https://doi.org/10.48047/AFJBS.6.16.2024.898-912

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

UPLC-ESI-MS/MS Profile and Cytotoxic, Anti-inflammatory and Alpha-Amylase Activities of the Ethanolic Extracts of Three Different *Zygophyllum* Species

Abdeldjabbar Messaoudi*[1,2], Messaouda Dekmouche[2], Abdennour Gheriani[3], Walid Boussebaa[4]

[1] Department of Drilling and Mechanics of Oil Fields, University of Kasdi Merbah Ouargla.
[2] Laboratory of Valorization and Promotion of Saharan Resources (VPRS), University of KasdiMerbah Ouargla.
[3] Pollution and Waste Treatment Laboratory, Faculty of Mathematics and Matter Sciences, University of Kasdi Merbah Ouargla.
[4] Scientific and Technical Research Center in Physic-chemical Analysis, Ouargla, Algeria.

***Corresponding Author E-mail: messaoudiabdeldjabbar@gmail.com**

Volume 6, Issue 16, Dec 2024

Received: 25 Oct 2024

Accepted: 15 Nov 2024

Published: 04 Dec 2024

doi: 10.48047/AFJBS.6.16.2024.898-912

Abstract
Zygophyllum is one of the most important genus of the plant family Zygophyllaceae that grows in the desert and dry regions, is well-known for its anti-inflammatory, antioxidant, antibacterial and anticancer properties. three predominant species of genus Zygophyllum which grows in southern Algeria (Z. album, Z.cornutum and Z.gaetulum) (ZAl, ZCo and ZGa) were were analyzed for phenolic components by UPLC/MS-MS. Nineteen phenolic compounds were identified, including Esculin Hydrate, Catechin and Thymol as major constituents. The cytotoxicity assay of three Zygophyllum ethanolic extracts on Saccharomyces cerevisiae showed that ZAl and ZCo extracts are non-toxic at all tested concentrations. In contrast, the ZGa extract significantly decreased cell viability by about 50% at the highest concentration (10,000 µg/ml). The anti-inflammatory activity of Zygophyllum extracts varied significantly across different concentrations. Aspirin, used as a reference, showed a dose-dependent effect with a maximum inhibition of 72.7% at 20 mg/ml. At the same concentration, ZGa exhibited the strongest effect at 50.77%, followed by ZCo at 27.22% and ZAl at 17.70%. The IC50 values from the Alpha-amylase assay indicated significant differences in the potency of the tested samples. ZAl showed the highest potency with an IC50 of 3.7, indicating effectiveness but requiring more concentration than Acarbose.

Keywords: Zygophyllum, ethanolic, cytotoxicity, anti-inflammatory, Alpha-amylase.

Introduction

Zygophyllaceae is an ancient family of approximately 25 genera that grows in arid, semiarid, and saline deserts worldwide and contains 240 species adapted to difficult climates[1,2].

Species belonging to the genus *Zygophyllum* represent an important group of the plant family *Zygophyllaceae*, succulent plants that are very drought-resistant, also salt-tolerant, and live in dry climatic conditions[3,4]. The *Zygophyllum* family comprises small trees and herbaceous plants with attractive star-shaped flowers and some are toxic[5] as well as to their unpalatability[6]. The growth of *Zygophyllum* species is attributed to their ability to adapt to the salt, the chemical nature of the soil (also soils polluted)[7] and the drought, and they have broad-spectrum resistance to many heavy metals like Pb, Cu and Zn[8]. The largest genus *Zygophyllum* consists of one hundred species, distributed in desert and steppe habitats from the Mediterranean to central Asia arriving in Australia and South Africa[9]. Many species of genus *Zygophyllum* are widespread in the deserts of Algeria (Sahara) as in cases *Z.album*, *Z.cornutum* and *Z.gaetulum*[10].

The local population for various ailments, such as gout[11], rheumatism[12], diuretic[13], diabetes[14], and asthma[15], has utilized them in traditional medicine. Biological studies on *Zygophyllum* species have indicated significant Antihemolytic[16], Antioxidant[17], Antidiabetic[18], Antifungal[19], Cytotoxic[20], Antibacterial[21], Antiepileptic[22] and Anti-hyperlipidaemic[23]. Phenolic compounds may have a role in these activities due to the presence of many flavonoids and phenolic acids[24].

This paper foregrounds the richness of some species from the genus *Zygophyllum* of the phytochemical bioactive compounds (phenolic compounds) responsible for the wide uses of these species in traditional medicine and pharmaceutical such as cytotoxicity, anti-inflammatory and Alpha-amylase.

EXPERIMENTAL PART

Plant material

The aerial parts of *Z.album*, *Z.cornutum* and *Z.gaetulum* were collected in April from the Sahara of Algeria, The identification was done according to the basis of Quezel and Santa by Professor Youcef Halis researcher in Touggourt- Algeria.

Preparation of the extracts

20 g of air-dried aerial parts of *Z.album*, *Z.cornutum* and *Z.gaetulum* were macerated at room temperature with 5 volumes of EtOH (CH_3CH_2OH) for 12h[25]. After the step of filtration, then the filtrate was evaporated to dryness and kept at 4C°.

Ultra-performance liquid chromatography-mass spectrometry (UPLC–ESI–MS/MS)

Chromatographic analysis of *Z.album*, *Z.cornutum* and *Z.gaetulum* was conducted using the Shimadzu 8040 UPLC-MS-MS equipped electrospray ionization with binary bump (Nexera XR LC-20AD). Gradient elution was carried out as follows with the mobile phase consisting of A: (H_2O and 0.1 % HCOOH) and B: Methanol (CH_3OH) amounting to a total flow of 200 uL/min with a set injection volume of 5ul. A Restek Ultra C18 analytical column (2.1×150 mm, 3 μm) was used for chromatographic separation. The tune page parameters for the ionization source were the following: 6 kV for the spray voltage with nebulizing gas flow 3 l/min, 250 ◦C for the source temperature and 400◦C for the desolvation temperature. Elution was started at 85 % solvent A at 0 min to 1min, afterwards 5 % A at 1 min to 10 min, following 5 % A at 10 min to 18 min and finally 85 % A at 18 min to 23min. Large-scale analysis of phenolic compounds by shotgun mass spectrometry starts with the measurement of the mass-to-charge ratio (m/z) to identify each compound, after that, it was compared with their respective standard such as Esculin Hydrate, Ferulic Acid, 8-hydroxyquinoline, beta-carotene, Curcumin, Rutin, Oleanolic Acid, Kojic Acid, Chrysin, Riboflavin, Quercetine, Vanillin, Catechin, Thymol, Caffeic Acid, Chlorogenic Acid, Vanillic Acid, Gallic and Salycilic acid.

Cytotoxicity tests by yeast cells

The cytotoxic effects of various concentrations of *Z.album*, *Z.cornutum* and *Z. gaetulum* extracts (Z_{Al}, Z_{Co}, Z_{Ga}) on the growth of Saccharomyces cerevisiae were evaluated using a growth sensitivity assay[26]. Initially, the ethanolic extracts were serially diluted in two-fold increments to achieve concentrations ranging from 1.25×10^3 to 10×10^3 µg. Yeast cells at a density of 2×10^7 cells/ml in 100 mM potassium phosphate buffer (pH 7) were exposed to these concentrations for 30 min at 37°C in a 96-well plate—cells without treatment served as controls. Following treatment, 3µl of each culture was spotted onto YPD-agar plates supplemented with 2% glucose, both with and without the *Zygophyllum* extracts at varying concentrations. After that, all the plates were then incubated at 37°C for 48 hours. Finally, Growth outcomes were documented using an Epson® scanner to assess the impact of the three *Zygophyllum* extracts on yeast viability.

Anti-inflammatory activity

This protocol is meticulously crafted to evaluate the inhibitory effects exhibited by various concentrations of *Zygophyllum* ethanolic extracts on the protein denaturation, utilizing a modified egg white model specifically designed for microplate assays[27]. First, a 10% egg white protein solution is prepared by diluting fresh egg white with distilled water. For the assay, 50 µL of this solution is added to each microplate well. Then, 10 µL of each plant extract, with concentrations ranging from 10,000 to 12,500 µg/ml, is incorporated into the wells. The three samples are subsequently incubated at temperatures ranging from 70 to 80°C for 15 to 20 min, optimized to promote protein denaturation. The extent of protein denaturation is measured using UV-visible spectrophotometry at a wavelength of 280 nm, where the absorbance of undenatured proteins usually reaches its maximum.

Aspirin is utilized as a comparative control at varying concentrations under the same assay conditions to evaluate its standard anti-denaturing effects against those demonstrated by the extracts.

Alpha-amylase inhibitory assay

The Alpha amylase inhibitory activity of Z_{Al}, Z_{Co} and Z_{Ga} extracts with different concentrations was determined Iodine method[28]. Firstly, the alpha-amylase enzyme was prepared in phosphate buffer (pH 7), an iodine reagent in potassium iodide solution and a 1% w/v starch solution in distilled water. Briefly, 40µl of different concentrations of *Zygophyllum* extracts and 40µl of starch solution are added to each well of the microplate, followed by 20 µL of the pre-diluted alpha-amylase enzyme solution. The mixture is then incubated at 37°C for 10 minutes, allowing the enzyme to interact with the starch in the presence of the Z_{Al}, Z_{Co}

and Z_{Ga} extracts. The reaction is terminated by adding 20 µL of iodine reagent, which reacts with any undigested starch to create a blue-black complex, and the absorbance was measured at 620nm. For controls, wells are set up with starch and enzyme without *Zygophyllum* extract as a negative control, and wells with enzyme and starch but no iodine reagent as a positive control to measure full enzyme activity. Additionally, acarbose, a well-known alpha-amylase inhibitor, is used as a reference drug to establish a baseline for enzyme inhibition.

Results

Ultra-performance liquid chromatography–mass spectrometry (UPLC–ESI–MS/MS) analysis of *Zygophyllum* extracts

The phytochemical composition of *Zygophyllum* was studied in 3 species (*Z.album*, *Z.cornutum* and *Z.gaetulum*) of the family *Zygophyllaceae* for the first time as a comparative study using UPLC/MS-MS. The comparative analysis allowed 19 diversity of compounds to be identified in these species. A total of phenolic compounds for the extractions of three species of *Zygophyllum* were identified and quantified with area, including 8 acids and 11 flavonoids by using UPLC–ESI–MS/MS (Figure1) .

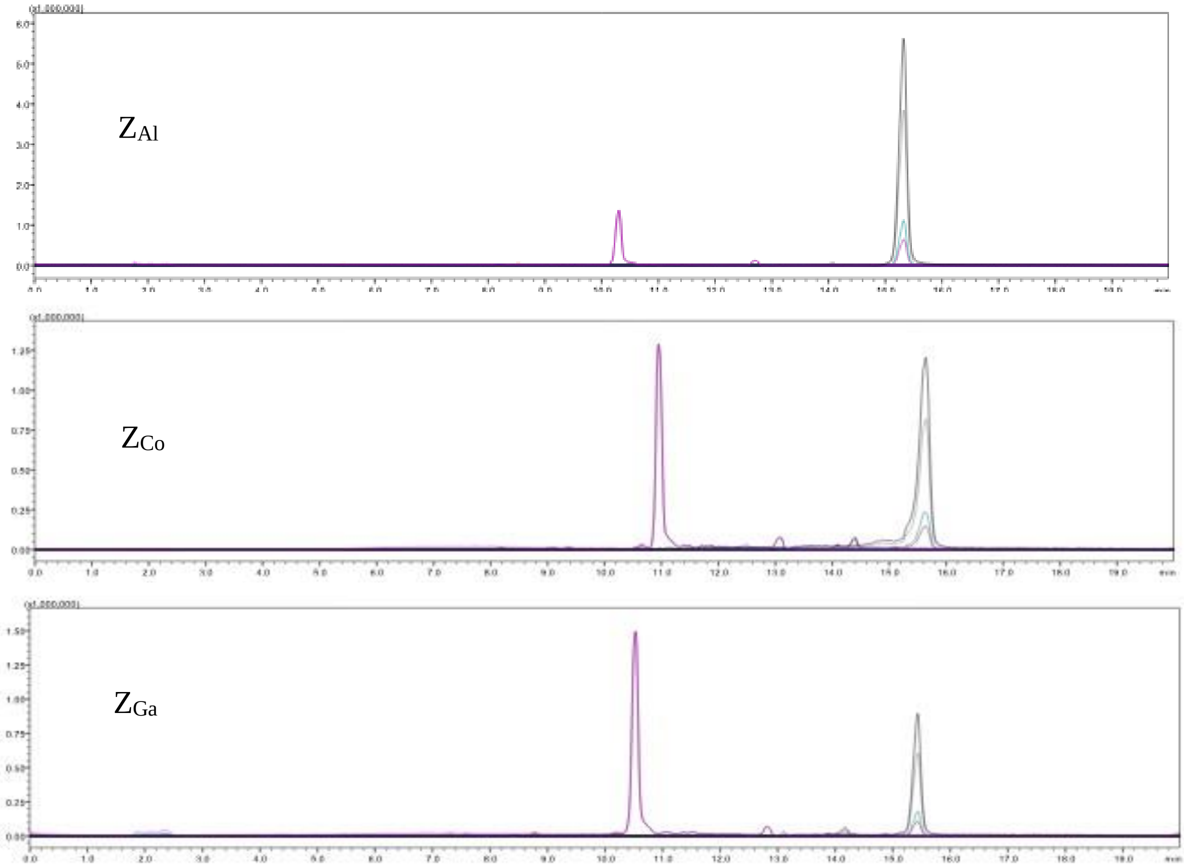

Figure 1: UPLC–ESI–MS/MS chromatograms of Z_{Al}, Z_{Co} and Z_{Ga} extracts.

The obtained results demonstrated that the *Z.album*, *Z.cornutum* and *Z.gaetulum* extracts have different phenolic composition profiles and concentrations (area). The common phenolic constituents within the different extracts for Z_{Al}, Z_{Co} and Z_{Ga} were detected as the Esculin Hydrate are significantly higher than the other constituents (Esculin Hydrate is in higher concentration in the extract of Z_{Al}), also Ferulic Acid, beta-carotene, Curcumin, Riboflavin and Quercetine. Rutin, Vanillin, Thymol and Salicylic acid were found in just *Z.album* and *Z.cornutum* extracts (Catechin is the highest among them). The remaining constituents are found in one or two extracts, with almost insignificant amounts. A previous study reported the presence of Rosmarinic acid, Sinapic acid, Catechin, Caffeic acid, Caffeic acid and Chlorogenic acid in specie of *Zygophyllum*[29]. Another study revealed the presence of Catechol, Coumarin and Resorcinol in another specie of *Zygophyllum*[30]. Another study also revealed the presence of gallic acid, p-coumaric acid, chlorogenic acid, caffeic acid, vanillic acid, vanillin, quercetin, rutin, naringin and quercetin in *Zygophyllum album*[31]. This report confirmed the presence of many phenolic components such as acids and flavonoids in 3 species of *Zygophyllum* (Table 1).

Table 1: Results of the molecules detected in Z_{Al}, Z_{Co} and Z_{Ga} extracts by UPLC-ESI-MS/MS analysis

Compound name	Molecular Weight	Species extract	m/z	Retention Time	Area
Esculin Hydrate	358.3	*Z.album*	359.1	15.323	36382222
		Z.cornutum		15.641	10470600
		Z.gaetulum		15.435	5518942
Ferulic Acid	194.18	*Z.album*	194.8	ND	
		Z.cornutum			
		Z.gaetulum			
8-hydroxyquinoline	145.16	*Z.album*	146.2	8.170	24352
		Z.cornutum		ND	
		Z.gaetulum		ND	
beta-carotene	536.87	*Z.album*	536.9	ND	
		Z.cornutum			
		Z.gaetulum			
Curcumin	368.4	*Z.album*	368.9	ND	
		Z.cornutum		12.172	16507
		Z.gaetulum		ND	
Rutin	610.5	*Z.album*	611.2	ND	
		Z.cornutum			
		Z.gaetulum			

Oleanolic Acid	456.7	*Z.album*	457.1	12.810	24608
		Z.cornutum		ND	
		Z.gaetulum		ND	
Kojic Acid	142.11	*Z.album*	142.8	ND	
		Z.cornutum			
		Z.gaetulum			
Chrysin	254.24	*Z.album*	254.8	ND	
		Z.cornutum			
		Z.gaetulum			
Riboflavin	376.4	*Z.album*	368.9	ND	
		Z.cornutum			
		Z.gaetulum			
Quercetine	302.23	*Z.album*	302.4	12.988	138799
		Z.cornutum		12.490	67201
		Z.gaetulum		13.102	137162
Vanillin	152.15	*Z.album*	153.2	8.538	78672
		Z.cornutum		9.417	49202
		Z.gaetulum		ND	
Catechin	290.27	*Z.album*	291.1	14.074	227713
		Z.cornutum		14.395	341127
		Z.gaetulum		ND	
Thymol	150.22	*Z.album*	151.1	9.985	209247
		Z.cornutum		10.655	321938
		Z.gaetulum		ND	
Caffeic Acid	180.16	*Z.album*	No peak	ND	
		Z.cornutum		ND	
		Z.gaetulum		10.217	212418
Chlorogenic Acid	354.31	*Z.album*	No peak	ND	
		Z.cornutum			
		Z.gaetulum			
Vanillic Acid	168.15	*Z.album*	No peak	7.676	19246
		Z.cornutum		ND	
		Z.gaetulum		ND	
Gallic acid	170.12	*Z.album*	No peak	ND	
		Z.cornutum			
		Z.gaetulum			
Salicylic acid	138.12	*Z.album*	137	6.642	147107
		Z.cornutum		8.165	73101
		Z.gaetulum		ND	

Cytotoxicity assay

The cytotoxicity assay results on Saccharomyces cerevisiae using three different *Zygophyllum* extracts show varying effects on yeast cell viability. Z_{Al} and Z_{Co} Extracts showed no significant cytotoxic effects across all tested concentrations, with cellular viability similar to that of the untreated control. This suggests that these two extracts are non-toxic to the yeast cells under the tested conditions, potentially indicating a benign biochemical profile concerning S. cerevisiae. In contrast, Z_{Ga} extract exhibited a significant cytotoxic effect at the highest concentration tested (10,000 µg/ml), where there was approximately a 50% reduction in cell viability compared to the untreated control. This decrease in viability was distinctly in the semiquantitative spot assay, highlighting a strong inhibitory effect on yeast growth at this concentration. At lower concentrations, beginning at 5000 µg/ml, the growth of the yeast cells was not significantly affected, showing full recovery and 100% viability relative to the untreated sample (Figure 1).

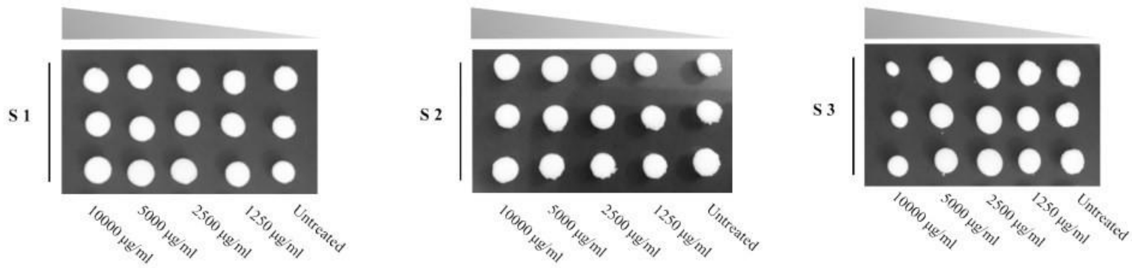

Figure 2: Assessment of *Zygophyllum* extracts on Saccharomyces cerevisiae growth: a semi-quantitative analysis using a spot-assay technique, (S1: Z_{Al} extract, S2: Z_{Co} extract, S3: Z_{Ga} extract).

These findings suggest that while Extracts Z_{Al} and Z_{Co} extracts can be considered safe for yeast cells within the experimental concentration range, but Z_{Ga} extract may be toxic or contains compounds that are potentially toxic or have antifungal properties that warrant further investigation to identify the active constituents and understand their mode of action. Based on these results and what has been studied in the studies of each of Elbadry et al., and Mohammed et al. who have demonstrated variation in toxicity against many cells such as: and against MCF-7, HCT-116, HepG2 cell-lines, and HeLa and MCF-7 cell[32,33].

Anti-inflammatory activity

The anti-inflammatory activity results demonstrated significant variations in the percentage of inhibition across different concentrations of the *Zygophyllum* extracts and Aspirin. Aspirin, used as a reference anti-inflammatory drug, presented a dose-dependent effect with a maximum effect of 72.7% at the dose of 20 mg/ml. At the same concentration, the extract of Z_{Ga} exhibited the strongest inhibitory effect at 50.77%, followed by Z_{Co} at 27.22% and Z_{Al} at 17.70%. As the concentration decreased to 5 mg/ml, inhibition rates remained relatively high for Z_{Ga} (42.18%) and Z_{Co} (25.65%), while Z_{Al} showed a reduction to 13.41%. At lower concentrations (2.5 mg/ml and below), the inhibitory effects diminished, with Z_{Ga} still showing 35.38% inhibition at 0.625 mg/ml which is considered very close to the rate of inhibition of Aspirin 38.55% (Figure 3).

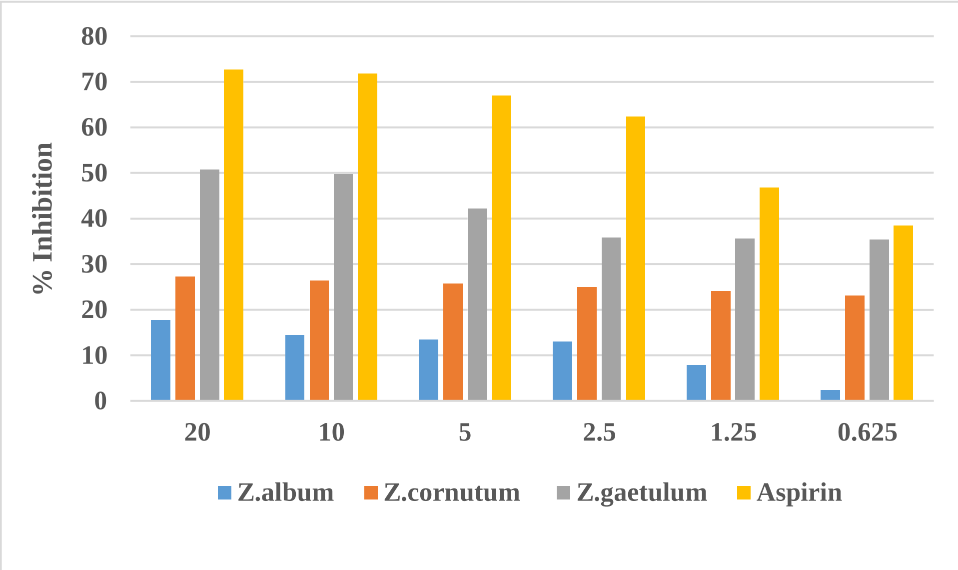

Figure 3: Effect of different plant extracts on inhibition of protein (egg albumin) denaturation. Aspirin was used as a control.

Anti-inflammatory activity of *Zygophyllum* extracts have been reported in several studies such as the study of Shawky et al., Bahlil et al. and Abdelhameed et al. have proven the presence of anti-inflammatory efficacy, since they contain numerous active compounds that contribute to inhibiting inflammation, extracts from various types of *Zygophyllum* can demonstrate significant potential for therapeutic use in inflammatory conditions[34-36].

The mechanism of protein denaturation is complex and involves modifications in bonds, leading to the formation of autoantigens in conditions like rheumatoid arthritis, cancer and diabetes, which are directly associated with inflammation. Therefore, by inhibiting protein denaturation, inflammatory activity can be inhibited[37].

The egg albumin method offers a cost-effective alternative and is quick for testing the anti-inflammatory activity of herbal medicines using the denaturation technique, and this approach should be validated through further studies.

Alpha-amylase inhibitory assay

The IC50 values obtained from the Alpha-amylase assay reveal significant differences in the potency of the tested samples. Acarbose (an oral medication used to manage type 2 diabetes) exhibited the highest potency, with an IC50 of 0.417 mg/ml, indicating its effectiveness in inhibiting the target activity at low concentrations. In comparison, the extract Z_{Al} showed a higher IC50 of 3.7 mg/ml, demonstrating notable effectiveness but requiring more concentration than Acarbose. Z_{Ga} extract also displayed moderate potency with an IC50 of 9.5 mg/ml, while Z_{Co} had the least potency among the samples, with an IC50 of 16.3 mg/ml. These results suggest that Z_{Al} is the most effective anti-inflammatory agent tested of the extracts, followed by Z_{Ga}, with Z_{Co} requiring significantly higher concentrations for similar inhibitory effects (Figure 3).

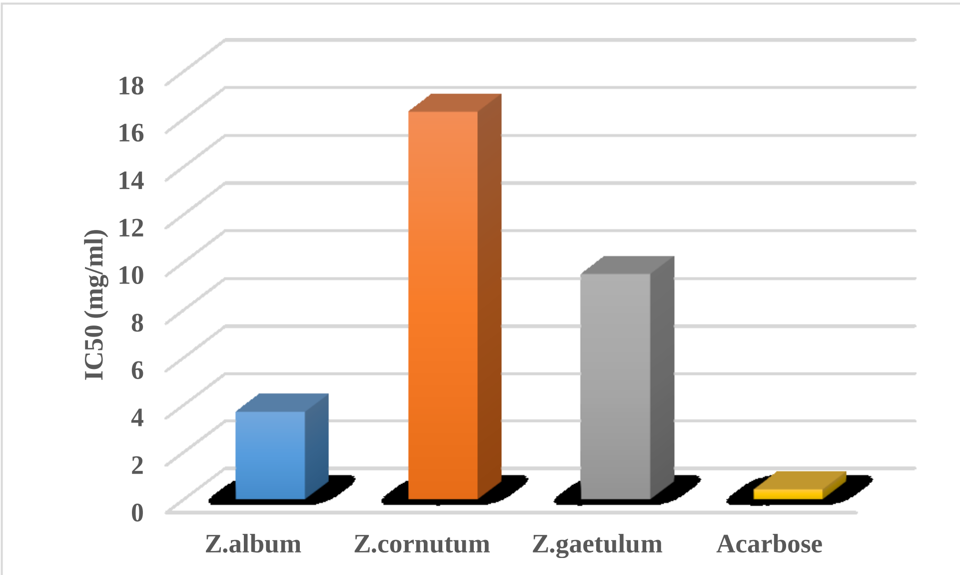

Figure 3: α-Amylase inhibitory activity of the different extracts tested: comparison of IC50 values.

Several studies have shown that certain spices *Zygophyllum* extracts can lower blood sugar levels by evaluating their activity against α-amylase and α-glucosidase, like Mnafgui et al., Al-Omar et al., Kchaou et al. and Medjdoub et al. supports these findings, highlighting the important potential of many species *Zygophyllum* as a natural anti-diabetic agent[38-41].

α-Amylase inhibitors, work by binding to the enzyme's active site, preventing it from acting on dietary carbohydrates, and this leads to decreased glucose absorption[42].

Overall, the inhibition of α-amylase by species of *Zygophyllum* extracts presents a promising avenue for the development of natural anti-diabetic therapies. Understanding the mechanisms and knowing the active compounds involved will be critical for harnessing their full potential in clinical applications. Future studies should focus on isolating the compounds that Contribute to this activity and exploring their effects in vitro and in vivo to further elucidate their role in diabetes management.

Conclusion

The chemical constituents profiling through UPLC-ESI-MS/MS analysis of *Z.album, Z.cornutum* and *Z.gaetulum* was achieved, and ninteen compounds belonging to two different chemical classes phenolic acids and flavonoids. The study of species *Zygophyllum* extracts reveals varying degrees of cytotoxicity, anti-inflammatory and Alpha-amylase activity, highlighting their potential as therapeutic agents whether as pharmaceutical medications or as medicinal herbs. Z_{Al} and Z_{Co} extracts demonstrated non-toxic effects on Saccharomyces cerevisiae, while Z_{Ga} exhibited significant cytotoxicity at higher concentrations. In terms of anti-inflammatory potency, Z_{Al} extract was identified as the most effective, followed by Z_{Ga}, with Z_{Co} extract showing the least potency. The IC50 values established *Zygophyllum* extracts as relatively promising candidates for further investigation in managing diabetic conditions, particularly given their varying effects on alpha-amylase activity and cell viability. To gain clearer insights into the effectiveness of species of genus *Zygophyllum*, future research should focus on isolating and characterizing the active compounds within these extracts. This separation will help optimize their therapeutic applications and elucidate the mechanisms of action potentially leading to more targeted and effective treatments.

References

1- Touaibia M, Dhoha A. Biochemical characterization, antioxidant and antidiabetic properties of Zygophyllum geslini Coss extracts. International Journal of Biological and Chemical Sciences. 2024; 18(3): 1105-1114.

2- Abdel-Hamed AR, Wahba AS, Khodeer DM, Abdel-Kader MS, Badr JM, Mahgoub S, Hal DM. Metabolomic Profiling and In Vivo Antiepileptic Effect of Zygophyllum album Aerial Parts and Roots Crude Extracts against Pentylenetetrazole-Induced Kindling in Mice. Metabolites. 2024; 14(6): 316.

3- Souddi M, Bouallala MH. Floristic Diversity of Communities Associated with Water Towers in the Algerian Sahara. Advanced Research in Life Sciences. 2023; 7(1): 125-134.

4- Fu G, Li J, Li L, Wu S, Wu Y, Wang Y, ... Zhang L. The cell cycle arrested results in the premature advent of apical leaflets development cessation in Zygophyllum xanthoxylon. Trees. 2023; 37(2): 223-237.

5- Merıem D, Sekıa I, Ameni D, Ikessoulen T, Baghıanı A. Impact of toxic heavy metals and their concentration in Zygophyllum species, Mentha longifolia, and Thymus vulgaris traditional medicinal plants consumed in Setif-Algeria. European Journal of Biology. 2023; 82(1): 109-123.

6- El-zohri M, Al-moshaddak A, Alsamadany H, Alzahrani HS. Comparative phytochemical study on three tetraena species (zygophyllaceae) growing at different salinity levels. Pak. J. Bot. 2024; 56(4): 1233-1243.

7- Brahim S, Noureddine S, Abdelmadjid C, Haroun B. Spatio-Temporal Floristic Study Of The Saharan Rangelands Of South-East Algeria Case Of The Souf And Ouedrigh Regions. Journal of Survey in Fisheries Sciences. 2023; 1188-1193.

8- Dhaouadi S, Ghannem S, Kanzari S, Bilal E. Ecological risk assessment of heavy metals accumulation in soil and zygophyllum album album: a case study of industrial phosphate vicinity, Tunisia. Earth Environ. Sci. 2023; 18: 447-459.

9- Mohammedi Z. Phytochemical, Antidiabetic and Therapeutic Properties of Zygophyllum. Herbal Medicines Journal (Herb Med J). 2020; 5(4).

10- Souddi M, Bouallala MH. Floristic Diversity of Communities Associated with Water Towers in the Algerian Sahara. Advanced Research in Life Sciences. 2023; 7(1): 125-134.

11- Hemmami H, Seghir BB, Zeghoud S, Ben Amor I, Kouadri I, Rebiai A., ... Atanassova M. Desert endemic plants in Algeria: A review on traditional uses, phytochemistry, polyphenolic compounds and pharmacological activities. Molecules. 2023; 28(4): 1834.

12- Aissa I, Bussmann RW, Elachouri M. Zygophyllum arabicum (L.) Christenh. & Byng.; Zygophyllum bruguieri (DC.) Christenh. & Byng.; Zygophyllum creticum (L.) Christenh. & Byng; Zygophyllum glutinosum (Delile) Christenh. & Byng; Zygophyllum molle (Delile) Christenh. & Byng; Zygophyllum indicum (Burm. f.) Christenh. & ByngZygophyllaceae. Ethnobotany of Northern Africa and Levant. 2023; 1-7.

13- Naji WA, Enaya SH, Malih HR. Studying the Effect of Flavonoids Extracted from Haloxylon salicornium and Zygophilium coccinium Plants on Burn Healing in Diabetic White Rabbits. The Egyptian Journal of Hospital Medicine. 2023; 90(1): 1542-1557.

14- Medjdoub H, Bouhada Y, Boufeldja T. Antidiabetic Effect of Zygophyllum geslini aerial Part: in vivo and in vitro studies. Phytothérapie. 2023; 21(4): 194-198.

15- Abdel-Hamed AR, Wahba AS, Khodeer DM, Abdel-Kader MS, Badr JM, Mahgoub S, Hal DM. Metabolomic Profiling and In Vivo Antiepileptic Effect of Zygophyllum album Aerial Parts and Roots Crude Extracts against Pentylenetetrazole-Induced Kindling in Mice. Metabolites. 2024; 14(6): 316.

16- Ouffai K, Rachid AZZI., Abboou F, LAHFA FB. Antihemolytic and antioxidant activities of aerial parts extracts of Zygophyllum album L. and Globularia alypum L. from Algeria. Journal of Natural Product Research and Applications. 2022; 1(03): 41-55.

17- Bahlil Y, Krouf D, Taleb-Dida N. Zygophyllum album aqueous extract reduces oxidative damage in red blood cells and attenuates pro-inflammatory plasma markers in hypercholesterolemic diabetic rats. Int J Phytomed. 2021; 12: 86-96..

18- Arbi B, Bouchentouf S, El-Shazly M. Investigation of the Potential Antidiabetic Effect of Zygophyllum Sp. by Studying the Interaction of its Chemical Compounds with Alpha-Amylase and DPP-4 Enzymes using a Molecular Docking Approach. Current Enzyme Inhibition. 2023; 19(2): 100-108.

19- Belmimoun A, Meddah B, Meddah ATT, Gabaldon J, Sonnet P. Antifungal activity of Myrtus communis and Zygophyllum album extracts against human pathogenic fungi. European Journal of Biological Research. 2020; 10(2): 45-56.

20- El-Afify SM, El-Metwaly MA, Abbas MA, El-Amier YA. In Vitro Assessment of Antioxidant and Cytotoxic Activities of Zygophyllum coccineum L. Methanolic Extract. Environment. 2024; 7:8.

21- Ahmed SN, Al Touby SS, Hossain MA. Isolation and evaluation of significant antibacterial fraction of methanol and its derived fractions from the leaves extract of Zygophyllum simplex. Advances in Biomarker Sciences and Technology. 2023; 5: 1-7.

22- Abdel-Hamed AR, Wahba AS, Khodeer DM, Abdel-Kader MS, Badr JM, Mahgoub S, Hal DM. Metabolomic Profiling and In Vivo Antiepileptic Effect of Zygophyllum album Aerial Parts and Roots Crude Extracts against Pentylenetetrazole-Induced Kindling in Mice. Metabolites. 2024;14(6): 316.

23- Rehman AU. Chemical profile and in vivo anti-hyperlipidaemic activity of chloroform fraction of Zygophyllum indicum in Triton X-100 induced hyperlipidaemic rats. Natural Product Research. 2023; 1-11.

24- Sikron-Persi N, Granot G, Batushansky A, Toubiana D, Grafi G, Fait A. Mass spectrometry-based metabolite profiling reveals functional seasonal shifts in the metabolome of Zygophyllum dumosum Boiss and its relation to environmental conditions. Planta. 2023; 258(1): 10.

25- Komes D, Belščak-Cvitanović A, Horžić D, Drmić H, Škrabal S, Miličević B. Bioactive and sensory properties of herbal spirit enriched with cocoa (Theobroma cacao L.) polyphenolics. Food and Bioprocess Technology. 2012; 5: 2908-2920.

26- Elsztein C, Lucena RM, Morais MA. The resistance of the yeast Saccharomyces cerevisiae to the biocide polyhexamethylene biguanide: involvement of cell wall integrity pathway and emerging role for YAP1. BMC Molecular Biology. 2011; 12: 1-10.

27- Chaiya P, Senarat S, Phaechamud T, Narakornwit W. In vitro anti-inflammatory activity using thermally inhibiting protein denaturation of egg albumin and antimicrobial activities of some organic solvents. Materials Today: Proceedings. 2022; 65: 2290-2295.

28- Xiao Z, Storms R, Tsang A. A quantitative starch? Iodine method for measuring alpha-amylase and glucoamylase activities. Analytical biochemistry. 2006; 351(1): 146-148.

29- Barzegar R, Safaei HR, Masoumzadeh MS. Chemical composition and antibacterial activity of Zygophyllum qatarense Hadidi leaf extract. Future Natural Products. 2018; 4(4): 23-32.

30- Ali OT, Mohammed MJ. Bioactive phenolic compounds from natural Zygophyllum fabago plant: Isolation, purification and characterization. Journal of Global Pharma Technology. 2019; 7(11): 794-800.

31- Mesbahi MA, Ouahrani MR, Rebiai A, Amara DG, Chouikh A. Characterization of Zygophyllum album L monofloral honey from El-Oued, Algeria. Current Nutrition & Food Science. 2019; 15(5): 476-483.

32- Elbadry MA, Elaasser MM, Elshiekh HH, Sheriff MM. Evaluation of antimicrobial, cytotoxic and larvicidal activity of Zygophyllum coccineum North Sinai, Egypt. Medicinal and Aromatic Plants. 2015; 4(5): 214.

32- Mohammed HA, Khan RA, Abdel-Hafez AA, Abdel-Aziz M, Ahmed E, Enany S., ... Hegazy MM. Phytochemical profiling, in vitro and in silico anti-microbial and anti-cancer activity evaluations and Staph GyraseB and h-TOP-IIβ receptor-docking studies of major constituents of Zygophyllum coccineum L. Aqueous-ethanolic extract and its subsequent fractions: An approach to validate traditional phytomedicinal knowledge. Molecules. 2021; 26(3): 577.

34- Shawky E, Gabr N, El-gindi M, Mekky R. A comprehensive review on genus Zygophyllum. Journal of Advanced Pharmacy Research. 2019; 3(1): 1-16.

35- Bahlil Y, Krouf D, Taleb-Dida N. Zygophyllum album aqueous extract reduces oxidative damage in red blood cells and attenuates pro-inflammatory plasma markers in hypercholesterolemic diabetic rats. Int J Phytomed. 2021; 12: 86-96.

36- Abdelhameed RF, Fattah SA, Mehanna ET, Hal DM, Mosaad SM, Abdel-Kader MS, ... Eltamany EE. Zygo-albuside a: new saponin from Zygophyllum album L. with significant antioxidant, anti-inflammatory and antiapoptotic effects against methotrexate-induced testicular damage. International Journal of Molecular Sciences. 2022; 23(18): 10799.

37- Sangeetha G, Vidhya R. In vitro anti-inflammatory activity of different parts of Pedalium murex (L.). inflammation. 2016; 4(3): 31-36.

38- Mnafgui K, Kchaou M, Hamden K, Derbali F, Slama S, Nasri M, ... Elfeki A. Inhibition of carbohydrate and lipid digestive enzymes activities by Zygophyllum album extracts: effect on blood and pancreas inflammatory biomarkers in alloxan-induced diabetic rats. Journal of physiology and biochemistry. 2014; 70: 93-106.

39- Al-Omar MS, Mohammed HA, Mohammed SA, Abd-Elmoniem E, Kandil YI, Eldeeb HM, ... Khan RA. Anti-microbial, anti-oxidant, and α-amylase inhibitory activity of traditionally-used medicinal herbs: a comparative analyses of pharmacology, and phytoconstituents of regional halophytic plants' diaspora. Molecules. 2020; 25(22): 5457.

40- Kchaou M, Ben Salah H, Mnafgui K, Abdennabi R, Gharsallah N, Elfeki A., ... Allouche N. Chemical composition and biological activities of Zygophyllum album (L.) essential oil from Tunisia. 2018.

41- Medjdoub H, Bouhada Y, Boufeldja T. Antidiabetic Effect of Zygophyllum geslini aerial Part: in vivo and in vitro studies. Phytothérapie. 2023; 21(4): 194-198.

42- Ramu R, Shirahatti PS, Deepika TH, Bajpe SN, Sreepathi N, Patil SM, Prasad N. Investigating Musa paradisiaca (Var. Nanjangud rasa bale) pseudostem in preventing hyperglycemia along with improvement of diabetic complications. J. Appl. Biol. Biotechnol. 2022; 10: 56-65.