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Short Communication

Biochemical properties of some medicinal plants used as dye plants

[Propiedades bioquímicas de algunas plantas medicinales utilizadas como plantas colorantes]

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Abstract: The biochemical contents of plants may vary depending on soil and climatic conditions. Under unsuitable conditions, the resistance mechanism of plants is determined by the biochemical properties of the plants. For this reason, it is of great importance to determine the biochemical contents of some plants that are naturally grown and generally used as dye plants. For this purpose, *Salix alba* L., *Verbascum thapsus* L., *Urtica dioica* L., *Digitalis lanata* Ehrh, *Galium aparine* L., *Lythrum salicaria* L., *Hypericum perforatum* L., *Hypericum scabrum* L., *Quercus robur* L. subsp. *robur*, *Glycyrrhiza glabra* L. plants were collected. Antioxidant enzyme activity, hormone, organic acid, total phenolic substance and some other biochemical analyzes were made in these plant samples. As a result of the study, depending on the plant varieties, total antioxidant, total phenolic content and organic acid levels showed significant differences. According to the results of this study, *Verbascum thapsus* L. plant had high antioxidant enzyme activity, while *Digitalis lanata* Ehrh. plant was found to be higher in terms of hormone and total organic acid content. The highest amount of carotenoid, abscisic acid and hydrogen peroxide were determined in Urtica dioica L. plant. Different results were obtained in other plant varieties, and it was observed that a different biochemical content was more effective in each plant. For this reason, it has been determined that the biochemical contents of these plants, which have different uses. The usage area of this plant should be created after the main active ingredient is determined.

Keywords: Dye plants; Organic acid; Phenolic contents; Antioxidant enzyme; Medicinal plants.

Resumen: El contenido bioquímico de las plantas puede variar según el suelo y las condiciones climáticas. En condiciones inadecuadas, el mecanismo de resistencia de las plantas está determinado por las propiedades bioquímicas de las plantas. Por esta razón, es de gran importancia determinar el contenido bioquímico de algunas plantas que se cultivan de forma natural y se utilizan generalmente como plantas colorantes. Con este fin, *Salix alba* L., *Verbascum thapsus* L., *Urtica dioica* L., *Digitalis lanata* Ehrh, *Galium aparine* L., *Lythrum salicaria* L., *Hypericum perforatum* L., *Hypericum scabrum* L., *Quercus robur* L. subsp. *robur*, *Glycyrrhiza glabra* L., fueron recolectadas. En estas muestras de plantas se realizaron análisis de actividad enzimática antioxidante, hormona, ácido orgánico, sustancia fenólica total y algunos otros análisis bioquímicos. Como resultado del estudio, dependiendo de las variedades de plantas, el el contenido fenólico total, antioxidante total, y los niveles de ácidos orgánicos mostraron diferencias significativas. Según los resultados de este estudio, la planta *Verbascum thapsus* L. tuvo una alta actividad enzimática antioxidante, mientras que la planta *Digitalis lanata* Ehrh. se encontró que era más alta en términos de contenido hormonal y ácido orgánico total. La mayor cantidad de carotenoides, ácido abscísico y peróxido de hidrógeno se determinó en la planta *Urtica dioica* L. Se obtuvieron resultados diferentes en otras variedades vegetales, y se observó que el contenido bioquímico diferente resultó más efectivo en cada planta. Por este motivo, se ha determinado que los contenidos bioquímicos de estas plantas, que tienen diferentes usos. El área de uso de esta planta debe crearse después de determinar el ingrediente activo principal.

Palabras clave: Plantas de tinte; Ácido orgánico; Contenido fenólico; Enzima antioxidante; Plantas medicinales.

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INTRODUCTION

Natural dyes are derived from natural sources such as animals, plants and minerals, but the main source of natural dyes is plants. Natural dyes have been used for years for coloring food substrate, leather and natural protein fibers such as wool, silk and cotton (Samanta & Agarwal, 2009; Dias *et al*., 2013). Natural dyes are generally preferred because they are environmentally friendly, anti-allergic, biodegradable and less toxic than synthetic dyes (Ali *et al*., 2007; Muthu, 2014). These color pigments in the plant are used instead of synthetic dyes due to their health benefits (Saxena & Raja, 2014). As a result of knowing the phytochemical properties of plants, the contents of bioactive substances in the plant can be determined. Some of these secondary metabolites to be determined in the plant are flavonoids, saponins and antioxidants (Alapati *et al*., 2015). Natural dyestuffs obtained from plants contain bioactive compounds with antimicrobial, analgesic and anti-inflammatory properties. In a number of studies, the properties of dye plants were tried to be examined (Radhia *et al*., 2018). However, some biochemical contents such as antioxidant and phenolic substance contents were not investigated. It is of great importance to know the biochemical contents of the plant from which it is taken, in order to prevent microbial infection of natural dyestuffs to be obtained from plants, reduce bad odor and maintain good hygienic condition of textile (Gökhale *et al*., 2004; Santhosh & Vaithiyanathan, 2018).

The incompatibility between antioxidants and free radicals causes oxidative stress that leads to cellular damage. This oxidative damage, which occurs under adverse conditions, can lead to many diseases such as cancer and neurodegenerative disorders (Nooman *et al*., 2008; Li *et al*., 2013). In this study, the antioxidant contents, some biochemical properties and enzyme activities of some dye plants were examined and their potential properties were tried to be determined.

MATERIALS AND METHODS

Materials

Plants used in this study included some dye plant species *Salix alba* L., *Verbascum thapsus* L., *Urtica dioica* L., *Digitalis lanata* Ehrh, *Galium aparine* L., *Lythrum salicaria* L., *Hypericum perforatum* L., *Hypericum scabrum L., Quercus robur* L. subsp. *robur, Glycyrrhiza glabra* L. to be analyzed the leaves of the plants were separated. And then, the samples were weighed and stored at -20°C for biochemical analyzes.

Antioxidant enzymes analysis

To determine the antioxidant content of the plant samples, the plant samples were homogenized with phosphate buffer. Frozen cell samples were pulverized with liquid nitrogen and then treated with phosphate buffer containing EDTA+PMSF+PVP mixture. Then, the amount of POD, CAT, SOD and AxPOD in the plant was determined by measuring with spectrophotometer (Li *et al*., 2015).

Hydrogen peroxide and lipid peroxidation concentration

The amount of hydrogen peroxide in the plant was measured according to the method specified by Loreto & Velikova (2001). For this purpose, plant samples were first homogenized in trichloroacetic acid. Then, the supernatant taken from the homogenized sample was measured in a spectrophotometer at a wavelength of 532 nm (Du *et al*., 2010).

Organic Acid

In plant samples, for the determination of organic acid, the plant samples were homogenized in distilled water and then centrifuged. The solutions formed as a result of centrifugation were filtered and read in HPLC, and the amount of organic acid in the plant was determined.

Hormone

Amount of hormone in plant samples Kuraishi et al. (1991). It was made according to Battal & Tilekliоğlu (2001). After the fresh plant samples were extracted with methanol, they were homogenized. Homogenized samples were incubated in the dark for 24 hours and then filtered through 0.45 m mesh filters. Afterwards, the extract prepared after some processing was analyzed using HPC.

RESULTS

Hormone contents

In dye plants, changes in gibberellic acid (GA), salicylic acid (SA), indole acetic acid (IAA) and abscisic acid (ABA)

depending on plant varieties were investigated. When the hormone contents of dye plants with different characteristics were examined, the hormone contents of the plants were statistically different depending on the cultivars. In dye plants, GA 352.78-647.91 ng μl^{-1}; SA 26.75-65.88 ng μl^{-1}; IAA 11.38-24.82 ng μl^{-1} and ABA hormone varied between 0.32-0.48 ng μl^{-1} (Table No. 1A & 1B). When the dye plants were examined as a variety, the highest GA amount was obtained in *Galium aparine* L. plant, while the lowest GA amount was determined in *Hypericum perforatum* L. plant. The highest amounts of SA and IAA were obtained from *Digitalis lanata* Ehrh. plant. However, the lowest amount of SA was determined in *Galium aparine* L. plant, and the lowest amount of IAA was determined in *Salix alba* L. plant. The highest amount of ABA was in *Urtica dioica* L. and *Quercus robur* L. subsp. *robur* plants; the lowest was determined in *Glycyrrhiza glabra* L. plant.

Table No. 1A
Hormon contents and antioxidan ezyme activity of dye plants

Species	GA	SA	IAA	ABA	CAT
	ng μl^{-1}				EU gr $leaf^{-1}$
***Salix alba* L.**	587.79^{b}	38.64^{c}	11.38^{d}	0.36^{d}	918.53^{e}
***Verbascum thapsus* L.**	567.42^{b}	48.45^{b}	14.31^{c}	0.40^{b}	1888.76^{a}
***Urtica dioica* L.**	400.70^{e}	30.39^{d}	15.24^{c}	0.48^{a}	516.18^{f}
***Digitalis lanata* Ehrh.**	463.63^{d}	65.88^{a}	24.82^{a}	0.41^{b}	1131.72^{d}
***Galium aparine* L.**	647.91^{a}	26.75^{e}	22.01^{a}	0.37^{cd}	1394.39^{c}
***Lythrum salicaria* L.**	639.76^{a}	34.10^{c}	17.46^{b}	0.33^{e}	1244.18^{cd}
***Hypericum perforatum* L.**	352.78^{f}	44.51^{b}	23.78^{a}	0.38^{c}	1361.27^{c}
***Hypericum scabrum* L.**	452.63^{d}	31.95^{d}	11.95^{d}	0.36^{d}	655.67^{f}
Quercus robur* L. subsp. *robur	511.86^{c}	31.45^{d}	13.47^{c}	0.48^{a}	1386.37^{c}
***Glycyrrhiza glabra* L.**	458.65^{d}	46.52^{b}	18.77^{b}	0.32^{e}	1414.65^{b}

GA: Giberallic acid, SA: salicyclic acid, IAA: indole acetic acid, ABA: absisic acid, CAT: catalase

Table No. 1B

Species	POD	SOD	AxPOD	MDA	H_2O_2
	ng μl^{-1}			nmol g^{-1} fw	EU gr $leaf^{-1}$
***Salix alba* L.**	201.71^{d}	39.96^{d}	25.89^{f}	55.71^{e}	3.52^{d}
***Verbascum thapsus* L.**	447.07^{a}	40.85^{d}	43.21^{a}	128.61^{a}	2.61^{e}
***Urtica dioica* L.**	87.00^{f}	75.21^{a}	17.39^{g}	40.45^{f}	9.90^{a}
***Digitalis lanata* Ehrh.**	280.86^{cd}	35.31^{e}	30.76^{e}	73.68^{d}	2.79^{e}
***Galium aparine* L.**	391.07^{b}	31.20^{f}	36.55^{d}	97.44^{b}	2.21^{f}
***Lythrum salicaria* L.**	230.59^{d}	52.32^{b}	30.61^{e}	73.54^{d}	4.16^{c}
***Hypericum perforatum* L.**	303.10^{c}	34.76^{e}	31.71^{e}	89.47^{bc}	2.35^{ef}
***Hypericum scabrum* L.**	95.15^{f}	53.61^{b}	17.35^{g}	33.64^{g}	5.30^{b}
Quercus robur* L. subsp. *robur	164.95^{e}	49.41^{c}	41.01^{b}	84.29^{c}	2.37^{ef}
***Glycyrrhiza glabra* L.**	351.07^{b}	44.14^{cd}	38.45^{c}	92.10^{b}	3.49^{d}

POD: peroxidase, AxPOD: ascorbate peroxidase, SOD: superoksid dismutase, MDA: malondialdehyte, H_2O_2: hydrogen peroxide

Antioxidant enzyme activity

POD, CAT, SOD, AxPOD, MDA and H_2O_2 contents of some dye plants were examined (Table No. 1). Statistically examined parameters were found to be significant. The highest CAT, POD, AxPOD and MDA were measured from *Verbascum thapsus L.* (1888.76; 447.07; 43.21 EU g $leaf^{-1}$, 128.61 nmol g^{-1} fw, respectively). The highest SOD enzyme activity and H_2O_2 were determined from *Urtica dioica* L. (75.21 EU g $leaf^{-1}$ and 9.90 mikromol g^{-1} fw, respectively). Although the highest SOD enzyme activity was measured in Urtica dioica L. plant, CAT and POD enzyme activity were measured at a lower level than the other examined plants. The lowest amount of SOD and H_2O_2 was determined in *Galium aparine* L. plant, and the lowest amount of AxPOD and MDA in *Hypericum scabrum* L. plant.

Organic acid contents

As a result of the analysis carried out to determine the amount of organic acid contained in some dye plants, significant differences were determined between the species (Table No. 2). The highest propionic, tartaric, malic and succinic acid were determined in the *Salix alba* L. (8.52, 14.30, 21.42 and 82.62 ng mL^{-1} respectively). The highest malonic, lactic and fumaric acid were determined in the *Glycyrrhiza glabra* L. (55.42, 29.25 and 26.55 ng mL^{-1} respectively). The highest oxalic acid was determined in the *Galium aparine* L. and *Hypericum perforatum* L. plants (8215.47 ng mL^{-1}). When other organic acids were evaluated, butyric acid was measured most in *Lythrum salicaria* L. plant, citric acid in *Hypericum perforatum* L. plant, maleic acid in *Hypericum scabrum* L. plant. Due to the different organic acid levels in the dye plants, the total organic acid amounts varied. The highest organic acid content obtained from the *Digitalis lanata* Ehrh. plant (Figure No. 1). The lowest amount of organic acid was obtained from *Galium aparine* L. plant.

Table No. 2A
Organic acid content of dye plants

Species	Oxalic	Propionic	Tartaric	Butyric	Malonic
	ng μl^{-1}				
***Salix alba* L.**	15.50^{a}	8.52^{a}	14.30^{a}	18.05^{d}	23.80^{g}
***Verbascum thapsus* L.**	8.68^{e}	4.89^{d}	11.07^{b}	22.35^{c}	45.69^{b}
***Urtica dioica* L.**	11.55^{c}	6.94^{b}	8.85^{d}	18.39^{d}	33.63^{e}
***Digitalis lanata* Ehrh.**	13.10^{b}	3.31^{e}	11.89^{b}	25.02^{b}	42.27^{c}
***Galium aparine* L.**	15.47^{a}	5.29^{c}	7.29^{d}	18.02^{d}	31.65^{ef}
***Lythrum salicaria* L.**	10.53^{d}	1.81^{g}	10.51^{c}	27.09^{a}	34.15^{e}
***Hypericum perforatum* L.**	15.47^{a}	4.01^{d}	10.75^{c}	23.25^{c}	29.56^{f}
***Hypericum scabrum* L.**	10.38^{d}	2.26^{f}	7.19^{d}	22.82^{c}	37.34^{d}
Quercus robur* L. subsp. *robur	11.85^{c}	2.07^{f}	8.02^{d}	22.82^{c}	28.76^{f}
***Glycyrrhiza glabra* L.**	10.02^{d}	1.67^{g}	7.65^{d}	23.36^{c}	55.24^{a}

Table No. 2B

Species	Malic	Lactic	Sitric	Maleic	Fumaric	Succinic
	ng μl^{-1}					
***Salix alba* L.**	21.42^{a}	11.90^{e}	35.81^{c}	20.26^{c}	9.79^{g}	82.62^{a}
***Verbascum thapsus* L.**	8.39^{f}	23.25^{c}	28.17^{d}	26.04^{b}	13.05^{de}	49.43^{f}
***Urtica dioica* L.**	14.58^{c}	14.35^{d}	32.60^{c}	25.96^{b}	12.53^{e}	55.24^{e}
***Digitalis lanata* Ehrh.**	13.03^{d}	14.11^{d}	75.19^{a}	17.19^{d}	14.69^{d}	72.24^{c}
***Galium aparine* L.**	10.36^{e}	8.65^{f}	50.43^{b}	14.63^{e}	17.65^{c}	50.19^{f}
***Lythrum salicaria* L.**	12.63^{d}	15.01^{d}	45.80^{b}	22.45^{bc}	21.52^{b}	66.39^{d}
***Hypericum perforatum* L.**	11.25^{e}	14.39^{d}	72.70^{a}	15.48^{de}	11.03^{f}	78.54^{b}
***Hypericum scabrum* L.**	14.01^{c}	26.79^{b}	26.39^{d}	33.31^{a}	11.03^{f}	52.11^{ef}
Quercus robur* L. subsp. *robur	17.71^{b}	23.47^{c}	49.41^{b}	14.52^{e}	25.42^{a}	47.58^{f}
***Glycyrrhiza glabra* L.**	16.52^{b}	29.25^{a}	46.06^{b}	16.50^{d}	26.55^{a}	40.16^{g}

DISCUSSIONS

Reactive oxygen species accumulate in plants under different stress conditions. In order to eliminate the negative effects of these reactive oxygen species, antioxidant enzymes such as catalase, peroxidase, ascorbate peroxidase and superoxide dismutase are secreted by the plant. Depending on this increased enzyme activity in the plant, the level of resistance to oxidative damage increases (Verma & Dubey, 2003; Lee *et al.*, 2007). SOD protects plants from the toxicity of reactive oxygen species. In order to reduce the level of toxic effect, the toxic superoxide radical is converted to the less toxic hydrogen peroxide by the SOD enzyme (Vangronsveld & Clijsters, 1994). The ascorbate peroxidase enzyme, on the other hand, increases the plant resistance mechanism by eliminating the harmful effects of hydrogen peroxide produced by the SOD enzyme on the plant (Noctor & Foyer, 1998).

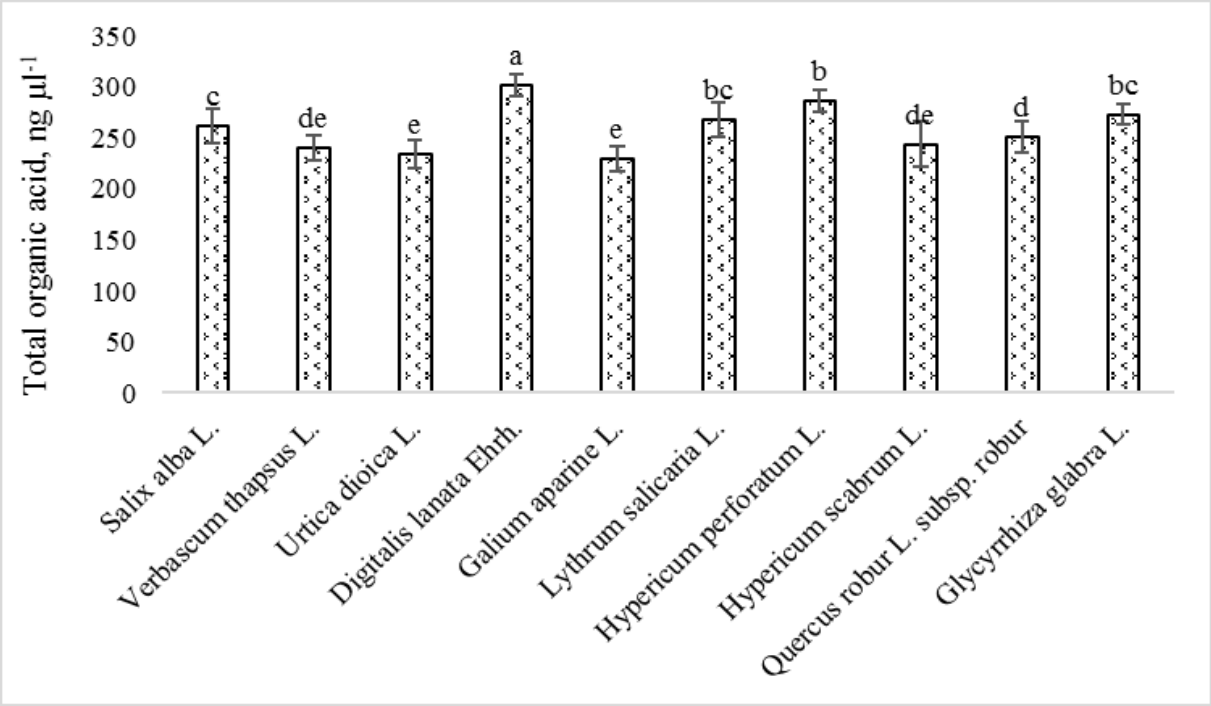

Figure No. 1
Total organic acid contents of dye plants

CONCLUSIONS

The antioxidant and organic acid amounts of some plant species commonly used as dye plants showed significant differences depending on the species. When the purpose of use and areas of use are determined according to the contents of such plants, the benefit rate to be obtained from these plants will increase. According to the results of this study, *Verbascum thapsus* L. plant had high antioxidant enzyme activity, while *Digitalis lanata* Ehrh. plant was found to be higher in terms of hormone and total organic acid content. In other plant groups, it is estimated that there may be an increase in biochemical contents in terms of different characteristics, and the biochemical contents of plants may vary according to climate, soil and growing conditions. For this reason, it was concluded that it would be appropriate to determine the biochemical contents of such plants before use.

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