

EFFECTS OF TOPICAL APPLICATION OF DRY EXTRACTS ON ALLERGIC CONTACT DERMATITIS INDUCED BY 2,4-DINITRO-FLUOROBENZENE, IN BALB/c MICE

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Abstract

The study investigated the efficacy of dry hydro-alcoholic extracts obtained from the leaves of *Amaranthus hypochondriacus* (AMR), *Cynara scolymus* (CYN), *Plantago lanceolata* (PLG), *Sambucus nigra* (SAM) and *Rubus idaeus* (RUB) topically administered, in an experimental model of contact hypersensitivity induced by 2,4-dinitrofluorobenzene (DNFB) in BALB/c mice. Hypersensitivity was induced by application of DNFB dissolved in a vehicle of acetone: olive oil 4: 1 (AOO 4:1), initially on the back of animal and then on the left ear. Extracts were suspended in the same vehicle (0.15%) and applied to the left ear two hours after DNFB. The effect of the extracts was compared with a control group, which received no allergenic substance, a positive control group (DNFB) treated with vehicle and a reference group (DNFB + DEX) treated with dexamethasone dissolved in vehicle (0.0375%). Allergic phenomenon was assessed by measuring ear thickness with a digital micrometer. Ear thickness was determined initially and at different times of the experiment over a period of 32 days. For the group DNFB was observed a delayed hypersensitivity reaction on days 10, 28, 30 and 32. Topical administration of dexamethasone reduced the hypersensitivity reactions. AMR and SAM applied topically, did not show efficacy in reducing allergic dermatitis. PLG, CYN and RUB applied topically, reduced occasionally the delayed hypersensitivity reaction. Further research will try to establish extraction fractions obtained from PLG, CYN and RUB in order to identify the active principles useful in the topical treatment of diseases with allergic component.

Rezumat

În această cercetare ne-am propus investigarea eficacității extractelor hidro-alcoolice uscate obținute din frunzele de *Amaranthus hypochondriacus* (AMR), *Cynara scolymus* (CYN), *Plantago lanceolata* (PLG), *Sambucus nigra* (SAM) și *Rubus idaeus* (RUB) administrate topic, într-un model experimental de hipersensibilizare de contact, indusă de 2,4-dinitrofluorobenzen (DNFB), la șoareci BALB/c. Hipersensibilizarea a fost indusă prin aplicarea DNFB dizolvat într-un vehicul format din acetona:ulei de măsline 4:1, inițial pe spatele animalelor și apoi pe urechea stângă. Extractele au fost suspendate în același vehicul (0,15%) și aplicate pe urechea stângă la două ore după DNFB. Efectul extractelor a fost comparat cu un lot martor, care nu a primit substanța alergenă, un lot martor pozitiv (DNFB) tratat cu vehicul și un lot de referință (DNFB + DEX), tratat cu dexametazonă dizolvată în vehicul (0,0375%). Fenomenul alergic a fost evaluat prin determinarea grosimii urechilor cu ajutorul unui micrometru digital. S-a determinat grosimea urechilor inițial și în diferite momente ale experimentului, pe parcursul a 32 de zile. Pentru lotul DNFB s-a evidențiat o reacție de hipersensibilizare întârziată în zilele 10, 28, 30 și 32. Administrarea topică a dexametazonei a redus apariția reacției de hipersensibilizare. AMR, SAM aplicate topic, nu au prezentat eficacitate în reducerea dermatitei alergice. PLG, CYN și RUB aplicate topic, au redus ocazional reacția de hipersensibilizare întârziată. Cercetări ulterioare vor încerca să stabilească fracții de extracție obținute din PLG, CYN și RUB în vederea identificării principiilor active utile în tratamentul topic al afecțiunilor cu componentă alergică.

Keywords: collagen, minocycline, spongioid delivery systems, freeze-drying

Introduction

Dermatoses are immunologically mediated medical conditions of the epidermis which are manifested through urticaria (hives, rash) and eczemas/dermatitis. The mechanisms involved are anaphylactic reactions or immediate hypersensitivity and delayed hyper-

sensitivity reactions, mainly due to the increase of IgE antibody type which cause the releasing of chemical mediators, especially histamine [17].

Conventional treatment of atopic dermatitis is oriented towards the common symptoms in dermatoses, namely: pruritus, inflammation and erythema [11, 21]. Thus, specific corticosteroids, calcineurin inhibitors and coal

tar are used to treat the inflammation and pruritus and emollients to restore the natural barrier of the skin. Other agents without a direct mechanism on inflammation such as menthol, N-palmitoylethanolamine, capsaicine, doxepine and naltrexone are used for their antipruriginous properties [9, 19].

The topical use of plant products in the treatment of cutaneous allergic conditions is limited due, on the one hand, to the insufficient research on their pharmacological effects and, on the other hand, on the lack of information on their safety. The common plant compounds useful in dermatological conditions, including the treatment of symptoms of urticaria and dermatitis belong to lipids, phenolic compounds, iridoids and alkaloids [2]. Topical administration of plant extracts with polyphenols has been proven beneficial in the treatment of dermatitis. In this sense it has been demonstrated that extracts from *Illicium verum* and *Camellia sinensis*, *Houttuynia cordata* and *Ulmus davidiana* var. *japonica* directly applied on lesions induced in animal experimental models have significantly reduced erythema, oedema, lichenification and cutaneous lesions, as compared with the control group [15, 16, 22, 23].

Researches on animal models help us find useful treatment in pathologies that do not have effective treatment [1, 3, 12]. Animal experimental models are widely used in screening of anti-dermatitis agents such as newly synthesized compounds and plant extracts. The hypersensitivity, after contact with allergens observed in experimental animals, is equivalent to eczema or atopic dermatitis observed in patients [5]. The animal model relies on cell-mediated immune response, due to skin cells contact with the hapten that induces a delayed hypersensitivity reaction, which can be measured and quantified. Hypersensitivity reaction has two phases: the initial phase, sensitization to allergen and delayed phase, challenged phase (swelling, oedema).

Various compounds with allergic potential were used in animal models (2,4,6-trinitrochlorobenzen, oxazolone, 2,4-dinitrofluorobenzene) [10]. After the study of specialized literature we noticed that there were used mainly female mice BALB/c, aged 6-10 weeks [4, 6, 13, 14, 22].

In this research we aimed to investigate the effect of five dry extracts (*Amaranthus hypochondriacus*, *Plantago lanceolata*, *Cynara scolymus*, *Sambucus nigra*, *Rubus idaeus*) containing active ingredients (flavonoids and polyphenols) that may reduce the hypersensitivity after the contact with compounds with allergic potential [7, 20, 24].

Materials and Methods

Preparation of extracts

The extracts were prepared from *Amaranthus hypochondriacus* (AMR), *Plantago lanceolata* (PLG),

Cynara scolymus (CYN), *Rubus idaeus* (RUB) leaves and *Sambucus nigra* (SAM) leaves and flowers using the method described in our previous works [7, 8, 18]. Briefly, the grounded plant materials were extracted under reflux with 10 parts of solvent (water:ethanol 1:1 (v/v)), followed by concentration and lyophilisation. The dry crude extracts were further used in the animal experiments.

Animals

Female 6-8 week-old BALB/c mice (16.29 ± 1.69 g, N = 45) were purchased from INCDMI Cantacuzino (Bucharest, Romania) and housed under specific conditions. Standard rodent food and water were available *ad libitum*. After arrival, they were allowed to acclimatize five days before they were used in the experiment. The temperature was between 21°C and 24°C and the relative humidity was generally maintained at 45 - 60%. All procedures were carried out in accordance with the European Directive 2010/63/UE/22.09.2010 regarding the protection of animals used for experimental and other scientific purposes and were approved by Animal Care and Ethics Committees of the Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

Induction of allergic contact dermatitis by 2, 4-dinitrofluorobenzene and experimental design

Induction of experimental atopic dermatitis was carried out as previously described by Gaspari *et al.*, 2001 using DNFB [10].

The active sensitization was induced by topically applying 20 µL DNFB (0.5%, v/v) dissolved in acetone and olive oil (AOO, 4:1) on the shaved back of the mice (D0). Six days later, each mouse (except the control group) was elicited by topically applying 10 µL x 2 DNFB (0.2%, v/v) in AOO the both sides of left ear, in the following days: D5, D7, D9, D19, D25, D27, D29, D31. The control animals were treated with AOO, 4:1 alone.

For topical applications, dexamethasone (DEX) (75 µg/ear) and plant extracts (300 µg/ear) were dissolved in AOO (0.0375%, v/v; 0.15%, v/v) and applied 10 µL x 2 onto the ventral and dorsum of the left ear two hours after DNFB (D5, D7, D9, D10 - D31). Twenty-four hours later, the ear swelling was appraised by measuring the right and left ears in their thickness (measured by using an engineer's electronic micrometer). Ear thickness was determined initially and at different times of the experiment over a period of 32 days (D0, D6, D8, D10, D18, D20, D25, D26, D28, D30, D32).

The control group (Control) received no treatment, the mice were treated with vehicles (AOO) (n = 5). Mice from the following groups were sensitized and challenged with DNFB, and received different treatments. DNFB group (DNFB) was without any treatment (n = 5). DEX 75 µg/ear in AOO (n = 5) was applied as a positive control treated animals

(DNFB + DEX). Vegetal extracts were administered with 300 µg/ear: DNFB + AMR (n = 5); DNFB + CYN (n = 5); DNFB + PLG (n = 5); DNFB + SAM

(n = 5); DNFB + RUB (n = 5). The experimental design is briefed in Table I.

Table I

Stages in the experimental design

Days after sensitization	Sensitization phase	Elicitation phase	Topical applications of treatment	Measuring the right and the left ears
0				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				
32				

Statistical analysis

Data were showed as mean $\pm$ SE (standard error) of 5 animals *per* group. We have established distribution in the groups using the D'Agostino & Pearson test. Mann-Whitney test or Kruskal-Wallis followed by Dunn's test was used for assessing significance between control group, DNFB group and extracts treated groups.

All results were considered statistically significant when $p < 0.05$. Results were statistically processed using GraphPad Prism 5 for Windows (GraphPad Software, San Diego, California, SUA, www.graphpad.com).

Results and Discussion

For the control group there were no changes in the thickness of the left ear. Thus, the vehicle used,

AOO did not modify the parameter (ear thickness), which evaluated the swelling due to the allergic reaction of 2,4-dinitrofluorobenzene (Figure 1).

After measuring the thickness of the left ear for DNFB group, there were higher mean values compared to baseline and control groups. Statistical significance occurred on the 10th day (146.25%, $p < 0.05$ vs. baseline and 153.48%, $p < 0.01$ vs. control group), the 28th day (151.25%, $p < 0.05$ vs. baseline and 153.66%, $p < 0.05$ vs. control group), the 30th day (375%, $p < 0.001$ vs. baseline and 5%, $p < 0.01$ vs. control group) and the 32nd day (231.25%, $p < 0.001$ vs. baseline and 234.86%, $p < 0.05$ vs. control group). This highlights the hypersensitivity reaction induced by DNFB on days 10, 28, 30 and 32 (Figures 1 and 2).

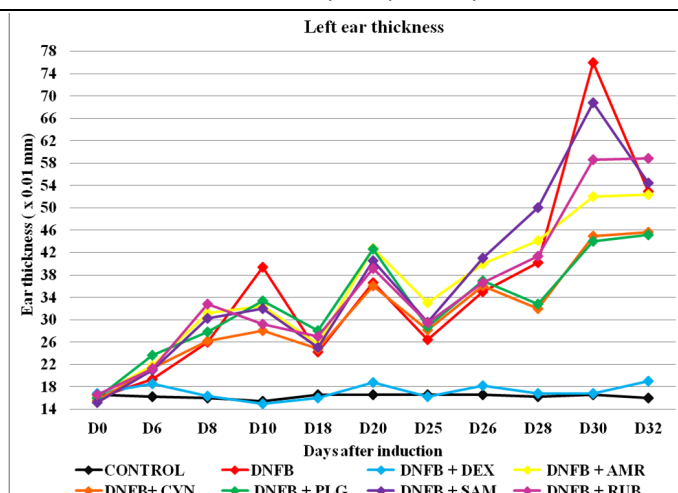


Figure 1.

Evolution of left ear thickness hallmark of swelling and vascular permeability in DNFB-induced allergic contact dermatitis mice

Data are showed as mean (n = 5 per group). AMR - *Amaranthus hypochondriacus* extract, PLG - *Plantago lanceolata* extract, CYN - *Cynara scolymus* extract, RUB - *Rubus idaeus* extract, SAM - *Sambucus nigra* extract, DNFB - 2,4-dinitrofluorobenzene, DEX – dexamethasone.

Following topical administration of DEX (75 µg/20 µL), no significant changes were recorded regarding the thickness of the left ear compared to baseline and to the control group. Compared with DNFB group (positive control), DEX delayed the hypersensitivity reactions, statistically significant effect on the 10th day (-156.96%, $p < 0.01$); the 28th day (-151.25%, $p < 0.05$); the 30th day (-375%, $p < 0.01$) and the 32nd day (218.15%, $p < 0.01$). These results confirm the efficacy of topical corticosteroids applied to

phenomena of hypersensitivity due to contact with allergens (Figures 2 and 3).

Following topical application of the extracts (AMR, PLG, CYN, SAM and RUB) at a dose of 300 mg/20 µL, there were statistically significant increases in the thickness of the left ear compared with baseline thickness and thickness measured in the control group on days 10, 20, 28, 30 and 32 (Figures 1 and 2).

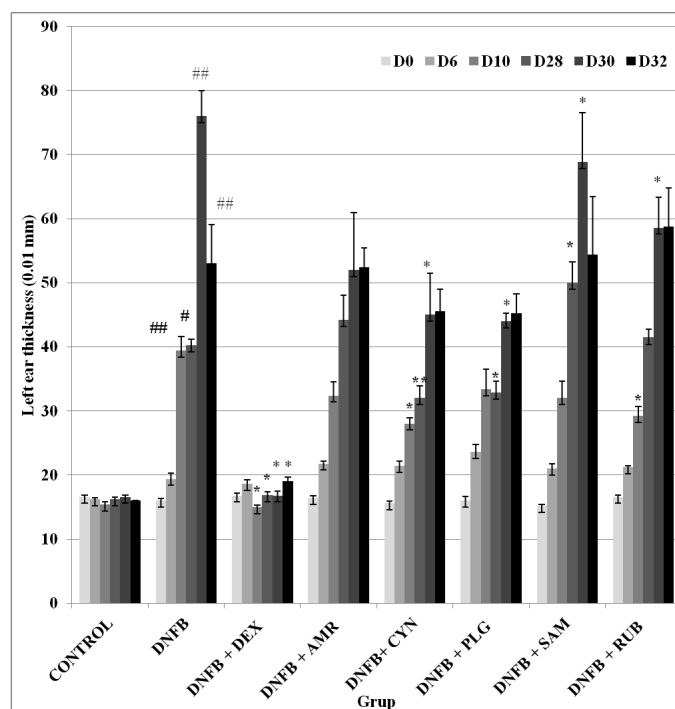


Figure 2.

Effects of DEX and vegetal extracts on the ear swelling and vascular permeability in DNFB-induced allergic contact dermatitis mice

Data are showed as mean ± standard error (S.E.) (n = 5 per group) ([#] $p < 0.05$; ^{##} $p < 0.01$ vs. control; * $p < 0.05$; ** $p < 0.01$ vs. DNFB).

Compared with DNFB group (positive control) these vegetal extracts reduced the delayed hypersensitivity reaction: CYN (-46.12%, $p < 0.01$ - day 28); PLG

(46.25%, $p < 0.05$ - day 28; -13.46%, $p < 0.05$ - day 30); RUB (-111.85%, $p < 0.05$ - day 30) (Figure 3).

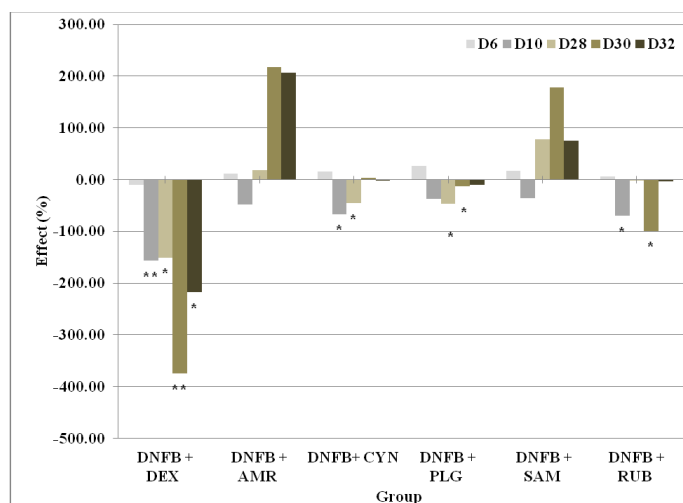


Figure 3.

Effects of treatment with DEX and vegetal extracts on contact hypersensitivity induced by DNFB

Data are expressed as percentage vs. DNFB group (* $p < 0.05$; ** $p < 0.01$).

The experimental results show a possible potential of dry extracts of *Cynara scolymus*, *Plantago lanceolata* and *Rubus idaeus* in use as a topical treatment in diseases with allergic component (Figures 2 and 3). There were no significant differences between the right and left ear thickness in the groups treated with vegetal extracts. Only for the group DNFB + DEX were recorded decreases in the right ear thickness compared with their original size (statistically significant at days 26, 28, 30 and 32). This was correlated with decreasing body weight, which only occurred in this group. Vegetal extracts used did not cause changes in body weight.

Conclusions

We assessed the potential of the vegetal extracts of *Amaranthus hypochondriacus*, *Plantago lanceolata*, *Cynara scolymus*, *Sambucus nigra* and *Rubus idaeus* as a therapeutic benefit on allergic contact dermatitis. Repeated application of DNFB induced ear swelling and ear erythema, which are the stamps of skin inflammation. However, treatment with DEX suppressed the increase in ear thickness induced by DNFB.

Vegetal extracts of *Plantago lanceolata*, *Cynara scolymus* and *Rubus idaeus* (300 $\mu\text{g}/20 \mu\text{L}$), applied topically, occasionally reduced the delayed hypersensitivity reaction. This may attempt to further use extraction fractions of these extracts in order to identify the active principles useful in reducing allergic dermatitis.

Extracts of *Amaranthus hypochondriacus* and *Sambucus nigra* (300 $\mu\text{g}/20 \mu\text{L}$), applied topically, did not show efficacy in reducing allergic dermatitis.

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References

1. Badea M., Pătrașcu F., Cerc Korošec R., Bukovec P., Raita M., Chifiriuc M.C., Maruțescu L., Bleotu C., Velescu B.Ș., Marinescu D., Uivarosi V., Olar R., Thermal, spectral, magnetic and biologic characterization of new Ni(II), Cu(II) and Zn(II) complexes with a hexaazamacrocyclic ligand bearing ketopyridine moieties. *JTAC*, 2014; 118(2): 1183-1193.
2. Burlando B., Verotta L., Cornara L., Bottini-Massa E., Herbal Principles in Cosmetics: Properties and Mechanisms of Action. CRC Press, Boca Raton, 2010; 37.
3. Ciotu I.C., Lupuliasa D., Chiriță C., Zbarcea C.E., Negreș S., The antihyperalgesic effect of memantine in a rat model of paclitaxel induced neuropathic pain. *Farmacia*, 2016; 64(5): 809-812.
4. Christensen A.D., Skov S., Haase C., The role of neutrophils and G-CSF in DNFB-induced contact hypersensitivity in mice. *Immun. Inflamm. Dis.*, 2014; 2(1): 21-34.
5. Corsini A.C., Bellucci S.B., Costa M.G., A simple method of evaluating delayed type hypersensitivity in mice. *J. Immunol. Meth.*, 1979; 30(2): 195-200.
6. Curzytek K., Kubera M., Majewska-Szczepanik M., Szczepanik M., Marcińska K., Ptak W., Duda W., Leśkiewicz M., Basta-Kaim A., Budziszewska B., Lasoń W., Maes M., Inhibition of 2,4-dinitrofluorobenzene-induced contact hypersensitivity reaction by antidepressant drugs. *Pharmacol. Rep.*, 2013; 65(5): 1237-1246.

7. Dinu M., Olaru O.T., Dune A., Popescu C., Nitulescu G.M., Ancuceanu R.V., The obtaining and characterization of a rich-phenolic extract from *Amaranthus hypochondriacus* L. *Rev. Chim. (Bucharest)*, 2016; 67(5): 880-883.
8. Dinu M., Anghel A.I., Olaru O.T., Seremet O.C., Calalb T., Cojocaru-Toma M., Negreş S., Hovanet M.V., Zbarcea C.E., Ancuceanu R.V., Toxicity investigation of an extract of *Amaranthus retroflexus* L. (*Amaranthaceae*) leaves. *Farmacia*, 2017; 65(2): 289-294.
9. Eschler D.C., Klein P.A., An evidence-based review of the efficacy of topical antihistamines in the relief of pruritus. *J. Drugs Dermatol.*, 2010; 9(8): 992-997.
10. Gaspari A.A., Katz S.I., Contact hypersensitivity. *Curr. Protoc. Immunol.*, 2001; Chapter 4: Unit 4.2.
11. Hong J., Buddenkotte J., Berger T., Steinhoff M., Management of itch in atopic dermatitis. *Semin. Cutan. Med. Surg.*, 2011; 30(2): 71-86.
12. Hovaneţ M.V., Oprea E., Ancuceanu R.V., Duţu L.E., Budura E.A., Şeremet O., Ancu I., Moroşan E., Wound healing properties of *Ziziphus jujube* Mill. leaves. *Rom. Biotech. Lett.*, 2016, 21(5): 11842-11849.
13. Kim S.R., Choi H.S., Seo H.S., Ku J.M., Hong S.H., Yoo H.H., Shin Y.C., Ko S.G., Oral administration of herbal mixture extract inhibits 2,4-dinitrochlorobenzene-induced atopic dermatitis in BALB/c mice. *Mediators Inflamm.*, 2014; 2014: 1-10.
14. Lee H.J., Jo S., Ryu J., Jeong H.S., Lee G., Ryu M.H., Jung M.H., Kim H., Kim B.J., Effects of *Schisandra chinensis* Turcz. fruit on contact dermatitis induced by dinitrofluorobenzene in mice. *Mol. Med. Rep.*, 2015; 12(2): 2135-2139.
15. Lim Y.M., An S.J., Kim H.K., Kim Y.H., Youn M.H., Gwon H.J., Shin J., Nho Y.C., Preparation of hydrogels for atopic dermatitis containing natural herbal extracts by gamma-ray irradiation. *Radiat. Phys. Chem.*, 2009; 78(7): 441-444.
16. Noh S.U., Cho E.A., Kim H.O., Park Y.M., Epigallocatechin-3-gallate improves *Dermatophagoides pteronissinus* extract-induced atopic dermatitis-like skin lesions in NC/Nga mice by suppressing macrophage migration inhibitory factor. *Int. Immunopharmacol.*, 2008; 8(9): 1172-1182.
17. Novak N., New insights into the mechanism and management of allergic diseases: atopic dermatitis. *Allergy*, 2009; 64(2): 265-275.
18. Olaru O.T., Venables L., van de Venter M., Niţulescu G.M., Margina D., Spandidos D.A., Tsatsakis A.M., Anticancer potential of selected *Fallopia Adans* species. *Oncol. Lett.*, 2015; 10(3): 1323-1332.
19. Schneider L., Tilles S., Lio P., Boguniewicz M., Beck L., LeBovidge J., Novak N., Atopic dermatitis: A practice parameter update 2012. *J. Allergy Clin. Immunol.*, 2013; 131(2): 295-299.
20. Stef D.S., Iosif G., Ioan-Trasca T., Stef L., Pop C., Harmanescu M., Biron R., Pet E., Evaluation of 33 medicinal plant extracts for the antioxidant capacity and total phenols. *J. Food Agric. Environ.*, 2010; 8(3-4): 207-210.
21. Steinhoff M., Cevikbas F., Yeh I., Chong K., Buddenkotte J., Ikoma A., Evaluation and management of a patient with chronic pruritus. *J. Allergy Clin. Immunol.*, 2012; 130(4): 1015-1016.
22. Suga H., Sugaya M., Miyagaki T., Ohmatsu H., Okochi H., Sato S., CXCR3 deficiency prolongs Th1-type contact hypersensitivity. *J. Immunol.*, 2013; 190(12): 6059-6070.
23. Sung Y.Y., Yang W.K., Lee A.Y., Kim D.S., Nho K.J., Kim Y.S., Kim H.K., Topical application of an ethanol extract prepared from *Illicium verum* suppresses atopic dermatitis in NC/Nga mice. *J. Ethnopharmacol.*, 2012; 144(1): 151-159.
24. Viskelis P., Rubinskienė M., Bobinaitė R., Dambrauskienė E., Bioactive compounds and antioxidant activity of small fruits in Lithuania. *J. Food Agric. Environ.*, 2010; 8(3-4): 259-263.