

Phytochemical Analysis of Xerohalophyte *Zygophyllum coccinum* and its Antioxidant Activity

Ahmed Kheir¹, El-Sayed F. El-Halawany¹, Suzan M. Hussien¹, Yasser A. El-Amier^{1*}

¹Botany Department, Faculty of Science, Mansoura University, Mansoura - 35516, Egypt

* Correspondence to: yasran@mans.edu.eg; Tel. +201017229120

Received: 10/11/2023
Accepted: 18/12/2023

Abstract : Halophytes, plants that thrive in saline environments, have gained attention in the field of medicine due to their unique biochemical composition and potential health benefits. The objective of this study was to ascertain the optimal *Zygophyllum coccinum* extracts and evaluate if their combination exhibits superior antioxidant activity compared to individual extracts. The plant material was subjected to extraction using methanol as the solvent, and subsequent analysis was conducted on the resultant extract. The research used quantitative methodologies to investigate the levels of alkaloids, tannins, saponins, flavonoids, and phenols. Furthermore, a range of phytochemical assays with different levels of sensitivity were used to detect the existence of bioactive substances. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) test was used to assess the antioxidant efficacy of the extract. Based on the findings, it was observed that the extract derived from the aerial portions had the highest level of antioxidant scavenging activity, as shown by an IC₅₀ value of 35.64 mg/L and RSA value of 60.44% at 1000 mg/l. It is vital to acknowledge, yet, that this specific endeavor surpasses the functionality of catechol at doses beyond an IC₅₀ threshold of 19.07 mg/ml. The presence of antioxidant phytochemicals in these plants lends support to the hypothesis that they were first used for therapeutic purposes.

keywords: *Zygophyllum coccinum*; Biological activity; Desert; DPPH, Phytochemical.

1. Introduction

In the twentieth century, public health initiatives began to place more emphasis on addressing bacterial infections, cancer, and heart disease due to the emergence of the epidemiological shift and the increasing prevalence of infectious diseases. The implementation and use of preventive methods in several developed countries have yielded substantial reductions in neonatal mortality rates [1]. The increase in life expectancy seen throughout the twentieth and early twenty-first century has been attributed to the achievements in public health, including the implementation of vaccination initiatives and the eradication of several infectious diseases such as polio, diphtheria, yellow fever, and smallpox. Such improvements included disinfecting drinking water with chlorine, filtering and treating wastewater, which led to a decrease in the death rate due to infectious diseases resulting from

water, such as cholera and intestinal diseases [2,3].

Pathogenic bacteria are bacteria that cause bacterial infections. Although most bacteria are not pathogenic or beneficial, a small percentage are considered pathogenic. One of these bacterial diseases is tuberculosis, caused by *Mycobacterium tuberculosis*, which kills two million people every year [4]. Pathogenic bacteria play a role in causing important diseases common in this era, for example pneumonia, which can be caused by *Streptococcus* or *Pseudomonas* bacteria. Likewise, diseases caused by food poisoning can be caused by *Salmonella*, *Shigella*, or *Campylobacter* (bacteria). Infective bacteria also cause sicknesses such as tetanus, typhoid fever, diphtheria, syphilis, and leprosy. Koch's hypotheses are a measure upon which to determine the relationship between the pathogenic microbe and the disease [5].

An antioxidant is a molecule capable of slowing down or preventing the oxidation of free radicals in the living body, and oxidation is a chemical reaction that transfers electrons from a specific substance into an oxidizing agent, which may damage some cells. Antioxidants terminate this chain of reactions by completely removing the essential stray intermediate and preventing other oxidation reactions from oxidizing themselves. Consequently, antioxidants often eliminate oxygen-reactive substances, such as thiols or polyphenols [6].

Approximately 80% of the worldwide population depends on it due to its significance in traditional medicine and herbal therapy across various regions, its widespread availability, cost-effectiveness, user-friendly nature, and potential effectiveness. Additionally, the potential risks linked to the utilization of chemical medications contribute to its popularity [7]. While most medicinal herbs are generally considered harmless, there exists a small subset that has the potential to cause fatality in both humans and animals upon ingestion. Scientific estimations suggest that the Earth has a considerable range of medicinal plants, with estimates ranging from 250,000 to 500,000 species. One classification of botanical organisms is comprised of plants that possess medical properties, which are sometimes ingested by both humans and other members of the animal kingdom [8].

Zygophyllum coccineum is a facultative halophyte widespread in n desert wadis and coastal areas of Egypt and grows in diverse habitats and different soil types. The plant is very common in the limestone wadis and plains of the Eastern (Arabian) desert and tolerant of saline soils. It dominates a community of widespread occurrence there [9]. *Z. coccineum* has medicinal value as an antidiabetic and antioxidant, and ecological value as a bio-accumulator for heavy metals from the soil and water [10-13] The purpose of this research was to analyze the wild plant *Zygophyllum coccineum*, which was obtained from the Northern sector of Nile Delta, with the purpose of determining its chemical analysis and assessing its antioxidant and antimicrobial activities.

2. Materials and Methods

2.1. Collection of Plant material

Zygophyllum coccineum was successfully collected in May 2023 from the northern Nile Delta in Egypt (at 29° 5'29.37"N and 32°17'58.38"E, to be exact). Tackholm [14] and Boulos [9] were consulted in order to aid in the identification of plants. After the sample was cleaned by hand, it was rinsed three times in distilled water to get rid of any remaining dust and pollutants. After that, full desiccation was attained by leaving the sample out in the shade at a room temperature of 25±4 °C for several days. The dried sample was eventually pulverized into a powdery form. The samples were then carefully placed in paper bags and stored at room temperature, away from heat and light, until further examination could be performed.

2.2. Extraction

The conventional approach for acquiring botanical therapeutics is using solvent extraction [15]. The word "extraction" refers to the procedure by which water-soluble constituents of plants are separated from the insoluble cellular residue inside a plant. The dried plant portions, with a total weight of 200 grams, were immersed in an 85% methanol solution for a duration of three days at room temperature [15]. In order to liberate phytochemicals that are soluble in water, the cell walls undergo a delicate process of disintegration and fragmentation. The use of the filtered solution might commence after a duration of three days. In general, the transport of heat occurs via the processes of convection and conduction, whereas the extraction of samples is facilitated using various solvents [16]. After undergoing filtration, evaporation, and dissolution in DMSO, the extracts were deemed suitable for use.

2.3. Phytochemical constituents

2.3.1. Qualitative phytochemical screening

The identification of phytochemical components was conducted using established methodologies as described by Farnsworth [17], Harborne [18], Sofowora [19], and Evans [20].

2.3.2. Quantitative determination of phytochemicals

The measurement of tannin, saponin, flavonoid, alkaloid, and total phenol concentrations in this investigation followed the protocols outlined by Sadasivam and Manickam [21], Harborne [18], Boham and Kocipai-Abyazan [22], and Obadoni and Ochuko [23].

2.4. Antioxidant activities

The evaluation of free radical scavenging activities was conducted using an approach like the one reported by Bibi *et al.* [24]. After the introduction of 180 microliters of DPPH solution dissolved in methanol, the sample solution in DMSO achieved a final concentration of 100 grams per milliliter. The measurement of absorbance for the samples was conducted at a wavelength of 517 nm using a microplate reader after a 15-minute incubation period at a temperature of 37°C in a light-restricted environment.

$$\text{Scavenging activity (\%)} = 100 \times [1 - (\text{Absorbance}_{\text{sample}} / \text{Absorbance}_{\text{blank}})]$$

3. Results and Discussion

3.1. 3.1. Qualitative phytochemical screening

Qualitative phytochemical screening provides an overview of the presence and quality of these compounds. It aids in determining plant sources containing specific compounds, contributing to directing pharmaceutical research for the development of

Table 1. Studying the phytochemistry of inland desert (northern part of the Eastern Desert) *Zygophyllum coccinum*.

Plant sample	Screening test								
	Tannins	Saponins	Alkaloids	Flavonoids	Phenols	Steroids	Glycosides	Anthraquinones	Terpenes
<i>Zygophyllum coccinum</i>	+++	+++	++	++	++	+	++	-	+

3.2. Quantitatively analysis of Some Secondary metabolites

Quantitative phytochemical analysis is a crucial process aimed at examining and determining the chemical components in plants accurately, emerged as one of the first branches within the area of organic chemistry. There exists a substantial need to describe and identify plant-derived compounds that possess therapeutic potential [26]. The unique attributes

more effective drugs. The analysis of the data presented in Table 1 reveals that *Zygophyllum coccineum* extract exhibits significant quantities of alkaloids, tannins, flavonoids, terpenoids, etc., which are recognized as prominent secondary metabolites. Plant secondary metabolites play a crucial role in medicine due to their diverse biological activities and therapeutic potential. These compounds, produced by plants for various ecological functions, have been harnessed for their medicinal properties [25].

In the context of qualitative screening, well-established methodologies are used to determine the presence or absence of phytochemicals in different extracts (Table 1). The phytoconstituent assessment in this study was assigned a numerical value ranging from -4 to +4. The development of this scale was influenced by the degree of color change, or the amount of precipitation seen. Hence, it was possible to conduct a qualitative investigation of the bioactive chemicals present in the native plant. The quantities of alkaloids, flavonoids, phenols, saponins, and tannins present in the samples are recorded in Table 1. Nevertheless, it is worth noting that the presence or lack of phytoconstituents has been identified in various samples, as shown in Table 1. Based on the findings of this investigation, it can be concluded that the examined plant species lack the presence of anthraquinones.

of the plant under investigation, together with the diverse range of phytoconstituents that exhibited variability across different plant samples, were revealed via a meticulous analysis of the analytical data pertaining to *Zygophyllum coccinum*. The examination further revealed that the plant under investigation has a notable abundance of saponins (20.29 mg/g dry wt.), tannins (22.38 mg/g dry wt.), phenols (16.91 mg/g dry wt.),

flavonoids (7.10 mg/g dry wt.), and alkaloids (6.67 mg/g dry wt.). Alkaloids, saponins, tannins, and flavonoids are among the chemical groupings that have been acknowledged for their medicinal properties in treating a diverse range of disorders. The historical use of these pharmaceutical substances for addressing a diverse range of medical conditions is substantiated by the research outcomes of investigations undertaken by Hassan *et al.* (27) and Usman and Osuji (28).

Many secondary metabolites exhibit pharmacological activities, such as antimicrobial, anti-inflammatory, antioxidant, antiviral, and anticancer properties. These properties make them valuable candidates for the development of pharmaceutical drugs. Moreover, secondary metabolites in halophytes play important roles in their adaptation to saline conditions and may contribute to their ecological success [6]

Table 2. *Zygophyllum coccinum*'s organically active components (mg g⁻¹ dry wt.) were measured in the inland desert.

Plant sample	Active organic compounds				
	Alkaloids	Flavonoids	Phenols	Saponins	Tannins
<i>Zygophyllum coccinum</i>	6.67±0.05	7.10±0.09	16.91±1.04	20.29±0.98	22.38±1.53

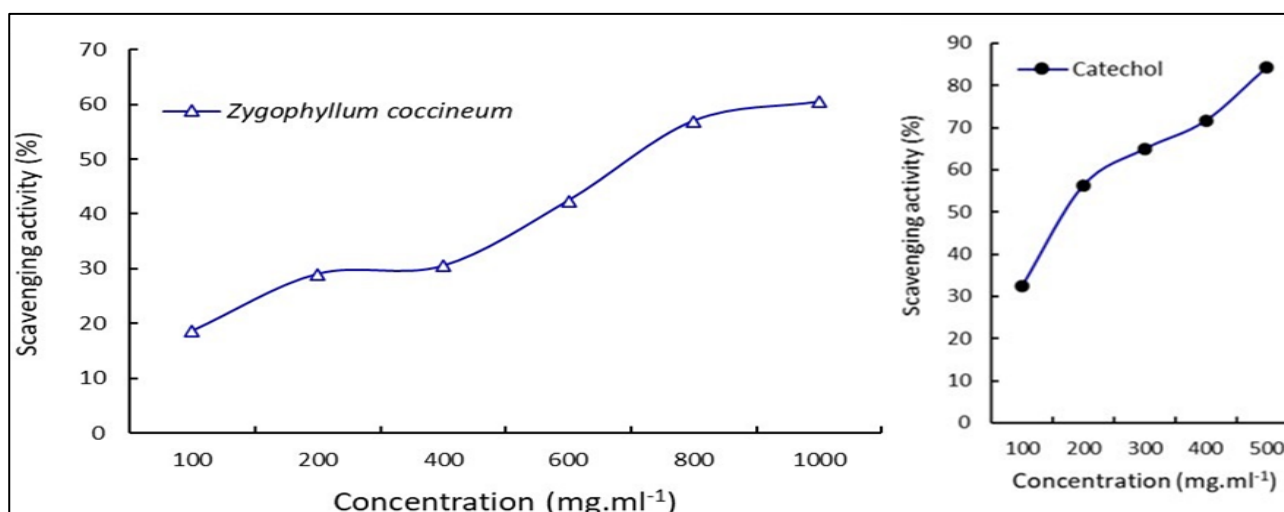


Figure 1. Standardization of DPPH radical-scavenging activity using a MeOH extract of the Egyptian desert plant *Zygophyllum coccinum* and the antioxidant compound catechol.

3.3. Antioxidant assay

Halophytes produce antioxidants such as flavonoids, phenolic compounds, and carotenoids to combat oxidative stress induced by high salt levels. These antioxidants help neutralize reactive oxygen species (ROS) generated under saline conditions. The essential function of antioxidants is to safeguard human cells from oxidative stress, hence reducing the likelihood of developing cancer [29]. In order to assess the antioxidant properties of the MeOH-extract of *Zygophyllum coccinum*, its ability to counteract DPPH free radicals was compared to that of catechol. The relative scavenging effectiveness of plant extracts and the gold standard was assessed using half-maximal inhibitory concentration (IC₅₀) values. The findings are shown in Figure 1. A

reduction in the IC₅₀ value indicates an enhancement in the efficacy of DPPH radical scavenging. The following examination of the data revealed that the extract obtained from the aboveground portions had the most potent antioxidant scavenging action, with an IC₅₀ value of 35.64 mg/L and RSA value of 60.44% at 1000 mg/l. However, when the concentration exceeds the IC₅₀ value of 19.07 mg/ml, the activity of the substance exceeds that of catechol. The findings presented in this study about *Zygophyllum coccinum* are consistent with the research conducted by Abd-ElGiawad *et al.* (30), Corenira *et al.* (31), and Leoanti (32).

The primary aim of this study is to examine and contrast the antioxidant efficacy of the shoot extract derived from *Zygophyllum*

coccinum with other extracts produced from indigenous plants coming from several geographical locations. Multiple studies have reported results indicating that the antioxidant properties of plants are influenced by the concentration of bioactive chemicals, namely phenolic constituents such as flavonoids, phenolic acids, ascorbic acid, and carotenoids [33]. The production of antioxidants in halophytes is part of their adaptive strategy to thrive in saline environments. It allows them to counteract the detrimental effects of salt-induced oxidative stress, ensuring their survival and growth in challenging habitats.

4. Conclusion

Halophytes are plants that thrive in saline environments, such as coastal areas or salt marshes. The outcomes of this investigation provide corroborating evidence for the use of certain botanical extracts. The botanical classification, the process of extraction, and the technique for assessment all influence the characteristics of the bioactive compounds detected in botanical samples. The strong correlation seen between antioxidant capacity and bioactive molecules implies that the latter are primarily accountable for the remarkable antioxidant properties shown by plants. These findings provide an opportunity for further investigation into the medicinal capabilities of this plant species and others, as well as the exploration of an appropriate solvent for the extraction of economically valuable bioactive chemicals.

4. References

1. Barrett, R., (2021). Emerging infectious diseases. The Wiley Blackwell Companion to Medical Sociology, pp.431-446.
2. Fielding, J.E., (1999). Public health in the twentieth century: advances and challenges. Annual review of public health, **20**(1), pp.xiii-xxx.
3. Bedford, J., Farrar, J., Ihekweazu, C., Kang, G., Koopmans, M. and Nkengasong, J., (2019). A new twenty-first century science for effective epidemic response. Nature, **575**(7781), pp.130-136.
4. Chai, Q., Zhang, Y. and Liu, C.H., (2018). Mycobacterium tuberculosis: an adaptable pathogen associated with multiple human diseases. Frontiers in cellular and infection microbiology, **8**, p.158.
5. Khaneghah, A.M., Abhari, K., Eş, I., Soares, M.B., Oliveira, R.B., Hosseini, H., Rezaei, M., Balthazar, C.F., Silva, R., Cruz, A.G. and Ranadheera, C.S., (2020). Interactions between probiotics and pathogenic microorganisms in hosts and foods: A review. Trends in Food Science & Technology, **95**, pp.205-218.
6. Yadav, A., Kumari, R., Yadav, A., Mishra, J.P., Srivatva, S. and Prabha, S., (2016). Antioxidants and its functions in human body-A Review. Res. Environ. Life Sci, **9**(11), pp.1328-1331.
7. Srivastava, J., Lambert, J. and Vietmeyer, N., (1996). Medicinal plants: An expanding role in development (Vol. **320**). World Bank Publications.
8. Babich, O., Sukhikh, S., Pungin, A., Ivanova, S., Asyakina, L. and Prosekov, A., (2020). Modern trends in the in vitro production and use of callus, suspension cells and root cultures of medicinal plants. Molecules, **25**(24), p.5805.
9. Boulos, L. , (2002) Flora of Egypt; Al Hadara Publishing: Cairo, Egypt.
10. Mansour, H.A.; Newairy, A.S.A.; Yousef, M.I.; Sheweita, S.A. (2002) Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. Toxicology, **170**, 221–228 .
11. AbouZid, S.; Elshahaat, A.; Ali, S.; Choudhary, M.I. (2008) Antioxidant activity of wild plants collected in Beni-Sueif governorate, Upper Egypt. Drug Discov. Ther., **2**, 286–288 .
12. Amin, E.; El-Hawary, S.S.; Fathy, M.M.; Mohammed, R.; Ali, Z.; Tabanca, N.; Wedge, D.E.; Becnel, J.J.; Khan, I.A. (2011) Triterpenoidal Saponins: Bioactive Secondary Metabolites from *Zygophyllum coccineum*. J. Med. Plant Nat. Prod. Res., **77**, 488–491 .
13. El-Sherbiny, M.M.; Ismail, A.; EL-Hefnawy, M.E (2019). A preliminary assessment of potential ecological risk and soil contamination by heavy metals around a cement factory, western Saudi Arabia. Open Chem., **17**, 671–684 .

14. Tackholm V. (1974). Students' Flora of Egypt. Cairo University Press, Cairo, Egypt.
15. Handa, S.S. (2008). An overview of extraction techniques for medicinal and aromatic plants. *Extraction Technologies for Medicinal and Aromatic Plants*, 1: 21-40.
16. Edo, G.I., Samuel, P.O., Ossai, S., Nwachukwu, S.C., Okolie, M.C., Oghenegueke, O., Asaah, E.U., Akpogheli, P.O., Ugbune, U., Owhero, J.O. and Ezekiel, G.O., (2023). Phytochemistry and pharmacological compounds present in scent leaf: A review. *Food Chemistry Advances*, 3, p.100300.
17. Farnsworth, N. R., (1996). Biological and phytochemical screening of plants. *J Pharm Sci Mar*; **55(3)**:225-76.
18. Harborne, J.B. (1973). *Phytochemical Methods*, London. Chapman and Hall, Ltd., 49-188.
19. Sofowora, A. (1993). Screening plants for bioactive agents. *Medicinal Plants and Traditional Medicinal in Africa*, **2**: 134-156.
20. Evans, W. C. (1999). *Trease and Evans Pharmacognosy* 14th Edition W. B. Saunders Company Limited, New York pp1-340.
21. Sadasivam, S. and Manickam, A. (2008). *Biochemical Methods*. 3rd ed. New Age Intern., Limited, New Delhi.
22. Boham, B.A. and Kocipai-Abyazan, R. (1974). Flavonoids and Condensed Tannins from Leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. *Pacific Science*, **48**: 458-463.
23. Obadoni, B.O. and Ochuko, P.O. (2001). Phytochemical Studies and Comparative Efficacy of the Crude Extracts of some Homeostatic Plants in Edo and Delta States of Nigeria. *Global Journal of Pure Applied Science*, **8**: 203-208.
24. Bibi, G., Haq, I., Ullah, N., Mannan, A. and Mirza, B., (2011). Antitumor, cytotoxic and antioxidant potential of *Aster thomsonii* extracts. *African Journal of Pharmacy and Pharmacology*, **5(2)**, pp.252-258.
25. Ali, S.S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A. and Bora, U., (2008). Indian medicinal herbs as sources of antioxidants. *Food research international*, **41(1)**, pp.1-15.
26. Oszahin AD, Kirecci OA (2016). Antioxidant properties, characterization of nutrients, and phytochemistry of seven medicinal plants. *Chemistry of Natural Compounds*; **52(6)**:1081-1083.
27. Hassan MM, Oyewale AO, Amupitan JO, Abdullahi MS, Okonkwo EM. (2004) Preliminary Phytochemical and antibacterial investigation of crude extracts of the root bark of *Detarium microcarpum*. *J Chem Soc Nigeria*; **29**: 26–29 .
28. Usman H, Osuji JC. (2007) Phytochemical and in vitro antimicrobial assay of the leaf extract of *Newbouldia leavis*. *Afr J Trad CAM*; **4(4)**: 476–480.
29. Abd-ElGawad, A.M.; El-Amier, Y.A.; Assaeed, A.M.; Al-Rowaily, S.L., (2020) ,Interspecific variations in the habitats of *Reichardia tingitana* (L.) Roth leading to changes in its bioactive constituents and allelopathic activity *Saudi Journal of Biological Sciences* **27**, 489-499.
30. Cornara, L.; La Rocca, A.; Marsili, S.; Mariotti, M., (2009) Traditional uses of plants in the Eastern Riviera (Liguria, Italy) *Journal of Ethnopharmacology*, **125**, 16-30.
31. Leonti, M. (2006) Local Mediterranean food as a source of novel nutraceuticals. in *In Pharmaceutical Soc Great Britain.. Pharmaceutical Press-Royal Pharmaceutical Soc Great Britain*.
32. Sytařová, I.; Orsavová, J.; Snopek, L.; Mlček, J.; Byczyński, Ł.; Mišurcová, L., (2020) Impact of phenolic compounds and vitamins C and E on antioxidant activity of sea buckthorn (*Hippophaë rhamnoides* L.) berries and leaves of diverse ripening times *Food chemistry*, **310**, 125784.