

Chemical Composition, Cytotoxic, Antimicrobial and Antihemolytic Activities of *Zygophyllum cornutum* Methanolic Extract

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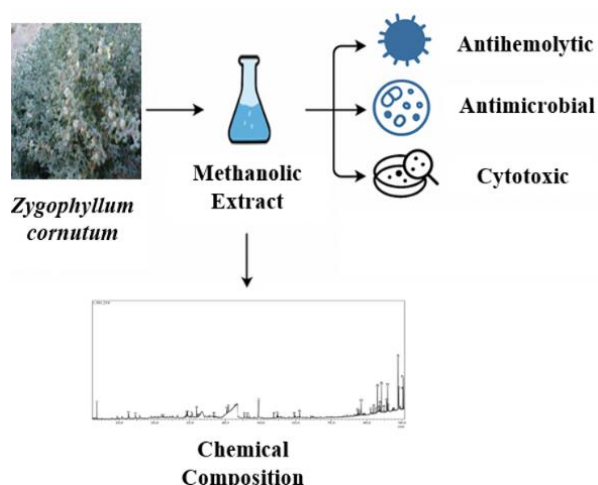
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Graphical abstract



Abstract

Zygophyllum cornutum is recognized for its traditional medicinal use among certain populations in the northern Sahara of Algeria for different conditions, including hypertension and diabetes. The current study sought to ascertain the chemical composition and efficacy of *Zygophyllum cornutum* methanolic extract (ZcME) regarding cytotoxicity, antihemolytic properties, and antibacterial activity by agar diffusion experiment. Thirty-four bioactive phytochemical components were discovered in the methanolic extract of *Zygophyllum cornutum*. The identification of phytochemical substances relies on retention duration, molecular weight, and molecular formula. The study's findings indicate that the methanolic extract of *Zygophyllum cornutum* is non-toxic at concentrations up to 20 mg/ml. ZcME had substantial antimicrobial activity against *Escherichia coli*, displaying increasingly bigger inhibition zones at higher concentrations (from 8 mm at 5 mg/mL to 23 mm at 40 mg/mL), with all MIC/MBC values at 1, signifying the

extract's bactericidal characteristics. At a dose of 1.25 mg/mL, ZcME and ascorbic acid exhibit comparable antihemolytic activity (26% vs. 28%), suggesting that, at this low dosage, ZcME may have equivalent efficacy to ascorbic acid in protecting red blood cells from lysis.

Keywords: chemical composition, Extract, *Zygophyllum cornutum*, Antimicrobial, Antihemolytic.

1. Introduction

Since decades ago, medicinal plants have maintained their prominent position in maintaining human health and preventing disease due to their significant value (Sen & Samanta, 2014). Many people are searching for natural substitutes for synthetic medications (Li & Gal, 2024), utilizing customs from popular culture and tradition that emphasize the therapeutic qualities of these common plants (Dean, 2024; Jamal, 2023). New medicines made from natural sources, such as extracts or pure compounds, have been discovered and validated as a result of scientific advancements that have shown the medicinal potential of many species and plant families (Chaachouay & Zidane, 2024; Najmi, Javed, Al Bratty, & Alhazmi, 2022). Furthermore, according to estimates from the World Health Organization (WHO), four out of five people worldwide use plant extracts or their active ingredients as traditional medicine in traditional therapies (Saggar *et al.*, 2022).

The xerophyte plant *Zygophyllum cornutum* (*Z. cornutum*), sometimes referred to as agaya, is a member of the *Zygophyllaceae* (genus: *Zygophyllum*) and grows in dry and semi-arid environments (Xi *et al.*, 2018). In addition to being an important part of traditional medicine, *Z. cornutum* may also be a significant food source for animals because of its dilated fruits on top of a free section of carpels that are recurved into hooks as long as the welded section portion (Hal *et al.*, 2022). While the

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Algerian people has effectively employed *Z. cornutum* to treat diabetes, rheumatism, dermatitis, asthma, and gout (Belguidoum *et al.*, 2015), certain research have shown its significance. Numerous investigations verified *Zygophyllum cornutum*'s anti-oxidant (Touaibia & Abdellali, 2025), anti-inflammatory (Harchaoui, Ouafi, Meguellati, & Saad, 2025), and anti-diabetic (Mohammedi, 2020) properties. In recent years, numerous modern analytical approaches, such as optimization algorithms and environmental data modeling, have gained considerable traction in research on natural products and environmental monitoring (Prajul Mohandas, Subramanian, & Surendran, 2025a, 2025b). Although these studies focus on environmental data, they reflect a growing trend towards using computational techniques to enhance accuracy and predictive power in many scientific studies (P Mohandas, Subramanian, & Rajendran, 2025; Sivasubramanian, Venkatesan, Thanarajan, & Rajendran, 2025), complementing research related to medicinal plants by improving data interpretation and enhancing the screening of bioactive compounds.

2. Material and Methods

2.1. Plant material

For this study, *Zygophyllum cornutum* was collected in the Biskra province, which is situated on Algeria's southeast coast. Doctor Halis Youcef, a researcher at the Center for Scientific and Technical Research on Arid Regions (CRSTRA) - Station Touggourt, identified the plant based on morphological and botanical characteristics. After meticulously cleaning the aerial components to get rid of any debris, they were left to naturally ventilate for several days at room temperature in a shaded area away from the sun. The plant material was manually ground into a fine powder once it had dried.

2.2. Preparation of the extract

Zygophyllum cornutum's aerial portions were set to air dry in the shade. In this investigation, maceration extraction (Chigayo, Mojapelo, Mnyakeni-Moleele, & Misihairabgwi, 2016) a traditional solvent extraction technique was employed. 60 g of *Z. cornutum* aerial parts were extracted in 200 ml of organic solvent in a 1L flask at room temperature as part of the maceration process. One day of maceration was enough to recover the phenolic compounds from the aerial portion of the plant. The filtrate was dried by evaporation and stored at 4°C after being filtered through filter paper (Whatman No. 1).

2.3. GC-MS analysis

The examination of the *Zygophyllum cornutum* methanolic extract (ZcME) was conducted at the Technical Research Centre for Physico-Chemical Analyses (PTAPC-CRAPC) in Laghouat, Algeria, utilizing instruments fitted with a fused Rxi®-5ms capillary column measuring 30 m × 0.25 mm, 0.25 µm, and composed of 5% diphenyl and 95% dimethylpolysiloxane. Helium, possessing a purity of 99.995%, functioned as the carrier gas at a flow rate of 1 mL/min. The injector temperature was sustained at 250°C,

but the detector temperature was established at 310°C. A volume of 0.5 µL of the ZcME was injected in split mode at a ratio of 1:10. The column's initial temperature was set at 50°C for 2 minutes, then increased to a final temperature of 310°C at a rate of 3°C/min. The analysis was conducted at a mass spectrometry ionization voltage of 70 eV, with an ion source temperature of 200°C, and electron ionization mass spectra (EI-MS) were obtained spanning the mass range of 45–600 m/z.

2.4. Cytotoxic assay by yeast cells

The impact of *Zygophyllum cornutum* methanolic extract (ZcME) on the proliferation of *Saccharomyces cerevisiae* was assessed utilizing a growth sensitivity analysis (Elsztein, de Lucena, & de Morais Jr, 2011). The extract of *Z. cornutum* was initially prepared at a concentration of 20 mg/mL and thereafter subjected to two-fold serial dilutions, resulting in final concentrations ranging from 20 mg/mL to 1.25 mg/mL. Yeast cells, calibrated to a final concentration of 2×10^7 cells/mL in 100 mM potassium phosphate buffer at pH 7, were exposed to varying concentrations of ZcME in a 96-well plate for thirty minutes at 37°C. Furthermore, utilize untreated cells as controls. For a semi-quantitative spot test, 2.5 µL of each treated culture from the 96-well plate was applied to solid YPD-agar plates containing 2% glucose, both with and without ZcME at the specified doses. The plates were subsequently incubated at 37°C for a duration of 48 hours. Following incubation, yeast proliferation was documented utilizing an Epson® scanner, contrasting treated samples at several doses with the control to evaluate cytotoxic effects.

2.5. Antimicrobial Activity

Escherichia coli ATCC 25922 was tested as a Gram-negative strain, *Staphylococcus aureus* ATCC 25923 as a Gram-positive strain, and one reference specimen of *Candida albicans* ATCC 10231 was used.

This method is frequently employed to evaluate the antimicrobial properties of substances, such as extracts, through the agar diffusion or well method (Bonev, Hooper, & Parisot, 2008).

A suspension of 106 cells/mL, prepared from a fresh culture of either yeast or bacterial cells, is aseptically inoculated into Petri dishes containing Sabouraud dextrose agar supplemented with 2% glucose (for testing against yeasts) and Mueller-Hinton agar (for testing against bacteria). Inoculation is accomplished by uniformly swabbing the agar surface. After the agar surface has dried, the upper end of a Pasteur pipette is used to construct wells at the center of each plate, ensuring that the wells are equally sized. Subsequently, each well is filled with approximately 50 µL of *Z. cornutum* methanolic extract (ZcME) at varying concentrations (5, 10, 20, and 40 mg/mL). Plates inoculated with yeast cultures are incubated at 37°C for 48 hours, while those inoculated with bacterial cultures are incubated at 37°C for 24 hours. The formation of an inhibition is indicative of the antimicrobial activity of each concentration of extract. The antimicrobial potency is assessed by measuring the

diameter of the inhibition zones in millimeters. If the diameter of the inhibition zone exceeds 6 mm, methanolic *Z. cornutum* extract is deemed to possess antimicrobial activity. This method allows for a simple comparison of the antimicrobial effects of methanolic extract against bacterial and yeast cultures, thereby providing a dependable indication of their potential as antimicrobial agents.

2.6. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The broth microdilution technique was employed to ascertain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Weinstein, 2018).

Initially, the bacterial and yeast suspensions are formulated. The bacterial strains are cultivated on Mueller-Hinton agar (MHA) and subsequently injected into cation-adjusted Mueller-Hinton broth (MHB). The cultures are incubated until they exhibit visible turbidity, after which they are diluted to a turbidity level of 0.5 McFarland (1.5×10^8 CFU/mL) utilizing the BioMerieux DensiCHEK Plus for VITEK 2 Systems. The plant extract solution is subsequently made by dissolving the extract in dimethyl sulfoxide (DMSO) to achieve a concentration of 20 mg/mL. The solution is subsequently homogenized by vortexing for one minute. Each well of the microtiter plate is prepared by adding 100 μ L of the plant extract solution, followed by the addition of 50 μ L of the bacterial or yeast culture. A growth control (without antibiotic or xenobiotic) and a sterile control (MHB alone) are incorporated for all isolates (Schwalbe, Steele-Moore, & Goodwin, 2007). The microtiter plate is thereafter incubated at 37°C for 18 to 24 hours. Post-incubation, the MIC is identified as the minimal concentration of plant extract that suppresses bacterial growth (Wayne, 2011). The determination of the Minimum Bactericidal Concentration (MBC) utilizing the spot test method with microplates containing methanolic *Z. cornutum* extract comprises several distinct procedures. Initially, various quantities of the extract are formulated in a broth medium within numerous wells of a microplate. A standardized bacterial suspension is thereafter introduced to each well, followed by incubation under appropriate conditions to facilitate bacterial proliferation. Subsequently, a tiny aliquot (3 μ L) from each well is deposited onto agar plates without the plant extract and incubated once again, utilizing Sabouraud dextrose agar for yeast and Mueller-Hinton agar for bacteria. The lack of bacterial proliferation on these plates signifies the bactericidal efficacy of the extract at particular concentrations. The MBC is defined as the minimal concentration of the plant extract at which no observable bacterial growth is present, indicating efficient bacterial eradication. This method is very effective for the efficient evaluation of the bactericidal activities of plant extracts against diverse bacterial strains in a high-throughput format (Suppi *et al.*, 2015).

2.7. Antihemolytic activity

The anti-hemolytic efficacy of the methanolic *Z. cornutum* extract was assessed in vitro using the methodology outlined by Berroukeche *et al.* (2022) (Berroukeche *et al.*, 2022), with minor changes.

Blood was collected in EDTA tubes and centrifuged at 2000 rpm for 10 minutes to extract erythrocytes, which were subsequently resuspended in phosphate-buffered saline (PBS) to formulate a 10% erythrocyte hemolytic solution. ZcME was synthesized at various concentrations ranging from 0.625 mg/mL to 20 mg/mL. Each well received 20 μ L of the erythrocyte solution, followed by 40 μ L of extract or ascorbic acid as a positive control. Hemolysis was produced by the addition of 40 μ L of hydrogen peroxide (H_2O_2) and incubated at 37°C for 180 minutes. Subsequent to incubation, 80 μ L of PBS and 30 μ L of a 20% sucrose solution were introduced to each well. Absorbance was quantified at 540 nm to assess hemolysis utilizing the 96-well plate format for optimal analysis efficiency. The percentage of inhibition was calculated by using the following formula:

$$\text{Inhibition \%} = 100 - \left(\frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100$$

3. Results and Discussion

3.1. GC-MS analysis

Thirty-Seven compounds accounting for 100% of the analysis methanolic extract were identified (**Table 1 and Figure 1**). The main components were Acetyl betulinaldehyde (16.24%) and 1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1,7-diol (9.95%). Besides, other constituents were discovered in lower quantities like gamma-Sitosterol 7.59%, 28-Norolean-17-en-3-ol (7.52%) and Acetic acid (7.25%). The quantitative and qualitative compositions demonstrated in the current investigation are very different from those published in the literature. As reported by Shah *et al.*, 2020 *Zygophyllum luntii* methanolic extract was distinguished by the dominance of octacosane (43.05%) (Shah *et al.*, 2020). Another study of Altameme, 2021 showed that the major constituents of Methanolic extract of *Zygophyllum coccineum* was Morphinan (12.29 %) and Thiophene,tetrahydro-2-methyl (27.27%) in Methanolic extract of *Z. coccineum* Methanolic extract of *Zygophyllum fabago* (Altameme, 2021).

3.2. Cytotoxic assay by yeast cells

The evaluation of the cytotoxicity of the methanolic *Zygophyllum cornutum* extract on *Saccharomyces cerevisiae* was conducted using a cytotoxicity spot-test method. The findings indicated no detrimental impact on yeast viability at all of the concentrations examined (20 to 1.25 mg/mL) (**Figure 2**). The spot test on YPD-agar plates demonstrated uniform growth patterns, with no notable variations in colony density or size relative to the control group, indicating an absence of cytotoxic effects.

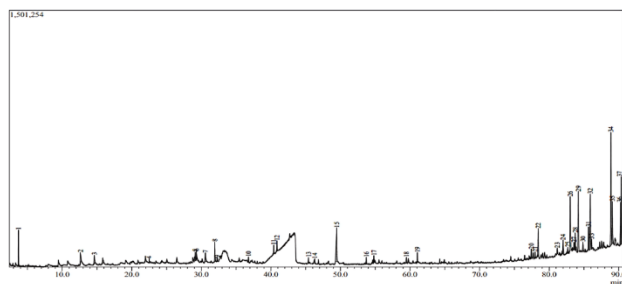


Figure1: GC-MS chromatogram of *Zygophyllum cornutum* methanolic extract

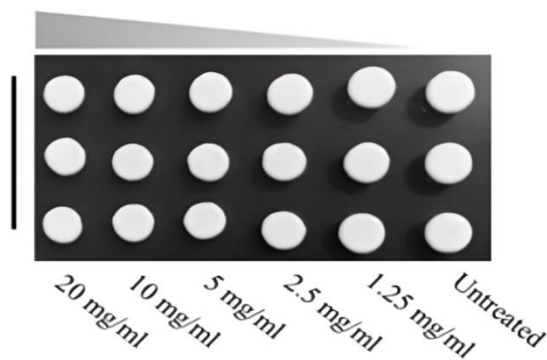


Figure 2. Cytotoxic assay for ZcME on *saccharomyces cerevisiae* using cytotoxicity spot-test assay.

Table 1. Chemical composition of *Zygophyllum cornutum* methanolic extract.

Peak	RetentionTime	Area%	Name
1	3.712	2.30	Toluene
2	12.623	1.49	Proceroside
3	14.614	1.15	2,4(1H,3H)-Pyrimidinedione,
4	22.496	0.29	Linalyl acetate
5	29.115	1.00	1a,2,5,5-Tetramethyl-cis-1a,4a,5,6,7,8-hexahydro-gamma-chromene
6	29.282	0.87	4-Methoxybenzene-1,2-diol
7	30.567	1.63	Apocynin
8	31.934	2.04	2H-Pyran-2-one, 5,6-dihydro-6-pentyl-
9	32.261	0.20	Apocynin
10	36.770	0.49	4-(2,4,6-trimethylphenyl)-2-butanol
11	40.411	0.82	Cyclopentanecarboxylic acid
12	40.875	1.07	2H-Pyran-2-one
13	45.394	0.49	Neophytadiene
14	46.312	0.26	1,2-Benzenedicarboxylic acid
15	49.455	6.36	Palmitic acid
16	53.704	0.72	2-Methylhexacosane
17	54.808	0.72	9-Octadecenoic acid
18	59.477	0.58	2-Methylhexacosane
19	61.041	1.01	9-Octadecenamide
20	77.439	1.25	28-Norolean-17-en-3-one
21	77.984	0.63	79 Cholesta-4,6-dien-3-ol, benzoate, (3.β.)-
22	78.422	3.30	β.-Sitosterol acetate
23	81.169	0.80	Campesterol
24	82.005	2.03	Germanicol
25	82.679	0.60	Ergosta-5,7,25(27)-trienol
26	83.02	7.59	γ.-Sitosterol
27	83.578	0.91	Kolavenol acetate
28	83.736	2.05	28-Norolean-17-en-3-one
29	84.186	7.52	28-Norolean-17-en-3-ol
30	84.860	1.13	Cholesta-3,5-dien-7-one
31	85.652	3.00	γ.-Sitostenone
32	85.900	7.25	Acetic acid
33	86.094	1.17	Methanesulfonic acid
34	88.881	16.24	Acetyl betulinaldehyde
35	89.090	5.39	Betulinaldehyde
36	90.275	5.70	Silane
37	90.377	9.95	1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1,7-diol
		100	

The results demonstrate that ZcME is biocompatible with *Saccharomyces cerevisiae*, exhibiting no harmful effects at all tested dosages (ranging from 20 mg/mL to 1.25 mg/mL). The extract's non-toxic characteristics and

complete vitality endorse its prospective utilization in many applications necessitating compatibility with yeast or analogous biological systems.

Research indicates that medicinal plants, particularly those from the Sahara rich in phenolic compounds, may aid in the formulation of certain pharmaceuticals due to the non-toxic nature of many of these plants. The identification of plant-derived pharmaceuticals predominantly resulted in the development of non-toxic treatments and continues to facilitate new avenues in clinical trials (Saklani & Kutty, 2008).

The extract derived from *Zygophyllum cornutum* exhibits a noncytotoxic action on *Saccharomyces cerevisiae*. Mohammed *et al.* reported that the aqueous-ethanolic extract of *Zygophyllum coccineum* exhibited cytotoxic activities against MCF-7, HepG2, and HCT-116 cell lines (Mohammed *et al.*, 2021). This outcome aligns with the **Table 2**. Zone of inhibition (mm) of different bacterial strains at various concentrations of *Z. cornutum* methanolic extract

Concentration (mg/mL)	Zone of inhibition (mm)		
	Microbial inhibition		Anti-Candida activity
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
5	8	NI	NI
10	11	NI	NI
20	13	NI	NI
40	23	11	NI

NI = No Inhibition

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for *Zygophyllum cornutum* Methanolic Extract on *Escherichia coli* and *Staphylococcus aureus*.

<i>Z. cornutum</i> methanolic extract	MIC (mg/mL)	MBC (mg/mL)	MIC/MBC
<i>Escherichia coli</i>	40	40	1
<i>Staphylococcus aureus</i>	40	40	1

For *Escherichia coli*, the inhibition zones measured 23 mm at 40 mg/mL, 13 mm at 20 mg/mL, 11 mm at 10 mg/mL, and 8 mm at 5 mg/mL. For *Staphylococcus aureus*, inhibition was observed only at 11 mm with 40 mg/mL.

The findings indicate that *Escherichia coli* and *Staphylococcus aureus* exhibit susceptibility to the methanolic extract derived from *Zygophyllum cornutum*. This observation aligns with the susceptibility of other bacterial strains, including *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Arcanobacterium haemolyticum*, and *Staphylococcus saprophyticus*, to various plant extracts from *Zygophyllum* species such as *Z. eurypterum*, *Z. fabago*, *Z. megacarpum*, and *Z. propinquum*, as reported by Alamholo en 2024(Alamholo, 2024). The findings align with those reported by Shah *et al.* 2020, which indicated that the crude extracts (from the stem, leaves, and roots) and the oil extracted from the leaves of *Zygophyllum luntii* are effective in reducing microbial activity across various bacterial strains, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The study conducted by Elbadry *et al.* 2015 demonstrated the significant effectiveness of various extracts from *Z. coccineum*, including Acetone, Ethanol, Ethyl acetate, Petroleum ether, Methanol, and water, against several bacterial strains (Elbadry *et al.*, 2015). These strains include *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Alcaligenes faecalis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Escherichia coli*, each exhibiting varying degrees of susceptibility. The presence of

findings of Elbadry *et al.*, who investigated the cytotoxic activity of acetone, ethanol, ethyl acetate, petroleum ether, methanol, and water extracts derived from *Zygophyllum coccineum*, evaluated in vitro against HeLa and MCF7 cells (Elbadry, Elaasser, Elshiekh, & Sheriff, 2015).

3.3. Antimicrobial Activity

This study presents the antimicrobial activity of methanolic *Z. cornutum* extract against one Gram-negative bacterium and one Gram-positive bacterium, specifically *Escherichia coli* and *Staphylococcus aureus*, respectively. The antimicrobial activity results of the ZcME are presented in **Table 2**.

Table 2. Zone of inhibition (mm) of different bacterial strains at various concentrations of *Z. cornutum* methanolic extract

biologically active compounds in plant extracts suggests potential efficacy against the microorganisms tested.

3.4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The results obtained indicate that the ZcME has a bactericidal effect on all four bacterial strains tested (*Escherichia coli* and *Staphylococcus aureus*) at a concentration of 40 mg/mL (**Table 3**).

MBC/MIC ratio of ≤ 4 is typically indicating a bactericidal compound, whereas an MBC/MIC ratio of >4 is suggesting a bacteriostatic effect (Makade *et al.*, 2024).

For all examined bacterial strains, the MBC/MIC ratio is 1. This indicates that The ZcME eradicates the bacteria at the same concentration that it suppresses their development. Consequently, the extract demonstrates bactericidal action; nevertheless, the efficacy is significantly lower than that of conventional bactericidal agents. The reduced ratio may suggest that the methanolic extract of *Zygophyllum cornutum* is less strong than certain other bactericidal drugs, however it demonstrates partial efficacy in both slowing bacterial growth and eliminating the bacteria. This aligns with certain findings reported by Alamholo, 202. In his work, he discovered that MIC/MBC=1 in numerous extracts, indicating the presence of bactericidal chemicals.

3.5. Antihemolytic activity

Through **Figure 3**, the methanolic *Z. cornutum* extract showed low effective in protecting erythrocytes from hemolysis by oxidizing agents (H2O2 radical).

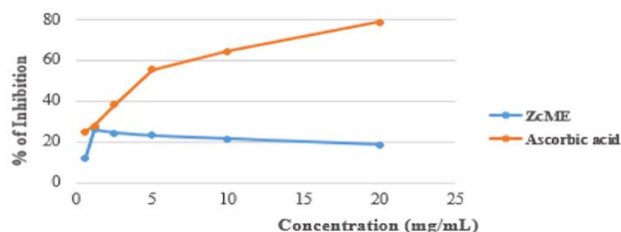


Figure 3. Influence of various methanolic *Z. cornutum* extract concentrations on hemolysis inhibition percentages

ZcME shows a gradual increase in anti-hemolytic activity as the concentration decreases, with the highest inhibition (26%) in at 1.25 mg/mL, it is very close to the inhibition rate at the same concentration of Ascorbic acid and the lowest (12%) at 0.625 mg/mL. Ascorbic acid shows a higher inhibition percentage overall, with the highest inhibition (79%) at 20 mg/mL, which decreases, reaching 25% at 0.625 mg/mL. It is double the percentage at the same concentration of methanolic extract.

4. Conclusion

The chemical ingredient profiling of *Z. cornutum* was conducted using GC-MS analysis, revealing thirty-seven chemicals, predominantly phenolic acids. Biological evaluations of *Z. cornutum* extract demonstrated the absence of cytotoxicity in *Saccharomyces cerevisiae* at all five tested concentrations, hence affirming its biocompatibility and safety. The *Z. cornutum* extract shown antibacterial activity, especially against *Escherichia coli*, with a distinct bactericidal impact (MIC/MBC ratio = 1). The anti-hemolytic activity, while considerably lower than that of ascorbic acid, exhibited a concentration-dependent protective impact on erythrocytes.

Collectively, these significant findings suggest that the methanolic extract of *Z. cornutum* exhibits non-toxic, antibacterial, and slightly anti-hemolytic characteristics, underscoring its potential as a source of natural medicinal compounds. Additional research especially in vivo investigations and the extraction of active compounds is necessary to thoroughly examine its pharmacological potential and its use in pharmaceutical formulations.

Future research should explore extensive investigations on this plant family due to its unique biological and pharmacological properties; this plant family may provide novel bioactive compounds with medicinal promise. To understand their mechanisms, this species' active phenolic chemicals must be isolated, purified, and characterized, and their biological effects studied. Analytical and computational modeling should also be used to correlate these compounds' chemical structures with their biological activities to predict and optimize their therapeutic potential.

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