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PHYTOCHEMICAL SCREENING OF POLYHERBAL COMPOSITION BASED ON *Portulaca oleracea* AND IT'S EFFECT ON MACROPHAGE OXIDATIVE METABOLISM

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The aim of the work was to explore phytochemical characteristics of water extract from polyherbal composition based on *Portulaca oleracea* and it's effect on oxidative metabolism of murine peritoneal macrophages. The qualitative phytochemical analysis was conducted by colorimetric method. Quantitative analysis of phenols was performed in the test with gallic acid as a standard. Murine peritoneal macrophages were isolated without previous sensitization. Leukotoxicity of the water extract from polyherbal composition leukotoxicity was evaluated in MTT test. Reactive oxygen species generation was assayed by the nitroblue tetrazolium reduction method. Phytochemical analysis revealed the presence of water-soluble and insoluble phenols, tannins, saponins, flavonoids, cardiac glycosides and coumarins in the studied plant mixture. The water extract from polyherbal composition used in a range of concentration 1–1000 μg/ml (lyophilisate in distilled H_2O) didn't exhibit any toxic effects on murine peritoneal macrophages. Water extract from polyherbal composition caused statistically significant dose-dependent increase in oxidative metabolism of murine peritoneal macrophages. The lack of toxicity and increase in macrophage oxidative metabolism suggest modulatory effect of studied water extract from polyherbal composition on innate immunity cells.

Key words: water extract from polyherbal composition, *Portulaca oleracea*, peritoneal macrophages, reactive oxygen species.

Use of complementary and alternative medicine, particularly herbal remedies is known as a beneficial approach for curing the different ailments. The growing number of studies report the anti-inflammatory, antioxidant, anticancer, antidiabetic, immunomodulatory and other effects of medicinal plants, which make them as a natural, innocuous and reliable remedy for treatment of wide spectrum of human diseases, as well as attractive objects for the biotechnology [1–3].

Portulaca oleracea is a medicinal plant with a broad range of therapeutic activities, and one of the most commonly used medicinal plants according to the World Health Organization [4]. The most common biologic effect of *P. oleracea* and its isolated constituents is the prevention and resolution of inflammation [5, 6]. In addition, *P. oleracea* extracts have an ability to restore suppressed immune reactivity in animal models [7]. Traditional anti-inflammatory effects of this plant can be implemented through the immunosuppressive or immune-stimulant effects and there are lack of obvious and clear characteristics of its impact on different effector cells of immune system, including phagocytes which are key players of majority immune responses [8, 9].

In most cases, *P. oleracea* based phytopreparations are used in the form of herbal remedies which are prepared from only this plant. Meanwhile, herbal mixtures containing a combination of different plant species have been reported to have better biological activities than monocomponent formulations because of synergistic effects of phytochemicals from

different medicinal plants [10, 11]. The use of such multicomponent formulations offer advantages due to the effect on different targets or improving bioavailability of crude herb extracts. There are reports showing the presence of especially prominent therapeutic effects of herb combinations that were unachievable when each plant extract was administered alone, making the study of such mixtures containing multiple plant products especially promising [12].

In this study, we aimed to explore phytochemical characteristics of water extract from polyherbal composition (WEPC) with *P. oleracea* and it's effect on oxidative metabolism of murine peritoneal macrophages (PMs).

Materials and Methods

Polyherbal composition. A total of 8 plant species were used: *Helichyrsum arenarium, Mentha piperita, Calendula officinalis, Taraxacum officinale, Polygonum aviculare, Matricaria recutita, Portulaca oleracea,* and *Hypericum perforatum* (Table 1). The material collected was identified by the standards of classical taxonomy. All plant species were collected from different regions of Azerbaijan. The flowers of species of *Asteraceae* family were collected in the region of Yardimli in June–July. The leaves of *Mentha piperita* were collected in the region of Tovuz from June until late September. The flowers of *Calendula officinalis* were collected in the region of Quba. The well-formed roots of *Taraxacum officinale* were taken up in the autumn in the region of Quba-Khachmaz. The flowers, leaves and stems of *Polygonum aviculare* were collected in Lankaran region from July until late September. The flowers of *Matricaria recutita* were used and collected in the region of Kura-Aras. Leaves and seeds of *P. oleracea* were collected in the region of Tovuz-Qazakh in July and August. The flowers and stems of *Hypericum perforatum* were collected in Lerik region in July–August.

All parts of the plants were dry out in the shade with good air ventilation, away from direct sunlight until use. To prepare herbal tea, all herbs were added in equal weight parts.

Preparation of the aqueous extract of medicinal plants and its lyophilization. WEPC was prepared according to [13]. 10 g of polyherbal composition were homogenized with a jar mill to a powdered state. The powder was poured with boiled distilled water in a volume of 200 ml and were infused for 30 min, after which it was filtered through a filter paper and liophilized.

Phytochemical screening. The freshly prepared WEPC was qualitatively tested for the presence of chemical constituents. The qualitative phytochemical analysis was performed using corresponding specific color reactions with following reagents and chemicals: Alkaloids with Dragendorff's and Mayer's reagent, flavonoids with the use of Mg and HCI; tannins with ferric chloride and potassium dichromate solutions, coumarins with 10% aqueous NaOH solution [13], and saponins with ability to produce suds. For the analysis of the presence of triterpenes, a modified Salkowski test [14] was used. To determine the presence of polysteroids, a modified Liebermann-Burchard test [15] was

Table 1. **Components of polyherbal composition**

	Botanical name	Family	Morphological part of plant used in WEPC	% in whole mixture
1	*Helichrysum arenarium*	*Asteraceae*	Flowers	12.5%
2	*Portulaca oleracea*	*Portulacaceae*	Leaves and seeds	12.5%
3	*Mentha piperita*	*Lamiaceae*	Leaves	12.5%
4	*Calendula officinalis*	*Asteraceae*	Flowers	12.5%
5	*Taraxacum officinale*	*Asteraceae*	Roots	12.5%
6	*Polygonum aviculare*	*Polygonaceae*	Flowers, leaves and stems	12.5%
7	*Matricaria recutita*	*Asteraceae*	Flowers	12.5%
8	*Hypericum perforatum*	*Hypericaceae*	Flowers and stems	12.5%

applied. The presence of phenolic groups was examined using modified Folin Ciocalteu test [16]. Water-soluble and water-insoluble phenolic compounds were examined as described by Cock I.E., Kukkonen L. [17]. Cardiac glycosides were detected by the modified Keller-Kiliani method [16]. Modified Kumar and Ajaiyeoba tests were used to determine the presence of anthraquinones [14, 15]. Positive reactions indicate the presence of various biologically active substances in the investigated extract.

Quantitative analysis of phenols in WEPC. The total amount of phenolic compounds in WEPC was determined by Siddhuraju P. et. al. method [18] with minor modifications. 1 ml of 10% aqueous Folin Ciocalteu solution was added to 20 µl of the EMHT extract or standard and mixed thoroughly. The mixture was incubated for 2 h, and then the optical density at 765 nm wavelength was measured. Gallic acid (C_6H_2 (OH) 3COOH) at concentrations of 20–200 mg/ml was used as a standard to plot a calibration curve. The results were expressed in mg of the equivalent of gallic acid per gram of dry matter of the WEPC. For this purpose, a standard solution of gallic acid was prepared: 0.01 g of gallic acid was dissolved in a 10 ml volumetric flask with distilled water to the mark. Next, dilution of the standard gallic acid solution from 1 mg/ml to 0.1 mg/ml was prepared. Up to 0.02 ml of each dilution of a standard gallic acid solution of different concentrations was added to 1.58 ml of H_2O and 0.1 ml of Folin reagent and kept 8 min in a dark place. After the addition of 0.3 ml of saturated Na_2CO_3 solution sample was again set in a dark place for 2 hours. Studied samples were prepared in a similar way. The optical density was measured at a wavelength of 760 nm on a Hitachi U-2810 spectrophotometer. 3 measurements were performed to ensure reliability. A standard curve was made according to the results.

Peritoneal macrophage isolation. PMs were isolated without preliminary stimulation as described earlier [19]. Intact C56Bl/6 mice (male, 8 weeks old, bred at animal facility of ESC "Institute of Biology and Medicine") were sacrificed and PMs were harvested using phosphate buffered saline containing 100 U/mL of heparin. Cells were then centrifuged at 300 g for 5 min at 4 °C, washed thrice with serum–free DMEM, and resuspended in DMEM containing 10% FCS and 40 µg/mL gentamycin.

Viability assay. Cytotoxic effect of WEPC on murine peritoneal macrophages (PMs) was determined using the MTT test [20]. PMs were seeded in 96-well plates (4×10^5 at the volume of 200 ml) and treated with 1, 10, 100, and 1000 µg/ml WEPC for 24 hours at 37 °C in the presence of 5% CO_2. Ten microliters of MTT solution (5 mg/ml) (Sigma, USA) was added to each well and followed by 2 h incubation. Media was then aspirated and 100 µl of dimethyl sulfoxide (Sigma, USA) was added. The optical density was determined at 560 nm using a Multiscan RC.

Reactive oxygen generation assay. O-2 generation was assayed by the nitroblue tetrazolium (NBT) reduction method as described earlier [19]. In a 5% CO_2 atmosphere PMs (2×10^5/well) were incubated for 1 h at 37 °C in Hanks buffered saline solution containing 1 mg of NBT per ml, with or without zymosan as a stimulator of oxidative burst. The optical density at 540nm in each well was examined with a plate reader. The modulation coefficient (MC) was evaluated after the treatment of PMs with zymozan and was calculated by the formula:

$$MC= (S - B/B) \times 100,$$

where S — index value after treatment with zymozan; B — index value of untreated cells (baseline value).

Statistical analysis. Each sample was assayed for generation in triplicate, and results are presented as mean ± SE. The statistical significance of the experimental results was determined by Student's *t* test. For all analyses, $P < 0.05$ was accepted as a significant probability level.

Results and Discussion

Investigated polyherbal composition includes medicinal plants with proven immunomodulatory properties. In particular, *Calendula officinalis* was found to inhibit bacterial LPS-induced pro-inflammatory phagocyte activity [21]. *Hypericum perforatum* is a medicinal plant with anti-inflammatory and immunostimulating properties. Aghili et al. (2014) demonstrated that *H. perforatum* affects leukocyte, monocyte and neutrophil migration [22]. *Taraxacum officinale* (dandelion herb) is a well-known medicinal plant with numerous therapeutic properties including immunomodulation. Lee et al. (2012) reported that dandelion extract potentiates effect of interferon-gamma and increase nitric oxide production by peritoneal macrophages [23]. Chamomille (*Matricaria recutita*) activates immunoregulatory cells and

increases sensitivity of effector cells to helper signals [24]. The *Polygonum* genus includes species containing diverse pharmacologically active constituents with various properties [25]. Immunomodulatory potential of *P. aviculare*, as well as of other plants from this genus, hasn't been extensively studied. However, George et al. (2014) showed that aqueous extract of *P. minus* caused significant increase in phagocytic activity of blood-derived phagocytes *in vivo* [26]. Peppermint is popular herb in traditional medicine that also has immunomodulatory effects [27, 28]. Cosentino et al. (2009) demonstrated the ability of peppermint oils to increase stimulated oxidative burst of peripheral blood monocytes and to decrease IL-4 production [29].

The abovementioned plants produce huge amount of different secondary metabolites playing protective role against infection or other harmful stimuli. A large number of these plant-derived secondary metabolites, also known as phytochemicals, possess immunomodulatory activity [1]. Among others, phenolic compounds are one of the most abundant phytochemicals. They can regulate immune system by targeting various receptors expressed on the surface of lymphoid cells and activating signaling pathways to initiate immune responses [30].

WEPC phytochemical charateristics. The results of qualitative WEPC phytochemical screening are given in Table 2.

Qualitative phytochemical analysis demonstrated the presence of water-soluble and insoluble phenols, tannins, saponins, flavonoids, cardiac glycosides and coumarins. Quantitative phytochemical analysis showed that studied WEPC contained 165 μg of phenolic compounds expressed in terms of gallic acid per 1 g of dry matter. Considering exceptional macrophage functional plasticity [31], these cells represent perfect targets for immunoregulatory impact of mentioned phytochemicals. Tannins and saponins are known to increase phagocytic activity [32, 33]. Saponins have the ability to stimulate cell mediated immune system as well as to enhance antibody production [34]. Flavonoids and other phenolic compounds are able to stimulate cellular and humoral immune response, lymphocyte proliferation, interferon secretion and phagocytic activity [2, 35–38].

Ye et al. (2004) showed enhancement of mitogen induced proliferation of murine splenocytes cultured *in vitro* in presence of three cardiac glycosides [39]. Coumarins obtained from vegetables and spices increased activation and IFN-γ secretion of lymphocytes [40].

Major constituents of *P. oleracea* are flavonoids, alkaloids, coumarins, monoterpene glycoside and fatty acids including Omega-3 [4]. Combination of *P. oleracea* with other medicinal plants allows to supplement this herbal remedy with other potent phytochemicals which can promote its immunomodulatory effect.

Table 2. **Phytochemical characteristics of WEPC**

Qualitative test	Positive reaction	Studied WEPC
Test for tannins	Dark blue color	+
Test for saponins	Persistent foam	+
Test for flavonoids	Yellow color with precipitate	+
Test for polysteroids	Reddish-brown color	–
Test for triterpenes	Red-violet color	–
Test for phenols	Blue-gray color	+
Test for water-soluble phenolic compounds	Red color	+
Test for water-insoluble phenolic compounds	Change in color of the mixture	+
Test for cardiac glycosides	Pink color	+
Test for anthraquinones	Bright pink color	–
Test for coumarins	Yellow color	+

Note: "-" — absence; "+" presence of the phytochemical.

The effect of WEPC on macrophage viability. Despite the great therapeutic potential of phytochemicals, they can exert adverse toxic effects resulting in the development of different pathologic conditions such as liver failure, contact dermatitis, hemolytic anemia, cancer and so on [41, 42]. Therefore, it was necessary to investigate potential cytotoxic activity of studied WEPC. MTT assay was used to assess toxic effect of WEPC on peritoneal macrophages *in vitro*. Cells were treated with increasing concentrations of studied aqueous phytoextract. As it can be seen from Fig. 1, there weren't any significant changes in the number of viable cells in samples of murine peritoneal macrophages that had been exposed to WEPC over a range of concentrations from 1 μg/ml to 1000 mg/ml. These results indicate on safety of the studied plant mixture.

The effect of WEPC on macrophage oxidative metabolism. Reactive oxygen species (ROS) production is an important mechanism used by phagocytic cells to defend host from pathogens. It is known that in addition to toxic effect on bacterial cells ROS also activate other antimicrobial defenses due to their role as secondary messengers of cellular signaling. ROS affect migration of phagocytes, regulation of their differentiation and phagocytic activity [43, 44]. In addition, ROS are involved in tissue regeneration and wound healing [45]. We used NBT test to evaluate the influence of WEPC on the ability of murine PMs to produce ROS. WEPC at all concentrations caused statistically significant dose-dependent increase in spontaneous ROS production by murine peritoneal macrophages

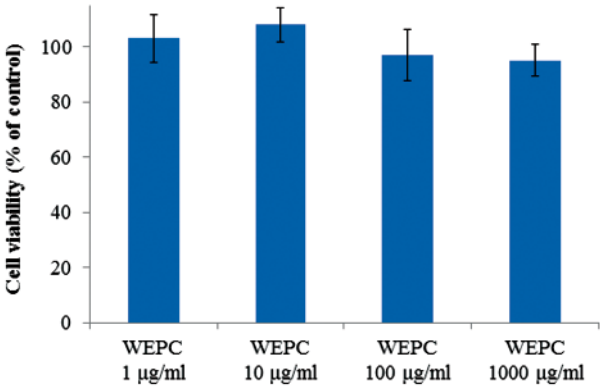

Fig. 1. **The effect of WEPC on the viability of murine peritoneal macrophages *in vitro*:** nonsensitized murine peritoneal macrophages were isolated and treated with varying concentrations of WEPC for 24 hours. Cell viability was measured by the MTT test. Control: peritoneal macrophages without WEPC. Data are expressed as the mean ± SE

(Fig. 2, *A*). Particularly, WEPC at the concentration of 100 μg/ml increased baseline oxidative burst by 2 times compared to control.

Treatment PMs with zymosan allowed us to evaluate the effect of WEPC on reactivity reserve (the remaining capacity of a cell to fulfill given metabolic activity under stress) of phagocyte oxidative metabolism, that was characterized by MC (see Materials and Methods). Treatment with WEPC PMs which were previously treated with zymosan resulted in the decrease of reactivity reserve of this phagocyte function (Fig. 2, *B*). However, WEPC at the concentration of 50 μg/ml didn't affect reactivity reserve of macrophage oxidative metabolism. Data concerning ROS-

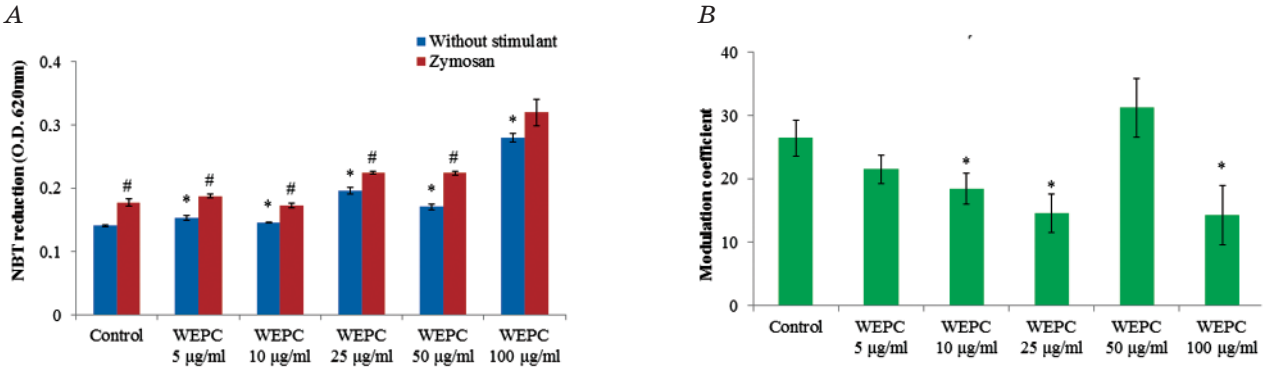

Fig. 2. **Effect of WEPC on the production of ROS by murine peritoneal macrophages:**
A — Spontaneous and stimulated nitroblue tetrazolium reduction:
* — $P < 0.05$ were considered significant compared to control without stimulant
# — $P < 0.05$ were considered significant compared to the same concentration of WEPC without stimulant.
B — Modulation coefficient of zymosan treatment:
* — $P < 0.05$ were considered significant compared to control

stimulating properties of phytochemicals are controversial and scarce. Cosentino et al. (2009) demonstrated increase in stimulated oxidative burst of polymorphonuclear cells stimulated with essential oils of *Mentha piperita* [29]. Conversely, Lee et al. described ROS-scavenging (antioxidant) properties of phytoextracts [46]. The effects of different herb extracts on ROS equilibrium may depend on their composition, determined by presence of different species of plants, their organs used in preparation of the extract, as well as the nature of solvent (water, methanol etc.).

In conclusion, studied WEPC didn't exhibit any leukotoxic effects and dose-dependently stimulated oxidative burst of murine peritoneal macrophages. Further research is needed to elucidate mechanisms of action of multiple compounds that have been detected in studied WEPC, as well as to explore immunomodulatory activity of this extract *in vivo*.

REFERENCES

1. *Venkatalakshmi P., Vadivel V., Brindha P.* Role of phytochemicals as immunomodulatory agents: a review. *Int. J. Green Pharm*. 2016, 10 (1).
2. *Li F. S., Weng J. K.* Demystifying traditional herbal medicine with modern approach. *Nat. Plants*. 2017, V. 3, P. 17109. https://doi.org/10.1038/nplants.2017.109
3. *Leonti M., Verpoorte R.* Traditional Mediterranean and European herbal medicines. *J. Ethnopharmacol.* 2017, V. 199, P. 161–167. https://doi.org/10.1016/j.jep.2017.01.052
4. *Iranshahy M., Javadi B., Iranshahi M., Jahanbakhsh S. P., Mahyari S., Hassani F. V.* A review of traditional uses, phytochemistry and pharmacology of *Portulaca oleracea* L. *J. Ethnopharmacol.* 2017, V. 205, P. 158–172.
5. *Catap E. S., Kho M. J. L., Jimenez M. R. R. In vivo* nonspecific immunomodulatory and antispasmodic effects of common purslane (*Portulaca oleracea* Linn.) leaf extracts in ICR mice. *J. Ethnopharmacol*. 2018, V. 215, P. 191–198.
6. *Li C. Y., Meng Y. H., Ying Z. M., Xu N., Hao D., Gao M., Zhang W. J., Xu L., Gao Y. C., Stien D., Ying X. X.* Correction to Three Novel Alkaloids from *Portulaca oleracea* L. and Their Anti-inflammatory Effects. *J. Agric. Food Chem*. 2017, 65 (4), 993–994. https://doi.org/10.1021/acs.jafc.6b05659
7. *Park Y. M., Lee H. Y., Kang Y. G., Park S. H., Lee B. G., Park Y. J., Lee H. M., Kim O. J., Yang H. J., Kim M. J., Lee Y. R.* Immune-enhancing effects of Portulaca oleracea L.-based complex extract in cyclophosphamide-induced splenocytes and immunosuppressed rats. *Food Agric. Immunol.* 2019, 30 (1), 13–24.
8. *Dey A., Allen J., Hankey-Giblin P. A.* Ontogeny and polarization of macrophages in inflammation: blood monocytes versus tissue macrophages. *Front Immunol.* 2015, V. 5, P. 683. https://doi.org/10.3389/fimmu.2014.00683
9. *Zhao Y., Zou W., Du J., Zhao Y.* The origins and homeostasis of monocytes and tissue-resident macrophages in physiological situation. *J. Cell Physiol*. 2018, 233 (10), 6425–6439. https://doi.org/10.1002/jcp.26461
10. *Dev S. K., Choudhury P. K., Srivastava R., Sharma M.* Antimicrobial, anti-inflammatory and wound healing activity of polyherbal formulation. *Biomed. Pharmacother*. 2018, V. 111, P. 555–567. https://doi.org/10.1016/j.biopha.2018.12.075
11. *Moukette B. M., Ama Moor V. J., Biapa Nya C. P., Nanfack P., Nzufo F. T., Kenfack M. A., Ngogang J. Y., Pieme C. A.* Antioxidant and Synergistic Antidiabetic Activities of a Three-Plant Preparation Used in Cameroon Folk Medicine. *Int. Sch. Res. Notices*. 2017, V. 2017, P. 9501675. https://doi.org/10.1155/2017/9501675
12. *Yuan H., Ma Q., Cui H., Guancheng L., Zhao X., Li W., Piao G.* How can synergism of traditional medicines benefit from network pharmacology? *Molecules*. 2017, V. 22, P. E1135.
13. *Deyab M., Elkatony T., Ward F.* Qualitative and Quantitative Analysis of Phytochemical Studies on Brown Seaweed, Dictyota Dichotoma. *IJEDR*. 2016, 4 (2), 674–678.
14. *Djipa C. D., Delmée M., Quetin-Leclercq J.* Antimicrobial activity of bark extracts of *Syzygium jambos* (L.) Alston (Myrtaceae). *J. Ethnopharmacol.* 2000, V. 71, P. 307–313.
15. *Duraipandiyan V., Ayyanar M., Ignacimuthu S.* Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complement. Altern. Med.* 2006, V. 6, P. 35.
16. *Oyedapo O. O., Sab F. C., Olagunju J. A.* Bioactivity of fresh leaves of *Lantana camara*. *Biomed. Lett.* 1999, V. 59, P. 175–183.
17. *Cock I. E., Kukkonen L.* An examination of the medicinal potential of Scaevola spinescens: Toxicity, antibacterial, and antiviral activities. *Pharmacognosy Res.* 2011. 3 (2), 85–94.
18. *Siddhuraju P., Becker K.* The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extract. *Food Chem*. 2007, V. 101, P. 10–19.
19. *Fedorchuk O., Susak Y., Rudyk M., Senchylo N., Khranovska N., Skachkova O., Skivka L.* Immunological hallmarks of cis-DDP-

resistant Lewis lung carcinoma cells. *Cancer Chemother. Pharmacol.* 2018, 81 (2), 373–385. https://doi.org/10.1007/s00280-017-3503-6
20. *Jang H. H., Cho S. Y., Kim M. J., Kim J. B., Lee S. H., Lee M. Y., Lee Y. M.* Anti-inflammatory effects of *Salvia plebeia* R. Br extract *in vitro* and in ovalbumin-induced mouse model. *Biol. Res.* 2016, 49 (1), 41.
21. *Verma P. K., Raina R., Agarwal S., Kour H.* Phytochemical ingredients and pharmacological potential of *Calendula officinalis* Linn. *Pharm. Biomed. Res.* 2018, 4 (2), 1–17.
22. *Aghili T., Arshami J., Tahmasbi A., Haghparast A.* Effects of *Hypericum perforatum* extract on IgG titer, leukocytes subset and spleen index in rats. Effects of *Hypericum perforatum* extract on IgG titer, leukocytes subset and spleen index in rats. *Avicenna J. Phytomed.* 2014, 4 (6), 413–419.
23. *Lee B. R., Lee J. H., An H. J.* Effects of *Taraxacum officinale* on fatigue and immunological parameters in mice. *Molecules.* 2012, V. 17, P. 13253–1326.
24. *Gupta V., Mittal P., Khokra S. I., Kaushik D.* Pharmacological potential of Matricaria recutita: a review. *Int. J. Pharmaceut. Sci. Drug Res.* 2010, 2 (1), 12–16.
25. *Nkuete A. H. L., Migliolo L., Wabo H. K., Tane P., Franco O. L.* Evaluation of multiple functions of *Polygonum* genus compounds. *Eur. J. Med. Plant.* 2015, 6 (1), 1–16.
26. *George A., Chinnappan S., Choudhary Y., Bommu P., Sridhar M.* Immunomodulatory activity of an aqueous extract of *Polygonum minus* huds on Swiss albino mice using carbon clearance assay. *Asian Pac. J. Trop. Dis.* 2014, 4 (5), 398–400.
27. *Adel M., Abedian Amiri A., Zorriehzahra J., Nematolahi A., Esteban M. Á.* Effects of dietary peppermint (*Mentha piperita*) on growth performance, chemical body composition and hematological and immune parameters of fry Caspian white fish (Rutilus frisii kutum). *Fish Shellfish Immunol.* 2015, 45 (2), 841–847.
28. *Arab Ameri S., Samadi F., Dastar B., Zerehdaran S.* Effect of Peppermint (*Mentha piperita*) Powder on Immune Response of Broiler Chickens in Heat Stress. *Iran. J. Appl. Anim. Sci.* 2016, 6 (2), 435–445.
29. *Cosentino M., Bombelli R., Conti A., Colombo M. L., Azzetti A., Bergamaschi A., Marino F., Lecchini S.* Antioxidant properties and in vitro immunomodulatory effects of peppermint (Mentha x piperita l.) Essential oils in human leukocytes. *J. Pharm. Sci. & Res.* 2009, 1 (3), 33–43.
30. *Ding S., Jiang H., Fang J.* Regulation of Immune Function by Polyphenols. *J. Immunol. Res.* 2018, V. 2018, P. 1264074.
31. *Murray P. J.* Macrophage polarization. *Ann. Rev. Physiol.* 2017, V. 79, P. 541–566.
32. *Okwu D. E., Okwu M. E.* Chemical composition of *Spondias mormbia* Linn plant parts. *J. Sust. Agric. Eviron.* 2004, V. 6, P. 140–147.
33. *Punturee K., Wild C. P., Kasinrerk W., Vinitketkumnuen U.* Immunomodulatory activities of *Centella asiatica* and *Rhinacanthus nasutus* extracts. *Asian Pac. J. Cancer Prev.* 2005, V. 6, P. 396–400.
34. *Rajput Z. I., Hu S. H., Xiao C. W., Arijo A. G.* Adjuvant effects of saponins on animal immune responses. *J. Zhejiang Univ. Sci. B.* 2007, 8 (3), 153–161.
35. *Akbay P., Basaran A. A., Undeger U., Basaran N. In vitro* immunomodulatory activity of flavonoid glycosides from *Urtica dioica* L. *Phytother. Res.* 2003, V. 17, P. 34–37.
36. *Chiang L. C., Ng L. T., Chiang W., Chang M. Y., Lin C. C.* Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of Plantago species. *Planta Med.* 2003, V. 69, P. 600–604.
37. *Yadav V. S., Mishra K. P., Singh D. P., Mehrotra S., Singh V. K.* Immunomodulatory effects of curcumin. *Immunopharmacol. Immunotoxicol.* 2005, V. 27, P. 485–497.
38. *Abd-Alla H. I., Moharram F. A., Gaara A. H., El-Safty M. M.* Phytoconstituents of *Jatropha curcas* L. leaves and their immunomodulatory activity on humoral and cell-mediated immune response in chicks. *Z. Naturforsch. C.* 2009, V. 64, P. 495–501.
39. *Ye Y., Li X., Sun H., Chen F., Pan Y.* Immunomodulating Steroidal Glycosides from the Roots of Stephanotis mucronata. *Helvetica Chimica Acta.* 2004, V. 87, P. 2378–2384.
40. *Cherng J. M., Chiang W., Chiang L. C.* Immunomodulatory activities of common vegetables and spices of Umbelliferae and its related coumarins and flavonoids. *Food. Chem.* 2008, 106 (3), 944–950.
41. *Galati G., O'Brien P. J.* Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free Radic. Biol Med.* 2004, 37 (3), 287–303.
42. *Bode A. M., Dong Z.* Toxic phytochemicals and their potential risks for human cancer. *Cancer. Prev. Res. (Phila).* 2015, 8 (1), 1–8.
43. *Abais J. M., Xia M., Zhang Y., Boini K. M., Li P. L.* Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? *Antioxid. Redox Signal.* 2015, 22 (13), 1111–1129.
44. *Zhang J., Wang X., Vikash V., Ye Q., Wu D., Liu Y., Dong W.* ROS and ROS-mediated cellular signaling. *Oxid. Med. Cell Longev.* 2016, V. 2016, P. 4350965.
45. *Dunnill C., Patton T., Brennan J., Barrett J., Dryden M., Cooke J., Leaper D., Georgopoulos N. T.* Reactive oxygen species (ROS) and

wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. *Int. Wound. J.* 2017, 14 (1), 89–96. https://doi.org/10.1111/iwj.12557

46. *Lee M. T., Lin W. C., Yu B., Lee T. T.* Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals — A review. *Asian-Australas J. Anim. Sci.* 2017, 30 (3), 299–308.

ФІТОХІМІЧНИЙ СКРИНІНГ ЗБОРУ ЛІКАРСЬКИХ РОСЛИН НА ОСНОВІ *Portulaca oleracea* ТА ЙОГО ВПЛИВ НА ОКСИДАТИВНИЙ МЕТАБОЛІЗМ МАКРОФАГІВ

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Метою роботи було дослідити фітохімічні характеристики водного екстракту збору лікарських рослин, до складу якого входить *Portulaca oleracea* та його вплив на оксидативний метаболізм перитонеальних макрофагів мишей. Якісний фітохімічний аналіз проводили колориметричним методом, кількісний аналіз фенолів здійснювали в тесті з використанням галової кислоти як стандарту. Перитонеальні макрофаги мишей виділяли без попередньої сенситизації. Лейкотоксичний ефект водного екстракту збору лікарських рослин оцінювали в МТТ-тесті. Генерацію реактивних форм кисню досліджували в тесті з нітросинім тетразолієм. Фітохімічний аналіз виявив у складі водного екстракту збору лікарських рослин присутність водорозчинних і водонерозчинних фенолів, танінів, сапонінів, флавоноїдів, серцевих глікозидів і кумаринів. Водний екстракт досліджуваного збору лікарських трав у діапазоні концентрацій 1–1000 мкг/мл (ліофілізату в дистильованій воді) не чинив токсичного ефекту на перитонеальні макрофаги мишей, спричинював статистично достовірне дозозалежне посилення їх оксидативного метаболізму. Відсутність токсичної дії та здатність посилювати оксидативний метаболізм перитонеальних макрофагів дають підстави припустити модуляторні властивості досліджуваного водного екстракту збору лікарських рослин щодо ефекторів вродженого імунітету.

Ключові слова: водний екстракт збору лікарських рослин, *Portulaca oleracea*, перитонеальні макрофаги, реактивні форми кисню.

ФИТОХИМИЧЕСКИЙ СКРИНИНГ СБОРА ЛЕКАРСТВЕННЫХ РАСТЕНИЙ НА ОСНОВЕ *Portulaca oleracea* И ЕГО ВЛИЯНИЕ НА ОКСИДАТИВНЫЙ МЕТАБОЛИЗМ МАКРОФАГОВ

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Целью работы было исследовать фитохимические характеристики водного экстракта сбора лекарственных растений, в состав которого входит *Portulaca oleracea* и его влияние на оксидативный метаболизм перитонеальных макрофагов мышей. Качественный фитохимический анализ проводили колориметрическим методом, количественный анализ фенолов выполняли в тесте с использованием галловой кислоты в качестве стандарта. Перитонеальные макрофаги мышей выделяли без предварительной сенситизации. Лейкотоксический эффект водного экстракта сбора лекарственных растений оценивали в МТТ-тесте. Генерацию реактивных форм кислорода исследовали в тесте с нитросиним тетразолием. Фитохимический анализ выявил в составе водного экстракта сбора лекарственных растений присутствие водорастворимых и водонерастворимых фенолов, таннинов, сапонинов, флавоноидов, сердечных гликозидов и кумаринов. Водный экстракт исследуемого сбора лекарственных трав в диапазоне концентраций 1–1000 мкг/мл (лиофилизата в дистиллированной воде) не оказывал токсического эффекта на перитонеальные макрофаги мышей, вызывал статистически достоверное дозозависимое усиление их оксидативного метаболизма. Отсутствие токсического действия и способность усиливать оксидативный метаболизм перитонеальных макрофагов позволяют предположить модуляторные свойства исследуемого водного экстракта сбора лекарственных растений относительно эффекторов врожденного иммунитета.

Ключевые слова: водный экстракт сбора лекарственных растений, *Portulaca oleracea*, перитонеальные макрофаги, реактивные формы кислорода.