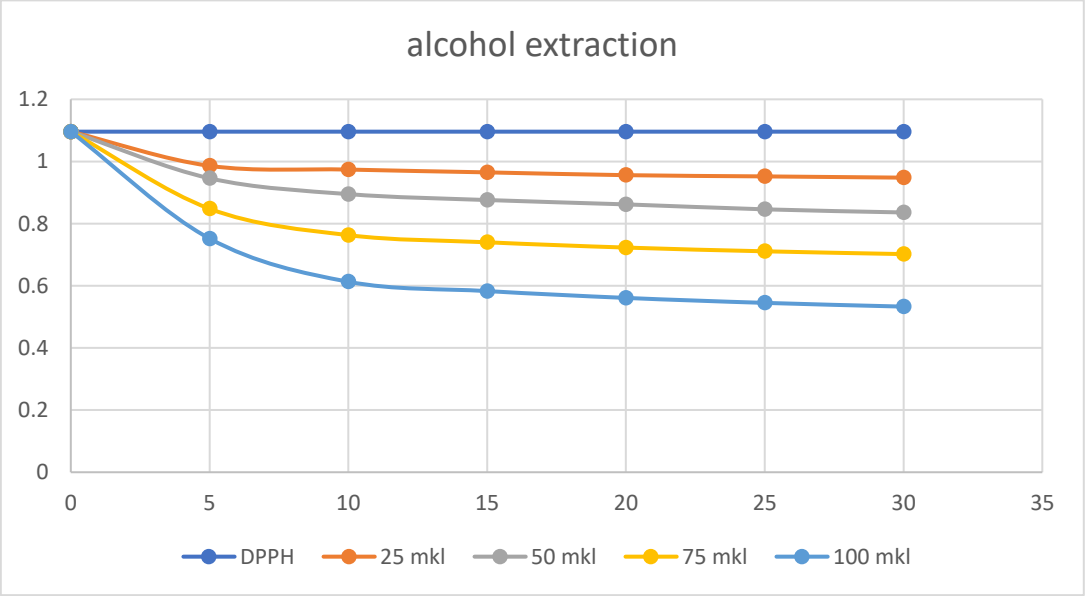

Figure 1. Graphical representation of the measured light absorption of blank and tested alcohol extracted sample solutions spiked with DPPH solution.

To calculate the IC_{50} of the samples - the concentration of inhibition of the DPPH solution by 50%, the following graph was constructed based on the antiradical activity (ARF%) values at 30 minutes in each experiment and the volume of alcohol samples added, and the trend line function applied to it was calculated.

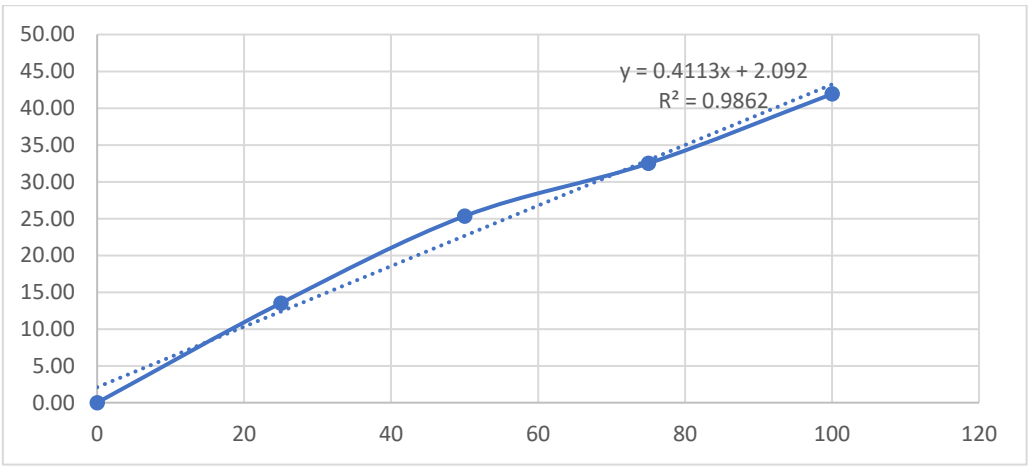

Figure 3. Graph of the relationship between ARF% and volumes determined at 10 minutes of an alcoholic extracted sample.

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The trend line plotted on the graph was calculated from the functional formula y=mx+b, based on the formula x=(y-b)/m, which represents the volume of 50% ARF% (IC_{50}):

$$IC_{50} = \frac{(50 - 0{,}13)}{0{,}5006} = 99{,}62\ \mu l$$

Analysis

The biological activity of *Ziziphora clinopodioides* subsp. *rigida* (commonly known as "Kiyik o'ti" in traditional medicine) was evaluated using a modern analytical method—DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. This method is widely recognized for assessing antioxidant potential by quantifying the capacity of natural compounds, particularly phenolic substances, to neutralize free radicals.

In the present study, ethanol-based plant extracts were prepared and tested at various volumes (25, 50, 75, and 100 µL). The antiradical activity of each sample was measured spectrophotometrically at 517 nm over a 30-minute period. The results indicated that the antioxidant activity increased proportionally with extract concentration. The highest observed Antiradical Activity (ARF%) was 51.37% at a sample volume of 100 µL. Additionally, the calculated IC_{50} value—the volume required to inhibit 50% of DPPH radicals—was determined to be 99.62 µL, suggesting that the extract demonstrates moderate antiradical activity.

The scientific importance of this study lies in its confirmation of the traditional use of *Ziziphora clinopodioides* through a standardized scientific approach. The presence of natural antioxidants within the extract was confirmed, providing a foundation for its potential use in developing natural health products, dietary supplements, or phytopharmaceuticals.

Moreover, such research contributes to the exploration and valorization of regional medicinal flora, supporting its integration into modern pharmacological applications. Given the growing global demand for safe, eco-friendly, and plant-derived antioxidants, this study underlines the relevance of *Ziziphora* species as a promising natural source of bioactive compounds.

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Conclusion

The study demonstrated that the phenolic compounds identified in *Ziziphora clinopodioides* subsp. *rigida* — including apigenin, rutin, salicylic acid, and quercetin — exhibit significant potential in supporting cardiovascular health, particularly in the management of hypertension. Each of these compounds contributes through distinct biochemical mechanisms: apigenin provides vasorelaxant and anti-inflammatory effects; rutin enhances capillary resistance and reduces vascular permeability; salicylic acid offers anti-inflammatory and mild anticoagulant properties; while quercetin acts as a powerful antioxidant and vasodilator.

Collectively, these bioactive compounds assist in lowering elevated blood pressure, stabilizing cardiac rhythm, improving peripheral and central blood flow, and mitigating oxidative stress-induced damage to vascular endothelium. Their synergistic activity contributes to the restoration of vascular elasticity and integrity, which are often compromised in hypertensive patients.

Given their natural origin, low toxicity, and multiple pharmacological actions, the phenolic constituents of *Ziziphora* extracts present promising therapeutic value as a basis for developing plant-based antihypertensive phytopreparations. Future in vivo studies and clinical trials are recommended to further validate these findings and to explore dosage standardization, pharmacokinetics, and long-term safety profiles.

These results also underline the broader potential of traditional medicinal plants in modern pharmacology, particularly in regions where access to synthetic drugs may be limited, and highlight the importance of integrating ethnobotanical knowledge with modern analytical techniques.

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