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Evaluation of antioxidant enzymes and elements content of *Centaurea kurdica* Reichardt and *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz

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Abstract

Chronic illnesses are quickly increasing worldwide. Nourishment and diet have an important place in the improvement and maintenance of health throughout the whole life. Biochemical and physiological change in the organism may result in overexpress of free radicals causing to oxidative injury to biomolecules. Recently, the use of medical plants has increased due to their useful properties such as antioxidants, anticancer and hypoglycaemic activities. *Centaurea kurdica* Reichardt and *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz (Asteraceae) used in this study taxa are endemic species naturally grown in various regions of Elazig. Many taxa of *Centaurea* are being traditionally used both in our country and across the world for medical purposes as well as nutrients. *Centaurea kurdica* Reichardt and *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz (Asteraceae) taxa were obtained from their natural habitats in Elazığ. Physiological changes such as heavy metal segregation, antioxidant enzymes superoxide dismutase (SOD), glutathione S transferase (GST), ascorbat peroxidase (AP) and catalase (CAT) activities in plant tissues were determined. Among the enzymes studied, SOD is the enzyme with the highest activity. It has been determined that the maximum accumulation of the accumulation is usually on the leaves. The activities of enzymatic antioxidants: superoxide dismutase (SOD), glutathione S transferase (GST), ascorbat peroxidase (APX) and catalase (CAT) activities were assayed and found significantly higher i.e. 1.315, 0.011, 0.031 and 0.007 (unit mg^{-1} fresh tissues) respectively in *Centaurea kurdica* than *Centaurea urvillei* DC. subsp. *hayekiana* , where *C. kurdica* was recorded next plant in order to be better-off in the entire enzyme activity assessed except ascorbate peroxidase.

Keywords: Antioxidant enzyme, *Centaurea*, endemic, element content

Entaurea kurdica Reichardt and *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz'nın Element İçeriği ve Antioksidan Enzimlerinin Belirlenmesi

Öz

Kronik hastalıklar dünya çapında hızla artmaktadır. Tüm yaşam boyunca sağlığın iyileştirilmesi ve sürdürülmesinde beslenme ve diyet önemli bir yere sahiptir. Organizmadaki biyokimyasal ve fizyolojik değişim, biyomoleküllerde oksidatif hasara yol açan serbest radikallerin aşırı artmasına neden olabilir. Son zamanlarda tıbbi bitkilerin kullanımı antioksidanlar, antikanser ve hipoglisemik aktiviteler gibi yararlı özellikler nedeniyle artmıştır. Bu çalışmada kullanılan, *Centaurea kurdica* Reichardt ve *Centaurea urvillei* DC. subsp. hayekiana Wagenitz (Asteraceae) Elazığ'ın çeşitli bölgelerinde doğal olarak yetişen endemik türlerdir. Centaurea'nın birçok taksonu geleneksel olarak hem ülkemizde hem de dünyada tıbbi amaçlarla ve besinler için kullanılmaktadır. *Centaurea kurdica* Reichardt ve *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz (Asteraceae) taksonları Elazığ'daki doğal yaşam alanlarından elde edilmiştir. Bitki dokularında ağır metal akümülasyonu, antioksidan enzimler süperoksit dismutaz (SOD), glutatyon S transferaz (GST), askorbat peroksidaz (AP) ve katalaz (CAT) aktiviteleri gibi fizyolojik değişiklikler tespit edilmiştir. Çalışılan enzimlerden SOD en fazla aktiviteye sahip olan enzimdir. Yapılan çalışma sonucunda en fazla akümülasyonun genellikle yapraklarda olduğu tespit edilmiştir. Antioksidan enzim aktiviteleri: süperoksit dismutaz (SOD), glutatyon S transferaz (GST), askorbat peroksidaz (APX) ve katalaz (CAT) değerlendirildi *Centaurea kurdica*'da sırasıyla 1.315, 0.011, 0.031 ve 0.007 unit mg^{-1} olarak tespit edildi. *C. kurdica*'nın *Centaurea urvillei* DC. subsp. *hayekiana*'dan askorbat peroksidaz dışında değerlendirilen tüm enzim aktivitelerinde daha iyi olduğu belirlenmiştir.

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Anahtar Kelimeler: Antioksidan enzim, c*entaurea*, endemik, element içeriği

1. Introduction

Although a great count of clinical substances have been advenced by the pharmaceutical industry, local ethno medinal therapies are still performed in many rural field (Abbasi et al., 2010). The large number of modern drugs have been derived from medicinal herbs. Plants generate primary and secondary metabolites, which have important implementations in contemporary treatment (Agbor and Naidoo, 2015). However have beneficial influences on health or play an active role in the curing of diseases. Phytochemical compounds are not necessary for the normal functioning of the body. Phytochemical efficacy in the treatment of different diseases may be due to antioxidant impacts (Akinmoladun et al., 2007). Medicinal herbs are wealthy in dissimilar elemental substance and by means of that supply a possible connection to the therapeutical effect of the medicine (Singh and Garg, 1997). Studies have shown that extreme grades of trace elements in traditional medicines have been shown to be one of the problems often encountered in plant treatment, leading to various health disorders (Arceusz et al., 2010). Live organisms require significant amounts of macroelemets (more than 100 mg daily) to fulfill their various functions (Murray et al., 2000). Some metals like copper (Cu), zinc (Zn), nickel (Ni), manganese (Mn) and iron (Fe) are essential trace elements for many structural and biochemical events such as plant growth and electron transport. However, there are non-essential metals such as cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg), which are poisonous to plants even at low concentrations (Shahid et al., 2017; Kabata-Pendias, 2000). The oxidative stress is deal with pathogenesis of diverse disorders like neurodegenerative disorders, atherosclerosis, cancer, hypertension, aging, liver diseases, AIDS and many more (Galli et al,2005). Superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase are the basic enzymes that convert reactive oxygen species to less reactive molecules (Zhu et al., 2008). The amount of oxidative stress in a cell determines the amount of H_2O_2, superoxide and hydroxyl radicals. Hence it is very important to maintain a equalize of CAT, SOD and APX activities to push down toxical ROS levels in a cell. Compensatory mechanisms will produce by the change of the equilibrium of the scavenge enzymes. For instance, when CAT activity decreased in plants, scavenging enzymes like GPX and APX increased. Confused effects may arise, as plants lacking both APX and CAT are less susceptible to oxidative stress when as compared with plants with suppressed CAT (Rizhsky et al., 2002). *Centaurea* L. is the third great genus, with regard to species numbers after *Verbascum* and *Astragalus*in Flora of Turkey (Davis, 1975). Also *Centaurea* is one of most important genera of the family Asteraceae. The genus *Centaurea* consist of 400 and 700 species (Dittrich, 1977; Wagenitz and Hellwig, 1996; Bancheva and Greilhuber, 2006) and many of them growing in Turkey (Davis, 1988; Guner et al., 2000). Some members of the genus have been used in Anatolian folk medicine. *Centaurea* is grown on roadsides, stony calcareous cliffs, seashores, vineyards ve on maritime limestone cliffs. Traditionally, plants have been used for medicinal goal in Turkey (Tetik et al., 2013). *C. kurdica* is used to treat rheumatism and sedative diseases in traditional medicine (Cakilcioglu and

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Turkoglu, 2010; Polat et al., 2013). Due to the fact that of the wealth of the content of antioxidant molecules medical plants have been the focus of many studies. This study was planned to research the fundamental element content of two medically significant endemic plants of Elazığ, to detect antioxidant enzyme activity and to stress toxicological evaluations of existing scientific gaps. This study will supply coming suggestions for secure and influential dosages of such medicinal plants for use by indigenous people.

2. Material and Methods

2.1. Plant material and metal estimation

Fresh samples of *Centaurea kurdica* Reichardt; *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz were taken from the Elazığ, Turkey. The plants are used for direct analysis or stored at -80 ° C until future work. Dried samples of *C. kurdica* and *C. urvillei* were digested with 10 mL of concentrated HNO_3, using a Millestone Start microwave digestion system. Volume of each sample was regulated to 25 mL utilizating double deionized water after digestion (Uruc Parlak 2016). In all samples, definition of element content (Magnesium, Calcium, Arsenic, Cadmium and Lead) was made in triplicate copy with Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

2.2. Enzyme extraction and total soluble protein estimation

Fresh samples texture (0.5 g) was homogenized with 5 mL of 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 15.000 g for 15 min at 4 °C (Hou et al., 2007). The supernatant liquid was utilized for enzyme assignation. Sum solvable protein ingredients of the enzyme extracts were evaluated in accordance with Bradford (1976) .

2.3. Superoxide dismutase (SOD)

Superoxide dismutase activity was analyzed by quantification the inhibition of photochemical nitroblue tetrazolium (NBT) degradation according to Beauchamp and Fridovich's method (1971). The analysis mixture included 20mM phosphate buffer, 10mM methionine, 0.1mM p-nitro blue tetrazolium chloride (NBT), 0.1mM EDTA, 0.005mM riboflavin and enzyme extract.

2.4.Catalase (CAT)

Catalase activity was determined spectrophotometrically at 240 nm with respect to the method of Aebi (1984). The reaction was begined by the adding of H_2O_2 and the reduce in the absorbance of H_2O_2 was registered at 240 nm for 3 min.

2.5. Glutathione S-transferase (GST)

Connection of GSH to CDNB catalysed by GST excited an increment in absorbance at 340 nm that was utilized to analysis GST activity as defined by Drotar et al. (1985). The enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient of NADPH ($6.2mM^{-1}$ cm^{-1} at 340 nm).

2.6. Ascorbate peroxidase (APX)

Ascorbate peroxidase activity was designated by the reduce in absorption of ascorbate at 290 nm, with respect to Nakano and Asada's method (1981). APX activity was calculated by using the extinction coefficient $2.8mM^{-1}$ cm^{-1}.

2.7. Guaiacol peroxidase

Guaiacol peroksidase activity, initiated by the addition of the enzyme, was determined by measuring the absorbance alteration at 470 nm with respect to Baycu et al. (2006).

2.8. Statistical Analysis

The whole work was performed in three replications. The values expressed in the figures show the mean values ± standard error (SE) for the enzyme activity. One-way analysis of variance (ANOVA) was performed to verify the variability of the dataand the validity of the results, and the Tukey test for significant differences between plant organs was determined.

3. Results

3.1. Metal accumulation

Living organisms are need a significant amount of macro elements in order to fulfill diversified functions (Murray et al., 2000). Although trace elements have definite important functions in alive organisms, they exhibit toxic effect after they surpass internationally allowable levels. The quantities of elements in *C. kurdica* and *C. urvillei* are briefly described as below (Table 1 and 2). The highest concentrations of calcium (Ca) were beholded in the leaves of *C. kurdica* (202.686 $mgkg^{-1}$) and leaves of *C. urvillei* (198.946 $mgkg^{-1}$). The content of magnesium (Mg) in *C. kurdica* and *C. urvillei* ranges from 2.236 $mgkg^{-1}$ to 21.780 $mgkg^{-1}$. The amount of Arsenic (As) was highest in leaves of *C. kurdica* (2.012 $mgkg^{-1}$), followed by root of *C. kurdica* (1.168 $mgkg^{-1}$). In the plants in this study, concentrations of cadmium (Cd) ranged from 0.031 $mgkg^{-1}$ (stem of *C. kurdica*) to 0.431 $mgkg^{-1}$ (stem of*C. urvillei*). Showing a high level of lead (Pb) element include stem of *C. kurdica* (10.31 $mgkg^{-1}$).

Table 1. The accumulation of elements in *C. kurdica.*

	The accumulation of elements ($mgkg^{-1}$ DW)				
	Mg	**Ca**	**As**	**Cd**	**Pb**
root	4,906±0,34	63,080±4,87	1,168±0,08	0,313±0,03	2,902±0,40
stem	2,236±0,04	35,040±1,81	0,317±0,02	0,031±0,06	10,31±0,23
leaf	21,780±0,81	202,686±21,13	2,012±0,19	0,404±0,03	2,174±0,23
soil	1,032±0,06	7,186±0,49	10,410±0,03	0,21±0,01	34,276±0,65

All worths are average ± standard deflection (n = 3).

Table 2. The accumulation of elements in *C. urvillei.*

	The accumulation of elements ($mgkg^{-1}$ DW)				
	Mg	**Ca**	**As**	**Cd**	**Pb**
root	8,114±0,10	76,473±2,48	0,336±0,05	0,274±0,06	0,288±0,16
stem	9,014±0,33	90,486±3,17	0,118±0,07	0,431±0,05	1,851±0,10
leaf	12,321±0,83	198,946±15,63	0,250±0,05	0,404±0,04	1,813±0,31
soil	11,547±0,47	213,516±13,23	8,743±0,04	2,026±0,05	34,363±0,28

3.2. Total Soluble Proteins and Antioxidant Enzymes Activity

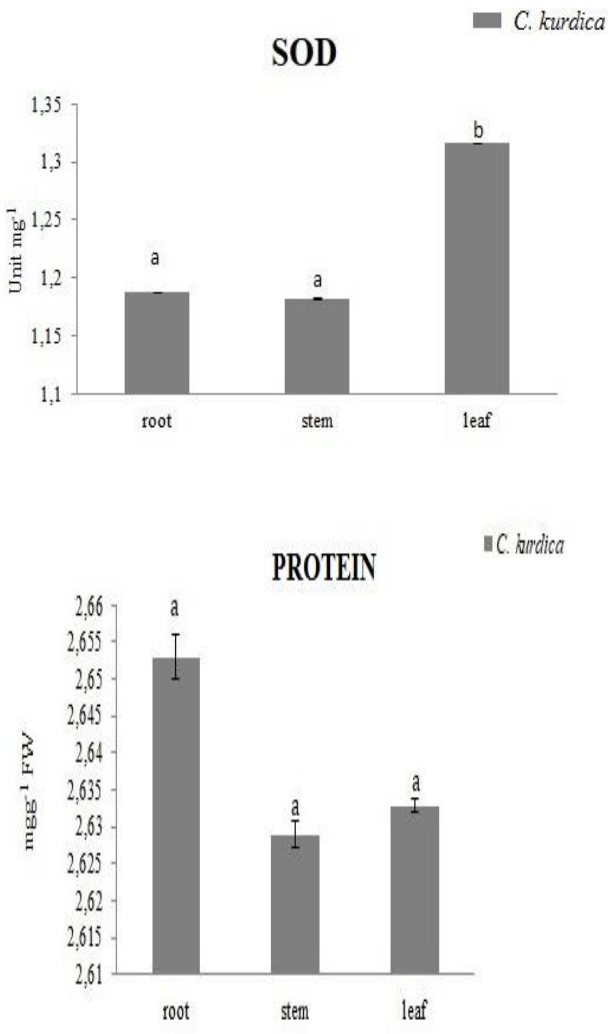

Figure 1a-b: The amount of protein in *C. kurdica* and *C. urvillei.*

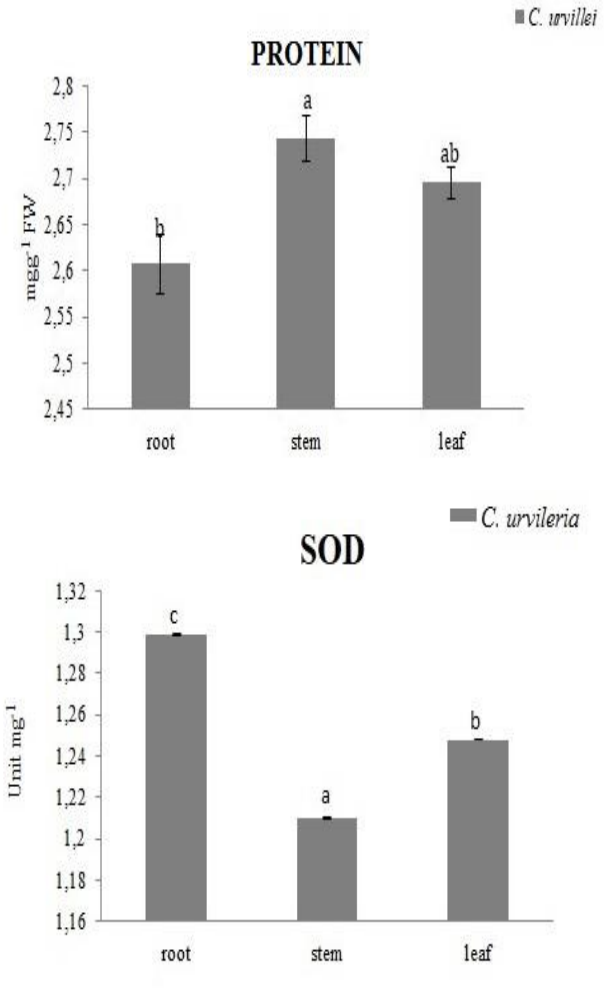

Figure 2a-b: Specific activity of SOD enzymes in *C. kurdica* and *C. urvillei.*

Cellular membranes and organelles can be protected only by the antioxidant enzymes, non-enzymatic antioxidants and secondary metabolites from the damaging effects of active oxygen species. It was observed that proteine amount was generally high in the stem, leaf and root of the plants (Figure 1a, 1b). The antioxidant enzymes appraised in the plants are presented in figures. At least SOD activity (Figure 2a) was assessed in stem of *C. kurdica* (1.181± 0.000 unit mg^{-1}), while SOD activity in root of *C. urvillei* (1.298± 0.000 unit mg^{-1}) was found to be significantly higher (Figure 2b).

Catalase activity was also recognized to be considerably higher ($P < 0.05$) in leaf of *C. kurdica* (0.007± 0.000 unit mg^{-1}) followed by leaf of *C. urvillei* (0.006 ± 0.000 unit mg^{-1}) (Figure 3a and 3b). It was observed that GST activity was generally high in the stem and leaf of the treated plants (Figure 4a and 4b). APX activity (Figure 5a and 5b) was also found to significantly higher ($P < 0.05$) in leaf of *C. urvillei* (0.076± 0.009 unit mg^{-1}) and lowest activity was observed to be in stem of *C. kurdica* (0,011± 0.000 unit mg-1). GPOX

(Figure 6a and 6b) was also found to significantly higher ($P < 0.05$) in leaf

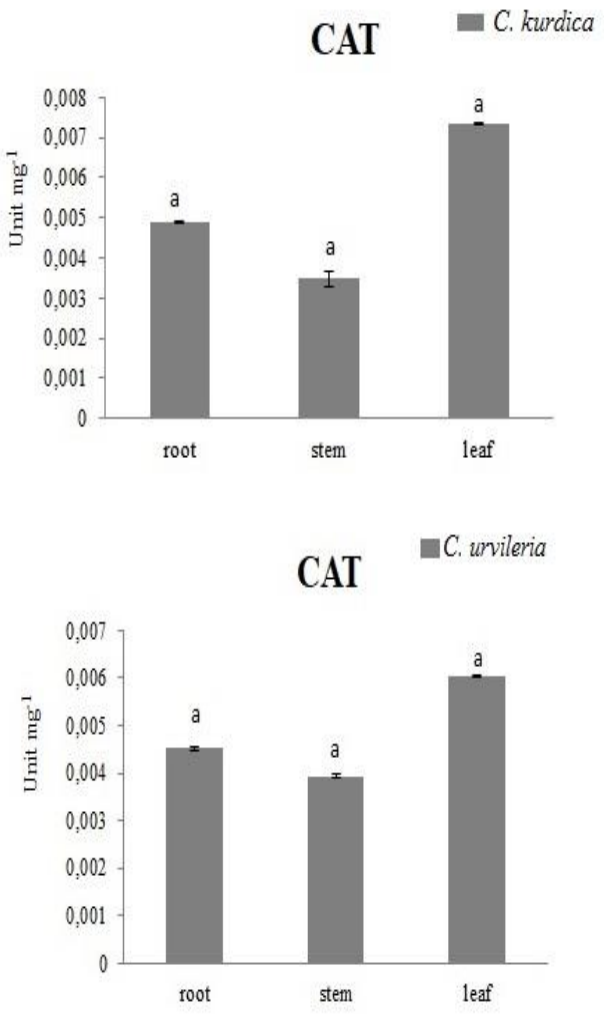

Figure 3a-b: Specific activity of CAT enzymes in *C. kurdica* and *C. urvillei*

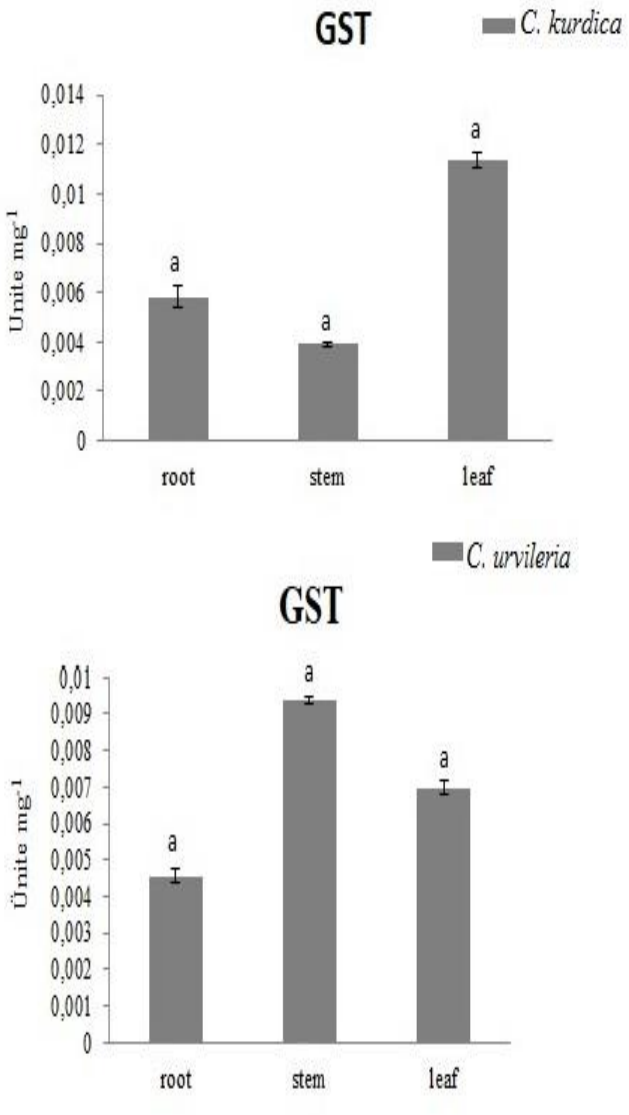

Figure 4a-b: Specific activity of GST enzymes in *C. kurdica* and *C. urvillei.*

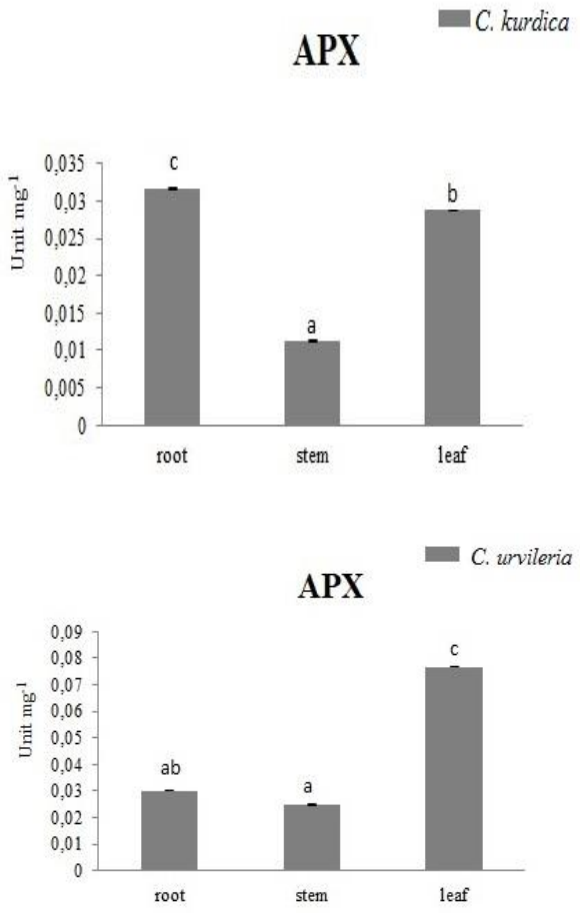

Figure 5a-b: Specific activity of APX enzymes in *C. kurdica* and *C. urvillei.*

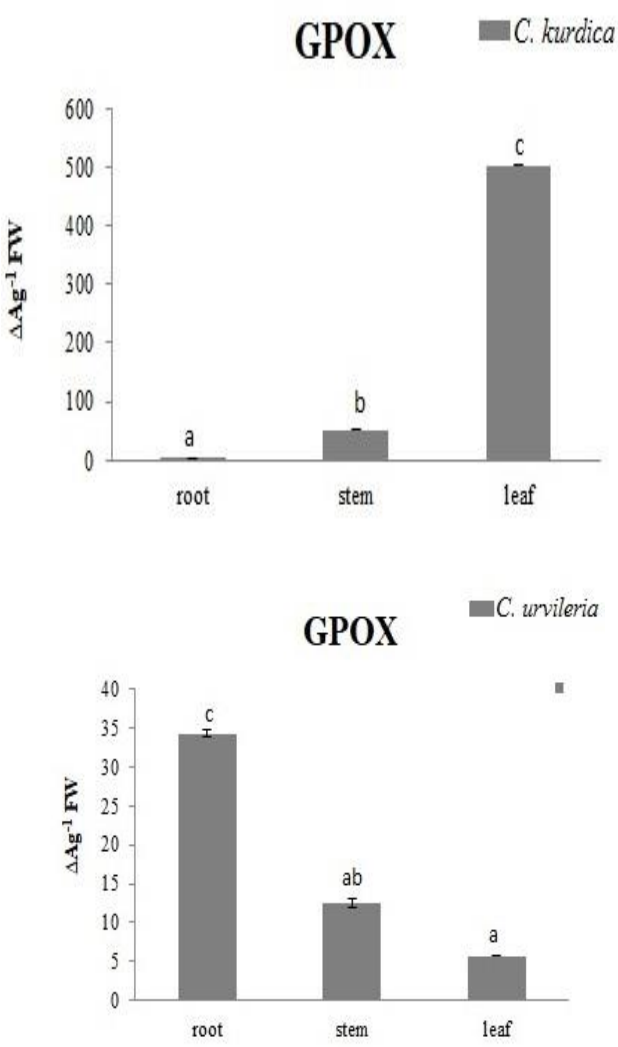

Figure 6a-b: Specific activity of GPOX enzymes in *C. kurdica* and *C. urvillei*

of *C. kurdica* (503.195±0.457 ΔAg^{-1}) and lowest activity was observed to be in leaf of *C. kurdica*(2,822 ± 0.001 ΔAg^{-1}).

4. Discussion

Different amounts of nutrients in plants are due to soil characteristics, soil pH, plant mineral transport mechanisms, developmental state of the plant, minerals uptake capacity of plant organs and genetic factors. Medical plants are affluent in divergent element contents and so supplies a probable connection to the therapeutic impact of the drug (Singh and Garg, 1997). Plants have the

capability of high uptake of various elemental substance of the soil in reaction to concentration gradients (Huang and Cunningham, 1996). Calcium (Ca) tide overs the troubles of high blood compression, premenstrual dysphoric disorder, heart attack, weak bones, column cancer, and threats of osteoporosis in elderliness (Murray et al., 2000). In a study Mihoc et al., (2012) reported the Ca concentration of *C. sativa* is higher than 9547 $mgkg^{-1}$, but in different research the concentration of Ca determined in *J. adhatoda* was 15,707 $mgkg^{-1}$ (Hossen et al., 2014). Magnesium (Mg) is necessaried for innumerable vital body functions like formation, accurate growing and function of bones and muscles, at the same time that is an effective ingredient of diverse enzyme systems (WHO, 2009). Lead (Pb) is one of the important heavy metals of the everytime and has obtained notable significance as a strong environmental contaminant. Pb is non-essential trace element for the development of organisms. Excessive Pb intake is quite detrimental to the human body. It contains *S. surattene* 52 $mgkg^{-1}$ and *S. marianum* 54 $mgkg^{-1}$ Pb concerning to *Asteraceae* and *Solanaceae* families according to Chizzola et al., (2003). Leaves according to fruits and seeds contain Pb in higher concentrations. For example Varalakshmi and Ganeshamurthy determined leafy vegetables accumulated maximum levels of Pb (Varalakshmi and Ganeshamurthy 2012). Cadmium (Cd) is a toxic element for a human body, animals or plants. Since Cd is easily dispersed and inhibits antioxidant enzymes, even low levels of Cd are anxiety. This may lead to increased oxidative stress due to membrane damage and enzyme loss (Soetan et al., 2010). Arsenic (As) is an ecological poison that is found inherently in whole soils. The metalloid participate in agriculture systems thanks to a variety of means that contain natural geochemical processes, use of pesticides, mining, irrigation and fertilization. As can seriously inhibit plant growth and biomass accumulation, as well as compromising plant reproductive capability (Garg and Singla 2011). Plants have been used since the earliest times in the treatment of diseases. Many of these medicinal plants have been used in the treatment of various diseases due to their antioxidant and antiinflammatory activity. Reactive Oxygen Species (ROS) or free radicals are undecided intermediates shaped from molecules via the fracture of a chemical bond such that each piece holds one electron, via cleavage of a radical to give another radical and, also via redox reactions (Pham et al., 2008). Free radicals and oxidative stress are seen in many diseases such as aging, asthma, cancer, diabetes and arthritis. Free radicals are also generated either from normal main metabolic processes in the human body or from outside resources likeheat, cold, heavy metals, herbicides and drought (Lobo et al.,2010). Scientific search indicates that ROS can increase the oxidative level through loss of cellular structure and function so they are detrimental to the cell (Lee et al., 2007). The free radicals can be cleaned by using the antioxidant system including non-enzymatic compenents and a series of antioxidant enzymes which of antioxidants are substances that can prevent or reduce oxidative damage even at low concentrations. Antioxidants can be given as nourishment reinforcements to arrange such conditions (Halliwell, 2002; Gutteridge and Halliwell, 2000). Studies have shown that antioxidants in human diets can significantly inhibit the damaging effects of reactive nitrogen species and reactive oxygen species on the human body, which cause oxidative stress (Soong and Barlow, 2004). Organisms develop antioxidant defensive system to hinder negative effects of oxidative stress on the cells (Briat, 2002). Antioxidative enzymes like glutathione reductase, superoxide dismutase, ascorbate peroxidase and catalase function as ROS detoxification agents in cells. The basic content of the

antioxidative defending system in plants, SOD converts toxical (poisoned) superoxide anion radicals into smaller toxic hydrogen peroxides (Vangronsveld and Clijsters, 1994; Shalini and Dubey, 2003). Catalase, which is required for detoxification of high ROS levels, is a tetrahedric protein consisting of four heme groups that catalyze hydrogen peroxide exchange (Scandalios, 1987; Willekens et al., 1997). In this studied medicinal plants *C. kurdica* showed higher catalase activity according to other plant, it maintains plant from biotic and abiotic stress. The antioxidative capability of the cells can be assessed by gauging APX activity. Hydrogen peroxide produced by superoxide dismutase can be scavenge using ascorbate by APX (Noctor and Foyer, 1998). Compared with catalase, APX has a high affinity for H_2O_2, permitting for the scavenging of minor quantity of H_2O_2 in more specific places (Radic etal.,2010; Asada, 1992). It is thought that glutathione, which is regenerated by GST activity from GST, is a crucial signaling molecule that functions between environmental stress and adaptable reactions. (Navari-Izzo et al.,1997). Peroxidases such as GPOX are relate to their corresponding oxidative reactions. Because of metal stress, there is detected increment in peroxides and free radicals in plant cells. This ROS is exterminated by the induction of specific enzymes for example GPOX, which are utilised as susceptable biomarkers of biotic and abiotic stresses in plants (Yürekli and Porgalı, 2006; Gill and Tuteja, 2010).

5. Conclusion

The present study show that *C. kurdica* Reichardt and *C. urvillei* DC. subsp. *hayekiana* have free radical scavenging activities and the variation in their antioxidant enzymes activities can be attributed to seconder metabolite. The amount of Ca, Mg and Pb determined on vegetation and in addition to this, it is thought that people could benefit from *C. kurdica* Reichardt and *C. urvillei* DC. subsp. *hayekiana* treatment of diseases related to oxidative stres taken into consideration enzyme activities like SOD, CAT and APX. Observations suggest that *C. kurdica* Reichardt and *C. urvillei* DC. subsp. *hayekiana*may be useful in the treatment of oxidative stress-related diseases.

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