

Comparative phytochemical studies on *Zygophyllum coccineum* L. from different habitats, Egypt

Abstract

Zygophyllum coccineum L. (Family Zygophyllaceae) was collected from two habitats namely Wadi Hagul and Nile Delta coast. Phytochemical analysis of plant leaves showed that the coastal plant contained higher content of Na^+ , K^+ , Ca^{+2} and Mg^{+2} compared to desert plant. 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 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.

Keywords: *Zygophyllum coccineum*, Secondary metabolite, Glucosinolate, Amino acids, Enzymes activities.

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 (Hammoda *et al.*, 2013).

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 (El-Ghazali *et al.*, 2010).

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 (El-Shora *et al.*, 2015).

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 (Wink, 2008).

Glucosinolates are natural plant products that have become increasingly important as flavor precursors, cancer prevention agents and crop protectants (Graser *et al.*, 2000). It has been reported that glucosinolates are found in all parts; however their quantities may vary considerably among organs (Font *et al.*, 2005)

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 (Tamura *et al.*, 2003). 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.

Material and Methods

Collection of plants

Plant species were collected from naturally growing population in coastal (Deltaic Mediterranean coast) and inland desert (Wadi Hagul) of 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 (AOAC, 1990).

Soil Analysis

Three soil samples (0-30 cm) were collected from each stand. The soil samples were pooled together to form one composite sample. They were spread, air dried, sieved (2 mm sieves) and finally packed in plastic bags to be ready for analysis. Physical and chemical analyses of soil samples were carried out according to Piper (1947), Jackson (1962) and Allen *et al.* (1974).

Determination of the various metabolites

Total phenol was determined by using the Folin-Ciocalteu reagent (MacDonald *et al.*, 2001). Total flavonoid content was determined by the methods of Cheng *et al.* (2002). Determination of alkaloids was carried out by the method of Ulubalen (2001). Tannin content determination was done according to AOAC (1990). The saponins content was determined according to Obadmi (2001). Glucosinolate determination was carried out according to Malabed *et al.* (2014).

Proline was estimated colorimetrically according to Bates *et al.* (1973). Total ascorbate was estimated according to Cakmak and Marschner (1992). Total glutathione was determined according to Anderson (1985). Soluble, insoluble and total proteins were determined by the methods of). Soluble, insoluble and total carbohydrates were determined according to Fales (1951). Amino acid analysis was carried out according to the method of Samir (2015).

Enzyme extraction and assays

The enzyme extract was prepared according to Bihzad and El-Shora (1996). Pyruvate kinase was assayed by the method of Plaxton (1988). Pyruvate

dehydrogenase was measured according to the method of Budde and Randall (1990). Glutamine synthetase was measured according to El-Shora and Khalaf, 2008). Phosphogluconate dehydrogenase was assayed according to Sindelar and Sindelarova (1999).

Results and Discussion

The major factor governing metal availability to plants in soils is the solubility and the thermodynamic activity of the uncomplexed ion (Jenne and Luoma, 1977).

Soil analysis revealed that the coastal desert attained significantly high pH than inland desert (Table 1). Clay, EC, Na^+ , K^+ , Ca^{2+} and Mg^{2+} showed moderate significant difference between coastal and inland desert. On the other hand, soil texture (sand and silt) and SO_4^{--} contents did not show significant differences. The plant uptake of chemical species in soil solution is also dependent on a number of plant factors. These factors include: root intrusion, water, kinetics of membrane transport, ion interactions and metabolic fate of absorbed ions (Rieuwerts *et al.*, 1998).

The majority of plants are exposed to water stress due to extreme soil water deficits in arid and semi-arid environment. The survival of land plants in such areas depends on water availability and their ability to adapt stress (Kramer, 1984).

The desert plant adaptation is attributed to their capability to maintain their water and their turgidity. The plants land to accumulate the inorganic solutes with high quantities within their tissues and tends to accumulate compatible solutes in cell cytoplasm to be able to balance the osmotic pressure within the cells (Kamel, 2007).

Zygophyllum exhibited appreciable content of total phenols (Fig. 1) and flavonoids (Fig. 2). Muscolo *et al.* (2001) reported that the phenolic compounds inhibited the enzymatic activity of all enzymes monitored. 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 (Arora *et al.*, 2000). Plant phenols have been reported to be induced by biotic and abiotic stress (Naczka and Shalidi, 2004)

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 (Nijveldt *et al.*, 2001). It has been reported that the interaction

between flavonoids and phenols with free radical gives the free radicals chemical stability than the original radicals (Parr and Bolwell, 2002).

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 (Smirnoff, 1996; Horemans *et al.*, 2000) and cell elongation (Blokhina *et al.*, 2003).

Zygophyllum leaves contained appreciable content of proline (Fig. 4). Proline acts as a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte. Moreover, it is considered as antioxidant defense molecule and signaling molecule (Hayat *et al.*, 2012).

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 (Patel *et al.*, 2011).

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 (Rosa, 1997). The content of glucosinolates is controlled by multiple genes and is complexly regulated in the cell (Uzunova *et al.*, 1995).

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.

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 (Sircelj *et al.*, 2005).

Alkaloids, saponins and tannins accumulated in *Zygophyllum* leaves (Fig. 7, Fig. 8 and Fig. 9). Alkaloids in plants have antioxidant activity (Maiza-Benabdessela *et al.*, 2007). Saponins are consisting of a sterol ring with attached sugars (Price *et al.*, 1987), 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 (Wink, 2003).

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 (2008).

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.

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), GA₃, and coumarin resulted in the enzyme activation and GA₃ was the best activator. The activation of the enzyme by GA₃ is in harmony with those of El-Shora and Metwally (2008).

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

References

- Allen, S.E., Grimshaw, H.M., Parkinson, J.A., Quarmby, C. and Roberts, J.D. (1974). Chemical analysis of ecological materials. Blackwell Scientific Publications. Osney, Oxford, London.
- Anderson, M.E. (1985). Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol.*, 113: 548-55.
- Aroram, A., Byrem, T.M., Nair, M.G., and Strasburg, G.M. (2000). Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. *Arch. Biochem. Biophys.* 73: 102–109
- Batanouny, K. H. and Ezzat, N. H. (1971). Eco-physiological studies on desert plants. 1. Autecology of Egyptian *Zygophyllum* species. *Oecologia*, 7: 170-183.
- Bates, L., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205-207.
- Iokhina, O., Virolainen, E., and Fagerstedt, K.V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann. Bot.*, 91:179-194.
- Bihzad, M. and El-Shora, H.M. (1996). Phosphoenolpyruvate carboxylase from *Rumex dentatus*, a C3-plant. *J. Plant Physiol.* 149: 669-676.
- Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*
- Budde, R.J.A. and Randall, D.D. (1990). Pea leaf mitochondria pyruvate dehydrogenase complex is inactivated in vivo in a light dependent manner. *Proc. Natl. Acad. Sci.* 87: 673-676.
- Cakmak, I. and Marschner, H. (1992): Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. *Plant. Physiol.* 98: 1222-1227.
- Chang, C., Yang, M., Wen, H., and Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Analysis*, 10: 178-182.
- Elgamal, M.H.A., Shaker, K.H., pollmann, K. and Seifert, K.H. (1995). Triterpenoid saponins from *Zygophyllum* species". *Phytochem.*, 40: 233-1236.
- El-Ghazali G.E., Al-Khalifa K.S., Saleem G.A and Abdallah E. M. (2010). Traditional medicinal plants indigenous to Al-Rass provinces, Saudi Arabia. *J. Med. Plants Res.*, 2010; 4: 2680-2683.
- El-Shora, H.M. and Abdel-Azeem, M.M. (2008). Production, purification and characterization of proteases from whey by some fungi. *Ann. Microbiol.*, 58: 495-502.

- El-Shora, H.M., El-Farrash, A.H., Kamal, H. and Abdelrazek, A. (2015). Enhancement of antioxidant defense system by UV radiation in fenugreek as medical plant. *Inter. J. Sci. Res.*, 3: 529-535.
- El-Shora, H.M and Khalaf, S.A. (2008). Activities and kinetic characteristics of glutamine synthetase from *Penicillium cyclopium*. *Ann. Microbiol.* , 4:691-96.
- Fales, F.W. (1951). The assimilation and degradation of carbohydrates of yeast cells. *J. Biol. Chem.* 193, 113–116.
- Font, R., Rio-Celestino, M.D., Cartea, E., and Harow-Bailon, A.D. (2005). Quantification of glucosinolates in leaves of leaf rape (*Brassica napus*) by near-infrared spectroscopy. *Phytochem.*, 66:175–185. doi: 10.1016/j.phytochem.2004.11.011.
- Graser, G., Schneider, B., Oldham, N.J. and Gershenzon, J. (2000). The methionine chain elongation pathway in the biosynthesis of glucosinolates in *Eruca sativa* (Brassicaceae). *Arch Biochem Biophys.* 378: 411–419.
- Hammoda, H.M., Ghazy, N.M., Harraz, F.M., Radwan, M.M., ElSohly, M.A., and Abdallah, I.I. (2013). Chemical constituents from *Tribulus terrestris* and screening of their antioxidant activity. *Phytochem.*, 92: 153-9.
- Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A. (2012). Role of proline under changing environments: a review. *Plant Signal. Behav.*, 7:1456–1466.
- Horemans, N., Foyer, C.H., and Asard, H. (2000). Transport and action of ascorbate at the plant plasma membrane. *Trends Plant Sci.* 5: 263–267.
- Jackson, M.L. (1962). Soil chemical analysis. International institute for tropical agriculture (IITA). Mannual Series No 1, pp 70.9999
- Jenne, E.A., and Luoma, S.N. (1977). Forms of trace elements in soils, sediments, and associated waters: An overview of their determination and biological availability. p. 110--143. In: R. Wilding and H. Drucker (ed.) Biological implications of metals in the environment. NTIS, Springfield, VA.
- Kamel, M. (2007). Osmotic adjustment in three succulent species of Zygophyllaceae. *Afr. J. Ecol.* 46: 96-104.
- Khani, M.N. and Heidari, R. (2008). Effects of drought stress on soluble proteins in two maize varieties. *Turk. J. Biol.* 32: 23-30.
- Kramer, P.J. (1984). Problems in water relations of plants and cells. In: International Review of cytology. (Ed.): P.J. Kramer. pp. 254-286.
- Maiza-Benabdesselam, F., Khentache, S, Bougoffa, K., Chibane, M., Adach, S., Chapeleur, Y., Max, H., and Laurain-Mattar, D. (2007). Antioxidant activities of

- alkaloid extracts of two Algerian species of *Fumaria* : *Fumaria capreolata* and *Fumaria bastardii*. Rec. Nat. Prod. 1: 28-35
- Malabed, R.S., Marissa G. N., Benjamin C. A. and Erika A. F. T. (2014). Characterization of the glucosinolates and isothiocyanates in mustard (*Brassica juncea* L.) extracts and determination of its myrosinase activity and antioxidant capacity.
- McDonald, S, Prenzler P.D., Autolovich M, and Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. Food Chem., 73:73-84.
- Muscolo, A., Pauccio, M.R., and Sidari, M. (2001). The effects of phenolic acids extracted from two different soils in Aspromonte on germination of *Pinus laricio* seeds. Fres. Environ. Bull., 10: 659–663.
- Naczki, M. and Shalidi, F. (2004). Extraction and analysis of phenolics in food. Chromatogr. J. 1054:95-111.
- Neha, T., Patel, U., Panchal, N. S., Pandey, I.B., Amar N. and Pandey, I. (2011). Implications of calcium nutrition on the response of *Acacia senegal* (Mimosaceae) to soil salinity. Anales de Biologia 33: 23-34.
- Nijveldt, R.J., van Nood, E., van Hoorn, D.E.C., Boelens, P.G., van Norren, K., and van Leeuwen, P.A.M. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. Amer. J. Clin. Nutr. 74: 418–425.
- 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 J. Pure. Appl. Sci. 8: 203-208.
- Parr, A.J. and Bolwell, J.P. (2002). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. J. Sci. Food Agric., 80: 985–1012.
- Piper, C.S. (1947). Soil and plant analysis. Interscience. Publishers, Inc. New York.
- Plaxton, W.C. (1988). Purification of pyruvate kinase from germinating castor bean endosperm. Plant Physiol. 86: 1046-1069.
- Price, K.R., Johnson, I.T. and Fenwick, G.R. (1987). The chemistry and biological significance of saponins in food and feeding stuffs. Crit. Rev. Food Sci. Nutr. 26: 27-133.
- Rieuwerts, J.S., I., Thornton, M.E., Farago, E. and Ashmore, M.R. (1998). Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. Chem. Speci. Bioavail., 10: 61-75.

- Rosa, E. (1997). Daily variation of glucosinolate concentration in the leaves and roots of cabbage seedlings in two constant temperature regimes. *J. Sci. Food Agric.*, 73: 364-368.
- Samir, A. (2015). Effect of humic acid on growth and biochemical composition of some algal species grown in fish farming. Master Thesis, Botany Department, Faculty of Science, Mansoura University, Egypt
- Sindelar, L. and Sindelarova, M. (1989). Changes in activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase isozymes upon potato virus Y infection in tobacco leaf tissues and protoplasts. *Plant Physiol. Biochem.* 37: 195-201.
- Sircelj, H., Tausz, M., Grill, D., and Batic, F. (2005). Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. *J. Plant Physiol.* 162: 1308-1318.
- Smirnoff, N. (1996). The function and metabolism of ascorbic acid in plants. *Ann. Bot.* 78: 661–669.
- Tamura, T., Hara, K., Yamaguchi, Y., Koizumi, N., and Sano, H. (2003). Osmotic stress tolerance of transgenic tobacco expressing a gene encoding a membrane-located receptor-like protein from tobacco plants. *Plant Physiol.*, 131: 454–62.
- Uzunova, M., Ecke, W., Weissleder, K. and Robbelen, G. (1995). Mapping the genome of rapeseed (*Brassica napus* L.). 1. Construction of of an RFLP linkage map and localization of QTLs for seed glucosinolate content. *Theor. Appl. Genet.* 90: 194–204.
- Wink, M. (1977). Evolution of secondary plant metabolism. *Encyclopedia of life Sci.* DOI: 10.1002/9780470015902.a0001922.pub2.
- Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochem.* 64: 3–19.

Table 1. Mean values and standard error of the soil parameters of the studied two phytogeographical regions. EC = electrical conductivity, *: values are significant at $P \leq 0.05$, **: values are significant at $P \leq 0.01$, ns: non-significant at $P > 0.05$.

Soil variables	Phytogeographical regions		P-value	LSD _{0.05}
	Coastal	Inland		
Sand %	87.53	86.99	0.64 ^{ns}	6.43
Silt %	10.00	11.49	0.95 ^{ns}	5.54
Clay %	2.39	1.55	0.04*	1.54
SO ₄ ²⁻ %	0.20	0.19	0.83 ^{ns}	0.18
EC (ms/cm)	1.72	0.47	0.04*	1.98
pH	8.56	8.00	0.000**	0.23
Na ⁺ (mg/100g dry soil)	1419.86	314.08	0.02*	1332.43
K ⁺ (mg/100g dry soil)	159.61	35.19	0.03*	140.13
Ca ²⁺ (mg/100g dry soil)	425.76	96.36	0.04*	366.5
Mg ²⁺ (mg/100g dry soil)	210.56	45.47	0.03*	185.74

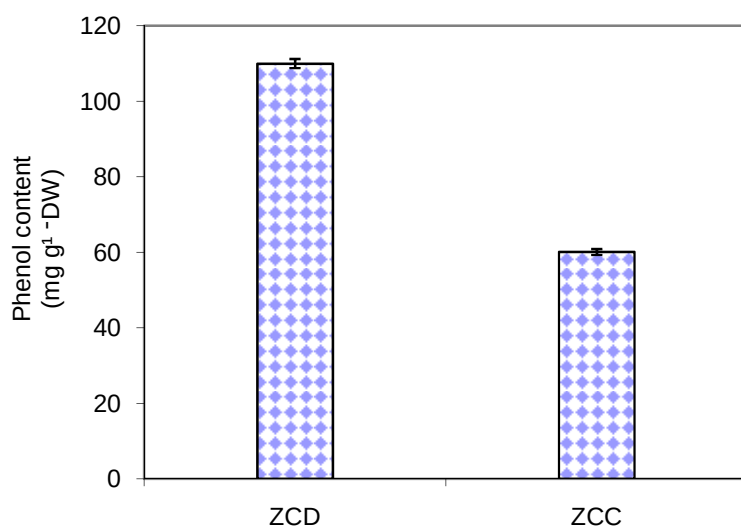


Fig. 1: Total phenol content of *Z. coccineum* leaves.

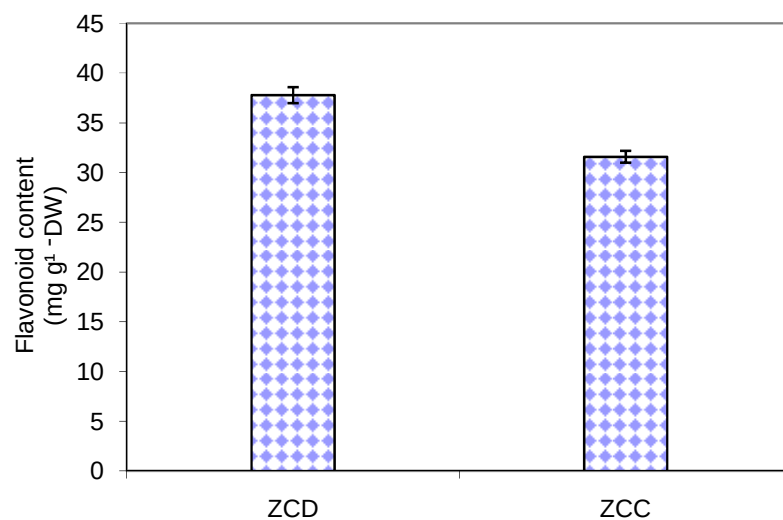


Fig. 2: Total flavonoid content of *Z. coccineum* leaves.

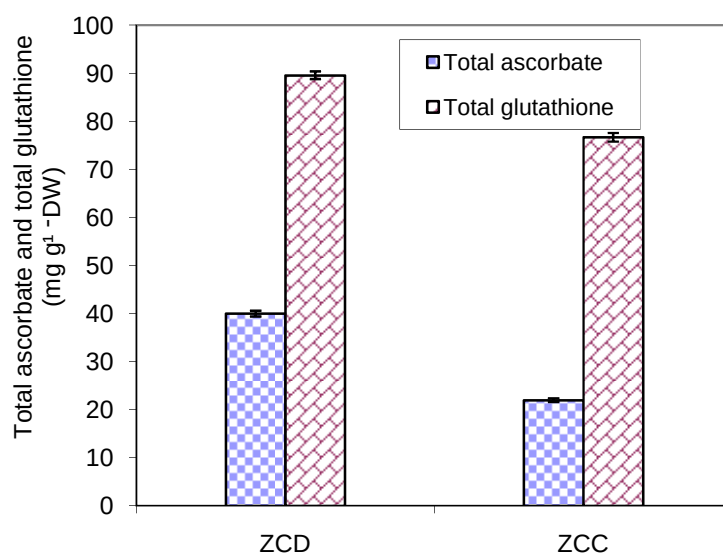


Fig. 3: Total ascorbate and glutathione content of *Z. coccineum* leaves.

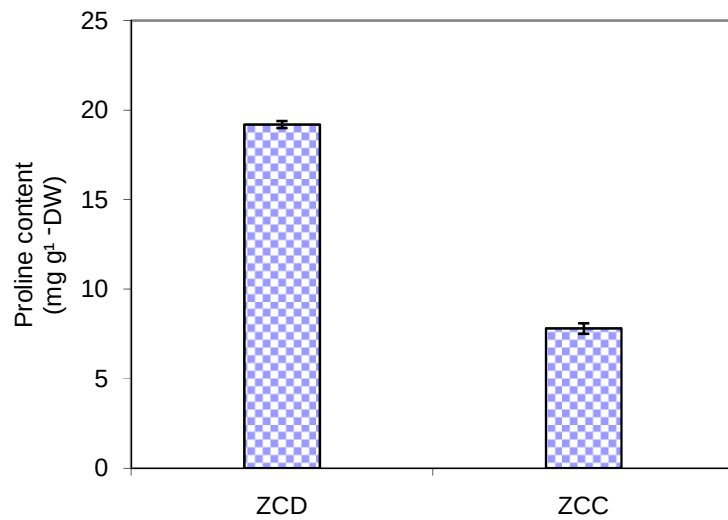


Fig. 4: Proline content of *Z. coccineum* leaves.

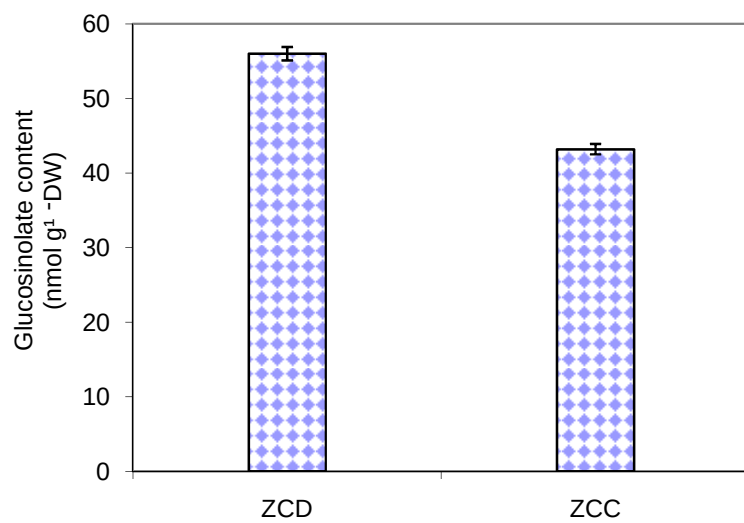
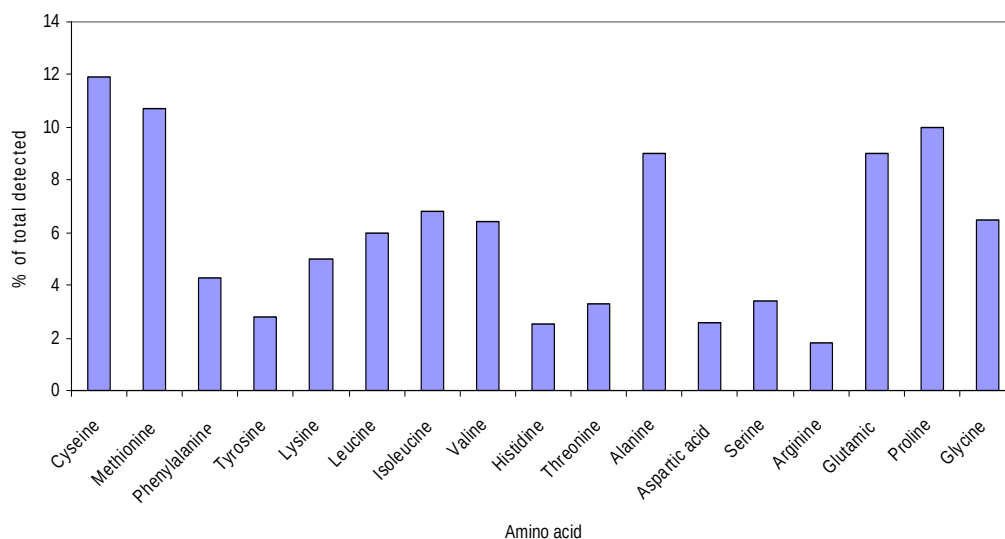
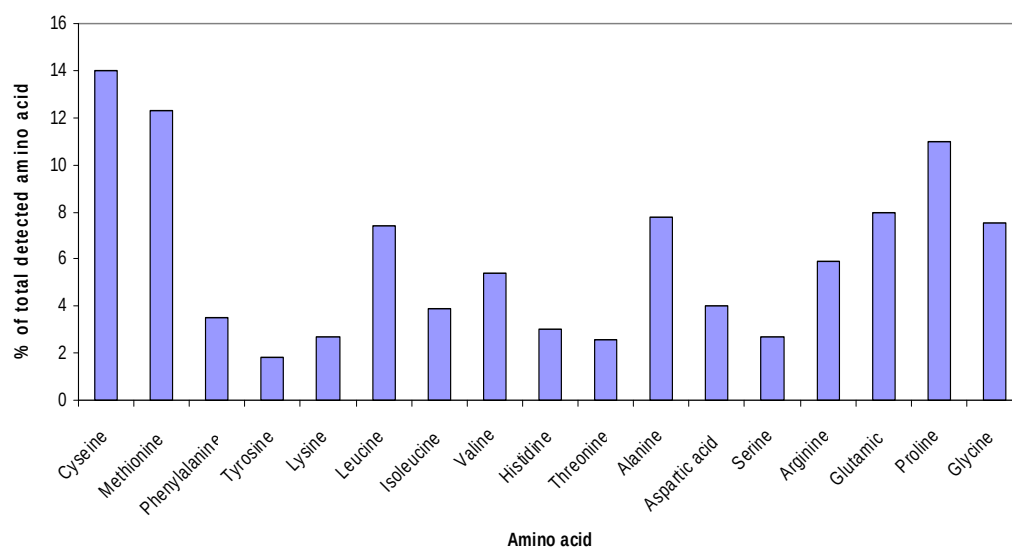


Fig. 5: Glucosinolate content of *Z. coccineum* leaves.



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



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

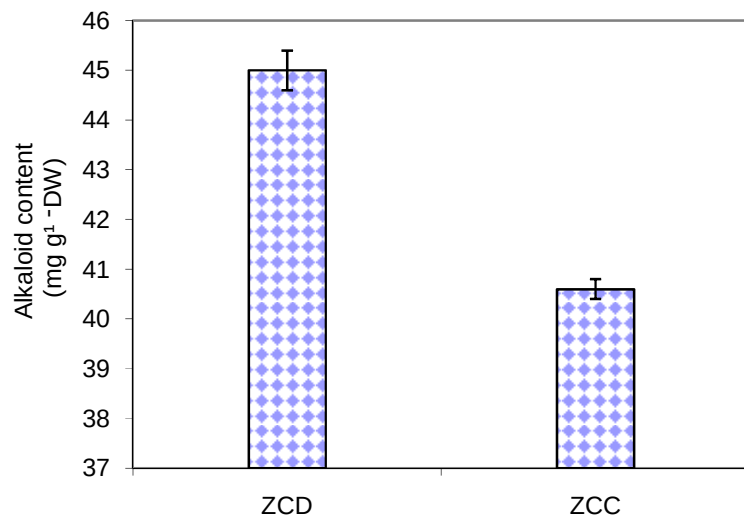


Fig. 7: Alkaloid content of *Z. coccineum* leaves.

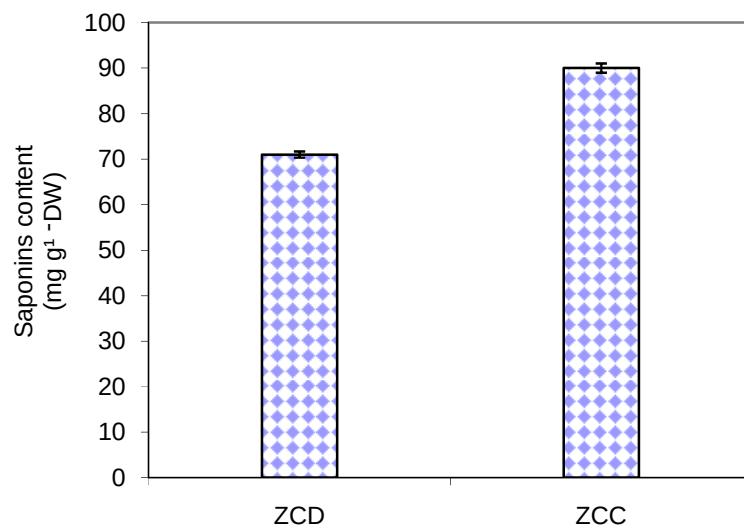


Fig. 8: Saponin content of *Z. coccineum* leaves.

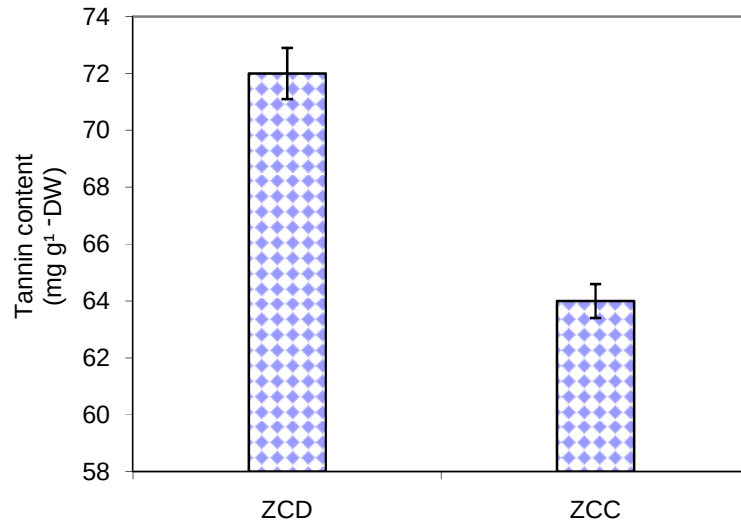


Fig. 9: Tannins content of *Z. coccineum* leaves.

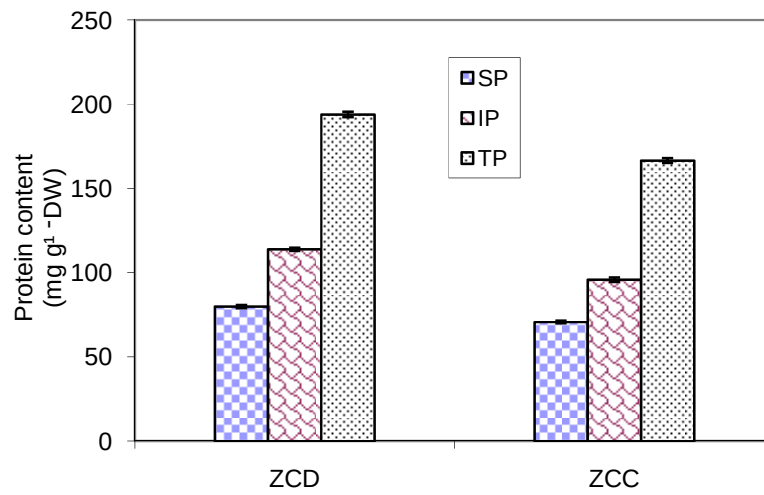


Fig. 10: Protein content of *Z. coccineum* leaves. SP: soluble protein; IP: insoluble protein and TP: total protein.

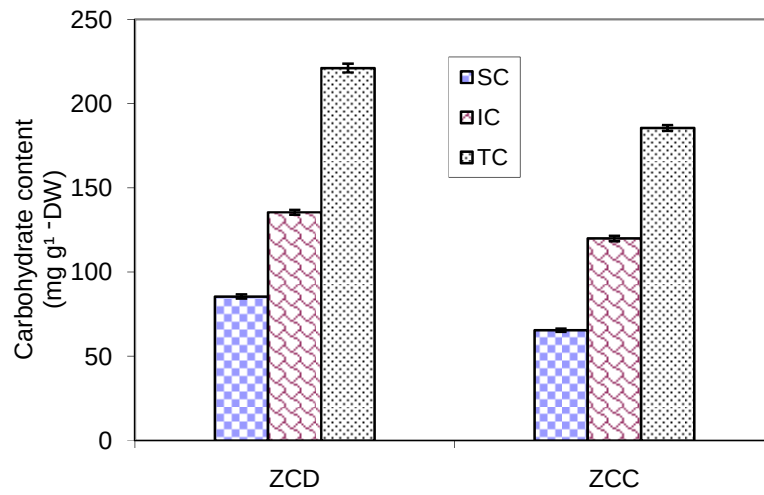


Fig. 11: Carbohydrate content of *Z. coccineum* leaves. SC:soluble carbohydrate; IC: insoluble carbohydrate and TC: total carbohydrate.

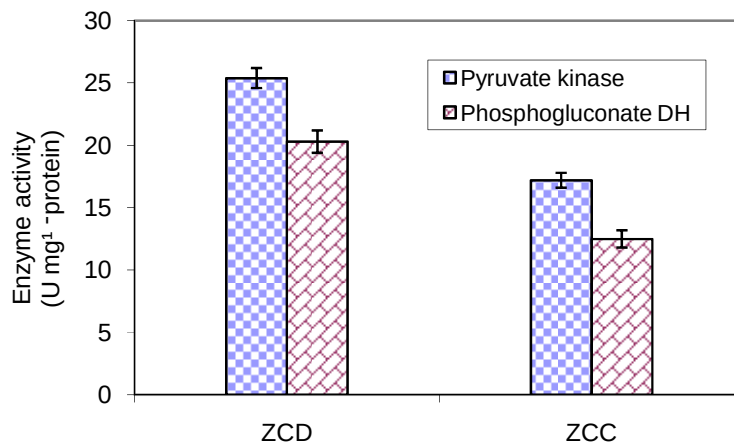


Fig. 12: Activities of pyruvate kinase and phosphogluconate dehydrogenase of *Z. coccineum* leaves.

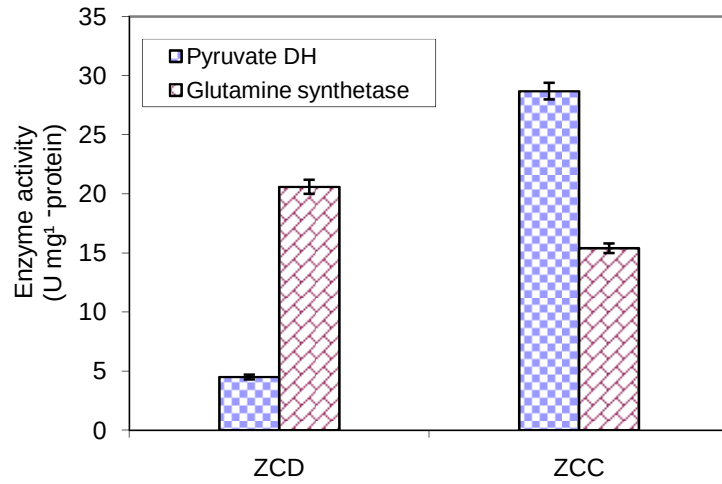


Fig. 13: Activities of pyruvate dehydrogenase and glutamine synthetase of *Z. coccineum* leaves.