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# Antimicrobial Activity and Allelopathic Potential of *Zygophyllum coccineum* L. on *Chenopodium album* L.

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***Authors' contributions***

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

*Zygophyllum coccineum* L. (Family Zygophyllaceae) was collected from two locations namely Wadi Hagul and Deltaic Mediterranean coast. Methanol, acetone and ethanol extracts prepared from Z. *coccineum* leaves and they were tested for their antibacterial activity. The bacterial strains tested were *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Bacillus cereus,* and *Pseudomonas aeruginosa*. Methanol extract from desert plant was the most potent extract against bacterial growth compared with other extracts. Also, the three extracts expressed antifungal activities against the tested fungi *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus.* Methanol extract from the desert plant was the strongest in retarding the fungal growth. The aqueous leaf extract of *Z. coccineum* from both desert and coastal areas inhibited seed germination and radical growth of *Chenopodium album* at 50 and 100 µgml$^{-1}$. The inhibition rate was remarkable with the desert plant compared to that of coastal one and this may be due to the higher contents of bioactive compounds.

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## 1. INTRODUCTION

About 80% individuals from developing countries depends on plant based preparations used in their traditional medicinal system and human health care [1-3]. Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicine [4]. Most of the pathogenic microorganisms are becoming resistant to many antimicrobial drugs. Natural products are a good source for obtaining low cost, highly safe and potent antifungal and antibacterial drugs. Thus, the antibiotic resistance of medically important bacteria became the major problem for human health. In addition, the antibiotics may also cause side effects on the host including hypersensitivity, immune-suppression and allergies. Thus, there is a need for developing alternative antimicrobial drugs for the treatment of infectious diseases [5,6]. Antimicrobials of plant origin have effective therapeutic potential. They are used in treating of infectious diseases and minimizing the side effects associated with synthetic antimicrobials [7].

The high cost of new antibiotics and their non-availability with limited effective span resulted in the increase in mortality and morbidity [8]. Therefore, there is a need to search for new drugs from other biological sources with proven antimicrobial activity [9].

Allelopathy is defined as direct or indirect effect, through which the chemicals and their metabolic products released by one plant affect the biochemical reactions of a neighboring plant [10].

The chemical compounds involved in the allelopathic effect are known as allelochemicals. Allelochemicals are the subsets of secondary metabolites not required for metabolism (growth and development) of the allelopathic organism. These allelochemicals can be released from plants by leaching from leaves, stems, seeds, flowers, buds, fruits, and disintegration of dead plant parts, exudation from roots and volatilization [11,3].

Allelopathy has been considered as a mechanism for the success of invasive plants through establishing monoculture and may contribute to the capability of exotic species to become dominants in particular invaded plant communities [12].

Allelochemicals as biological herbicides became alternative to current synthetic chemical approaches [13,14]. Weed competes with crop for nutrients, moisture, space as well as light and thus affect crop yields [15]. It has been reported that majority species of weed allelochemicals stop the crop production but sometimes also stimulate seed growth, germination and crop production [16,17].

*C. album* is responsible for important economic losses in agriculture around the world. Except in the extreme desert climate, *C. album* is found in all inhabited areas of the world where it thrives on all soil types and over a wide range of pH values [18]

*C. album* has been found to exhibit allelopathic effects on crop plants including soybeans, maize, cucumbers, carrots, onions, tomatoes, lettuce, squash and sunflowers and oats [19-21].

The main objectives of this research were: 1) to study the effects of leaf extracts from *Z. coccineum* on some pathogenic bacteria and fungi; 2) to investigate the influence of *Z. coccineum* aqueous leaf extract on the growth of *C. album*.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

*Zygophyllum coccineum* was collected from inland desert (Cairo-Suez desert road) as well as from coastal (Deltaic Mediterranean coast) desert of Egypt. The plant leaves were cleaned, washed with distilled water several times to remove dust and other residues. The leaves were then dried for several days in shaded place at room temperature for complete dryness followed by grinding into powder. The resulting powder was then kept in well stoppered bottles.

### 2.2 Preparation of Extract with Organic Solvents

The plant leaves was shade dried and ground into coarse powder. It was extracted by cold maceration twice (7 days each) using acetone, methanol and ethanol. The extracts obtained were dried using rotary evaporator and stored in air tight container in a refrigerator until further use.

## 2.3 Preparation of Aqueous Extract

The aqueous extract was prepared according to the method of El-Shora and Abd El-Gawad [14]. Leaves were washed several times with tap water and dried at 60℃ for 48h, and homogenized to fine powder by grinder. About 200 g of the homogenated material was soaked in 1 L of distilled water allowed on orbital shaker for 24h at room temperature (23-28℃) for extraction. The resulting extract was filtered and various concentrations were prepared.

## 2.4 Microbial Strains

The clinical bacterial strains of *E. coli, B. subtilis, S. aureus, B. cereus,* and *P. aeruginosa* were provided by Prof. Salwa A. Khalaf, Botany Department, Faculty of Science, Zagazig University, Egypt.

## 2.5 Microbial Inoculums

A 24 h microbial cultures grown in the bacteriological Mueller-Hinton Broth (MHB) at 37℃ and in the fungal Sabouraud Dextrose Broth (SDB) at 30℃ were adjusted at $2x10^{6}$ colony forming units (CFU $mL^{-1}$) and $2x10^{5}$ spores.

## 2.6 Antibacterial Activity

Antimicrobial activity of various plant extracts was determined by agar well diffusion method according to Shinde and Mulay [6]. Sample of 0.1 ml of freshly grown culture of test organisms was aseptically spread on the surface of sterile Muller Hilton agar plates. Wells of 6 mm diameter were made in agar plate using of sterile cork-borer. Fifty microliters of the various plant extracts and the same volume of extraction solvent for negative control were added in the wells with the help of micro pipette.

Ampicillin was used as positive controls for the tested organisms. Plates were kept for some time at 4℃ till the extract diffused in the medium with the lid closed and incubated for 24 h at 37℃. The plates were examined for the inhibition zone. Antibacterial activity effect was evaluated through measuring the diameter of the inhibition zone against the tested bacterial pathogens. Each assay in this experiment was replicated three times [22].

## 2.7 Antifungal Activity

Agar well-diffusion method [23] was followed to determine the antifungal activity. *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* were the test fungi. Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 h old-broth culture of respective fungi. Wells (10 mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of plant extract was prepared at a concentration of 1 mg/ml in different plant extracts of methanol, ethanol and acetone.

About 100 µl of various concentrations of plant solvent extracts were added by sterile syringe into the wells and allowed to diffuse at room temperature for 2h. Fluconazole was used as positive control. The plates were incubated at 28℃ for 48h for fungal pathogens. The diameter of the inhibition zone (mm) was measured.

## 2.8 Germination Bioassay

The germination of *Chenopodium album* was carried out according to El-Shora and Ali [24]. The germinated seeds were counted daily and the percentage of germination was calculated.

## 2.9 Allelopathic Experiment

This experiment was carried out according to El-Shora and Abd El-Gawad [14]. The germinated seeds in a control treatment with well-grown *C. album* roots were grown in plastic bowls containing 0.2 mM $CaCl_2$ solution and two different concentrations (50 and 100 µg $ml^{-1}$) of aqueous *Z. coccineum* leaf extract and vigorously aerated for 5 days.

## 2.10 Allelopathic Effect of Aqueous Leaf Extract of *Z. coccineum*

Seeds of *C. album* were treated with aqueous leaf extract of *Z. coccineum* for various time intervals (24, 48 and 72 h) followed by calculating the percentage of seed germination and measuring the radical length.

# 3. RESULTS AND DISCUSSION

In the present work, antimicrobial potential of leaf extracts of *Z. coccineum* has been investigated against five pathogenic bacteria. The leaf extracts were prepared by methanol, acetone and ethanol. The prepared leaf extracts were tested against *E. coli*, *B. subtilis*, *S. cereus* and *P. aeruginosa* (Fig. 1 and Fig. 2). Ampicillin was used as standard antibacterial.

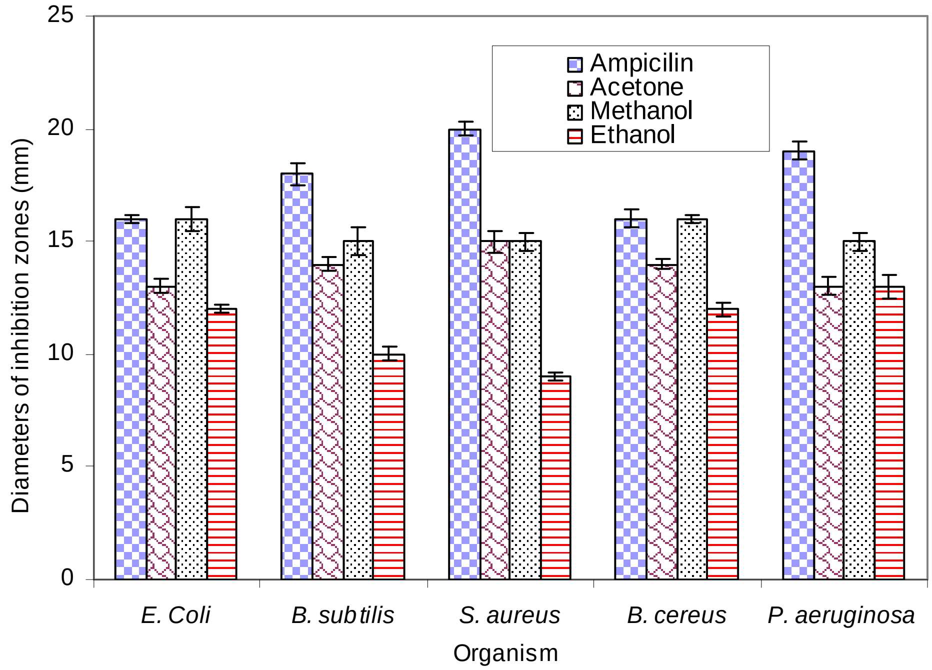


**Fig. 1. Antibacterial activity of ampicillin and leaf extracts of desert *Z. coccineum***

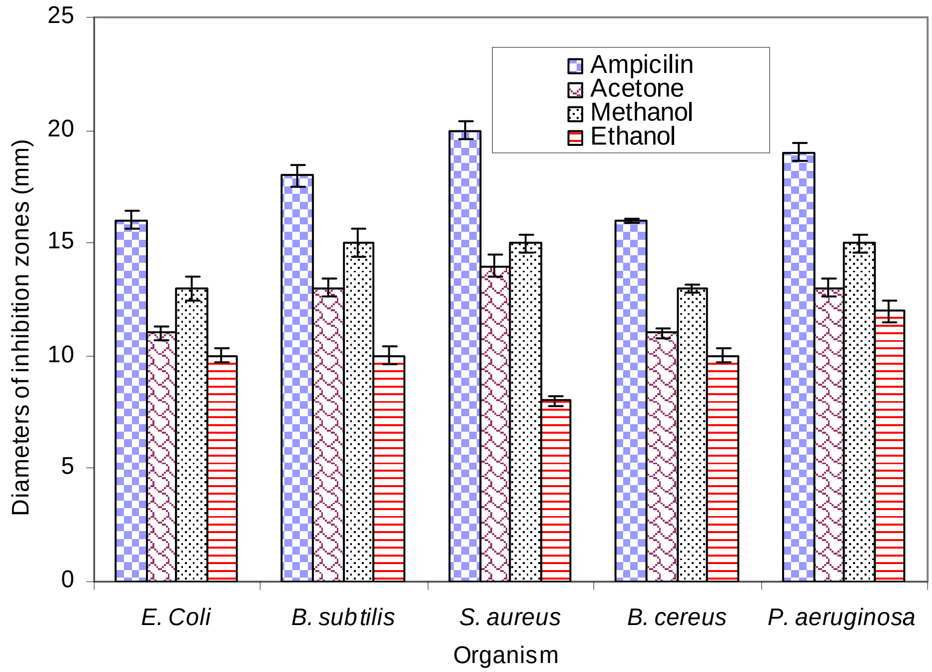


**Fig. 2. Antibacterial activity of ampicillin and leaf extracts of coastal *Z. coccineum***

Also, the antifungal effects of the various leaf extracts from *Z. coccineum* have been tested against *C. albicans*, *A. niger* and *A. flavus*. Fluconazole was tested against *C. albicans*, *A. niger* and *A. flavus* as standard antifungal (Fig. 3 and Fig. 4).

The various extracts from *Z. coccineum* leaf exhibited appreciable antimicrobial effect compared to control. Methanol extract was more effective as antimicrobial agents than ethanol and acetone extracts. This might be due to the high polarity of methanol. The antimicrobial activity of the various leaf extracts of *Z. coccineum* against both gram positive and gram negative bacteria is an indicator for the presence of broad spectrum of antimicrobial compounds in these extracts [25,26].

The effectiveness of a medicinal plant could be due to one main active compound or possibly due to the combined action of different compounds in the plant [10,27].

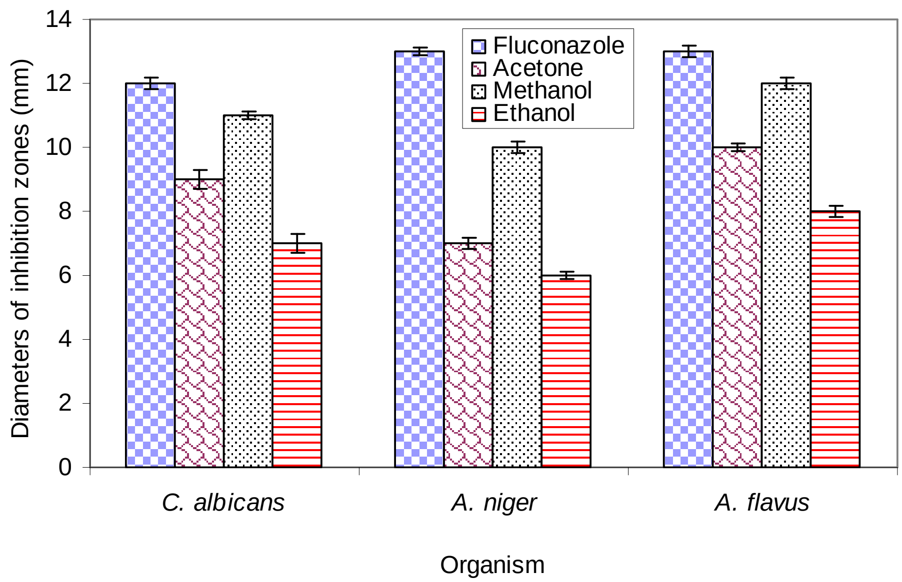


**Fig. 3. Antifungal activity of fluconazole and leaf extracts of desert *Z. coccineum***

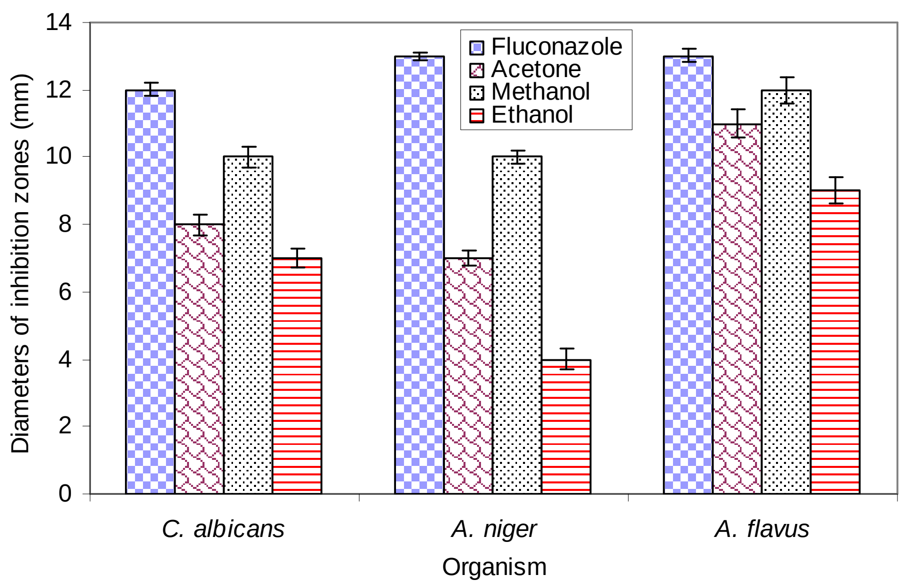


**Fig. 4. Antifungal activity of fluconazole and leaf extracts of coastal *Z. coccineum***

Most of the known medicinal plants exert antimicrobial potential. Flavonoids can form complex with soluble proteins and cell walls of bacteria [28]. Tannins bind with proteins rich with proline and can interfere with protein synthesis [29]. Antimicrobial effect of saponin could be attributed to its ability to cause protein leakage as well as certain enzymes from the cell [30]. Alkaloids have been suggested to function as antibacterial agents [31].

The present results confirm the validity of using *Z. coccineum* leaf extracts in traditional medicine and suggest that the plant leaf extracts contain compounds with antimicrobial properties. These compounds can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

Generally, the use of particular herb in medicine is quite safe compared to the chemically synthesized drug, but further studies should be carried out for enhancing the activity of plant extracts. It is also recommended to test safety and toxicity of any plant extract before its pharmaceutical application.

The present results revealed that aqueous extract of *Z. coccineum* at the two tested concentrations inhibited seed germination of (Fig. 5 and Fig. 6). The inhibitory effect of aqueous leaf extract was enhanced by increasing extract concentration. This agrees with the previous results of other investigators [32,33,14,34]. Allelopathy influences seed germination by two ways. Firstly, the chemical constituents hinder cell division. Secondly, these constituents inhibit the elongation of cells [35-37].

Soltani et al. [38] found that several allelopathic compounds could reduce the stimulating effect of some known growth hormones including indole acetic acid and gibberellins. Moreover, allelopathic compounds could disorder the different activities of plants through other mechanisms including disorder of oxidative phosphorylation, respiration and photosynthesis [35] and restriction of nutritive absorption [39].

Treatments of *C. album* seeds with aqueous leaf extract of *Z. coccineum* from the two habitats caused reduction of radical length compared to control (Fig 7 and Fig 8). Many investigators observed reduction in radical growth during their allelopathic studies [40-43]. Other investigators [32,33] support our results regarding the increase of allelopathic effect by increasing the extract concentration.

Plumule and radicle are the first plant organs emerge from the seeds during germination since their growth decreases if they are exposed to allelopathic compounds. Anaya [44] suggested that the reduction in the radicle length may be attributed to the decrease in cell division. Phenolic allelochemicals could alter the ultrastructure of the cells [45].

The decrease in radical length could be due to the presence of allelochemicals including flavonoids, tannins, and phenolic acids. Furthermore, the toxicity might be due to synergistic effect rather than single one [46].

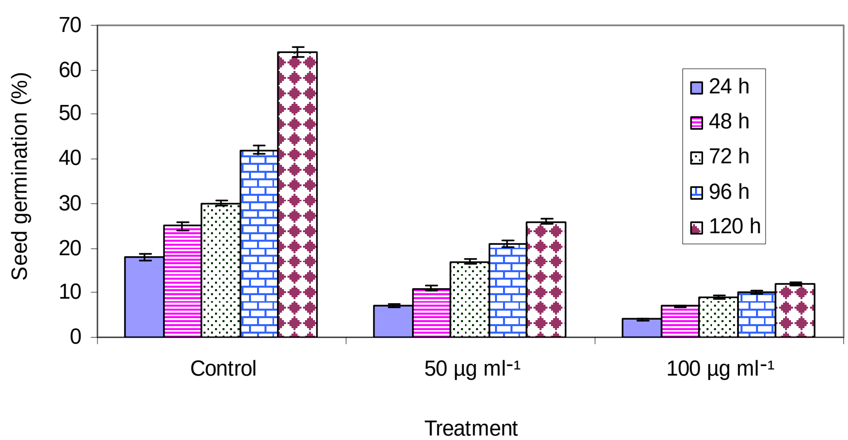


**Fig. 5. Allelopathic effect of aqueous leaf extract from desert *Z. coccineum* on seed germination of *Chenopodium album***

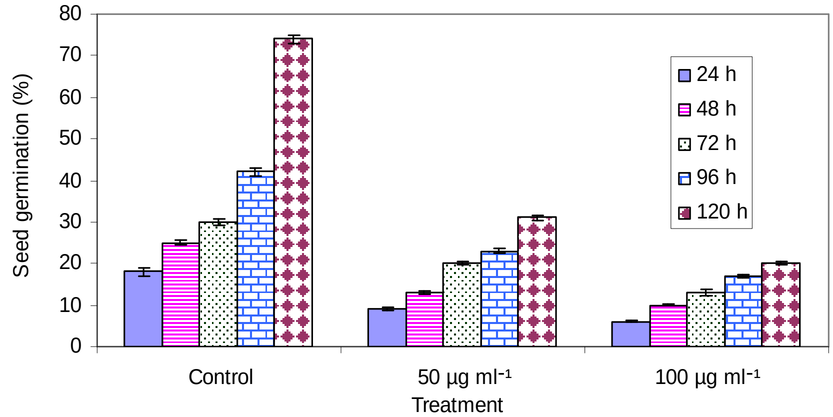


**Fig. 6. Allelopathic effect of aqueous leaf extract from coastal *Z. coccineum* on seed germination of *Chenopodium album***

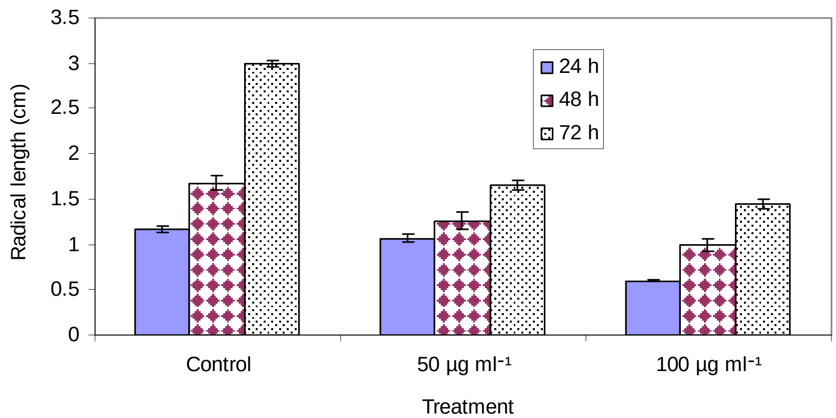


**Fig. 7. Allelopathic effect of aqueous leaf extract from desert *Z. coccineum* on radical length of *Chenopodium album***

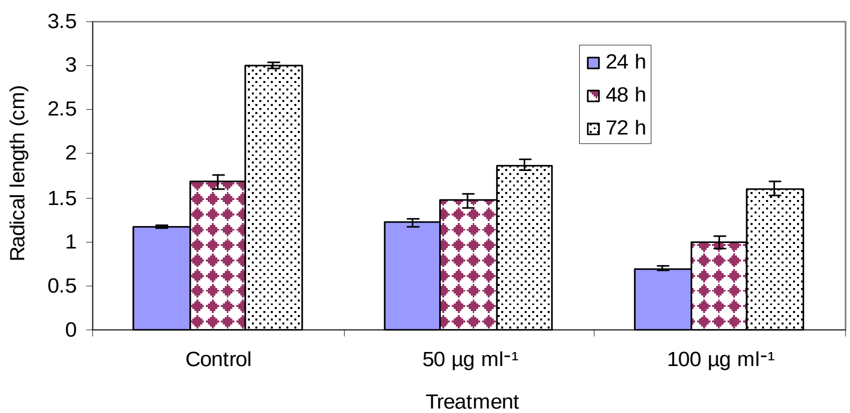


**Fig. 8. Allelopathic effect of aqueous leaf extract from coastal *Z. coccineum* on radical length of *Chenopodium album***

Phenolic acids in plant extracts have been investigated to exhibit a toxic effect on seed germination and various plant growth processes [47]. Phenolic acids inhibited protein synthesis in in roots of *Cicer arietinum* [14]. Phenolic allelochemicals could cause an increase in cell membrane permeability, inhibit the absorption of nutrients from the surroundings and consequently affect the normal growth of plants [48]. In addition, the inhibition of the key enzymes activity by allelochemicals in *Z. coccineum* could not be ruled out [34].

## 4. CONCLUSION

In conclusion, *Z. coccineum* leaf extract exhibited antimicrobial effect and allelopathic potential on *Chenopodium album*. Therefore, this extract can be used as antimicrobial agent and as bioherbicide for *Chenopodium album*.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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