

CHARACTERISATION, PHARMACOTOXICOLOGICAL AND BIOCHEMICAL STUDIES ON *MORUS ALBA* L. EXTRACT AND ITS FRACTIONS

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Abstract

The aim of the present study was the evaluation of the anti-allergic properties of an extract from mulberry (*Morus alba* L.) leaves and several fractions obtained by open column fractionation. The extract and its fractions were characterized by polyphenolic content, and their toxicity was evaluated using two methods: *Triticum aestivum* and *Daphnia magna* bioassays. The total extract was obtained from mulberry leaves by extraction with 50% ethanol. Six major fractions (A-F) were obtained using column fractionation. Because of the low yield of fractions A and B, they weren't further characterized. The IR analyses revealed a large band close to 3100 cm⁻¹, indicating the presence of polyphenols. Chlorogenic acid, p-coumaric acid, caffeic acid, epicatechin, rutin and kaempferol were identified by HPLC analyses in the extract and its fractions. The quantitative analysis showed a content of total polyphenols in the range of 2.64 to 7.33 mg, as gallic acid equivalents/g dry extract (DM) and a total flavonoid content of 0.95 to 2.39 mg, as quercetin equivalents/g. The acute toxicity assessment revealed a low toxic effect for the total extract and all tested fractions, in both *Triticum aestivum* and *Daphnia magna* models. The topical anti-allergic effect and the serum histamine levels were assessed in an experimental model of contact hypersensitivity induced by 2,4-dinitrofluorobenzene (DNFB) in BALB/c mice, over a period of 14 days. The total extract and fraction F reduced the hypersensitivity response all throughout the treatment and the most prominent antihistaminic effect was recorded for the groups of animals treated with C and D fractions and the total extract of *Morus alba* L.

Rezumat

Scopul acestui studiu a fost evaluarea proprietăților antialergice ale unui extract obținut din frunzele de dud (*Morus alba* L.) și a mai multor fracții obținute prin fracționare pe coloană deschisă. Extractul și fracțiunile acestuia au fost caracterizate prin conținut polifenolic, iar toxicitatea lor a fost evaluată utilizând două metode: testul *Triticum aestivum* și testul *Daphnia magna*. Extractul total a fost obținut din frunze de dud prin extracție cu etanol 50%. S-au obținut șase fracții majore (A-F), utilizând fracționarea pe coloană. Din cauza randamentului scăzut, fracțiunile A și B nu au fost caracterizate în continuare. În urma analizelor IR s-a evidențiat o bandă largă în apropiere 3100 cm⁻¹, acest lucru indicând prezența polifenolilor. Analizele HPLC ale extractului și ale fracțiilor sale au pus în evidență acidul clorogenic, acidul p-cumaric, acidul cafeic, epicatechina, rutina și kaempferolul. Analiza cantitativă a arătat un conținut de polifenoli totali cuprins între 2,64 - 7,33 mg, exprimați în echivalenți de acid galic/g extract uscat și un conținut total de flavonoizi cuprins între 0,95 - 2,39 mg, exprimați în echivalenți de quercetină/ g extract uscat. În urma evaluării toxicității acute s-a observat un efect toxic scăzut pentru extractul total și fracțiile sale, atât în cazul testului *Triticum aestivum*, cât și a testului *Daphnia magna*. Efectul antialergic și nivelele serice de histamină, consecutive administrării topice, au fost evaluate într-un model experimental de hipersensibilizare de contact, indusă de 2,4-dinitrofluorobenzen (DNFB), la șoareci BALB/c, pe o perioadă de 14 zile. Extractul total și fracțiunea F au redus răspunsul de hipersensibilitate pe tot parcursul tratamentului, iar efectul antihistaminic cel mai pregnant a fost înregistrat în cazul grupurilor de animale tratate cu fracțiunile C și D și cu extractul total de *Morus alba* L.

Keywords: atopic dermatitis, 2,4-dinitrofluorobenzene, BALB/c mice, *Morus alba* L., extract fractionation, polyphenols, histamine

Introduction

Allergic contact dermatitis (ACD) is an inflammatory condition that is characterized by a cell mediated

hypersensitivity reaction in the skin. The substances responsible for contact dermatitis, after single or multiple exposures, are non-protein chemicals, that react with self-proteins, resulting in a hapten-specific

immune response that induce skin inflammation, erythema, oedema, warmth, vesiculation and pruritus [8, 18].

ACD affects up to 20% of children and 3% of adults and the prevalence is still increasing, especially in low-income countries. Treatment achieves symptom control rather than cure and is frequent associated with cutaneous sensitization [10, 13]. Thus, there is a strong need to identify alternatives for ACD treatment. Histamine is an endogenous short-acting biogenic amine synthesized from the amino acid histidine and is widely distributed throughout the body. Histamine is an important mediator of immediate-type-allergic reactions. Usually, there is a minimal amount of histamine circulating in the human body. An allergic reaction can occur when a substance enters the body or there is a topical contact with a specific allergenic agent [17]. In response to the molecule, the organism will then release larger quantities of histamine, which starts a cascade of events and triggers an immune response. When histamine is released, part of the cascade of events generates inflammation [14].

Elevated plasma and tissue histamine levels have been frequently reported during anaphylaxis and experimental systems investigating the allergic responses of the skin and airways.

Topical administration of plant extracts containing polyphenols has been proven beneficial in the treatment of dermal allergy [19]. *Morus alba* L. is a well-known medicinal plant from the *Moraceae* family. The plant is found in temperate and sub-tropical regions, mainly in Northern hemisphere [5]. The leaves of this species are used since ancient times in China for silkworms growing, whereas the fruits are used mainly in alimentation [20]. Mulberry leaves, bark and fruits were traditionally used in medicine to treat fever, to protect the liver, improve eyesight, inflammation of the throat, cough, hypertension, and sleep disorders [16]. In the last decades, the leaves and fruits were intensively studied for their therapeutic properties which include anti-diabetic, antimicrobial, anticancer and antiulcer effects, with promising results [4, 21]. Moreover, these plant materials showed promising antioxidant properties which explained some of the biological activities. In a recent study on NC/Nga mice with induced atopic dermatitis, the treatment with a crude extract from mulberry leaves showed promising results [9]. Based on this research, in the present work we evaluated the anti-allergic topic effect of a total extract from mulberry leaves and its several fractions using the 2,4-dinitrofluorobenzene atopic-dermatitis model on BALB/c mice. The extract and its fractions were characterized by polyphenolic content and their toxicity was evaluated using two methods: *Triticum aestivum* and *Daphnia magna* bioassays. Considering the physiologic reactions consecutive to an allergic cascade, we performed the fluorometric quantitative

determination of serum histamine, in order to assess the biologic effects of *Morus alba* L. total extract and fractions.

Materials and Methods

Plant material and extract preparation

The extract was obtained from *Morus alba* L. leaves harvested in May - July from an ecological culture, (Hofigal SA, Bucharest, Romania, 2015). The leaves were dry under laboratory conditions (25°C, dark) and grounded. The extraction was performed using a 1:1 mixture of ethanol:water, and the plant material: solvent ratio was 1:10. After extraction under reflux for 30 min, the extractive solution was filtered and the plant material used for another extraction. The combined extractive solutions were concentrated at 40°C under vacuum using a rotary evaporator (RVO 004; Ingos, Czech Republic) and then lyophilized at -55°C (CoolSafe ScanVac 55; LaboGene, Denmark).

Extract fractionation

The total extract (TE) was subjected to column separation (silicagel-60 70 - 230 mesh, 63 - 200 µm) using diethyl ether, ethyl acetate, methanol, and 1% acetic acid in methanol. Fractionation was performed based on the UV-VIS spectra of each 100 mL fraction using the following solvents diethyl ether: ethyl acetate, ethyl acetate: methanol and methanol: 1% acetic acid in methanol, in step gradient (100:0, 25:75, 50:50, 75:25 and 0:100). The fractions with similar UV-VIS spectra were combined resulting afterwards 11 fractions [11]. A TLC (thin layer chromatography) analysis was performed on silicagel F60₂₅₄ 20 x 20 cm TLC plates (Merck) using as mobile phase a 72:7:7:14 mixture of ethyl acetate, anhydrous acetic acid, formic acid and water [12]. Based on the TLC results, the 11 fractions were further combine to yield six fractions and were tagged as: A (soluble in diethyl ether), B (soluble in diethyl ether: ethyl acetate and ethyl acetate), C (soluble in ethyl acetate), D (soluble in ethyl acetate: methanol), E (soluble in ethyl acetate: methanol) and F (methanol: 1% acetic acid in methanol). The fractions were concentrated at 40°C under vacuum using a rotary evaporator (RVO 004; Ingos, Czech Republic) and then lyophilized at -55°C (CoolSafe ScanVac 55; LaboGene, Denmark). The yields for fractions A and B were under 2% and therefore there were not included in further tests.

Chemical characterization of the extract and fractions

Chemical characterization was performed using qualitative methods, like FT-IR and HPLC fingerprint analysis and quantitatively by spectrophotometric determination of the total phenolic content.

Infrared analysis

Fourier Transform Infrared (FT-IR) spectra were measured according to the method described in the literature [3]. The FT-IR spectra were recorded in

duplicate using a JASCO FT/IR-4200 spectrometer with an ATR PRO450-S accessory, on the spectral range of 4000-400 cm^{-1} and a resolution of 4 cm^{-1} .

HPLC analysis

The HPLC analysis was performed using a Zorbax SB-18 analytical column 150 mm x 4.6 mm i.d. 3.5 μm particle (HiChrom) operated at 25°C and the mobile phase (phase A: methanol: acetonitrile 1:1 with 1% formic acid; phase B: water with 1% formic acid) in linear gradient. A 1 mL/min flow rate and a 20 μL injection volume were used. The detection was performed at 369 nm for flavonoids and at 320 nm for phenolic acids. The extractive solutions samples were mixed at a concentration of 10 mg/mL with the mobile phase and filtered through a 0.22 μm filter [3].

Total phenolic and flavonoid content

Total phenolic content was determined at 750 nm (spectrophotometer Halo DB-20-220 Dynamica Precisa, Germany) according to Folin - Ciocâlteu method and the total flavonoid content was measured by AlCl_3 method at 429 nm [15]. All determinations were performed in triplicate and expressed as the means $\pm$ standard deviation of the mean (SEM) of the experiments in mg gallic acid equivalents (GA equiv.) per gram of dry extract (DM) for total phenolic content and in quercetin equivalents (Q equiv.) per gram of dry extract (DM) for total flavonoid content, respectively.

Acute toxicity assessment

The extract (TE) and its fractions (C-F) were used in concentrations ranging from 50 to 1500 $\mu\text{g/mL}$ prepared in 1% DMSO distilled water solution (*Triticum* test) and a 1% DMSO medium solution (*Daphnia magna*) [1, 15].

Triticum bioassay

The biological material consisted of 20 wheat caryopses/each sample pre-soaked in distilled water for 24 h at $25 \pm 1^\circ\text{C}$, 75% humidity, in a plant room chamber (MLR 351H, Sanyo, Japan). After 24 h of exposure, the embryonic root length was measured using the application Image J version 1.46r (Wayne Rasband National Institutes of Health, USA). The values of root elongation were expressed in mm. The karyokinetic film modifications were observed using a Nikon Labophot 2 microscope (ocular 10 $\times$, ob. 40 $\times$, 100 $\times$) (Nikon, Chiyoda-ku, Tokyo, Japan) after staining the primary wheat root meristems according to the protocol described by Apostol *et al.* [1].

Daphnia magna bioassay

The bioassay was performed using the protocol described in literature [6] with some modifications [15]. The daphniids were exposed for 24 h to the samples in the same conditions described above for *Triticum* bioassay. Each determination was performed in duplicate, using 10 daphniids in cell culture 12-well plates. The experiment was performed in duplicate.

The daphniids were considered dead only if they did not move their appendages for 30 s during observations.

Data analysis

The inhibition effect (Efi%) and lethality percentage (L%) were calculated and IC₅₀ and LC₅₀ were calculated by interpolation on logarithm of the concentrations and the biologic effect curves plotted using the least squares fit method. All calculations were performed using GraphPad Prism version 5.01 software (GraphPad Software, Inc., La Jolla, CA, USA).

Anti-allergic effect evaluation

Animals. Female 6 - 8 week-old BALB/c mice (21.12 ± 1.21 g, n = 45) were purchased from "Cantacuzino" INCDMI (Bucharest, Romania) and housed under specific conditions with a 12 h light/dark cycle and free access to standard rodent food and water. After arrival, they were allowed to acclimatize for about a week before they were used in the experiment. The temperature and relative humidity were continuously monitored using an electronic hygro-thermometer. The temperature was 21°C - 24°C and the relative humidity was 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.

Experimental protocol. Atopic dermatitis was induced by the use of 2,4-dinitrofluorobenzene (DNFB) [7, 22]. The animals were divided into 9 groups (n = 5). Hypersensitivity was induced in all animals (except control group) by applying 20 μL 0.5% DNFB (Sigma-Aldrich, Germany), dissolved in acetone:olive oil, 4:1 (AOO), onto the mice shaved backs (D_0). Six days after sensitization (D_5), each mouse received 10 μL x 2 DNFB (0.2% in AOO) onto the ventral and dorsum of left ear. The process was repeated every 2 days, until day 13 of the experiment. The control animals received with AOO alone. Twenty-four h later (D_6 , D_8 , D_{10} , D_{12} and D_{14}), the right and left ears thickness was determined by using a digital micrometer, with a sensitivity of 0.01 mm (Brüder Mannesmann Werkzeuge, Germany).

Between day 5 and 13 of the experiment, the animals received (2 hours after application of the allergen, respectively after measuring the thickness of the ears), onto the ventral and dorsum of left ear, 2 x 10 μL of: AOO for the control (C) and positive control group (DNFB), 0.375% solution of dexamethasone (Zentiva, Romania) in AOO, for the reference group (DNFB + DEX) and 1.5% suspension of the plant extracts in AOO, for the tested groups (DNFB + TE, DNFB + B, DNFB + C, DNFB + D, DNFB + E, DNFB + F).

At the end of the experiment, blood was collected from the test animals and further used for biochemical determinations.

The fluorometric assay of serum histamine

Briefly, serum histamine was determined using a fluorometric method: histamine reacts with ophthalaldehyde (OPA) at alkaline pH to form fluorescent conjugation products, having the excitation wavelength at 360 nm and emission at 450 nm. The reaction is stopped upon acidification with sulfuric acid [2].

Extraction of histamine from total blood. The total blood collected from the test animals was treated with 0.4 N HClO₄ (v/v) and centrifuged for 10 min., at 1400g. The supernatant was treated with 5N NaOH and n-butanol, followed by centrifugation at 1400g. The obtained supernatant was treated, in the next step, with H₂SO₄ 0.1 N and n-heptane, and after centrifugation, the aqueous layer was used for further procedures.

The fluorimetric assay of histamine. The samples to be analysed were treated with 1N NaOH in a ratio of 5/1 (v/v), then with 1% OPA (in methanol, prepared *ex tempore*) in 20/1 (v/v) ratio. The fluorescence intensity of the samples was registered at $\lambda_{\text{ex}} = 360$ nm, $\lambda_{\text{em}} = 450$ nm. The histamine calibration curve was also performed using standard histamine (Sigma-

Aldrich, Germany) in the concentration range of 0.02 µg/mL - 1 µg/mL.

Statistical analysis. The normality was established by the use of Kolmogorov-Smirnov test. ANOVA or Kruskal-Wallis tests, followed the appropriate post-tests, were 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 version 5.01 software.

Results and Discussion

Chemical characterization of total extract and fractions

Infrared analysis. The infrared spectra were used to evaluate the different fractions and the TE fingerprints. The statistically relevant differences between the IR spectra indicate the differences between chemical compositions of each sample. The large band close to 3100 cm⁻¹ was registered for each extract and is considered an indicator of polyphenols content. The IR spectra are presented in Figure 1.

Statistical analysis based on Pearson coefficient (r) of the IR spectra indicate a high degree of correlation for TE and fractions B (r = 0.849), D (r = 0.890), E (r = 0.806) and significant differences between TE and fractions C (r = 0.596) and F (r = 0.600).

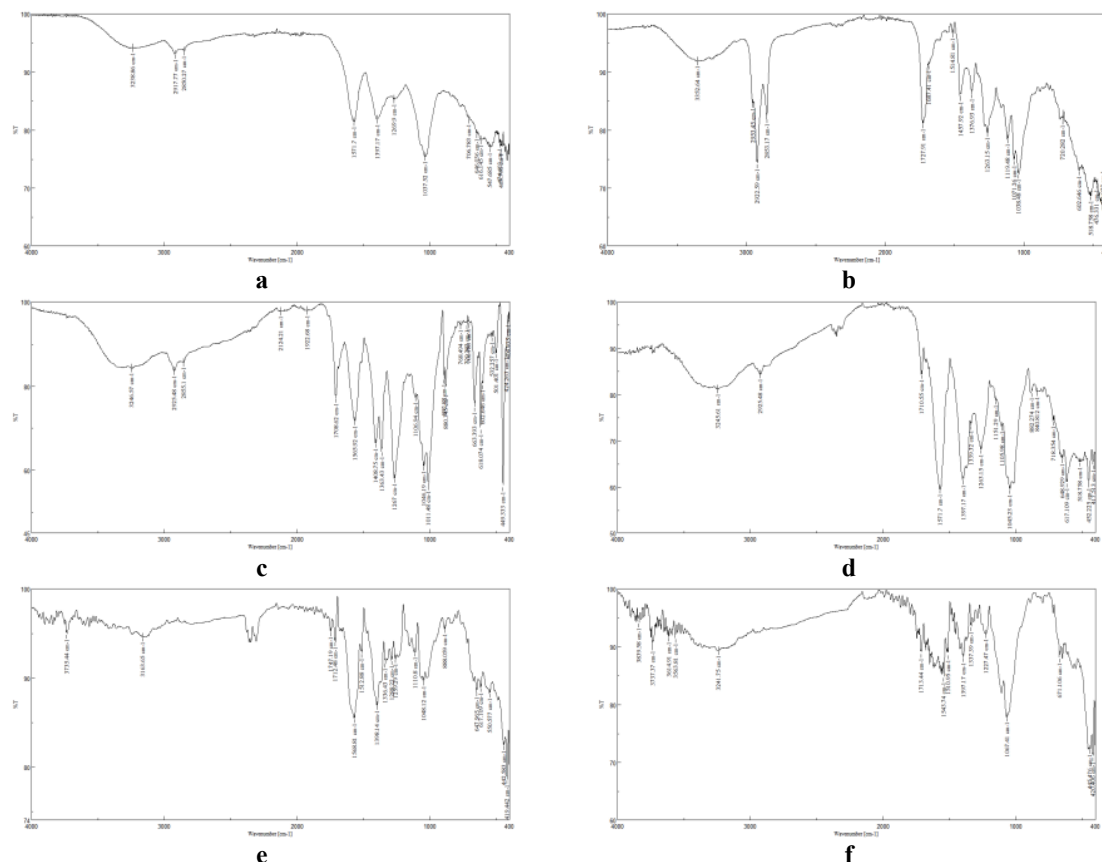


Figure 1.
IR analysis of the TE (a) and its fractions: B (b), C (c), D (d), E (e) and F (f)

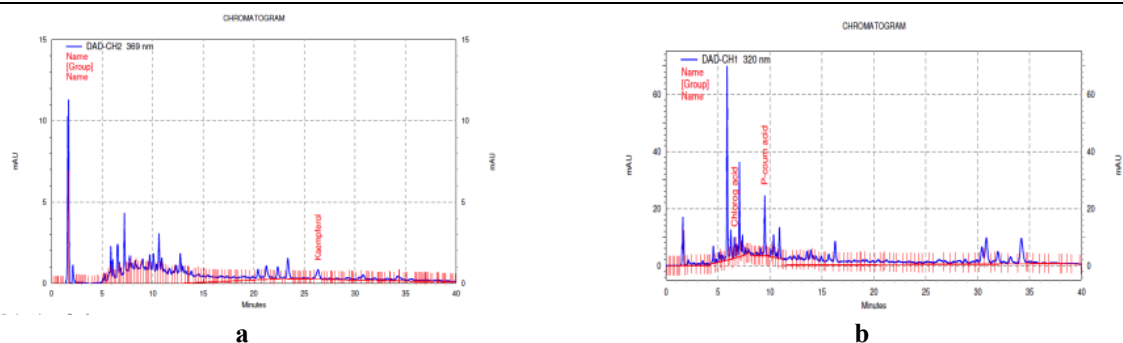


Figure 2.

HPLC chromatogram of the total extract from *Morus alba* L. leaves: a. chromatogram of flavonoids; b. chromatogram of phenolic acids

HPLC analysis. The HPLC analysis revealed the presence of flavonoids and phenolic acids (Table I). In Figure 2 are presented representative chromatograms of TE. The compounds are well - known phenolics and can be used in the future standardization of the extracts. Moreover, the HPLC fingerprint can be used for the identification of the sample

Total phenolic and flavonoid content. The results of the quantitative analysis are presented in Table II. The total phenolic content varied from 2.64 to 7.33 mg GA equiv./g DM and total flavonoids from 0.95 to 2.39 mg Q equiv./g DM. TE showed the highest content of both, total phenolics and total flavonoids. Among fractions, fraction E is the richest in total phenolics and flavonoids.

Table I

HPLC analysis of TE from *Morus alba* L. leaves and its fractions

No.	Sample	Identified compound	RT
1	TE	Chlorogenic acid	6.573
		p-coumaric acid	9.497
		Kaempferol	26.343
2	A	p-coumaric acid	9.483
		Epicatechin	7.573
		Kaempferol	26.307
3	B	Chlorogenic acid	6.543
		Caffeic acid	7.553
		Rutin	9.470
		Kaempferol	26.240
4	C	Chlorogenic acid	6.543
5	D	Chlorogenic acid	6.550
6	E	Chlorogenic acid	6.537
7	F	Chlorogenic acid	6.550
		Kaempferol	19.607
		Quercetin	26.200

Table II

Polyphenols quantitative analysis

Sample	Total flavonoid content (mg Eq Q equiv. /g DM)	Total phenolic content (mg Eq GA equiv./g DM)
TE	2.396 ± 0.130	7.331 ± 0.062
C	0.952 ± 0.026	3.385 ± 0.464
D	1.063 ± 0.263	3.444 ± 0.114
E	1.718 ± 0.042	5.297 ± 0.169
F	1.384 ± 0.084	2.643 ± 0.228

DM = dry extract

Acute toxicity assessment

Triticum bioassay. The Efi% observed for TE varied from 4.5 and 21.16%, thus the toxicity induced is very low. Similar results were obtained after testing fractions D, E and F. Fraction C inhibition curve is presented in Figure 3. Its IC50 could be calculated because it induced a maximum Efi% of 70.96%. Thus, the IC50 of fraction C was 1113.0 µg/mL with a 95% confidence interval of 913 to 1357 µg/mL. Based on IC50 value, fraction C induced a low toxicity. The karyokinetic film modifications observed were in accordance with the Efi% value. Thus, fraction C at the highest concentration induced the

hypertrofiation of the nucleoli and the inhibition of mitosis. The roots exposed at concentrations 500 and 1000 µg/mL presented rare karyokinetic film modifications such as: metaphases, anaphases, telophases in tropokinesis and disorganized metaphases. For all other samples the microscopic examination revealed a similar profile with the untreated control sample.

Daphnia magna bioassay. Lethality curves of the determination are presented in Figure 3. Among the tested samples, the highest toxicity was induced by fraction C (LC50 = 270 µg/mL), followed by TE (LC50 = 962 µg/mL), fraction F (LC50 = 1031 µg/mL)

and E ($LC_{50} = 1428 \mu\text{g/mL}$). Fraction D showed no toxicity, even at highest concentration. Except for

fraction C, all tested samples presented low or even no toxicity on *D. magna*.

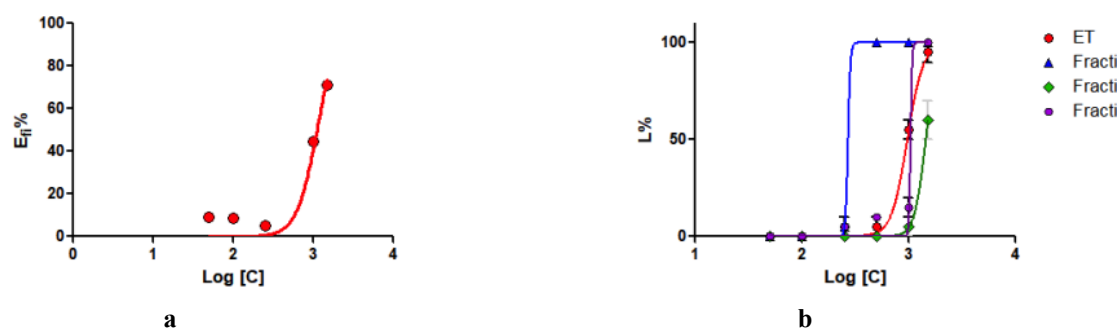


Figure 3.

Results of the acute toxicity evaluation: a. *Triticum aestivum* test - logarithm of concentration - inhibitory effect curves; b. *Daphnia magna* logarithm of concentration - lethality curves

Anti-allergic effect evaluation

For the animals in the control group there were no significant changes in the thickness of the left ear. DNFB produces a hypersensitivity, which is evidenced by the increased thickness of the left ear, for the positive control group, when compared with the control group, starting with day 6 of the experiment. The changes are statistically significant in day 12 (155.58%, $p < 0.001$) and day 14 (212.20%, $p < 0.001$) (Figure 4).

Following topical administration of dexamethasone ($75 \mu\text{g}/20 \mu\text{L}$), only small increases of the left ear thickness, when compared with the control group animals, are observed. When compared with the positive control group, dexamethasone reduced the delayed hypersensitivity reaction. The results were statistically significant from the eighth to the twelfth day (Figure 5).

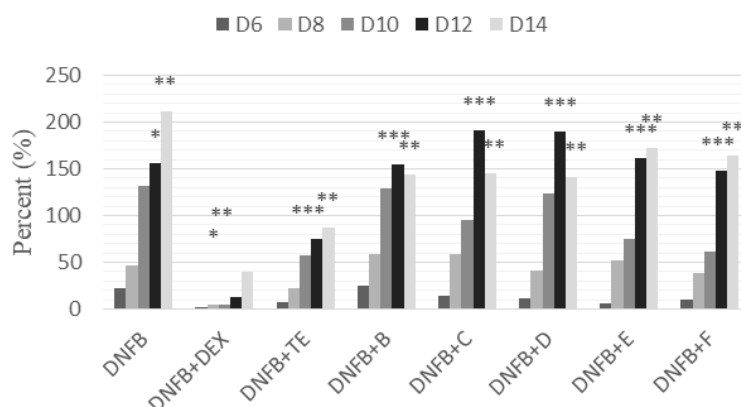


Figure 4.

Modification of left ear thickness during the experiment vs. control group, for the positive control, reference and tested groups and the statistical significance of the Kruskal-Wallis test with Dunn's post-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

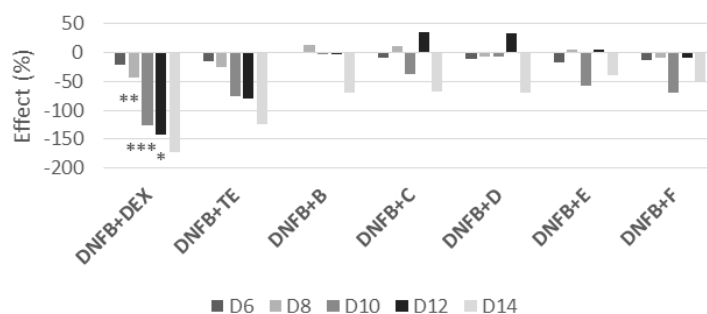


Figure 5.

Effects of treatment with DEX and the vegetal extracts on the hypersensitivity induced by DNFB and the statistical significance of the Kruskal-Wallis test with Dunn's post-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

Following the topical application of the total extract and its five fractions, statistically significant increases in left ear thickness compared to the thickness measured in mice in the control group, were observed, starting from day 12. When compared to the DNFB treated group, a decrease in the left ear thickness throughout the treatment was observed only for animals treated with total extract and fraction F.

For the tested groups, the reduction of the delayed hypersensitivity reaction induced by DNFB, in the last day of the experiment, decreases in the following order: TE (-124.39); D (-70.22%); B (-68.82); C (-66.52%); F (-48.56%) and E (-40.04%).

The experimental results confirm the potential of *Morus alba* L. total dry extract for the topical use in allergic disorders and identifies fractions of this total extract (especially fractions E and F) that contain active principles with therapeutic potential in atopic dermatitis.

The fluorometric quantification of serum histamine

The implication of histamine in allergic reactions is well described, so its assay on the allergic animal model implied in the study, is of high interest.

The murine histamine serum concentrations are expressed as μg histamine/mL blood, and are presented as mean $\pm$ SD in Table III and Figure 6.

Table III

Experimental results for serum histamine in the studied animals

Groups of tested animals	Histamine ($\mu\text{g/mL}$) $\pm$ SD
C	0.044 ± 0.0039
DNFB	0.063 ± 0.0051
DNFB + DEX	0.049 ± 0.0066
DNFB + TE	0.055 ± 0.0054
DNFB + B	0.059 ± 0.0044
DNFB + C	0.051 ± 0.0053
DNFB + D	0.053 ± 0.0058
DNFB + E	0.061 ± 0.0045
DNFB + F	0.057 ± 0.0038

As we can observe, the topical application of 2,4-dinitrofluorobenzene (DNFB) induced a high allergic reaction evidenced by the high serum histamine levels in the positive untreated control animals ($0.063 \pm 0.0051 \mu\text{g}$ histamine/mL, in the DNFB group), compared to the absolute control group ($0.044 \pm 0.0039 \mu\text{g}$ histamine/mL, in the C group) that only received the AOO, so the validity of the used animal model is also confirmed at molecular level. Performing a statistical analysis, the highest significance was evidenced for the group of animals treated with the C fraction, compared to the DNFB group ($p < 0.05$). Following the topical application of the total extract and its five fractions, the histamine serum levels decreased, revealing an anti-allergic pattern. The best results were registered for the C fraction of *Morus alba* L. extract ($0.051 \pm 0.0053 \mu\text{g}$ histamine/mL, in

the DNFB + C group), that counterpoised the allergenic effect of DNFB, and reduced the animals histamine release (Figure 6).

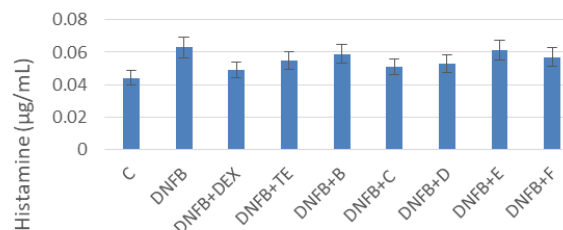


Figure 6.

Histamine serum levels on animals treated with DEX and *Morus alba* L. vegetal extracts after dermal sensitization induced by DNFB topical application

We calculated the percent effect of the administered extracts and the reference substance (dexamethasone) compared to the positive control - DNFB group (Figure 7), and to the absolute control - C group (Figure 8).

The reference substance had the most important anti-histaminic effect: -22.22% compared to DNFB group, closely mimicked by the C (-19.05%) and D (-15.87%) fractions and also by the total extract of *Morus alba* L. (-12.70%).

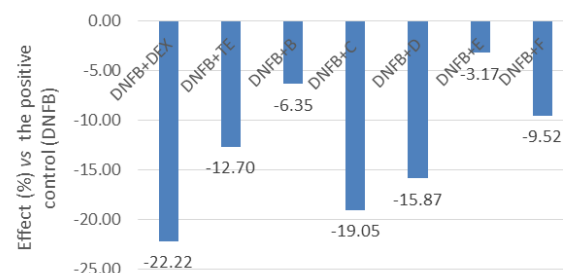


Figure 7.

The effect of the treatment with DEX and the *Morus alba* L. vegetal extracts on the histamine serum levels induced by DNFB topical application, compared to the positive control group of animals

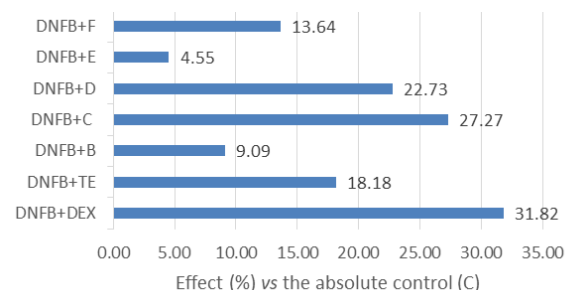


Figure 8.

The effect of the treatment with DEX and the *Morus alba* L. vegetal extracts on the histamine serum levels induced by DNFB topical application, compared to the absolute control group of animals

The animals that received the treatment with the C fraction of the extract (DNFB + C group) displayed the highest antihistaminic activity, besides the reference substance, depicted by its effect compared to the absolute control-C group of animals: 27.27%. *Morus alba* L. total extract and its fractions evidenced antihistaminic potential in the used experimental

model of atopic dermatitis. Their effects are, to a certain extent, very similar to that of dexamethasone, a classic anti-inflammatory agent from the glucocorticoids class, fact also revealed in Figure 9, as a comparison between the vegetal extracts and the synthetic glucocorticoid.

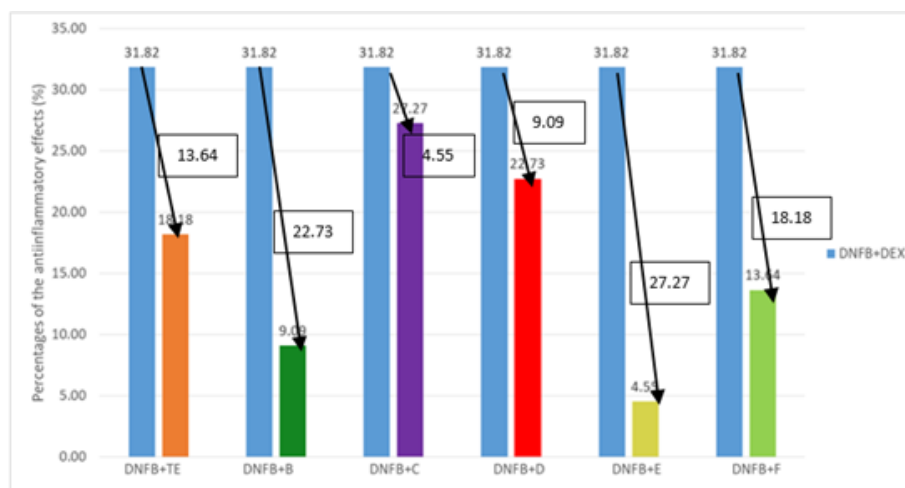


Figure 9.

Effects of the treatment with *Morus alba* L. vegetal extracts on histamine release after the sensitization with DNFB, as a comparison with the effect exerted by the reference anti-inflammatory agent, dexamethasone

The obtained results confirm the potential of *Morus alba* L. total dry extract as an antihistaminic potential candidate for the topical use in allergic disorders together with its fractions, from which fraction C proved the highest biologic activity.

Conclusions

In the present work we evaluated the polyphenolic content, toxicity and anti-allergic effect, after topical administration, of a total extract from mulberry leaves and its several fractions. The total extract showed the highest content of both, total phenolics and total flavonoids. Among fractions, fraction E was the richest in total phenolics and flavonoids. Except for fraction C, all tested samples presented low or even no toxicity in *Triticum aestivum* and *Daphnia magna* tests. The total extract and the E and F fractions exhibited anti-allergic properties, in the experimental model of atopic dermatitis, that uses the 2,4-dinitro-fluorobenzene, on BALB/c mice. Considering the antihistaminic potential of the employed extracts, the best results were displayed by fractions C, D and the *Morus alba* L. total extract.

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