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Antioxidant and healing properties of the extract *Glycyrrhiza glabra* L.

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Abstract: The antioxidant properties of licorice extract were studied on the leaves of common wheat (*Triticum aestivum* L.) seedlings exposed to toxic concentrations of Zn^{2+} in order to create an increased content of reactive oxygen molecules in the cell and chloroplasts. Based on changes in the characteristics of recombination of millisecond delayed fluorescence of chlorophyll *a* (ms DF Chl *a*) and the pigment pool, blocking of electron transfer reactions into the electron transport chain of photosystem II, to a greater extent, on its donor side, and suppression of the accumulation of photosynthetic pigments were shown. The highest resistance of Chl *a* 680 to this stress was shown. During the incubation of stressed seedlings in *Glycyrrhiza glabra* L. extract, restorati on of electron transfer (P680*–Q_A-Q_B) in the photosystem II chain and the absorption spectra of light harvesting antenna pigments was observed. The antioxidant activity of the extract was confirmed by the reaction of the stable DPPH radical. The pharmacological effect of the bioactive composition "Glysotrical" based on the extract of *Glycyrrhiza* was expressed in the regeneration of the skin of rabbits in case of chemical and thermal damage. Our results suggest that the protective effect of the *Glycyrrhiza* Tourn. ex. L. extract is due to its rich phytochemical composition, the main components of which are flavonoids and triterpene saponins, which prevent the development of a free radical state that protects the cell from damage. Possibly, being membrane-active compounds, saponins correct the lipid component of the damaged membrane.

Keywords: *effects, extracts, Glycyrrhiza glabra, antioxidant, electron transport, PS II, pigments, Triticum aestivum, zinc*

INTRODUCTION

It is now known that plants are one of the main sources of biologically active compounds, which also include antioxidants. The deteriorating ecological situation makes us think about the protection of valuable sources of natural antioxidants. Much attention is paid to medicinal plants from which herbal medicines are obtained for the treatment of a wide range of diseases [Kezetas et al., 2011; Kurkin, 2013; Jafarova, 2019]. With the advent of new technologies, scientists are conducting more in-depth research on well-known and widely used medicinal plants in order to identify new possibilities for their use. One of the valuable medicinal plants is licorice (*G. glabra* L.) [Pastorino et al., 2018; Sharifi-Rad et al., 2021; Ageeva et al., 2022]. *G. glabra* is a plant belonging to the legume family.Its main phytochemical compounds are flavonoids and triterpene glycyrrhiza and glycyrrhizic acid, which are actively studied for biological and pharmacological activity [Hasan et al., 2021]. However, negative environmental factors affect the physiological and biochemical functions of the plant. Reactive oxygen species accumulate in the plant cell, causing oxidative stress and damage to the membrane system, in particular chloroplasts [Järvi et al., 2015; Rantala et al., 2021; Zhang et al., 2019]. As a result of these damages, the activity of ion transport through the membranes of mitochondria and chloroplasts is disrupted, which disrupts energy metabolism and photosynthesis. To protect against reactive molecules, their rapid detoxification is necessary. Otherwise, the generation of reactive molecules increases and can lead to plant death [Foyer, Noctor, 2005; Foyer, 2018]. In plants, defense systems that counteract the development of oxidative stress are synthesized or activated. These are low molecular compounds, stress proteins, antioxidant enzymes, and other protective compounds that regulate the redox potential and ensure plant resistance to negative factors [Ganiyeva et al., 2009; Scandalios, 2005]. The purpose of this work was to determine the antioxidant and medicinal properties of the *G. glabra* extract.

There are about 21 species of the genus *Glycyrrhiza* in the world, 6 of which grow in the steppe and mountainous territories of the Republic of Azerbaijan and differ in their morphological and phytochemical parameters (Fig. 1).

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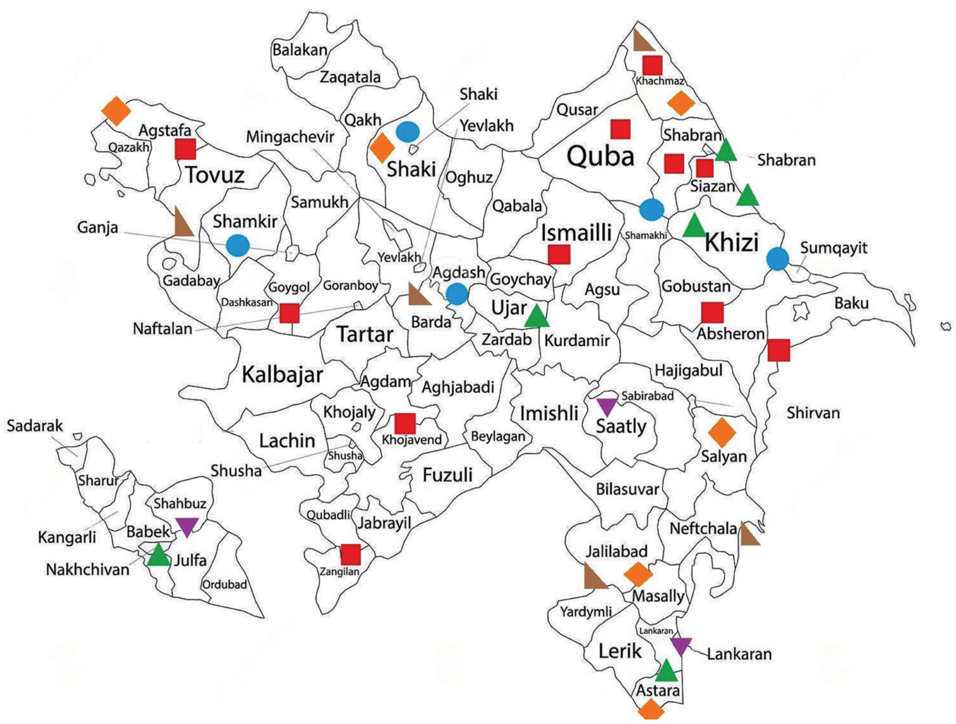

Figure 1. Map of *Glycyrrhiza* growth in the territory of the Republic of Azerbaijan: ■ – *Glycyrrhiza glabra* L.; ▲– *Glycyrrhiza echinata* L.; ● – *Glycyrrhiza aspera* - Pall.; ♦ – *G. foetidissima* Taush; ◣– *G. hirsuta* L. emend. Pall.; ▼– *G. glandulifer*a Waldst. and Kit.

MATERIAL AND METHODS

The object of research was 7-day-old seedlings of wheat (*T. aestivum* L.) grown under factorostatic conditions (24°C, humidity 80%) and subjected to the action of Zn ions ($ZnSO_4$+H_2O, “p.a.” Sigma-Aldrich) at a toxic concentration (10^{-3} M) for 24 hours, as well as 2 % alcohol extract of the roots of *G. glabra*. Antioxidant (AO) and free radical quenching were determined using the stable radical model DPPH (1,1 diphenil-2-picrylhydrazyl) [Molyneux, 2004]. The AO ability of the sample was determined using the computer program Perella Scientific Ins., Amherst, USA.

The kinetics of ms-DF was measured using a phosphoroscopeas described previously. A sample insilica suprasil cuvette was irradiated with continuous white light (250 W m^{-2} s^{-1}) passing through a 2 cm ofa $CuSO_4$ solution. The light was passing trough holes on the rotating wheel of the phosphoroscope (three holes, 120° apart) in such way that 0.3 ms of excitation was following by 1.25 ms of dark and 0.3 ms of registration of the delayed light emission (three cycles of excitation/dark/registrationper one full turnover of the phosphoroscope wheel). The delayed light was measured with a photomultiplier viared glass cut off KC9 filter (LOMO Russia, >680 nm). The signal passing through an amplifier was recorded byelectrical potential recorder [Rubin et al., 1984; Goltsev et al., 2009].

The absorption spectrum of native forms of chlorophyll in seedlings was determined on a Varian Cary 50 Scan spectrophotometer (Furye).

Animal experiments to determine the activity of Glysotrical were carried out in accordance with the guidelines of the Animal Welfare Act of 1966.

The official tincture of calendula (*Calendula officinalis* L.) was used as a control for comparison. The experiments were carried out on rabbits of *Chinchilla* Bennett breed.

Experiments were repeated 3 times and standard errors are shown in figures where appropriate.

RESULTS AND DISCUSSION

Experiment on animal. To determine the AO activity of the *G. glabra* extract, the standard Trolox-DPPH reaction was used. As can be seen from table 1, the study of the quenching of the stable radical DPPH trolox showed an inhibition concentration (IC) 50 =17.1 μg/ml.

The AO activity of the extract was confirmed on the basis of the kinetic curves of recambination fluorescence and the spectral characteristics of photosynthetic pigments.

Table 1. DPPH reaction of *G. glabra* root extract.

Samples	IC 50(μg/ml)*
Trolox	17,1±0,1
Glycyrrhiza glabra extract	11,9±0,1

*IC 50 (μg/ml) is the concentration of a substance that quenches (neutralizes) reactive oxygen species (ROS) by 50% in the DPPH reaction. Trolox is the standard antioxidant for DPPH reaction.

After creating an increased content of reactive oxygen species (ROS) in the cells of seedlings (*T. aestivum* L.) with Zn ions, the state of the electron transport chain within photosystem II (PS II) was determined (Fig. 2).

The inhibitory effect of Zn^{2+} on photosynthesis takes place on both the oxidative and donor sides of PSII electron

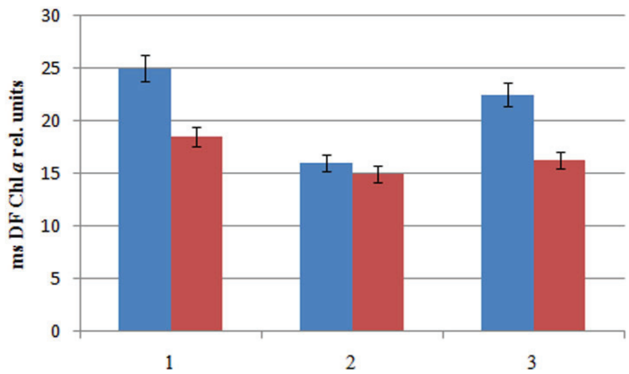

Figure 2. Changes in the activity of fast (■-fs.ph/s.ph) and slow (■-sl.ph/s.ph) fluorescence (ms DF Chl *a*) in seedlings of *T. aestivum* L. after 24 hours of action: 1-control; 2-$ZnSO_4$ (10^{-3}M); 3-$ZnSO_4$+ *G. glabra* extract.

transport chain (ETC) [Wang et al., 2009; Kondzior, Butarewicz, 2018].The activity of both fast and slow fluorescence (fs.ph/s.ph, sl.ph/s.ph) decreased as a result of blocking by the toxicant of recombination reactions of the excited Chl-reaction center (RC Chl *a*) with a positive equivalent on the PS II donor side (Fig. 2 fs.ph/s.ph). In addition, these processes under stress are in competition with direct electron transfer to PS II, which leads to damage to the stable electron transfer on the acceptor side of PS II at the level of quinone acceptors Q_A-Q_B (Fig. 2 sl.ph/s.ph) [Papageorgiou, Govindjee, 2004; Gasanov et al., 2007; Stefanov et al., 2018]. In stressed seedlings, the activity of fs.ph decreased by 40%, and the activity of sl.ph by 25% relative to the control. Incubation of stressed seedlings in *G. glabra* extract restored fs.ph activity by 37.5% and sl.ph by 20% relative to these parameters in stressed seedlings. Under stress, the destructive changes occurring in the pigments lowered their absorption capacity (Fig. 3).

The absorption capacity of Chl *a* 680 decreased by 25%, and Chl *b* 645 by 39%. Under the condition with

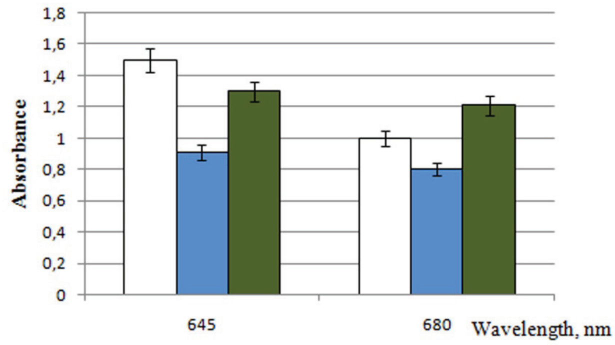

Figure 3. Changes in absorption of Chl *a* 680 and Chl *b* 645 forms in seedlings of *T. aestivum* L. upon exposure for 24 hours: 1. □- control; 2. ■-$ZnSO_4$ (10^{-3}M); 3. ■-$ZnSO_4$+*G. glabra* extract.

the extract, the resistance of pigments to the action of Zn increased. The absorption capacity of Chl *a* 680 was restored by 36%, and Chl *b* 645 by 30% relative to stress. The high AO activity of the extract is determined by the rich content of phytochemical compounds in *G. glabra*, the main of which are triterpene sapanins and flavonoids (glycyrrhizin, glycyrrhizic acid, isoliquiritigenin, liquiritigenin, lichalocone and glabridin), isoflavones etc. [Wahab et al., 2021].

Being low molecular weight antioxidants, they are able to extinguish ROS generated during stress. *G. glabra* has a broad pharmacological effect that is being studied at the molecular level. A biologically active composition based on the extract of *G. glabra* "Glysotrical" was obtained. The extracts included in it were purified from ballast substances [Mehraliyeva, 2008; Mehraliyeva et al., 2008, Litvinenko et al., 2014; Hosseinzadeh, Nassiri-Asl, 2015] and their toxicity was determined.

Experiment on animal. In the control group of animals with thermal and chemical damage to the skin, a necratic state of the tissue was observed. Violation of the vascular system led to swelling of the skin. In this group of animals, when wounds were treated with the official marigold tincture, tissue granulation began on days 42-48. In the experimental group of animals, after the treatment of

Table 2. Indicators of treatment of chemical and thermal damage to the skin in a rabbit with a biologically active composition "Glysotrical".

Animals (rabbits)	Thermalda mage	Chemical damage	Medicinal product	Treatment time (day)		Healing time (day)
control	+		Ointment from calendula flowers	39-42	Beginning ofgranulation	42
control		+	Ointment from calendula flowers	Beginning ofgranulation	40-48	48
experience	+		«Glysotrical»	14-16	Granulation and regeneration	17
experience		+	«Glysotrical»	19-22	–	24

wounds with Glysotrical, the formation of a vascular network was observed on days 5-6, and the granulation process was observed on days 16-17. Tissue healing was fixed at 22-24 days. The resulting effect suggests that the "Glysotrical" formulation has anti-inflammatory and regenerative properties (Tab. 2).

CONCLUSION

An increase in the content of ROS in a biological cell under stress leads to a violation of the barrier function of the membrane. Therefore, under the conditions of the toxic effect of Zn ions on the seedlings of *T. aestivum* L., we observe destructive changes in the pigment system and blocking of photochemical reactions in the PS II ETC. Adaptive reactions that occurin the plant organism in the presence of the extract lead to the restoration of the parameters under study due to the content of flavonoids in the extract, such as epichiin and epitechiumgallate, located near the membrane surface and protecting phospholipids from the action of free radicals [Faizal, Geelen, 2013; Mamedov, Egamberdieva, 2019; Polyakov, Leshina, 2023]. In addition, the main phytocomponent of licorice is saponin-glycyrrhizin, which has a wide range of biological and pharmacological properties. Being a membrane-active compound and participating in biochemical processes, it has the ability to correct the lipid component of the membrane damaged by oxidative stress [Farshad Ashraf et al., 2013; Likhatskaya, Anisimov, 1999; Selyutina et al., 2020]. In addition, being a low molecular weight antioxidant, it manages to extinguish reactive molecules formed when the barrier system of the membrane is broken and restore the balance between photosystems.

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Glycyrrhiza glabra ekstraktının antioksidant və müalicəvi xüsusiyyətləri

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Tədqiqatların əsas məqsədi toksiki Zn^{2+} ionların təsirinə məruz qalmış buğda cücərti yarpaqlarının (*Triticum aestivum* L.) hüceyrə və xloroplastlarında oksigenin reaktiv molekulyar tərkib artımının qarşısını alan antioksidant mənşəli *Glycyrrhiza glabra* ekstraktının xüsusiyyətinin tədqiqi olmuşdur. Flüoressensiyanın (Xl *a*-nın msan LF) rekombinasion xarakteristik və piqment fondunun dəyişkənliyi əsasında göstərilmişdir ki, fotosistem II-nin elektron nəqliyyat zəncirinin, əsasən donor tərəfindən elektron daşınma reaksiyasının blokadası və fotosintetik piqmentlərin akkumulyasiyasının zəifləməsi baş verir. Bu stresə qarşı Xl *a* 680 nm daha davamlıdır. Stresə məruz qalmış cücərtilərin *G. glabra* ekstraktı ilə inkubasiyası zamanı fotosistem II-nin elektron nəqliyyat zəncirində elektron nəqlinin ($P680^*–Q_A-Q_B$) və piqmentlərin işiq toplayan antenlərinin udma qabiliyyətinin bərpası müşahidə olunur. Ekstraktın antioksidant aktivliyi stabil radikal DPPH reaksiyası ilə təsdiqlənir. *G. glabra* əsasında “Glysotrical” bioaktiv kompozisiyanın farmakoloji effekti kimyəvi və termiki zədələnmə zamanı dovşanın dəri örtüyünün regenerasiyasında biruzə olunur. Alınan nəticələrdən fərz etmək olar ki, *G. glabra* ekstraktının müdafiə effektliyi onun zəngin fotokimyəvi tərkibindən asılıdır. Əsası flavonoidlər və triterpen saponinlər olan ekstrakt sərbəst radikalların artmasının qarşısını alır və hüceyrəni zədələnmədən qoruyur. Mümkündür ki, saponinlər membran aktiv birləşmə olub, stres zamanı zədələnən membranın lipid komponentini korrelyasiya edir.

Açar sözlər: *təsirlər, ekstraktlart, Glycyrrhiza glabra, antioksidant, elektron nəqli, FS II, piqmentlər, Triticum aestivum, sink*

Антиоксидантное и лечебное свойство экстракта *Glycyrrhiza glabra*

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Исследовали антиоксидантные свойства экстракта солодки на листьях проростков *Triticuma estivum* L. подвергнутых действию Zn^{2+} в токсических концентрациях с целью создания в клетке и хлоропластах повышенного содержания реактивных молекул кислорода. На основании изменения характеристик рекомбинационной флуоресценции (мсек ЗФ Хл *a*) и пигментного фонда показано блокирование реакций переноса электрона в электронтранспортной цепи фотосистемы II, в большей мере, на ее донорской стороне и подавление аккумуляции фотосинтетических пигментов. Показана наибольшая устойчивость Хл а 680 к данному стрессу. При инкубации стрессовых проростков в экстракте *Glycyrrhiza glabra* наблюдалось восстановление переноса электрона ($P680^*–Q_A$-Q_B) в цепи фотосистемы II и спектров поглощающей способности пигментов светособирающей антенны. Антиоксидантная активность экстракта подтверждалась реакцией стабильного радикала DPPH. Фармакологический эффект биоактивной композиции “Glysotrical” на основеэкстракта *Glycyrrhiza* выразился в регенерации кожного покрова кроликов при химическом и термическом повреждении. Наши результаты предполагают, что защитный эффект экстракта *Glycyrrhiza* обусловлен богатым фитохимическим составом основными компонентами которого являются флавоноиды и тритерпеновые сапонины, препятствующие развитию свободно–радикального состояния и защищающих клетку от повреждения. Возможно, являясь мембраноактивными соединениями, сапонины корригируют липидный компонент поврежденной мембраны.

Ключевые слова: *эффекты, экстракты, Glycyrrhiza glabra, антиоксидант, электронный транспорт, ФС, цинк II, пигменты, Triticum aestivum*