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Anti-inflammatory activity of American yam *Dioscorea trifida* L.f. in food allergy induced by ovalbumin in mice

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ABSTRACT

Tropical ecosystems are particularly rich in edible plant species with different bioactive substances. Among the plants with promising benefits for health are species from the genus *Dioscorea* (Dioscoreaceae), especially those named yam. Recent studies have shown the beneficial effects of different species of *Dioscorea*, and its main constituent, diosgenine, in the treatment of food allergy. In this study we evaluated the potential of *D. trifida*, the only yam native from South America, in the treatment of ovalbumin (OVA) induced food allergy in Balb/c mice. HPLC/DAD analysis showed the presence of three very distinctive groups of natural products in extracts and fractions: (I) very polar substances, including allantoin, (II) phenolic substances as flavonoids and phenolic acids and (III) diosgenin and derivatives. Sensitive mice received casein feed with supplementation of crude extract (CE) and fractions. The supplementation with all products from *D. trifida* reduced IGE, intestinal oedema and mucus production, parameters observed in OVA allergic mice. The results showed the potential of this food to prevent or treat this disease and the necessity to be better explored.

1. Introduction

The number of food allergy cases has increased over the last few decades in both developed and developing countries. There is no effective treatment for this condition and bioactive substances, present in vegetable foods, are considered to be an important alternative to management and/or treatment. Tropical ecosystems are particularly rich in edible plant species with different bioactive substances and constitute one-third of the botanical biodiversity of the planet (Cardellina, 2002; Desmarchelier, 2010; Devalaraja, Jain, & Yadav, 2011; Oliveira, Yamada, Fagg, & Brandão, 2012). Among the foods with promising benefits are species from the genus *Dioscorea* (Dioscoreaceae), commonly named yam.

The tubercles of the yam have been used in the human diet for centuries. Many species have economic importance, due the presence of diosgenin, which is used as a prototype for oral contraceptive in the pharmaceutical development (Hata, Reguero, Garcia, Buitrago, & Alvarez, 2003). The most important species cultivated for their edible tubers include *D. alata*, originating from Asia, *D. cayenensis* and *D. rotundata* from West Africa, and *D. trifida* from tropical America. The

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tubercles of these species contain several nutrients, including carbohydrates, amino acids, minerals, tiamine, riboflavin, niacine and ascorbic acid. Diosgenin and allantoin constitute the mainly secondary bioactive metabolites (Huang, Ku, & Jan, 2009; Yang, Lu, & Hwang, 2003), and have also been shown to possess anti-inflammatory and immunomodulatory properties (Lin & Yang, 2008; Jan, Wey, Kuan, Liau, & Wu, 2007; Lin, 2006; Macpherson & Uhr, 2004). Recent studies showed that *Dioscorea*, and its main constituent, diosgenine, are useful for the treatment of food allergies. Diosgenine reduced IgE and IL-4 production and increased IFN-α levels (Huang, Liu, & Jan, 2010; Huang et al., 2009). In vitro immunomodulatory activity was as well attributed to the alkaloid dioscorine (Liu, Shang, Wang, Hsu, & Hou, 2007), which also promoted the activation of macrophages, NK cells and increased production of IFN-α in vivo (Liu, Liu, Huang, Wang, & Shang, 2009). *D. batatas* (syn. *D. opposita*) is a well-studied species and exhibits antioxidant (Hou et al., 2001; Yang, Yoon, Chin, Park, & Kim, 2009) and immunomodulatory activities (Choi, Koo, & Hwang, 2004), and inhibits the enzyme nitric oxide synthase (NOS) (Jin et al., 2010; Yang et al., 2009).

Different species of yam ("inhame" in Portuguese) are cultivated and used as food in Brazil, but only *D. trifida* is native to South America. This species is cultivated in the northern region of the continent and in the Caribbean. It produces a group of small tubers that are 15–20 cm long. The flesh can be white, yellow, pink or purple (Purseglove, 1972). Nothing is known about the chemical composition and biological potential of this species. Therefore, we evaluated the activity of extracts and fractions obtained from the tubercles of *D. trifida* in the treatment of OVA-induced food allergy in Balb/c mice, and discuss about the participation of the detected bioactive compounds in its activity.

2. Materials and methods

2.1. Plant material

Tubercles of *D. trifida* L.f. were acquired from producers in Boa Vista, Roraima, in north of Brazil, in October of 2009. The samples were identified by J. Paula-Souza, and a voucher was deposited in DATAPLAMT, from UFMG (number DAT-125).

2.2. Extract and fractions preparation

Dried and pulverized tubercles were extracted by percolation with ethanol (80%) and the solution was evaporated in vacuum until dryness resulting in the crude extract (CE). The CE was diluted in water and extracted successively with dichloromethane and butanol saturated with water which resulted in two fractions. These fractions were evaporated to dryness to obtain dichloromethane (DF) and butanol fraction (BF) fractions, respectively. An aqueous fraction (AF) was obtained by freeze drying. The objective of this process was to obtain products enriched in substances of different polarities.

2.3. Chromatographic analysis of the CE and fractions

The CE and the fractions (DF, BF and AF) were analysed by high performance liquid chromatography (HPLC), using an Agilent 1100 with a DAD UV detector. The column used was a Lichrospher C18 (250 um, 4.6 mm, Merck, Darmstadt, Germany) at 30°C, acetonitrile (A): water (B) as mobile phase; gradient elution at 35–100% A for 0–45 min and then at 100% A for 45–50 min. Rebalancing: 100–35% A for 50–55 min, and then 35% A for 55 a 60 min; flow of 1 mL/min. The presence of allantoin and diosgenin in all products was confirmed by the co-analysis with reference standard of these substances (Sigma–Aldrich®, St-Louis, MO, USA). The other substances were identified by analysis of their respective UV spectra at 205 nm. The standards were prepared in acetonitrile/water (1:1) at concentration of 0.5 mg/mL and the products from *D. trifida* at 6 mg/mL. The volume of injection was 40 μL. The quantification of the substances was done by calculation of the area of each peak in the chromatograms.

2.4. Animals

Groups of five female BALB/c mice with weigh between 18 and 20 g, aged 6–8 weeks, were fed a balanced feed diet (casein diet, ANI93G) prepared in the laboratory. The animals were kept in 12/12 h light/dark rhythm and controlled temperature with free access to water and feed in cages with five animals each. The studies were approved by the Ethics Committee for Animal Experimentation at UFMG (CETEA/UFMG, number 010/2010).

2.5. Sensitisation of the mice

On day 0, the mice received a subcutaneous injection of 0.2 ml saline containing 10 μg Ova (five times crystallised hen's egg albumin; Sigma–Aldrich, St. Louis, MO, USA) and 1 mg $Al(OH)_3$, as previously described (Saldanha et al., 2004). A second subcutaneous injection of 10 μg soluble Ova was given on day 14. Seven days later (day 21), the animals were given a solution of Ova 20% administered in the drinking water for a period of 1 week (day 21–28). The non-sensitised group received in the subcutaneous injection only the adjuvant diluted in saline on day 0 and saline on day 14.

2.6. Doses and treatment

The CE and the fractions were added as supplement to the casein diet. CE was added at doses of 100 and 300 mg/kg/day (CE100 and CE300) and AF, BF and DF at 100 mg/kg/day. The control animals (non-treated, NT) received only casein feed without supplementation, and the positive control received casein feed supplemented with dexamethasone (DX-Decadron®) at a dose of 0.4 mg/kg/day (Cara, Conde, & Vaz, 1997). The final dose of substances administered to the animals was calculated by weighting the feed before the consumption and after the consumption of each group every day during the 28 days of experiment.

2.7. Body weight evaluation

Body weight was measured each week, and the results were obtained by calculating the means of the individual values of each group, expressed in grams (g).

2.8. Serum antibody determination

Serum samples used for anti-Ova IgE quantification were obtained from the animal groups sacrificed by cervical dislocation 7 days after oral antigen exposure. Anti-Ova IgE was measured by capture-ELISA using plates coated with rat anti-mouse IgE, as previously described (Russo et al., 2001). The plates were developed with *o*-phenylenediamine and H_2O_2 and were read at 492 nm on an automated ELISA reader (EL800, Bio-Tek Instruments, Inc., Winooski, VT, USA). The data are reported as the mean ± SEM for each group. The serum IgE samples were compared to a positive control serum, which was taken as 1000 arbitrary units (A.U.). Five animals were included in each group. $P < 0.05$ was considered statistically significant.

2.9. Intestinal tissue water content

Duodenum fragments (1 cm) were collected and weighed. The samples were dried at 60 °C for 24 h, and the dry weight was determined by the following formula: water content = (wet weight − dry weight)/dry weight. The data are reported as the mean ± SEM of each group, which was calculated using the following formula: water content = (wet weight − dry weight)/dry weight. Five animals were included in each group. $P < 0.05$ was considered statistically significant.

2.10. Histological analysis

The histological analysis was performed as previously described (Saldanha et al., 2004). Tissue samples were fixed in 10% formalin in PBS, embedded in paraffin and cut into 5-μm-thick sections. The sections were stained with periodic acid Schiff (PAS) for mucus analysis or with toluidine blue to evaluate the number of mast cells.

2.11. Mast cells evaluation

Histological sections stained with toluidine blue, and used to count mast cells were examined under an optical microscope. The count was conducted in 20 randomly selected fields observing at 40× (53.333 μm^2/field) and the result expressed as number of cells/field.

2.12. Evaluation of mucus containing neutral mucin

After staining for histological analysis, a microcamera was used to capture images of three fields in each small intestine section. The images were analysed using Image J software. To determine the caliciform cell volume, all green pixels were selected to create a binarised image and subsequently calculate the total area. The results were expressed in μm^2 P.A.S./Field.

2.13. Eosinophil peroxidase assay

A colorimetric assay for peroxidase was performed using the method described by Silveira et al. (2002). The eosinophil peroxidase (EPO) assay was used as an estimate of eosinophil numbers in intestinal tissue. After flushing the small intestine with 20 ml of PBS, 100 mg of the intestinal tissue was weighed, chopped, and homogenised in PBS 5% (w/v) by using a tissue homogenizer (Power Gen 125; Fisher Scientific, Pittsburgh, PA, USA). The homogenate was centrifuged (3000*g* for 10 min), the red blood cells in the pellet were lysed, and cells were resuspended in PBS (pH 7.4) containing 0.5% hexadecyl trimethyl ammonium bromide (HTAB; Sigma–Aldrich, St. Louis, MO, USA). The cell solution was homogenised again, and the homogenates were then subjected to freeze/thaw three times in liquid nitrogen and were stored at 20 °C until the assay execution. Then, samples of the intestinal tissue were spun down and supernatant was diluted 1:3 in PBS/HTAB. The assay was carried out in 96-well plates (Nalge Nunc International Co., Naperville, IL, USA). Each sample was tested in triplicate by adding 100 μl of the sample/well and 100 μl of OPD substrate (1.5 mM *o*-phenylalanine-diamine (Sigma–Aldrich, St. Louis, MO, USA) and 6.6 mM hydrogen peroxide in 75 mM Tris–HCl, pH 8.0)/well. The reaction was carried out at 20 °C for 30 min and was stopped with 4 M sulphuric acid solution. Plates were read at 492 nm on a microplate reader (Tittertek Multiskan). The data are reported as the mean ± SEM for each group, and the results expressed in arbitrary units (A.U.), $n = 5$ animals/group. $P < 0.05$ was considered statistically significant.

2.14. Periuterin fat, spleen and liver weight evaluation

This analysis was performed to verify whether the loss of body weight was obscured by fluid retention. The periuterin fat, spleen and liver from the mice in each group were collected and weighted to evaluate whether there was any water retention that could interfere with the body weight measurements.

2.15. Statistical analysis

Statistical significance was analysed using a one-way analysis of variance (ANOVA) followed by a Tukey test. Five animals were included in each group. $P < 0.05$ was considered statistically significant. The data are reported as the mean ± SEM. $^{*}P < 0.05$ with respect to the control (Ova−) groups. $^{**}P < 0.05$ compared to the non-treated (NT) allergic (Ova+) group.

3. Results

3.1. Chemical analysis

The analysis of CE by HPLC showed the presence of three very distinctive groups of natural products (Fig. 1a): (I) very polar substances, with retention time (Rt) from 0 to 10 min, including allantoin (Rt 2.14 min, Fig. 1b); (II) a group of phenolic substances, with Rt between 11 and 30 min, among them organic acids and flavonoids and (III) unpolar substances with Rt from 31 to 60 min, constituted by diosgenin (Rt 51.09 min, Fig. 1b) and its derivatives.

The chromatograms and UV spectra of AF, BF and DF showed that these fractions are each one enriched in the different types of substances, which were separated from CE by their polarity. AF is rich in substances with high polarity

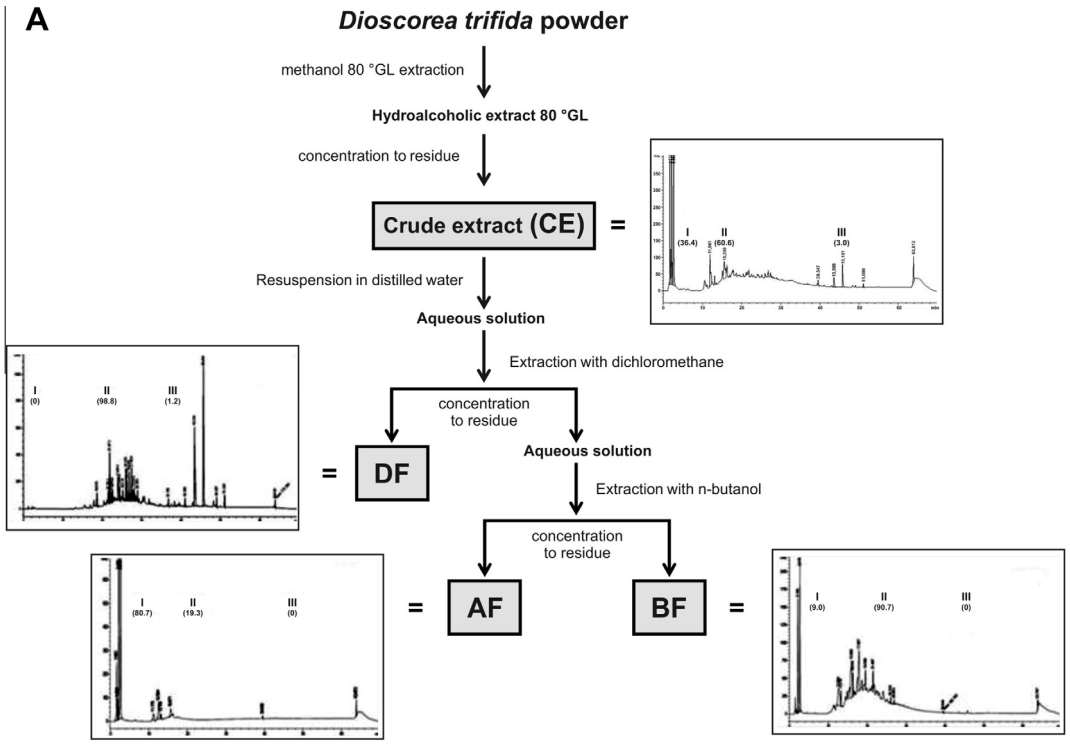

Fig. 1a – Route of obtention of crude extract (CE) and fractions (AF, BF, DF) from *D. trifida* roots, and corresponding chromatograms in HPLC/ DAD. Retention times: (I) Rt 0–10 min: very polar substances, allantoin at Rt 2.14 min; (II) Rt 11–30 min: phenolic substances (organic acids and flavonoids) and (III) Rt 31–60 min: unpolar substances, diosgenin at Rt 51.09 min. Concentrations of each class of substances are between [ ].

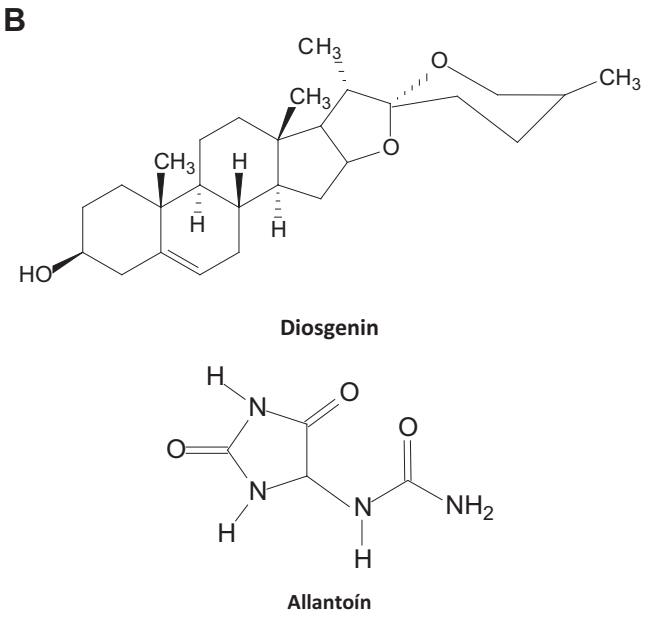

Fig. 1b – Chemical structure of the active components diosgenin and allantoin.

(group I) and does not have diosgenin and its derivatives. DF is rich in diosgenin and derivatives and does not have polar substances. The quantitative results were: CE = 36.6% of substances from group (I), 60.6% from (II) and 3.0% from (III); AF = 80.7% of (I), 19.3% of (II) and 0% of (III); BF = 9.0% of (I) and 90.7% of (II) and 0% of (III); DF = 0% of (I), 98.8% of (II) and 1.2% of (III).

3.2. *Consumption of a casein diet and body weight loss*

After oral challenge with Ova, allergic mice lost approximately 8% of their body weight (data not shown). The treatments did not affect body weight in the non-sensitised groups. In the allergic groups, only DX treatment was able to prevent weight loss.

3.3. *CE and fractions reduce anti-Ova IgE in the serum of allergic mice*

Sensitisation induced the production of anti-Ova IgE in mice. Fig. 2 shows that treatment with CE (CE100, CE300) and AF, BF, DF or DX reduced anti-Ova IgE compared with non-treated (NT) allergic mice ($P < 0.05$).

3.4. *CE and fractions reduce intestinal oedema*

The change in intestinal oedema in the allergic animals compared to that of the non-treated (NT) allergic mice ($P < 0.05$) was of −23% for CE100, −24% for CE300, −31% for AF, −32% for BF, and −30% for DF (Fig. 3).

3.5. *CE and fractions reduce mast cell hyperplasia*

All of the treatments reduced the hyperplasia of mast cells in allergic animals in comparison to non-treated (NT) allergic

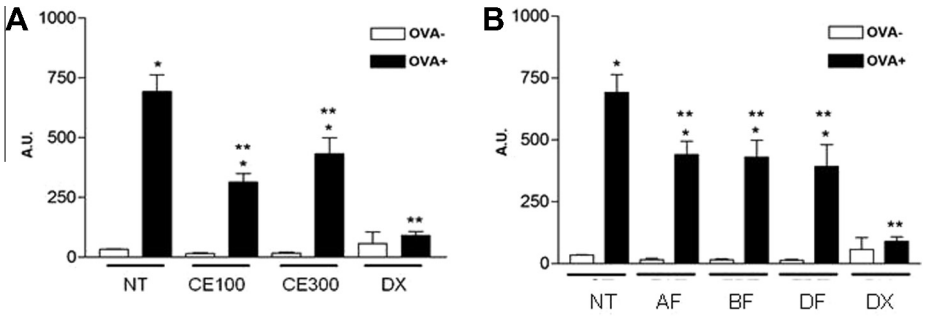

Fig. 2 – Effects of the CE (A) and fractions (B) of *D. trifida* on the serum concentration of anti-Ova IgE of mice sensitised and non-sensitised with OVA. Data are presented as mean ± SD ($n = 5$). $^{*}P < 0.05$ with respect to their respective control (Ova−) groups. $^{**}P < 0.05$ compared to the non-treated (NT) allergic (Ova+) group. NT, non-treated group; CE, animals that receive the crude extract of *D. trifida*; CE100, animals treated with 100 mg/kg/day of the crude extract; CE300 animals treated with 300 mg/kg/day of the CE; DX, animals treated with dexamethasone; AF, animals treated with 100 mg/kg/day of the aqueous fraction; BF, animals treated with 100 mg/kg/day of the buthanol fraction; DF, animals treated with 100 mg/kg/day of the diclorometane fraction.

mice ($P < 0.05$). In the allergic group, the number of mast cell in the intestinal submucosa increased ($P < 0.05$) when compared to the respective control group. The number of mast cells in allergic animals treated with CE (CE100, CE300), AF, BF and DF decreased when compared to NT animals of the allergic group ($P < 0.05$) (Fig. 4).

3.6. *CE and fractions decreased mucus secretion*

Mucus secretion was reduced by 33% for CE100, 31% for CE300, 22% for AF, 20% for BF, 31% for DF, and 38% for DX in comparison with non-treated (NT) allergic mice ($P < 0.05$) (Fig. 5).

3.7. *CE and fractions decrease eosinophil peroxidase*

All treatments were able to decrease eosinophil infiltration in treated animals compared to NT animals ($P < 0.05$) (Fig. 6).

3.8. *CE and fractions do not modify periuterine fat, liver and spleen weight*

No difference was observed ($P < 0.05$) in the liver, periuterine fat and spleen weight for any of the treated animals. Only the animals treated with the positive control DX showed a significant decrease ($P < 0.05$) when compared to NT animals (data not shown).

4. Discussion

The clinical symptoms of food allergy are characterised by a set of immune reactions, including the secretion of cytokines IL-4 and IL-13, which are associated with IgE production (Dourado et al., 2010; Sampson & Wang, 2009). When Ova-sensitised BALB/c mice are given an Ova solution as the only liquid option, they develop local signs of inflammation that are characterised by eosinophil infiltration, oedema, an increase in number of mast cells in the intestinal submucosa, mucus secretion, an increase in serum anti-OVA IgG1 and IgE, and weight loss (Dourado et al., 2011). Recent studies have demonstrated that many vegetal foods can exert beneficial effects on immunopathologies such as food allergy (Costa, Garcia-Diaz, Jimenez, & Silva, 2013; Shen, Hsu, Lee, Chang, & Wu, 2012; Tantoush et al., 2012; Zhang, Yang, Rupa, Jiang, & Minea, 2012). These activities are correlated to different classes of natural bioactive substances.

Firstly, it was necessary to determine which doses of the extracts of *D. trifida* would be suitable for the study. It is

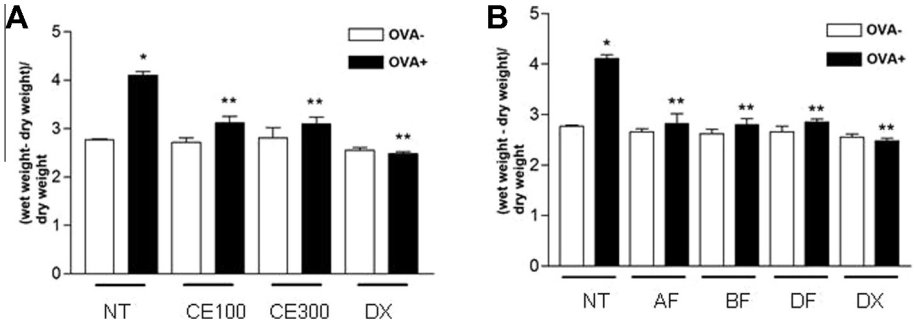

Fig. 3 – Effects of the CE (A) and fractions (B) of *D. trifida* on intestinal oedema of mice sensitised and non-sensitised with OVA. Data are presented as mean ± SD ($n = 5$). $^{*}P < 0.05$ with respect to their respective control (Ova−) groups. $^{**}P < 0.05$ compared to the non-treated (NT) allergic (Ova+) group. The groups are described in the legend to Fig. 2.

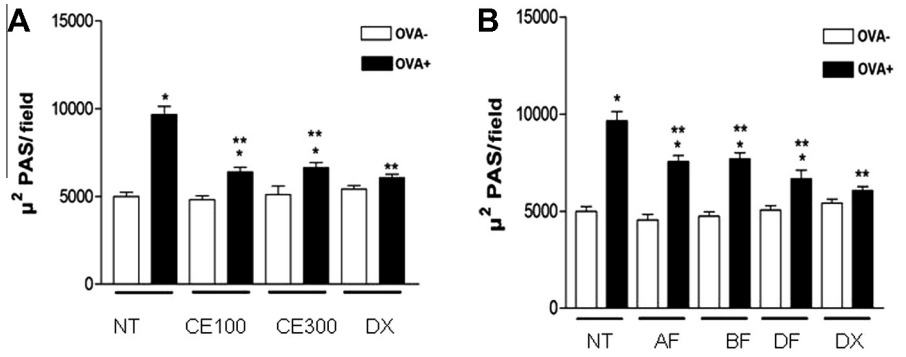

Fig. 4 – Effects of treatment with 100 and 300 mg/kg/day of the CE (A) and 100 mg/kg/day of AF, BF and DF (B) added to the casein feed, from day −1 to day 28 on mast cell hyperplasia. Data are presented as mean ± SD (n = 5). $^{*}P < 0.05$ with respect to their respective control (Ova−) groups. $^{**}P < 0.05$ compared to the non-treated (NT) allergic (Ova+) group. The groups are described in the legend to Fig. 2.

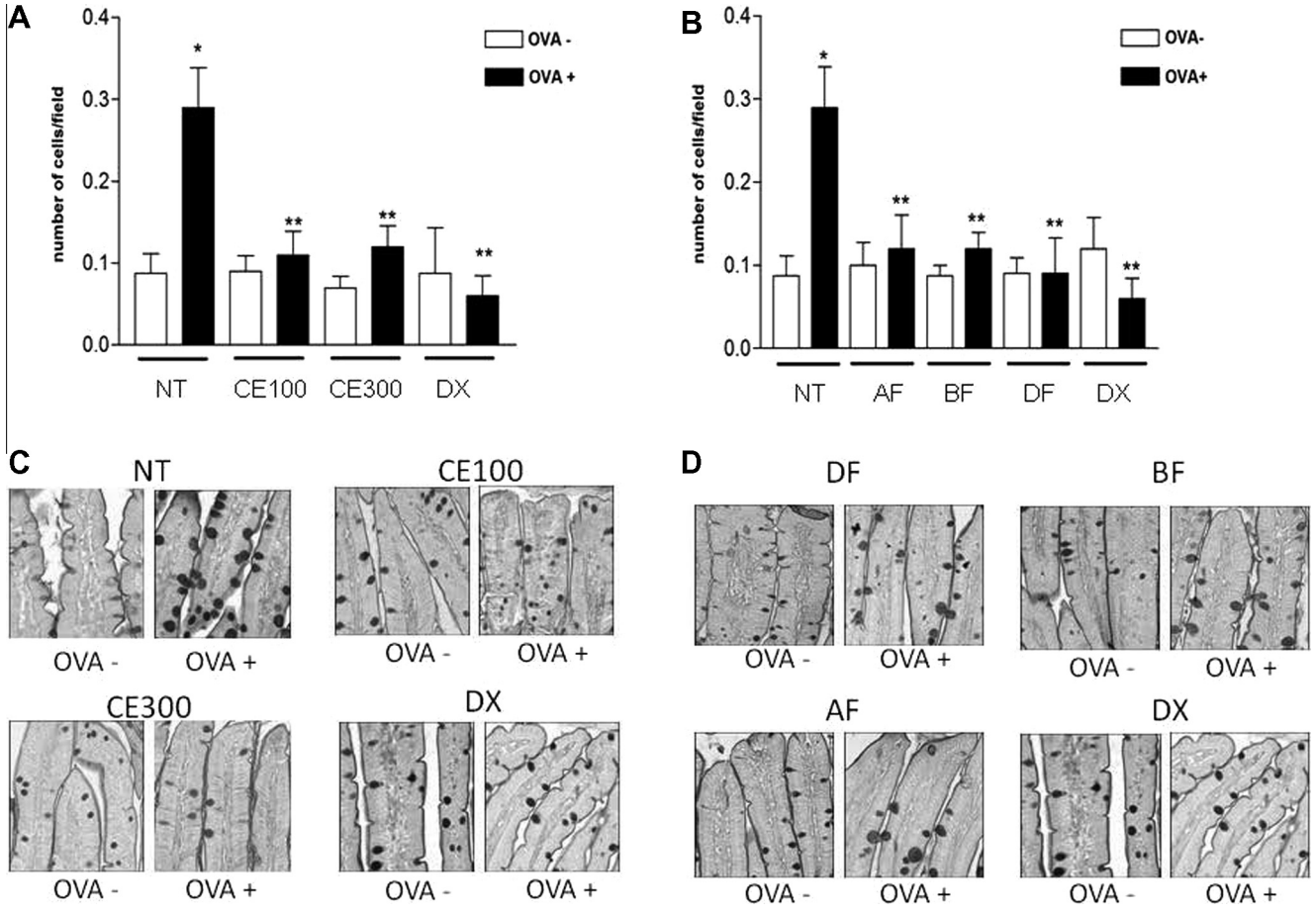

Fig. 5 – Effects of the CE (3.A) and fractions (3.B) of *D. trifida* on mucus production by caliciform cells of mice sensitised and non-sensitised with OVA. And morfometric images for comparison of mucus production by caliciform cells between Ova+ and Ova− in each group (3.C and 3.D). Data are presented as mean ± SD (n = 5). $^{*}P < 0.05$ with respect to their respective control (Ova−) groups. $^{**}P < 0.05$ compared to the non-treated (NT) allergic (Ova+) group. The groups are described in the legend to Fig. 2.

known that the species Dioscoreaceae has allantoin (Sagara et al., 1989; Niu, Chen, Wu, Cheng, & Wen, 2010). Allantoin (hydantoin 5-ureido) has been widely described in the literature as being responsible for numerous pharmacological activities, including wound healing, anti-irritating, moisturising, necrotic tissue remover, stimulating cell mitosis, as well as stimulation of epithelial promoter, analgesic and keratolytic (Shestopalov et al., 2006; Veraldi, Menter, & Innocenti, 2008). Due to these actions, allantoin has been used in medicinal and cosmetic preparations over the past 70 years for different

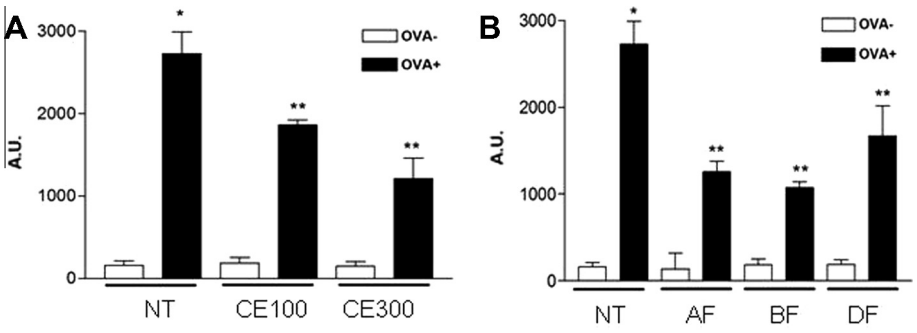

Fig. 6 – Effects of the CE (A) and fractions (B) of *D. trifida* on eosinophyl peroxidase of mice sensitised and non-sensitized with OVA. Data are presented as mean ± SD ($n = 5$). $^{*}P < 0.05$ with respect to their respective control (Ova−) groups. $^{**}P < 0.05$ compared to the non-treated (NT) allergic (Ova+) group. The groups are described in the legend to Fig. 2.

purposes, especially for wound healing. In an experimental study of respiratory allergy, pure allantoin was used in doses of 50 mg/kg/day and 25 mg/kg/day and it promoted suppressive effects on allergic inflammation. In the two doses tested, it caused a decrease in the number of eosinophils, in the production of mucus by goblet cells in lung tissue and production of IL-4, IL-5, anti-OVA IgE and total IgE (Lee et al., 2010). Other bioactive compound found in wild yam is diosgenin, but a previous study showed the absence of this substance in *D. trifida* (Rozanski, 1972) . In studies with pure diosgenin, doses of 100 mg/kg/day and 200 mg/kg/day promoted a reduction of intestinal inflammation, in the number of mast cells and IgE production (Huang et al., 2009). This same research group demonstrated that the use of diosgenin implicated in decrease of GATA-4 and IL-3 and increased production of IL-10, as a result of attenuating Th2 immune responses (Huang et al., 2010). In another study, which used a model of respiratory allergy and two doses of pure diosgenin (200 mg/kg/day and 400 mg/kg/day) Jan et al. (2007) found only a tendency of decrease in the production of total IgE and anti-OVA ($P > 0.05$). Our chemical analysis of crude extract from *D. trifida* (CE) shows that allantoin corresponds to 36.6%, a similar percentage of the tested substance, while diosgenin and correlated substance are present only in very low concentration (3.0%). According to these results and considering that crude extracts and not purified isolated compounds were used, it was determined that doses of crude extract of *D. trifida* should be tested as 100 and 300 mg/kg/day (which corresponds to 36.6 and 109.8 mg/kg/day of allantoin, and 3.0 and 9.0 mg of diosgenin correlated substances/kg/day, respectively). When testing extracts, we are trying to mimic the effect of the ingestion of food and its role as a functional food. In crude extracts there is a mixture of various bioactive substances in low concentrations. However, it has been well established that the complex mixture of bioactive compounds in fruits and vegetables can provide health benefits through the combination of additive and synergistic effects (Seeram, Adams, Hardy, & Heber, 2004).

One of the parameters used to analyse the development of allergic process is the dosage of isotype IgE antibodies. These immunoglobulins attach their respective FcεRI receptors to the surface of mastocytes/basophils, and following allergen exposure, inflammatory mediators are released to generate the allergic symptoms. All the tested products from *D. trifida* inhibited IgE production compared to not treated animals, and this activity is probably due to the presence of diosgenin and/or allantoin and correlated substances, as shown in previous studies (Huang et al., 2009, 2010; Lee et al., 2010). Since the reduction in IgE production regulates T cell differentiation, it can be suggested that diosgenin and allantoin modulate the food allergy response (a typical immune response to Th2). In our experiment, the inhibition tended to be higher in AF and BF, which contains allantoin, as DF with diosgenin.

During an allergic reaction, mediators such as histamine and TNF-α are released to promote vascular permeability and local blood flow (Bingham & Austen, 2000). This reaction causes the leakage of proteins (such as albumin) and liquid into the interstitium, causing inflammatory oedema (Brasileiro-Filho, 2006; Guo, Li, Lin, Samee, & Khalid, 2009). In animals treated with CE and its fractions, we observed a reduction in oedema, which was also probably due to the presence of diosgenin and allantoin. Huang et al. (2009) have shown that diosgenin reduced the production of anti-OVA IgE and the inflammatory infiltrate in allergic BALB/c mice intestines, resulting in a reduction in oedema. Tewtrakul and Itharat (2006, 2007) found that crude extracts of *D. membranaceae* and *D. birmania*, which is rich in sapogenins, inhibited the production of NO (possibly by inhibiting the iNOS) and TNF-α, leading to a reduction in intestinal oedema. A study by Olayemi and Ajaiyeoba (2007) has also associated the antiedematogenic activity of crude extract from *D. esculenta* to the presence of sapogenins. *In vivo* studies have demonstrated that oral administration of allantoin attenuates the production of IgE, IL-4 and IL-5 (Lee et al., 2010); has an anti-inflammatory effect and promotes healing (Olayemi & Ajaiyeoba, 2007).

Mastocytes are highly specialised cells that are activated when antigens create connections with IgE on specific membrane receptors. Degranulation occurs in seconds, releasing a series of pre-formed inflammatory mediators (Yu et al., 2001). These processes lead to an immediate increase in local blood flow and vascular permeability, causing oedema (Cara, Ebbert, & Mccafferty, 2004). As showed in Fig. 3 and 4, allergic animals treated with products from *D. trifida* had a reduced number of mastocytes and oedema. Huang et al. (2009) observed a reduction in mastocyte infiltration and its degranulation in the TGI of animals that were allergic to OVA when treated with diosgenin; this reaction might explain the observed reduction in

mastocytes induced by CE and DF. The reduction observed for AF and BF could also be due to the presence of allantoin, as observed previously by Lee et al. (2010).

In the food allergy model used in this study, BALB/c mice showed an increase in mucus production by the caliciform cells in the small intestine (Saldanha et al., 2004). Hypersecretion of mucus is a characteristic allergic response and is induced by IL-4 and IL-3; mucus is released to protect the intestinal cells by limiting the antigen absorption (Dourado et al., 2010; Saldanha et al., 2004). Animals treated with CE showed a reduction in production of mucus, and this result was also observed for AF, BF and DF. Previous studies showed that diosgenin and allantoin activities reduce mucus production in animals allergic to OVA (Huang et al., 2009; Lee et al., 2010). This finding can explain the results observed for *D. trifida*. IL-4 is involved in the hyperplasia and hypertrophy of caliciform cells (Blanchard et al., 2004; Dourado et al., 2010), and diosgenin reduced its production (Huang et al., 2009). Other substances, such as those found in the species of *Dioscorea*, could also be involved in IL-4 reduction. Glycoproteins from *D. batatas* (sin. *D. opposita*) also caused inhibition in IL-4 production *in vitro* (Oh & Lim, 2009).

The presence of eosinophil peroxidase (EPO) indirectly reflects the infiltration of eosinophils into intestinal mucosa. Animals treated with CE and its fractions showed a reduction of EPO. This reduction was probably due the presence of allantoin, since the fraction enriched in this substance showed better results. Pure allantoin had a similar effect in the respiratory allergy model, causing a reduction in the infiltration of inflammatory cells, including eosinophils (Lee et al., 2010).

In this study, we showed that products obtained from tubercles of *D. trifida* reduced all of the inflammatory parameters associated with food allergies in Ova-sensitised animals and this activity is correlated with the presence of allantoin and diosgenin correlated substances. The presence of both substances and its derivatives in the tested products can explain the activity observed in our studies. Phenolics are known for their antioxidant activity and ability to reduce reactive oxygen and nitrogen species generated by inflammation (Hou et al., 2001; Yang et al., 2009). Chemical analysis also showed the presence of phenolic compounds in all the tested products, but they may not be responsible for the activity. Despite being present in very high concentrations in BF (90.7%) and DF (98.8%), for example, the activity was the same as observed for AF, in which the concentration is very low (19.3%).

We have recently shown that the induction of food allergy in mice leads to adipose tissue inflammation and systemic metabolic alterations that contribute to the weight loss (Dourado et al., 2011). In the present experiment, there was no difference in consumption of feed between the animal groups, but the animals treated with Ova lost a slight amount of weight. This is a result of hyper-catabolism caused by the production of inflammatory cytokines. There is a decrease in the consumption of Ova solution related to immunological aversion (Cara et al., 1997; Dourado et al., 2010). To evaluate whether weight loss in allergic animals was obscured by water retention, as occurs in C57/BL6 families in OVA sensitisations studies (Moreira, 2006), we evaluated the weight of periuterine fat, but no differences were found. Jan et al., 2007 also did not find differences in animals treated with diosgenin at dosages of 200 mg/kg/day and 400 mg/kg/day compared with NT animals. Possible hepatic damage was also evaluated by weighing the liver, but there was no increase in weight and there were no morphologic alterations in any of the treated animals.

5. Conclusion

This study shows that *D. trifida* reduced inflammatory parameters associated with food allergies and has potential to prevent and treat this disease. New studies are necessary, however, to confirm the participation of each substance in the observed activities.

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REFERENCES

Bingham, C. O., & Austen, K. F. (2000). Mast-cell responses in the development of asthma. *Journal of Allergy and Clinical Immunology, 105*, 527–534.

Blanchard, C., Durual, S., Estiene, M., Bouzakri, K., Hein, M. H., Blin, N., & Cuber, J. C. (2004). IL-4 and IL-13 up-regulate intestinal trefoil factor expression: Requirement for STAT6 and de novo protein synthesis. *Journal of Immunology, 172*, 3775–3783.

Brasileiro-Filho, G. (2006). Patologia Bogliolo. 7th ed. Rio de Janeiro. Guanabara Koogan, pp. 1472.

Cara, D. C., Conde, A. A., & Vaz, N. M. (1997). Immunological induction of flavor aversion in mice II. Passive/adoptive transfer and pharmacological inhibition. *Scandinavian Journal of Immunology, 45*, 16–20.

Cara, D. C., Ebbert, K. V., & Mccafferty, D. M. (2004). Mast cell-independent mechanisms of immediate hypersensitivity: A role for platelets. *Journal of Immunology, 172*, 4964–4971.

Cardellina, J. H. (2002). Challenges and opportunities confronting the botanical dietary supplement industry. *Journal of Natural Products, 65*, 1073–1084.

Choi, E. M., Koo, S. J., & Hwang, J. K. (2004). Immune cell stimulanting activity of mucopolissacharide isolated from yam (*Dioscorea batatas*). *Journal of Ethnopharmacology, 91*, 1–6.

Costa, A. G. V., Garcia-Diaz, D. F., Jimenez, P., & Silva, P. I. (2013). Bioactive compounds and health benefits of exotic tropical red–black berries. *Journal of Functional Foods* 5, 539–549.

Desmarchelier, C. (2010). Neotropics and natural ingredients for pharmaceuticals: why isn't South American biodiversity on the crest of the wave?. *Phytotherapy Research, 24*, 791–799.

Devalaraja, S., Jain, S., & Yadav, H. (2011). Exotic fruits as therapeutic complements for diabetes, obesity and metabolic syndrome. *Food Research International, 44*, 1856–1865.

Dourado, L. P., Saldanha, J. P., Gargiulio, D. L., Noviello, M. L., Brant, C. C., Reis, M. L., Souza, R. M., Faria, A. M., Souza, D. G., & Cara, D. C. (2010). Role of IL-4 in aversion induced by food allergy in mice. *Cellular Immunology, 262*, 62–68.

Dourado, L. P., Noviello, M. L. M., Alvarenga, D. M., Menezes, Z., Perez, D. A., Batista, N. V., Menezes, G. B., Ferreira, A. V., Souza, D. G., & Cara, D. C. (2011). Experimental food allergy leads to

adipose inflammation, systemic metabolic alterations and weight loss in mice. *Cellular Immonology, 270*, 198–206.
Guo, Y., Li, Z., Lin, H., Samee, H., & Khalid, J. (2009). Studies on specific degranulation of mast cell sensitized by several allergens in vitro. *Cellular and Molecular Immunology, 62*, 149–153.
Hata, Y., Reguero, M. T., Garcia, L. A., Buitrago, G., & Alvarez, A. (2003). Evaluación del contenido de sapogeninas em variedades nativas de inhame (*Dioscorea* spp.) provenientes de la colección de La Universidad del Córdoba. *Revista Colombiana de Ciências Químicas e Farmacêuticas, 32*, 149–157.
Hou, W. C., Lee, M. H., Chen, H. J., Liang, W. L., Han, C. H., Liu, Y. W., & Lin, Y. H. (2001). Antioxidant activities of dioscorin, the storage protein of yam (*Dioscorea batatas* Decne) tuber. *Journal of Agricultural and Food Chemistry, 49*, 4956–4960.
Huang, C. H., Ku, C. Y., & Jan, T. R. (2009). Diosgenin attenuates allergen-induced intestinal inflammation and IgE production in a murine model of food allergy. *Planta Medica, 75*, 1300–1305.
Huang, C. H., Liu, D. Z., & Jan, T. R. (2010). Diosgenin, a plant-derived sapogenin, enhances regulatory T-cell immunity in the intestine of mice with food allergy. *Journal of Natural Products, 73*, 1033–1037.
Jan, T. R., Wey, S. P., Kuan, C. C., Liau, M. H., & Wu, H. Y. (2007). Diosgenin, a steroidal sapogenin, enhances antigen-specific IgG2a and interferon-gama expression in ovalbumin-sensitized BALB/c mice. *Planta Medica, 73*, 421–426.
Jin, M., Suh, S. J., Yang, J. H., Lu, Y., Kim, S. J., Kwon, S., Jo, T. H., Kim, J. W., Park, Y. I., Ahn, G. W., Lee, C. K., Kim, C. H., Son, J. K., Son, K. H., & Chang, H. W. (2010). Anti-inflammatory activity of bark of *Dioscorea batatas* Decne through the inhibition of iNOS and COX-2 expressions in RAW264.7 cells via NF-κB and ERK1/2 inactivation. *Food Chemical Toxicology, 48*, 3073–3079.
Lee, M. Y., Lee, N. H., Jung, D., Lee, J. A., Seo, C. S., & Lee, H. (2010). Protective effects of allantoin against ovalbumin (OVA)-induced lung inflammation in a murine model of asthma. *International Immunopharmacology, 10*, 474–480.
Lin, J. T. (2006). Effects of domestic processing on steroidal saponins in Taiwanese yam culturar (*Dioscorea pseudojaponica* Yamamoto). *Journal Agricultural of Food Chemistry, 54*, 9948–9954.
Lin, J. T., & Yang, D. J. (2008). Determination of steroidal saponins in different organs of yam (*Dioscorea pseudojaponica* Yamamoto). *Food Chemistry, 108*, 1068–1074.
Liu, Y. W., Shang, H. F., Wang, C. K., Hsu, F. L., & Hou, W. C. (2007). Immunomodulatory activity of dioscorin, the storage protein of yam (*Dioscorea alata* cv. Tainong No.1) tuber. *Food Chemical Toxicology, 45*, 2312–2318.
Liu, Y. W., Liu, J. C., Huang, C. Y., Wang, C. K., Shang, H. F., & Hou, W. C. (2009). Effects of oral administration of yam tuber storage protein, dioscorin, to BALB/c mice for 21 days on immune responses. *Journal of Agricultural and Food Chemistry, 57*, 9274–9279.
Macpherson, A. J., & Uhr, T. (2004). Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science, 303*, 1664–1665.
Moreira, L.F. (2006) Estudo dos componentes nutricionais e imunológicos na perda de peso em camundongos com alergia alimentar. (Mestrado). Departamento de Patologia, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, pp. 99.
Niu, C. S., Chen, W., Wu, H. T., Cheng, K. C., Wen, Y. J., Lin, K. C., & Cheng, J. T. (2010). Decrease of plasma glucose by allantoin, an active principle of Yam (Dioscorea spp.) in spteptozotocin-induced diabetics rats. *Journal of Agricultural and Food Chemistry, 22*, 12031–12035.
Oh, P. S., & Lim, K. T. (2009). Glycoprotein isolated from *Dioscorea batatas* Decne modulates expressions of IL-4 and IL-10 in primary-cultured mouse lynphocytes. *Cell Biochemistry, 27*, 316–322.
Olayemi, J. O., & Ajaiyeoba, E. O. (2007). Anti-inflammatory studies of yam (*Dioscorea esculenta*) extract on winstar rats. *African Journal of Biotechnology, 6*, 1913–1915.
Oliveira, V. B., Yamada, L. T., Fagg, C. F., & Brandão, M. G. L. (2012). Native foods from Brazilian biodiversity as a source of bioactive compounds. *Food Research International, 48*, 170–179.
Purseglove, J. W. (1972). *Dioscoreaceae. In Tropical Crops. Monocotyledons*. Harlow: Longman House. pp. 607.
Rozanski, A. (1972). A simplified method of extraction of diosgenin from Dioscorea tubers and its determination by gas-liquid chromatography. *Analyst, 97*, 968–972.
Russo, M., Nahori, M. A., Lefort, J., Gomes, E., de Castro, K. A., Rodriguez, D., Ribeiro, O. G., Adriouch, S., Gallois, V., Faria, A. M., & Vargaftig, B. B. (2001). Suppression of asthma-like responses in different mouse strains by oral tolerance. *American Journal of Respiratory Cell and Molecular Biology, 24*, 518–526.
Saldanha, J. C., Gargiulo, D. L., Silva, S. S., Carmo-Pinho, F. H., Andrade, M. C., Alvarez-Leite, J. I., Teixeira, M. M., & Cara, D. C. (2004). A model of chronic IgE-mediated food allergy in ovalbumin-sensitized mice. *Brazilian Journal of Medical and Biological Research, 37*, 809–816.
Sampson, H. A., & Wang, J. (2009). Food allergy: Recent advances in pathophisiology and treatment. *Allergy Asthma Immunology Research, 1*, 19–29.
Seeram, N. P., Adams, L. S., Hardy, M. C., & Heber, D. (2014). Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against tumor cell lines. *Journal of Agricultural Food Chemistry, 52*, 2512–2517.
Shen, S. R., Hsu, W. H., Lee, C. C., Chang, W. C., & Wu, S. C. (2012). Buckwheat extracts (*Fagopyrum tataricum*) and rutin attenuate Th2 cytokines production and cellular allergic effects in vitro and in vivo. *Journal of Functional Foods, 4*, 793–799.
Shestopalov, A. V., Shkurat, T. P., Mikashinovich, Z. I., Kryzhanovskaya, O., Bogacheva, M. A., Lomteva, S. V., Prokof'ev, V. P., & Gus'kov, E. P. (2006). Biological functions of allantoin. *Biological Bulletin, 33*, 437–440.
Silveira, M. R., Nunes, K. P., Cara, D. C., Souza, D. G., Corrêa, A., Jr, Teixeira, M. M., & Negrão-Corrêa, D. (2002). Infection with *Strongyloides venezuelensis* induces transient airway eosinophilic inflammation, an increase in immunoglobulin E, and hyperresponsiveness in rats. *Infection and Immunity, 70*, 6263–6272.
Tantoush, Z., Apostolovic, D., Kravic, B., Prodic, I., Mihajlovic, L., Stanic-Vucinic, D., & Velickovic, T. C. (2012). Green tea catechins of food supplements facilitate pepsin digestion of major food allergens, but hampers their digestion if oxidized by phenol oxidase. *Journal of Functional Foods, 4*, 650–660.
Tewtrakul, S., & Itharat, A. (2006). Anti-allergic substances from rhizomes of *Dioscorea membranacea*. *Bioorganic Medical Chemistry, 14*, 8707–8711.
Tewtrakul, S., & Itharat, A. (2007). Nitric oxide inhibitory substances from the rhizomes of *Dioscorea membranacea*. *Journal of Ethnopharmacology, 109*, 412–416.
Veraldi, S., Menter, A., & Innocenti, M. (2008). Treatment of mild to moderate seborrhoeic dermatitis with MAS064D (Sebclair), a novel topical medical device: Results of a pilot, randomized, double-blind, controlled trial. *Journal of the European Academy of Dermatology and Venereology, 22*, 290–296.
Yang, D. J., Lu, T. J., & Hwang, L. S. (2003). Isolation and identification of steroidal saponins in Taiwanese yam cultivar (*Dioscorea pseudojaponica* Yamamoto). *Journal of Agricultural and Food Chemistry, 51*, 6438–6444.

Yang, M. H., Yoon, K. D., Chin, Y. W., Park, J. H., & Kim, J. (2009). Phenolic compounds with radical scavenging and cicloxigenase-2 (COX-2) inhibitory activities from *Dioscorea opposita*. *Bioorganic and Medical Chemistry, 17*, 2689–2694.

Yu, L. C. H., Yang, P. P., Berin, C. M., Di Leo, V., Conrad, D. H., MCKay, D. M., Satoskar, A. R., & Perdue, M. H. (2001). Enhanced transepithelial antigen transport in intestine of allergic Mice is mediated by IgE/CD23 and regulated by interleukin-4. *Gastroenterology, 121*, 370–381.

Zhang, T., Yang, C., Rupa, P., Jiang, B., & Minea, Y. (2012). Inhibitory effects of *Quillaja saponin* on IgE-mediated degranulation of rat basophilic leukemia RBL-2H3 Cells. *Journal of Functional Foods, 4*, 864–871.