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Comparative Phytochemical Studies on *Zygophyllum coccineum* L. from Different Habitats, Egypt

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Authors' contributions

This work was carried out in collaboration between all authors. Author HMES designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed literature searches. Authors YAEA and MHA managed the analyses of the study and literature.

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ABSTRACT

Zygophyllum coccineum L. (Family Zygophyllaceae) was collected from two habitats namely Wadi Hagul and Nile Delta coast. Plant leaves from both habitats contained appreciable content of total phenols and total flavonoids. The contents of these compounds were remarkably higher in desert than coastal habitat. Alkaloids, saponins and tannins are detected in plant leaves from both habitats, however their contents varied. Tannins and alkaloids are found in desert habitat with higher content compared to the coastal one, however the content of saponins was higher in coastal than in desert plants. The leaves of *Z. coccineum* contained glucosinolates which was higher in desert than in coastal plants. Proline and total ascorbate were lower in coastal than the desert plants whereas total glutathione was higher in coastal than desert area. It was noticed that the soluble protein as well as the soluble carbohydrate were lower than their insoluble counterparts in the leaves. Amino acids analysis revealed that cysteine and methionine were the dominant amino acids in leaves of plants from the two habitats. Alanine content was higher in coastal than desert plants. The total chlorophyll

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was higher in desert plants than in coastal plants. Z. *coccineum* leaves from both habitats showed remarkable activities of pyruvate kinase, pyruvate dehydrogenase, glutamine synthetase and phosphogluconate dehydrogenase. The desert plant is the best source for the plant material for medical use.

Keywords: Zygophyllum coccineum; secondary metabolite; glucosinolate; amino acids; enzymes activities.

1. INTRODUCTION

Zygophyllaceae is a family includes 25 genera and 240 species. They are adapted to semi desert as well as Mediterranean climates**.** Species of this genus represent a group of succulent plants that are salt tolerant and/or drought resistant. The distribution and growth of *Zygophyllum* species because they are dependent on the soil chemical structure of its habitats (1).

Aqueous extract of the plant is documented to produce a lowering in blood pressure, and acts as a diuretic and antipyretic, local anesthetic, with antihistamine activity, stimulation and depression of isolated amphibian heart, relaxation of isolated intestine, contraction of uterus and vasodilation. The extract antagonized acetyl choline action on skeletal muscle, and acted additively to the muscle relaxant effect of d-tubocurarine [2].

Plant secondary metabolism produces products that aid in the growth and development of plants but are not required for the plant to survive. Secondary metabolism facilitates the primary metabolism in plants. This primary metabolism consists of chemical reactions that allow the plant to live. In order for the plants to stay healthy, secondary metabolism plays a pinnacle role in keeping all the of plants' systems working properly [3].

Secondary metabolites play important role in protection of plants stress and for protection against UV light and other physical stress. Secondary metabolites utilized for engage pollinators as well as seed dispersers as signals [4].

Glucosinolates are natural plant products that have become increasingly important as flavor precursors, cancer prevention agents and crop protectants [5]. It has been reported that glucosinolates are found in all parts; however their quantities may vary considerably among organs [6]. Many plant cope with the stress by synthesizing and accumulating some compatible solutes such as soluble amino acids and proline which are termed as osmoprotectants as osmolytes. They are small, electrically neutral molecules and non-toxic even at molar constructions [7]. The amino acids represent the building blocks of proteins and they play an essential role in the regulation of metabolism of living organisms.

The aim of the present work was to compare the level of the chemical components of *Zygophyllum coccineum* whether primary or secondary metabolites as well as the activities of some metabolic enzymes in the plants collected from the two different habitats.

2. MATERIALS AND METHODS

2.1 Collection of Plants

Plant species were collected from naturally growing population in coastal [Deltaic Mediterranean coast) and inland desert (Wadi Hagul) of Egypt and identified by Prof. Naser Barakat, Professor of Plant Ecology, Botany Department, Minia University, Egypt. The plant material was handily cleaned, washed several times distilled water to remove dust and other residues, dried at room temperature in shaded place for several day till complete dryness and ground into powder, then preserved in well stopped bottles.

2.2 Determination of the Various Metabolites

Tannin content determinationon was done according to Trease and Evans [8]. Total phenol was determined using Folin-Ciocalteu reagent [9]. Total flavonoid content was determined by the methods of Cheng et al. [10]. Determination of alkaloids was carried out by the method of John et al [11]. The saponins content was determined according to Obadmi [12]. Glucosinolate determination was carried out according to Malabed et al. [13].

Proline was estimated colorimetrically according to Bates et al. [14]. Total ascorbate was estimated according to Cakmak and Marschner [15]. Total glutathione was determined according to Anderson [16]. Soluble, insoluble and total proteins were determined by the methods of Bradford [17]. Soluble, insoluble and total carbohydrates were determined according to Fales [18]. Amino acid analysis was carried out according to the method of Samir [19].

2.3 Enzyme Extraction and Assays

The enzyme extract was prepared according to Bihzad and El-Shora [20]. Pyruvate kinase was assayed by the method of Plaxton [21]. Pyruvate dehydrogenase was measured according to the method of Budde and Randall [22] Glutamine synthetase was measured according to El-Shora and Khalaf [23]. Phosphogluconate dehydrogenase was assayed according to Sindelar and Sindelarova [24].

3. RESULTS AND DISCUSSION

Zygophyllum exhibited appreciable content of total phenols (Fig. 1) and flavonoids (Fig. 2). The phenolic compounds inhibited the enzymatic activity of all enzymes monitored [25]. The phenols are important for desert plant to alter peroxidation kinetics by modifying lipid packing order. They can stabilize cell membranes and hinder the harmful effect of free radicals produced because of drought stress [26]. Plant phenols have been reported to be induced by biotic and abiotic stress [27].

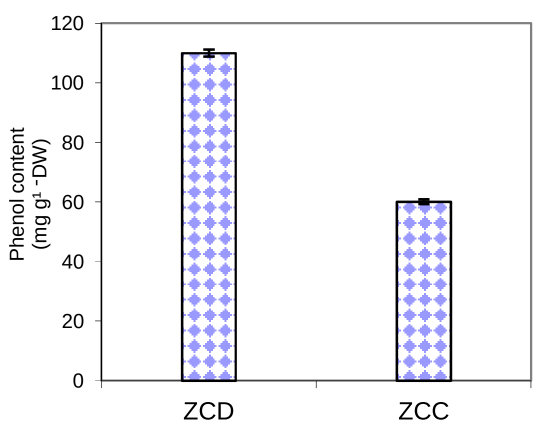

Fig. 1. Total phenol content of *Z. coccineum* leaves

Flavonoids play important role in the plant cell since it is considered as a non- enzymatic antioxidant compound to protect the cells from the harmful effect of reactive oxygen species [28]. It has been reported that the interaction between flavonoids and phenols with free radical gives the free radicals chemical stability than the original radicals [29].

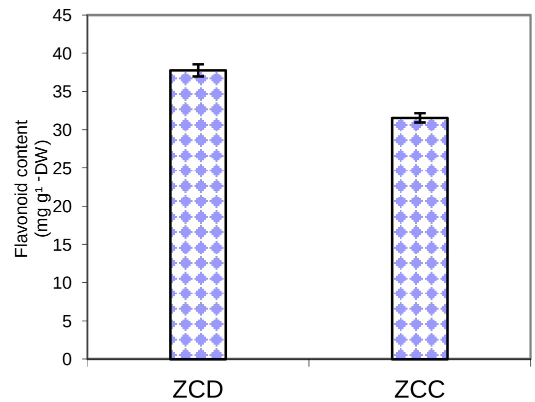

Fig. 2. Total flavonoid content of *Z. coccineum* leaves

Zygophyllum expressed remarkable content glutathione and ascorbate (Fig. 3). It is known that ascorbate carries out a number of antioxidant functions in the cell. It has been implicated in the regulations of the cell division, cell cycle progression [30,31] and cell elongation [32].

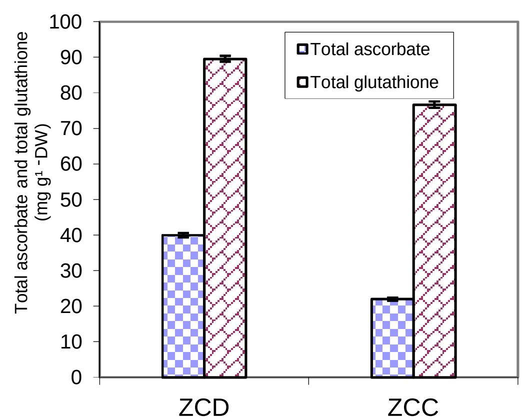

Fig. 3. Total ascorbate and glutathione content of *Z. coccineum* leaves

Zygophyllum leaves contained appreciable content of proline (Fig. 4). Proline acts as a free radical scavenger and may be more important in overcoming stress then in acting as a simple osmolyte. Moreover, it is considered as antioxidant defense molecule and singling molecule [33]. Proline accumulation in plants is caused by proline biosynthesis and the inactivation of proline degradation. Thereby, the increase in the level of accumulated proline in dehydrated plants is increased by simultaneous increase in the leaf water potential [34].

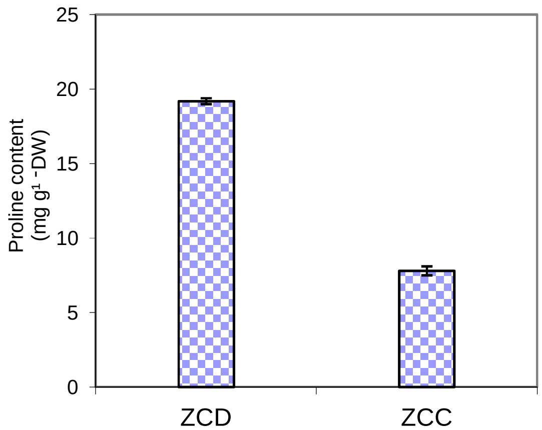

Fig. 4. Proline content of *Z. coccineum* leaves

Glucosinolates are sulfur and nitrogen containing compounds. The present results indicate the presence of glucosinolates in *Zygophyllum* collected from the desert as well as from coastal area (Fig. 5). However, the plants collected from the desert area demonstrated higher content compared to those from the coastal area. Variation in the amount and pattern of glucosinolates in *Zygophyllum* could be attributed to genetic and environmental factors including plant age, temperature, water stress and soil type [35]. The content of glucosinolates is controlled by multiple genes and is complexly regulated in the cell [36].

Analysis of amino acids composition of *Zygophyllum* leaves from the two habitats indicated the presence of several amino acids (Fig. 6). Amino acid composition of *Zygophyllum* from desert area (Fig. 6a) indicated that cysteine and methionine contents were the highest among all the detected amino acids, followed by proline and glutamic. Amino acid composition of *Zygophyllum* leaves from the coastal area (Fig. 6b) showed that similar results for cysteine, methionine, proline and glutamic. Cysteine and glutamic acids are the main component of glutathione which is antioxidant. Methionine is the precursor of polyamines which play a role as antioxidant.

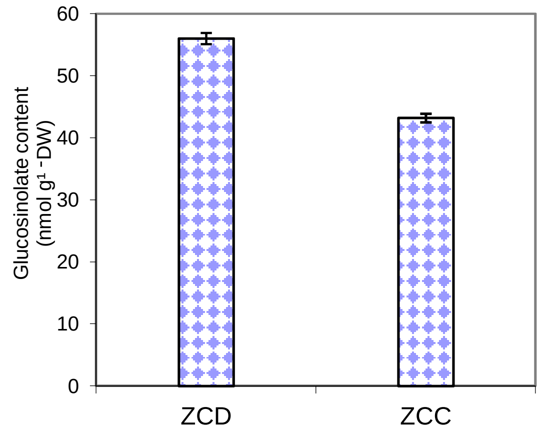

Fig. 5. Glucosinolate content of *Z. coccineum* leaves

The accumulation of amino acids in *Zygophyllum* leaves plays a vary role in drought tolerance, probably through osmotic adjustment, and it may be due to the high synthesis of amino acids from protein hydrolyses, in which the free amino acids are utilized by the plant to reduce the effect of water deficit through organic solute accumulation which in turn increased the water retention capacity [37].

Alkaloids, saponins and tannins accumulated in *Zygophyllum* leaves (Fig. 7, Fig. 8 and Fig. 9). Alkaloids in plants have antioxidant activity [38]. Saponins are consisting of a sterol ring with attached sugars [39] which have sensitive role in decreasing the amount of cholesterol in hypercholesterolemia organisms. Tannins may play the role in protecting *Zygophyllum* against herbivores. Also, these compounds can act as therapeutic agent against microbial infections [40].

Zygophyllum leaves expressed lower content of soluble protein compared to the insoluble content (Fig. 10). This might be due to a severe decrease in photosynthesis and consequently, the required materials for protein synthesis are not reduced; thus protein synthesis is reduced by Khani and Heidari [41].

Also, the soluble carbohydrate was lower than the insoluble carbohydrate than the soluble one in *Zygophyllum* leaves (Fig. 11). This phenomenon is similar to that of insoluble protein which could be attributed to the reduction in photosynthesis due to drought stress in the desert or salt stress in the coastal area.

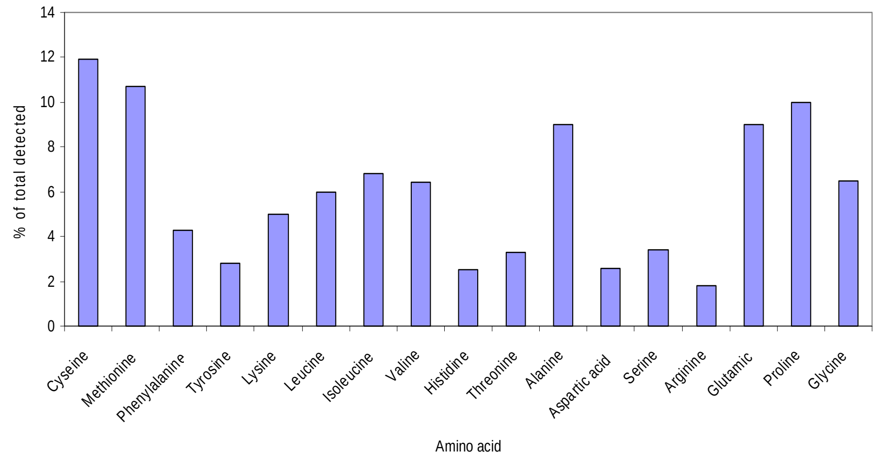

Fig. 6a. Amino acid composition of *Z. coccineum* leaves from the desert habitat

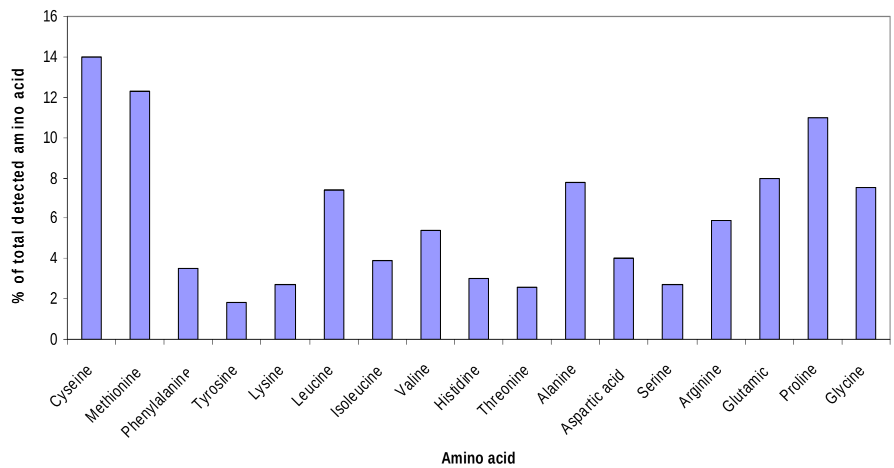

Fig. 6b. Amino acid composition of *Z. coccineum* leaves from the coastal habitat

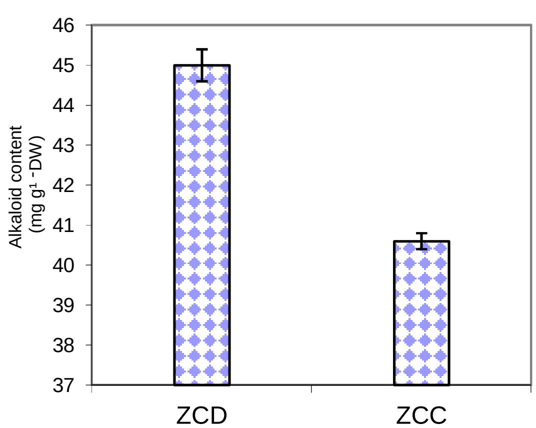

Fig. 7. Alkaloid content of *Z. coccineum* leaves

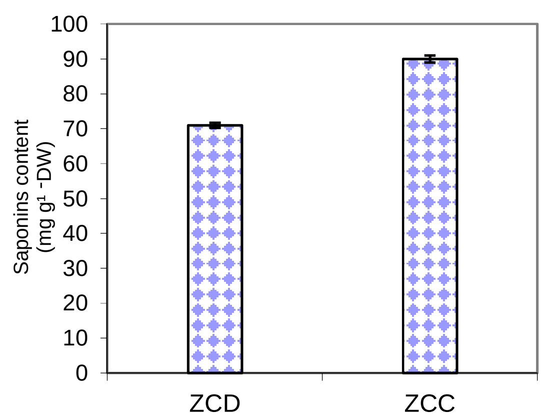

Fig. 8. Saponin content of *Z. coccineum* leaves

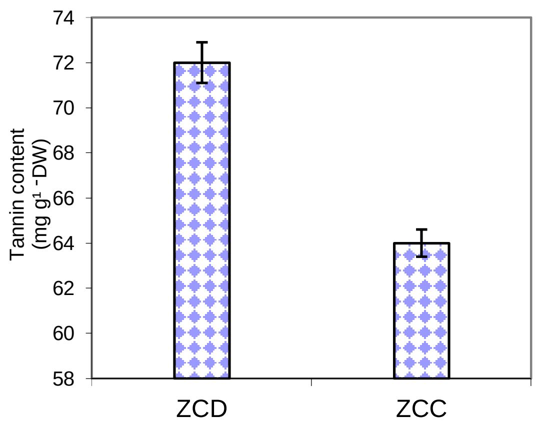

Fig. 9. Tannins content of *Z. coccineum* leaves

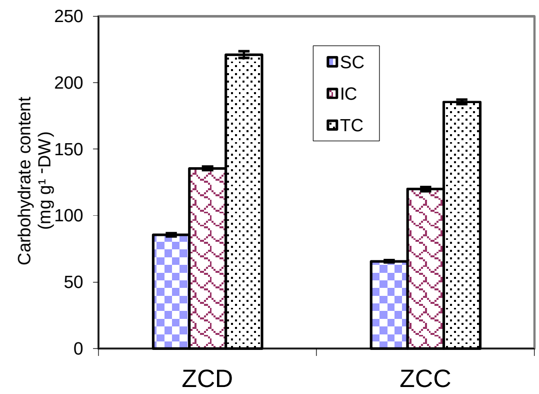

Fig. 11. Carbohydrate content of *Z. coccineum* leaves

SC: soluble carbohydrate; IC: insoluble carbohysrate and TC: total carbohydrate

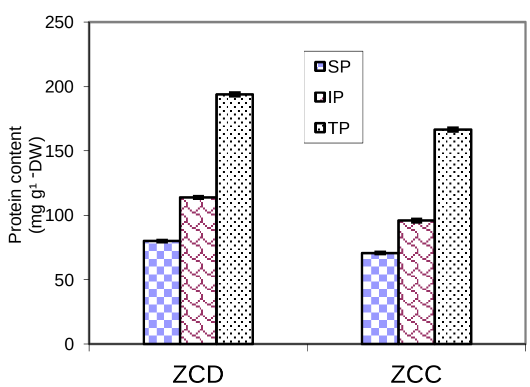

Fig. 10. Protein content of *Z. coccineum* leaves

SP: soluble protein; IP: insoluble protein and TP: total protein

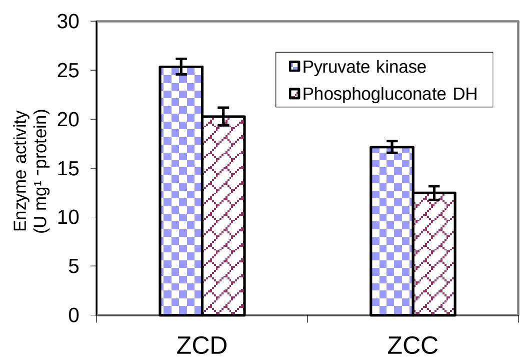

Fig. 12. Activities of pyruvate kinase and phosphogluconate dehydrogenase of *Z. coccineum* leaves

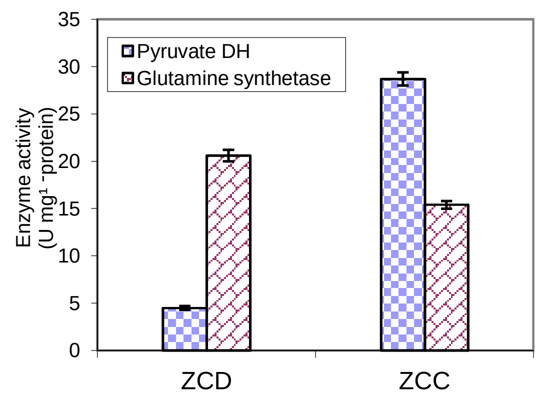

Fig. 13. Activities of pyruvate dehydrogenase and glutamine synthetase of *Z. coccineum* leaves

Regarding to the activities of the various enzymes there was some variations between the activity levels depending on the habitat (Fig. 12 and Fig. 13). All the tested enzymes in the desert plants expressed higher activities compared to the coastal plants. Glutamate dehydrogenase showed the highest activity compared to the other enzymes in plants from the two habitats. Treatment of glutamate synthase with indole acetic acid (IAA), gibberellic acid (GA_3), and coumarin resulted in the enzyme activation and GA_3 was the best activator. The activation of the enzyme by GA_3 is in harmony with those of El-Shora and Metwally [42].

4. CONCLUSION

In conclusion, *Zygophyllum* from both habitats under investigation seems to be rich with various secondary metabolites which can be used as antioxidants. In addition, this plant contained appreciable amount of glucosinolates which are important as flavor precursors, cancer prevention agents and crop protectants.

The desert plant exhibited higher contents of the various bioactive compounds than the coastal one, therefore the desert plant seems likely to be better than the coastal one in medicine purposes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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