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(71) Applicant: **BIOVICO Sp. z o.o.** [PL/PL]; ul. Hryniewickiego 6B/135, 81-340 Gdynia (PL).

(72) Inventors: **LEMKE, Krzysztof**; ul. Batalionów Chłopskich 2A, 81-452 Gdynia (PL). **LESZCZYŃSKA-WILOCH Magdalena**; ul. Pólnicy 24/12, 80-177 Gdańsk (PL). **KRZYCZKOWSKI, Wojciech**; Narcyzów 45/33, 05-509 Józefosław (PL). **BIDZIŃSKA, Joanna**; ul. Myśliwska 85A/3, 80-283 Gdańsk (PL). **OLDAK, Alicja**; ul. Polanowska 45/2, 76-100 Sławno (PL). **WILANDT, Artur**; Rzucewo 2, 84-100 Puck (PL).

(74) Agent: **KANCELARIA PRAWNO-PATENTOWA**; Małgorzata Matyka, ul. Kurierów AK 4A/7, 80-041 Gdańsk (PL).

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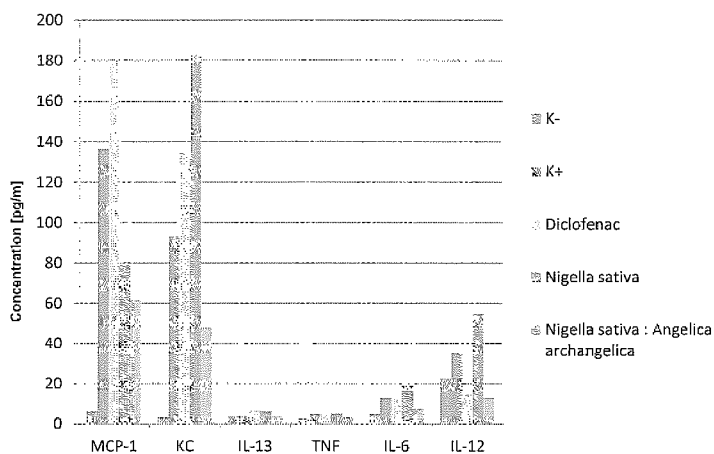
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(54) Title: A PLANT COMPOSITION WITH ANTI-INFLAMMATORY, ANTI-ALLERGIC, ANTI-ASTHMATIC AND/OR ANTI-BACTERIAL PROPERTIES AND ITS APPLICATION

Fig. 5.



(57) Abstract: The object of the invention is the plant composition of anti-inflammatory, anti-allergic, anti-asthmatic and/or anti-bacterial properties, which contains a combination of extracts demonstrating synergistic effects, obtained from the following plant material: the seed of Black cumin *Nigella sativa*, the root of Garden angelica *Angelica archangelica*, the herb of Meadowsweet *Filipendula ulmaria*, the herb of Korean mint *Agastache rugose*, and the cones of Hop *Humulus lupulus*. Furthermore, the object of the invention is the product containing plant compositions ranging between 1 and 100% of the composition described above.

WO 2016/178589 A1

5 **A plant composition with anti-inflammatory, anti-allergic, anti-asthmatic
and/or anti-bacterial properties and its application**

The object of the invention is the plant composition of anti-inflammatory,
10 anti-allergic, anti-asthmatic and/or anti-bacterial properties, which contains a
combination of extracts demonstrating synergistic effects, obtained from the
following plant material: the seed of Black cumin *Nigella sativa*, the root of
Garden angelica *Angelica archangelica*, the herb of Meadowsweet *Filipendula
ulmaria*, the herb of Korean mint *Agastache rugose*, the cones of Hop *Humulus
15 lupulus*. Furthermore, the object of the invention is the application of the
composition as well as the product containing the above mentioned plant
composition.

Inflammation is one of the oldest terms in the pathophysiology of
diseases and suffering. It is defined as the response to an external factor which
20 interferes in the natural homeostasis of the organism. Over the last twenty years
many mechanisms of this complex process have been found. Many
inflammatory mediators influencing the course and duration of the process are
engaged in the inflammatory reaction. The action profile of some mediators may
change depending on the coexisting molecular-cellular conditions in which
25 inflammation develops. In the early stage of the inflammation phagocyte and
endothelial cells secrete pro-inflammatory cytokines, which include interleukins
(IL): IL-1a/b, IL-6, IL-8 and the tumor necrosis factor TNF- α . The group of
antagonistic effects is made up of anti-inflammatory cytokines such as IL-4, IL-5,
IL-10, and IL-13 produced by lymphocytes Th2. The inflammatory process
30 involves yet another phenomenon, namely secretion of mediators of humoral
origin such as histamine and serotonin. These mediators are released from
mast cells in the degranulation process. Endotoxins present in the inflammatory
environment act on granulocytes, macrophages and platelets activating an
arachidonic acid cascade, which leads to the production of e.g. leukotrienes and
35 prostaglandins. The formation of those mediators may be inhibited by blocking

5 the cyclooxygenase-2 (COX-2), as is the case in i
anti-inflammatory drugs (NSAID).

Nowadays, allergy belongs to the most common diseases. Allergic
reactions type I, such as atopic skin inflammation, hay fever, food allergies or
10 asthma, are chronic conditions and require systematic, multidisciplinary
treatment. In case allergy type 1, the antibody-induced reaction appears within
20-60 minutes after the contact with the allergen. Th2 lymphocytes play the key
role in the occurrence of the allergic symptoms. They stimulate lymphocytes B
which recognize the allergising antigen to produce antibodies type IgE. These
15 antibodies coat the mast cells thus activating them. If contact with the antigen
reoccurs, the mast cells secrete mediators of the allergic reaction, mainly
histamine and pro-inflammatory cytokines. Thus, IL-4 produced by Th2
lymphocytes and mast cells plays a significant role. It takes part in the switch of
the immunoglobulins produced by lymphocytes class B to E, stimulates the
20 formation of Th2 cells from Th0, and increases the expression of the FcεRI
receptors on the surface of mast cells (mastocytes and basophils), thus causing
the positive reciprocity and intensification of the allergy symptoms.

Allergy related bronchial asthma is a disease characterized by chronic
inflammation of the respiratory system. Mastocytes, eosinophils and T
25 lymphocytes play the main role in the pathogenesis of asthma. The prime
mediators released by these cells are: histamine, prostaglandin D2 and
numerous cytokines including e.g. IL-4, TNF-α, IL-5, IL-1β, and IL-13. Other
triggers of bronchial asthma include immunological mechanisms dependent on
immunoglobulin IgE, closely related to allergy-induced diseases.

30 The extract demonstrating action on the cellular/biochemical pathways of the
inflammation process and allergy can be used in treating bronchial asthma.

Pathogenic bacteria may be responsible for various diseases. When
entering the host's organism, they trigger defensive reaction of the
immunological system. Pharyngitis, or sore throat, is often caused by infection.
35 Common respiratory viruses account for a vast majority of cases, usually self-
limited. However, bacteria are also major etiologic agents of 10–30% of cases.

- 5 *Streptococcus pyogenes* (group A Streptococcus)
important bacterial origin of pharyngitis.

Plants have for centuries been commonly used in folk medicine and phytotherapy as the source of active substances in treatment and prevention of many diseases. Many preparations are based on plant related extracts. They
10 have gained popularity and trust among the consumers thanks to their natural origin and no side effects as compared to the compounds obtained through the chemical synthesis. Plant compositions prove highly advantageous versus chemical compounds since the chemical structure of the active substances they contain is similar to that of human metabolic products, which facilitates their
15 absorption. Another advantage of plant compositions in therapies consists in lower toxicity of the proposed substances as compared to synthetic compounds. The synergistic effect obtained through combining several extracts in appropriate proportions enables applying lower therapeutic doses and frequently the attainment of better results. The compound mixtures contained in
20 the extracts very often act on different signaling pathways, carrying a synergistic effect with respect to the main compound and thus improving its therapeutic activity.

The process of isolating active compounds from the plant can be performed in many ways. Extraction methods are continuously optimized to
25 achieve maximum possible concentration of pure active compounds and to decrease the production costs whilst employing environmentally-friendly methods. Many classical methods are still applied, to name e.g. maceration or Soxhlet extraction, where separation of various compound groups is attained by using a solvent or mixture of solvents of specific polarity. In order to increase
30 the extraction efficiency, several methods have been developed to prepare the raw material for extraction, as well as intermediate steps intended to increase the process yield. The methods developed to that aim include e.g. application of microwaves, ultrasounds, supercritical fluid extraction (water or carbon dioxide) or extraction under increased pressure.

35 Garden Angelica (*Angelica arachangelica*) is a herb of the celery family. It grows wild in the mountainous and wet areas of Europe and Asia. It owes its

5 customary name of a medical plant to its applicatio
of garden angelica contains an essential oil composed mainly of monoterpenes
and coumarins. Numerous research studies have confirmed the healing
properties of the plant root, to name e.g. its sedative, antispasmodic and anti-
rheumatic effects. It is reached for especially frequently in treating digestive
10 system disorders such as feeling of fullness, stomach ulcers, appetite loss,
anorexia, or digestive problems.

Black cumin (*Nigella sativa*) is an annual herbaceous plant cultivated in
Eurasia and North Africa, in sub-tropical and moderate zones. As goes for
Poland, it is grown in the southern part of the country. Black cumin belongs to
15 the small group of plants used in treating autoimmune diseases such as
rheumatism or allergy. Seed extracts of *Nigella sativa*, particularly those
containing highly concentrated active compound, thymoquinone, prove
substantially antimicrobial, active against both Gram-negative and Gram-
positive bacteria and inhibiting fungus growth. The seed extract of Black cumin
20 may also be deemed a non-steroidal anti-inflammatory drug, since scientific
research has revealed that thymol, thymoquinone and its derivatives such as
dithymoquinone and thymohydroquinone can inhibit the activity of
cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) – the key enzymes
in the formation of mediators which initiate the inflammatory process. In
25 addition, the seed extracts lower the blood glucose levels and reduce the risk of
thromboses and embolisms.

Meadowsweet (*Filipendula ulmaria*) is a perennial plant of the
Rosaceae (Rosaceae) family, found in the northern hemisphere areas of
moderate climate. Traditionally, infusions and extracts prepared from the seeds
30 and herb are used to treat rheumatoid arthritis, upper respiratory tract
infections, and – externally – in skin infections and hardly healing wounds.

Korean mint (*Agastache rugosa*) is an ornamental annual plant of the
mint family. Research has shown that one of the main components of
Agastache rugosa demonstrates anti-inflammatory properties and may be used
35 in the treatment of atherosclerosis at its early stages. The compounds isolated
from the plant, especially the oils, have antibacterial and antifungal properties.

5 Hop (*Humulus lupulus*) belongs to perennials

Hop infusions are effective at initiating sleep, reducing hyperactivity, treating prostate diseases, and improving digestion. Cone extract of Hop is used for ulcers and chronic wounds because of its strong bactericidal properties. The latter are first and foremost due to the substance called xanthohumol – a
10 strongly antioxidant compound of the flavonoid group.

Known from description EP1709995A1 is the use of extracts as well as the seeds of black cumin *Nigella sativa* in the treatment and prevention of allergy and asthma in mammals, humans included. Another description -
15 US20080152736A1 discloses that the lipid fraction composed mainly of long chain fatty acids, sterols and essential oils demonstrates properties preventing and relieving skin and bacterial infections, accelerating wound healing and active in the treatment and prevention of diseases of the circulatory and respiratory systems. Patent application **US20030060508A1** discloses the use of
20 a fraction composed mainly of unsaturated fatty acids, oleic and linoleic acids in particular, in the prevention and treatment of hemorrhoids, allergies, inflammations, as well as fungal and bacterial skin infections. Known from description **EP2263664A1** is the fact that extract of black cumin, and especially thymoquinone it contains, stimulates opioid receptors, which contributes to
25 elimination of food allergy reactions. Patent application **US20110076346A1** claims the oils obtained from black cumin seed in supercritical extraction with carbon dioxide, containing specific quantities of thymoquinone, to use in the treatment of diseases caused by inflammation.

Patent **KR100825869B1** which concerns root extract of Garden Angelica
30 *Angelica archangelica* discloses that its alcoholic extract proves particularly active in inhibiting the secretion of interleukins -4 and -13, which may prove useful in the treatment and prevention of asthma. Also known from patent description **CA2851947A1** is that ethanolic extract of Meadowsweet *Filipendula* is an effective medicament in treating and preventing chronic pain. The
35 description of patent **WO2002074320** discloses the use of the Korean mint *Agastache rugosa* extract, or the main active ingredient of the Korean mint

- 5 extract - tiliarin, as a component of therapeutic com
inflammation and atherosclerosis.

Our research has shown that a combination of extracts of the
aforementioned plants demonstrate higher effectiveness in therapy than when
each of them used separately. Combining the obtained extracts in appropriate
10 proportions has proved to give a synergistic effect. This has translated to the
attainment of substantially more potent anti-inflammatory, anti-allergic, anti-
asthmatic and antibacterial properties of the mixtures compared to those of any
one extract used alone, the same dose applied.

The object of the invention is the plant composition of anti-inflammatory,
15 anti-allergic, anti-asthmatic and/or anti-bacterial properties, which contains a
combination of extracts demonstrating synergistic effects, obtained from the
following plant material: the seed of Black cumin *Nigella sativa*, the root of
Garden angelica *Angelica archangelica*, the herb of Meadowsweet *Filipendula
ulmaria*, the herb of Korean mint *Agastache rugosa*, and the cones of Hop
20 *Humulus lupulus*.

The plant composition, where the percentage content of the extracts is as
follows: the seed extract of Black cumin *Nigella sativa* (5-70%), the root extract
of Garden angelica *Angelica archangelica* (0.5-30%), the herb extract of
Meadowsweet *Filipendula ulmaria* (0.5-40%), the herb extract of Korean mint
25 *Agastache rugosa* (0.2-40%) and the cone extract of Hop *Humulus lupulus* (0.5-
50%).

The plant composition, where the weight ratio of the seed extract of Black
cumin *Nigella sativa* to the root extract of Garden Angelica *Angelica
archangelica* is 1:1 to 12:1 and 1:12.

30 The plant composition, where the weight ratio of the seed extract of Black
cumin *Nigella sativa* to the herb extract of Meadowsweet *Filipendula ulmaria* is
1:1 to 1:4 and 4:1.

The plant composition, where the weight ratio of the seed extract of Black
cumin *Nigella sativa* to the herb extract of Korean mint *Agastache rugosa* is 1:1
35 to 4:1 and 1:4.

5 The plant composition, where the weight ratio of cumin *Nigella sativa* to the cone extract of Hop *Humulus lupulus* is 1:1 to 8:1 and to 1:8.

The plant composition which further contains typical excipients, bulking agents, thickeners, antioxidants, vitamins, and emulsifiers.

10 The plant composition which contains a combination of plant extracts demonstrating synergistic effects, given the pharmaceutical form of creams, ointments, capsules, gels, emulsions, lozenges, powders, drops, syrups, and aerosols.

The application of the plant composition defined above, where the plant
15 composition is used to develop products intended for the treatment of inflammations, allergies and/or asthma in the pharmaceutical form of creams, ointments, capsules, gels, emulsions, lozenges, powders, drops, syrups, and aerosols.

The product containing plant compositions ranging between 1 and 100% of
20 the composition described above.

The product is classified in the following group: dietary supplement, food for special medical purposes, cosmetic, medical product, medicinal product.

The product is classified in the following group: creams, ointments, capsules, gels, emulsions, lozenges, powders, drops, syrups and aerosols.

25 The exemplary compositions of plant extracts can act on a number of metabolic pathways or attain a specific activity exerting an impact on a single biochemical pathway. The source of the properties lies in the complex chemical composition and the unique proportions of the compounds in the extracts.

30 Brief description of the drawings:

Fig. 1. – presents the impact of the extracts on the relative expression of mRNA IL-4/mRNA GADPH, expressed as % of positive control.

5 **Fig. 2.** – presents the impact of the extracts on the r
COX-2/mRNA GADPH, expressed as % of positive control.

Fig. 3. – presents the impact of the extracts on % inhibition of cell degranulation
versus positive control.

10 **Fig. 4.** – presents the impact of the extracts on the IL-6 secretion, expressed in
% versus positive control.

Fig. 5. – presents the impact of the seed extract of Black cumin *Nigella sativa*,
the composition of the seed extract of *Nigella sativa* Black cumin and the root
extract of Garden angelica *Angelica archangelica*, and reference medicament
– Diclofenac (NSAID) on pro-inflammatory cytokines.

15

The following exemplary embodiments are illustrative only, hence are not
intended to limit the scope of the present invention:

Example 1.

20 **Preparation of the herb extract of Korean mint *Agastache rugosa*.**

The herb of Korean mint *Agastache rugosa* was grounded in a disintegrator and
sieved to isolate the grain fraction of 160-500 microns size. 5 g of the plant
material prepared in this way was pre-extracted in a percolator with 200 ml of
ethanol 96°. Then, proper extraction was performed by the maceration method
25 using 70° ethanol (3×150 ml, 24 hours each time). The extracts were combined
and evaporated dry to obtain 757 mg of dry extract.

Example 2.

Preparation of the root extract of Garden Angelica *Angelica archangelica*.

30 The root extract of Garden Angelica *Angelica archangelica* was obtained in
accordance with the procedure described in Example 1. 825 mg of dry extract
was obtained.

5 **Example 3.****Preparation of the herb extract of Meadowsweet *Filipendula ulmaria*.**

The herb extract of Meadowsweet *Filipendula ulmaria* was obtained in accordance with the procedure described in Example 1. 1.72 g of dry extract was obtained.

10 **Example 4.****Preparation of the seed extract of Black cumin *Nigella sativa*.**

Black cumin *Nigella sativa* seeds of the dry mass content of 92.2% were ground in an electric grinder to obtain grains sized less than 400 microns. A 10 ml steel extraction vessel was filled with 4 g of ground seeds and subject to extraction
15 with supercritical carbon dioxide (scCO₂) on a Waters MV-10 ASFE device. The extraction conditions were as follows: scCO₂ flow of 10 ml/min, pressure of 150 bar, and temperature of 50°C. The extraction resulted in obtaining 450 mg of oil composed of: 50-53% linoleic acid, 23-25% oleic acid, 9-12% palmitic acid, 2.4-3.0% eicosadienoic acid, 2-3% stearic acid, and 0.5--6.0% thymoquinone, 2-6%
20 p-cymene, and 1-4% thymol.

Example 5.**Preparation of the cone extract of Hop *Humulus lupulus*.**

Dried cones of Hop *Humulus lupulus* were milled and granulated to the grain of approx. 1 cm. A 10 ml steel extraction vessel was filled with 3.5 g of granulated
25 cones of Hop *Humulus lupulus* and subjected to extraction with supercritical carbon dioxide (scCO₂) on a Waters MV-10 ASFE device. The extraction conditions were as follows: scCO₂ flow of 8 ml/min, pressure of 300 bar, and temperature of 40°C. The extraction resulted in obtaining 1.2 g of the extract.

Example 5.

30 **a) Preparation of Eucerin-based cream containing seed extract of Black cumin *Nigella sativa* and root extract of Garden angelica *Angelica archangelica*.**

5 Cream composition:

No	Ingredient	Amount [g]
1	Distilled water	30.0
2	Eucerin anhydrous	63.9
3	Seed extract of Black cumin <i>Nigella sativa</i>	3.0
4	Root extract of Garden angelica <i>Angelica archangelica</i>	1.0
5	D-panthenol	2.0
6	Vitamin E	0.1
Total mass		100

Table 1. The composition of the Eucerin-based cream containing seed extract of Black cumin *Nigella sativa* and root extract of Garden Angelica *Angelica archangelica*.

The cream was prepared in accordance with standard procedures.

10 **b) Preparation of Eucerin-based cream containing seed extract of Black cumin *Nigella sativa*, herb extract of Korean mint *Agastache rugosa*, and herb extract of Meadowsweet *Filipendula ulmaria*.**

Cream composition:

No	Ingredient	Amount [g]
1	Distilled water	50.0
2	Eucerin anhydrous	43.0
3	Seed extract of Black cumin <i>Nigella sativa</i>	4.0
4	Herb extract of Meadowsweet <i>Filipendula ulmaria</i>	1.9
5	Herb extract of Korean mint <i>Agastache rugosa</i>	1.0
7	Vitamin E	0.1
Total mass		100

15 **Table 2.** The composition of Eucerin-based cream containing seed extract of Black cumin *Nigella sativa*, herb extract of Meadowsweet *Filipendula ulmaria*, and herb extract of Korean mint *Agastache rugosa*.

The cream was prepared in accordance with standard procedures.

c) Preparation of Eucerin-based cream containing seed extract of Black cumin *Nigella sativa*, and herb extract of Meadowsweet *Filipendula ulmaria*.

5 Cream composition:

No	Ingredient	Amount [g]
1	Distilled water	30.0
2	Eucerin anhydrous	63.0
3	Seed extract of Black cumin <i>Nigella sativa</i>	3.0
4	Herb extract of Meadowsweet <i>Filipendula ulmaria</i>	1.0
5	D-panthenol	2.0
6	Vitamin E	0.1
7	Vitamin A	0.9
Total mass		100

Table 3. Composition of the Eucerin-based cream containing seed extract of Black cumin *Nigella sativa* and herb extract of Meadowsweet *Filipendula ulmaria*.

The cream was prepared in accordance with standard procedures.

Example 6.

- 10 a) Preparation of petrolatum-based ointment containing seed extract of Black cumin *Nigella sativa* and root extract of Garden Angelica *Angelica archangelica*.

Ointment composition:

No	Ingredient	Amount [g]
1	Petrolatum	85.78
2	Lanoline	5.00
3	Seed extract of Black cumin <i>Nigella sativa</i>	7.00
4	Root extract of Garden angelica <i>Angelica archangelica</i>	0.60
5	D-panthenol	0.50
6	Vitamin E	1.00
7	Vitamin A	0.10
8	BHT	0.02
Total mass		100

- 15 Table 4. The composition of the petrolatum-based ointment containing seed extract of Black cumin *Nigella sativa* and root extract of Garden Angelica *Angelica archangelica*.

The ointment was prepared in accordance with standard procedures.

- b) Preparation of petrolatum-based ointment containing seed extract of Black cumin *Nigella sativa* and herb extract of Korean mint *Agastache rugosa*.

5 Ointment composition:

No	Ingredient	Amount [g]
1	Petrolatum	85.78
2	Lanoline	7.00
3	Seed extract of Black cumin <i>Nigella sativa</i>	4.00
4	Herb extract of Korean mint <i>Agastache rugosa</i>	1.00
5	D-panthenol	0.50
6	Vitamin E	1.00
7	Vitamin A	0.70
8	BHT	0.02
Total mass		100

Table 5. The composition of the petrolatum-based ointment containing seed extract of Black cumin *Nigella sativa* and herb extract of Korean mint *Agastache rugosa*.

The ointment was prepared in accordance with standard procedures.

10 **c) Preparation of petrolatum-based ointment containing seed extract of Black cumin and herb extract of Meadowsweet *Filipendula ulmaria*.**

Ointment composition:

No	Ingredient	Amount [g]
1	Petrolatum	68
2	Lanoline	20
3	Seed extract of Black cumin <i>Nigella sativa</i>	2
4	Herb extract of Meadowsweet <i>Filipendula ulmaria</i>	8
5	D-panthenol	1
6	Vitamin E	1
Total mass		100

Table 6. The composition of the petrolatum-based ointment containing seed extract of Black cumin *Nigella sativa* and herb extract of Meadowsweet *Filipendula ulmaria*.

15 The ointment was prepared in accordance with standard procedures.

5 **Example 7.**

- a) Preparation of capsules containing seed extract of Black cumin *Nigella sativa* and root extract of Garden angelica *Angelica archangelica*.

Capsule contents:

No	Ingredient	Amount [mg]
1	Seed extract of Black cumin <i>Nigella sativa</i>	133.3
2	Root extract of Garden angelica <i>Angelica archangelica</i>	66.7
3	DL- alpha tocopheryl acetate	15.0
4	Excipients	405.0
Total mass of a capsule		620

10 **Table 7.** The contents of a capsule with seed extract of Black cumin *Nigella sativa* and root extract of Garden angelica *Angelica archangelica*.

The mixture was closed in a gelatin capsule. The capsulation process followed the standard procedure. The excipients in the capsule were: the typically used fillers (refined soybean oil), thickeners (silicon oxide) and emulsifier (soy
15 lecithin).

- b) Preparation of capsules containing seed extract of Black cumin *Nigella sativa* and herb extract of Meadowsweet *Filipendula ulmaria*.

Capsule contents:

No	Ingredient	Amount [mg]
1	Seed extract of Black cumin <i>Nigella sativa</i>	200
2	Herb extract of Meadowsweet <i>Filipendula ulmaria</i>	50
3	DL- alpha tocopheryl acetate	10
4	Excipients	360
Total mass of a capsule		620

20 **Table 8.** The contents of a capsule with seed extract of Black cumin *Nigella sativa* and herb extract of Meadowsweet *Filipendula ulmaria*.

The mixture was closed in a gelatin capsule. The capsulation process followed the standard procedure. The excipients used in the capsule were: the typically used fillers (refined soybean oil), thickeners (silicon oxide), and emulsifier (soy
lecithin).

- 5 **c) Preparation of capsules containing seed extract of *Nigella sativa* and herb extract of Korean mint *Agastache rugosa*.**

Capsule contents:

No	Ingredient	Amount [mg]
1	Seed extract of Black cumin <i>Nigella sativa</i>	300
2	Herb extract of Korean mint <i>Agastache rugosa</i>	100
3	DL- alpha tocopheryl acetate	10
4	Excipients	210
Total mass of a capsule		620

Table 9. The contents of a capsule with seed extract of Black cumin *Nigella sativa* and herb extract of Korean mint *Agastache rugosa*.

- 10 The mixture was closed in a gelatin capsule. The capsulation process followed the standard procedure. The excipients used in the capsule were: the typically used fillers (refined soybean oil), thickeners (silicon oxide), and emulsifier (soy lecithin).

- 15 **a) Preparation of lozenges containing seed extract of Black cumin *Nigella sativa* and cone extract of Hop *Humulus lupulus*.**

Lozenge composition:

No	Ingredient	Amount [mg]
1	Seed extract of Black cumin <i>Nigella sativa</i>	150
2	Cone extract of Hop <i>Humulus lupulus</i>	75
3	Sucrose	560
4	High fructose corn syrup	1200
5	Water	230
6	50% citric acid	30
Total mass of a lozenge		620

- 20 The mixture was formulated into a lozenge. The process followed the standard procedure.

5 **Example 8.**

Preparation of extracts for biological testing *in vitro*.

Dry extracts were dissolved in DMSO, ethanol or water, depending on their solubility, to the concentration of 20 mg/ml. In order to accelerate the process every solution was vortexed.

10

Example 9.

The impact of the extracts on the relative mRNA expression of interleukine-4 (IL-4) by Real-Time PCR.

Cell line:

15 Basophils RBL2H3 (ATCC 312, DSMZ) were cultured in the MEM medium containing 10% of inactivated fetal bovine serum and 1% of antibiotics. The cells were grown in a 5% CO₂ humidified incubator at 37°C.

RBL2H3 cells were seeded at the initial density of 7x10⁵ cells per 3 ml of the medium in 60 mm diameter Petri dishes. The DNP-IgE antibody was added to
20 the cells at the final concentration of 1 mg/ml and those were incubated for 16 hours. Once the cells were coated, the cell antibody was washed away with sterile saline. Then the tested extracts dissolved in a fresh medium were added and those were incubated for 1.5 hours. The extracts were prepared according to the description in Example 8. Then, 5 µg/ml of protein antigen DNP-BSA
25 (bovine albumin conjugated with dinitrophenyl) was added to the extract containing medium for 30 minutes. After the time, the supernatant was sampled, the cells washed with saline solution, scraped, spun, and the collected material was placed at -80°C until further analyses.

RNA was isolated from the collected material using the commercial Total RNA
30 Mini Plus set. cDNA was synthesized from the total RNA using the Transcriba set. The amplification reactions were conducted in the volume of 20 µl using the Real-time 2xPCR Master Mix Kit A set. All sets were used in accordance with the manufacturer instructions

35

5 The following primers were used:

Gene	Forward	Reverse
GADPH	5'-GTTACCAGGGCTGCCTTCTC-3'	5'-GATGGTGATGGGTTTCCCGT-3'
IL-4	5'-AAGGAACACCACGGAGAACG-3'	5'-AGACCGCTGACACCTCTACA-3'

Table 10. The sequences of the primers used in Real-Time PCR.

10 The reaction parameters: 3 min. at 95°C; 39 cycles: denaturation for 10 s at 95°C, annealing and extension for 30 s at 60°C; melting curve at 60°C to 95°C over 5 s in a thermocycler MiniOpticon (Biorad). The signals were normalized to the expression of the endogenous reference gene GADPH. The results of the relative expression of mRNA IL-4/mRNA GAPDH were presented as the percent of positive control.

No	Plant	Concentration [µg/ml]	Ratio	% K+
	Positive control (K+)			100
1	Root extract of Garden angelica <i>Angelica archangelica</i> (AA)	25		15
2	Herb extract of Meadowsweet <i>Filipendula ulmaria</i> (FU)	50		34
3	Seed extract of Black cumin <i>Nigella sativa</i> (NS)	25		21
4	Combination of extracts NS:AA	25	2:1	11
5	Combination of extracts NS:FU	25	4:1	1

15 Table 11. The impact of the extracts and their combinations on the relative expression of mRNA IL-4/mRNA GADPH, expressed as % of positive control.

The impact of the extracts on the relative expression of mRNA IL-4/mRNA GADPH, expressed as % of positive control, is illustrated on **Fig. 1**.

20 The obtained results evidently show that the reduction of the mRNA IL-4 level versus positive control is substantial when compositions of the said plants are applied instead of any of their extracts alone.

5 **Example 10**

The impact of the extracts on the cyclooxygenase 2 (COX-2) mRNA expression level by Real-Time PCR.

Cell line

10 RAW 264.7 (CLS 40039) cells were cultivated in the DMEM medium containing 10% of inactivated fetal bovine serum and 1% of antibiotics. The cultivation was conducted at 37°C, in 5% CO₂ atmosphere and 95% humidity.

The RAW 264.7 cells were seeded at the initial density of 1.5x10⁶ cells per 3 ml of culture medium in 60 mm diameter Petri dishes. After 24 h, the cells were re-suspended in a new culture medium and the tested extracts were added. After 4
15 h of incubation with extracts, 1 mg/ml of *E. coli* Lipopolysaccharide was added to the culture medium and the mixture was incubated for 24 h. The extracts were prepared according to the description in Example 8. After the time, the supernatant was collected for an ELISA bioassay. The cells were washed twice with phosphate buffer (PBS: 137 mM NaCl, 8.1 mM Na₂HPO₄, 1.5 mM
20 K₂H₂PO₄, 2.7 mM KCl, pH 7.4), scraped, and suspended in 3 ml of PBS, and centrifuged for 5 minutes at 300xg at 4°C. The collected cell pellets were snap frozen and stored at -80° C for analysis.

RNA was isolated from the frozen cell pellets using the Total RNA Mini Plus set. cDNA was synthesized from the total RNA using the Transcriba set. The
25 amplification reactions were conducted in the volume of 20 µl using the SensiFAST™ SYBR-No ROX set. All sets were used in accordance with the manufacturer instructions.

The following primers were used:

Gene	Forward	Reverse
GADPH	5'-AACGACCCCTTCATTGAC-3'	5'-TCCACGACATACTCAGCA-3'
COX-2	5'-CAGCACTTCACGCATCAGTT-3'	5'-CGCAGTTTACGCTGTCTAGC-3'

30 **Table 12.** The sequences of the primers used in the Real-Time PCR reactions.

The reaction parameters: 3 min. at 95°C; 39 cycles: denaturation for 10 s at 95°C, annealing and extension for 30 s at 60°C; melting curve at 60°C to 95°C over 5 s in a thermocycler MiniOpticon (Biorad). The signals were normalized to

- 5 the expression of the endogenous reference gene presented as the percent of positive control.

No	Plant	Concentration [ug/ml]	Ratio	% K+	SD
	Positive control (K+)			100	
1	Herb extract of Korean mint <i>Agastache rugose</i> (AR)	25		270	60
2	Root extract of Garden Angelica <i>Angelica archangelica</i> (AA)	25		81	27
3	Herb extract of Meadowsweet <i>Filipendula ulmaria</i> (FU)	25		98	22
4	Seed extract of Black cumin <i>Nigella sativa</i> (NS)	25		448	192
5	Composition of extracts NS:AR	25	1:1	73	2
6	Composition of extracts NS:AA	25	4:1	8	5
7	Composition of extracts NS:FU	25	1:1	53	5

Table 13. The impact of the extracts on the relative expression of mRNA COX-2/mRNA GADPH, expressed as % of positive control.

10

The impact of the extracts on the relative expression mRNA COX-2/mRNA GADPH, expressed as % of positive control, is illustrated on Fig. 2.

The obtained results evidently show that the reduction of the mRNA COX-2 level versus positive control is substantial when compositions of the said plants
15 are applied instead of any of their extracts alone.

Example 11.

Assessment of the cell degranulation level by monitoring β -hexosaminidase secretion.

Cells of the RBL2H3 line were seeded onto a 24-well plate at the initial density
20 of 1×10^5 . The anti-DNP-IgE antibody was added to the cells at the final concentration of 1 μ g/ml and those were incubated for 16 h. Once the cells were coated, the antibody was washed away twice with the HEPES buffer. Then, extracts dissolved in the HEPES buffer (pH=7.4) were added and those were incubated for 1.5 h. Subsequently, 5 μ g/ml of protein antigen DNP-BSA (bovine
25 albumin conjugated with 2-nitrophenyl) was added and this was incubated for 30 minutes. After the time, the supernatant was collected and placed in 96-well

- 5 plates adding 4-nitrophenyl-N-acetyl- β -D-glucosaminidase was incubated for 90 min at the temperature of 37°C. Next, carbonate buffer of pH 10.5 was added at the ratio of 1:1. The colorimetric measurement was taken at the wavelength of 405 nm using the BioTek microplate reader.

No	Plant	Concentration[ug/ml]	Ratio	%	SD
1	Root extract of Garden Angelica <i>Angelica Archangelica</i> (AA)	25		37	2
2	Herb extract of Meadowsweet <i>Filipendula ulmaria</i> (FU)	25		44	3
3	Seed extract of Black cumin <i>Nigella sativa</i> (NS)	25		24	3
4	Composition of extracts NS:AA	25	1:1	73	6
5	Composition of extracts NS:FU	25	1:1	78	6

Table 14. The impact of the extracts on the cell degranulation level vs positive control.

- 10 The impact of the extracts on the level of cell degranulation vs. positive control is illustrated on **Fig. 3**.

The obtained results evidently show that the said plant compositions demonstrate higher ability to inhibit cell degranulation as compared to that of any individual plant extract.

15

Example 12.

Assessment of the extracts' impact on the Interleukin 6 expression level.

Supernatants were collected following the procedure described in Example 10. The supernatants were centrifuged for 10 min.x2500g at 4°C and snap frozen to
 20 -80° C. They were dissolved as appropriate before testing. The IL-6 levels were tested in an ELISA assay in accordance with the procedure described by the manufacturer.

25

No	Plant	Concent [ug/ml]			
1	Herb extract of Korean mint <i>Agastache rugosa</i> (AR)	25		24	3
2	Root extract of Garden Angelica <i>Angelica Archangelica</i> (AA)	25		33	2
3	Herb extract OF Meadowsweet <i>Filipendula ulmaria</i> (FU)	25		45	7
4	Seed extract of Black cumin <i>Nigella sativa</i> (NS)	25		58	10
5	Composition of extracts NS:AA	25	1:1	75	2
6	Composition of extracts NS:FU	25	1:1	74	8
7	Composition of extracts NS:AR	25	4:1	66	4

5 **Table 15.** The impact of the extracts on IL-6 secretion, expressed as % vs. positive control.

The impact of the extracts on the secretion of IL-6 presented as the % vs. positive control illustrated by **Fig. 4**.

The obtained results clearly indicate that compositions of plants have a higher ability to inhibit the secretion of Interleukin 6 (IL-6) versus positive control than
10 any of the extracts applied alone.

Example 13.

Assessment of the extract effectiveness *in vivo*. The tests conducted on mice.

15

The assessment of the effectiveness of the seed extract of Black cumin *Nigella sativa* alone and in combination with the root extract of Garden Angelica *Angelica archangelica* at the ratio 4:1 was conducted on BALB/c mice (females 8-10 weeks old).

20 The tests were performed in 3 groups:

Negative control - placebo

Positive control (LPS)

1 – Diclofenac (NSAID)

2 – seed extract of Black cumin *Nigella sativa* (NS)

25 3 – combination of seed extract of Black cumin *Nigella sativa* and root extract of Garden angelica *Angelica archangelica* (NS:AA).

- 5 The aforementioned substances were administered mg/ml, once a day, for 7 consecutive days. Thereupon, the animals were treated intraperitoneally with LPS (3mg/kg) to cause inflammation. After 24 h, the blood serum was collected from the animals to assess the content of pro-inflammatory cytokines by the flow cytometric method (Luminex set).

	Control-	Control+	Diclofenac	NS	NS:AA
MCP-1 [pg/ml]					
Average	6.23	136.49	178.77	79.09	61.77
SD	0.68	50.28	90.72	18.74	34.08
KC [pg/ml]					
Average	3.46	93.38	134.74	182.84	47.93
SD	3.92	60.96	62.26	121.85	19.58
IL-13 [pg/ml]					
Average	3.82	4.00	6.69	6.22	3.75
SD	1.32	1.05	1.48	1.28	1.06
TNF [pg/ml]					
Average	2.58	4.98	4.63	5.21	3.22
SD	1.15	2.28	38.75	1.31	51.04
IL6 [pg/ml]					
Average	4.94	12.74	12.18	19.17	7.62
SD	0.68	4.23	3.17	17.1	2.25
IL-12 [pg/ml]					
Average	22.64	35.33	14.65	54.84	12.90
SD	6.91	14.40	5.48	30.61	6.92

10 **Table 16.** The impact of seed extract of Black cumin *Nigella sativa*, the combination of seed extract of Black cumin *Nigella sativa* and root extract of Garden Angelica - *Angelica archangelica*, and the reference product – Diclofenac (NSAID) on the pro-inflammatory cytokines.

The impact of the seed extract of Black cumin *Nigella sativa*, the combination of the seed extract of Black cumin *Nigella sativa* and the extract of Garden
 15 *Angelica Angelica archangelica*, versus the reference product – Diclofenac (NSAID) on the pro-inflammatory cytokines is illustrated on **Fig. 5**.

The obtained test results show that the composition made up of seed extract of Black cumin *Nigella sativa* and root extract of Garden Angelica *Angelica archangelica* reduces the level of all examined pro-inflammatory cytokines to a
 20 higher extent than the seed extract of Black cumin *Nigella sativa* alone, or the popular non-steroid anti-inflammatory drug - Diclofenac.

5 **Example 14.**

Assessment of extract toxicity *in vivo*. The tests conducted on mice.

Determination of the Maximum Tolerated Dose (MTD) was conducted on female, 8-10 weeks old BALB/c mice.

10 The acute toxicity test consisted in a single oral administration of the composition made up of seed extract of Black cumin *Nigella sativa* and root extract of Garden angelica *Angelica archangelica* at the ratio 4:1, and the composition of seed extract of Black cumin *Nigella sativa* and herb extract of Meadowsweet *Filipendula ulmaria* at the ratio 4:1, the mice body weight taken into consideration and the dose being 1x 2000 mg per kg of the body weight. 3
15 specimen mice were designated for each combination. The following stage came down to observation of the animals and taking their body weights (every 2-3 days) for 14 consecutive days. After the period and up to the experiment completion, the body weight was measured 2 times more.

The chronic toxicity test consisted in multiple oral administration of the pre-
20 determined volume of the composition made up of seed extract of Black cumin *Nigella sativa* and root extract of Garden angelica *Angelica archangelica* at the ratio of 4:1, and of the composition of seed extract of Black cumin *Nigella sativa* and herb extract of Meadowsweet *Filipendula ulmaria* at the same ratio, as appropriate for the mouse body weight. The dose was 1000 mg/kg x body
25 weight, administered daily for 14 days.

The mice of the experiment control group were treated with edible oil at the dose of 0.1 ml per mouse, for 14 days too. Each of the tested and control groups was made up of 3 specimen mice. The animals were observed and their weight taken daily for 14 consecutive days. After the period and up to the
30 completion of the experiment, the body weight was checked 2 more times.

The conducted acute and chronic toxicity tests did not yield any observation of a toxic effect of the above mentioned extract compositions on the animal organisms.

5 Example 15

Assessment of anti-bacterial properties against *Streptococcus pyogenes* PCM 465.

The assessment of anti-bacterial properties of the composition made up of seed extract of Black cumin *Nigella sativa* (NS) and cone extract of Hop *Humulus lupulus* (HL) against *Streptococcus pyogenes* PCM 465 was performed by the plate-diffusion method. The test was conducted in accordance with the MIK-10/SPR and MIK-3/SPR protocols recommended by National Reference Centre for Drug Sensitivity and Microorganisms.

Filter paper discs were saturated with specific concentrations of the tested extract compositions, antibiotics, and DMSO. The saturated filter paper disc were then placed directly on an inoculated agar culture medium LA with glucose added.

The inoculated plates were incubated at 37 °C for 24 hours.

Examined substance [ratio]	Concentration	Diameters of the bacterial growth inhibition zones [cm]
Ampicillin	10 µg/ml	2.5
Ampicillin	5 µg/ml	2
DMSO	100%	0.2
NS:HL [4:1]	800 mg/ml	3.2
NS:HL [4:1]	200 mg/ml	2.4
NS:HL [4:1]	100 mg/ml	2.2

Table 17. The impact of the extracts, DMSO, and the reference antibiotic (ampicillin) on growth inhibition of the *S. pyogenes* PCM 465 strain.

The diameter of the bacterial growth inhibition zone is directly proportional to the sensitivity of the bacterium to the antibiotic and the tested combination of the plants (NS:HL).

The extract combination proved significantly inhibiting to the *Streptococcus pyogenes* growth; moreover, it was dependent on the concentration of the NS/HL combination versus the used reference antibiotic.

Example 16

Assessment of anti-bacterial properties against *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus*.

The assessment of antibacterial properties of the composition of seed extract of Black cumin *Nigella sativa* (NS) and cone extract of Hop *Humulus lupulus* (HL)

- 5 against *Staphylococcus aureus* and the *Methicill. aureus*. Wells were formed in the center of each agar plate and filled with 30 μ l of the tested substance. The experiment was conducted on the Mueller-Hinton agar growth medium. Each composition was tested undissolved, as well as dissolved in water-DMSO mixture (2/8, v/v).
- 10 The plates were incubated at 37°C for 16 hours. After the time, it was possible to observe the inhibition zone.

Diameters of the bacterial growth inhibition zones [cm]												
Bacterial strain	Ratio NS:HL											
	10:1	8:1	6:1	4:1	2:1	1:1	1:2	1:4	1:6	1:8	1:10	0:1
<i>Staphylococcus aureus</i> 209P	6	6.3	6.2	6	6.2	5.6	5.8	5.8	5	4.6	4	
<i>Staphylococcus aureus</i> 15	4.2	4.2	4.7	4.5	4	3	2	3.1	2.3	2	2.6	2.6
<i>Staphylococcus aureus</i> 1397	5.3	4.6	5	5.2	4	3.5			4	3.2	3.5	3.2
<i>Staphylococcus aureus</i> 56A5	6.1	5.7	6.4	6.1	6	5.3	5.4	3.2	3.3	2.6	2.8	2.8
<i>Methicilin-resistant staphylococcus aureus</i> 12 144	6.5	6	5.4	5	6.7	5.4	5.5	4.4	4.1	3.4	4.2	
<i>Methicilin-resistant staphylococcus aureus</i> 6347	5	5	5.4	5.6	5.2	4.6	4.5	3.7	3.5	2.9	3	2.8
<i>Methicilin-resistant staphylococcus aureus</i> 411	7.2	6.4	5.8	6.8	4.8	4.9	5.4	4.3	3.1	2.6	3.1	2.6

Table 18. The impact of the extracts on growth inhibition of the *Staphylococcus aureus* bacterial strain and on the *Methicilin-resistant Staphylococcus aureus*

- 15 The obtained test results show that the NS/HL combination effectively inhibits the growth of all used clinical strains of *Staphylococcus aureus*, the multi-drug resistant MRSA strains included.

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5

Claims

1. A plant composition with anti-inflammatory, anti-allergic, anti-asthmatic and/or antibacterial properties which exert synergistic effects and comprise at least two extracts from the following plant material: the seeds of Black cumin *Nigella sativa* seeds, the root of Garden angelica *Angelica archangelica*, the herb of Meadowsweet *Filipendula ulmaria*, the herb of Korean mint *Agastache rugosa*, and the cone of Hop *Humulus lupulus*.
10
2. The plant composition according to claim 1, **characteristic in that** the percentage content of the extracts is as follows: the seed extract of Black cumin *Nigella sativa* (5-70%), the root extract of Garden angelica *Angelica archangelica* (0.5-30%), the herb extract of Meadowsweet *Filipendula ulmaria* (0.5-40%), the herb extract of Korean mint *Agastache rugosa* (0.2-40%) and the cone extract of Hop *Humulus lupulus* (0.5-50%).
15
3. The plant composition according to claim 1, **characteristic in that** the weight ratio of the seed extract of Black cumin *Nigella sativa* and the root extract of Garden angelica *Angelica archangelica* is 1:1 to 12:1 and 1:12.
4. The plant composition according to claim 1, **characteristic in that** the weight ratio of the seed extract of Black cumin *Nigella sativa* to the herb extract of Meadowsweet *Filipendula ulmaria* is 1:1 to 1:4 and 4:1
25
5. The plant composition according to claim 1, **characteristic in that** the weight ratio of the seed extract of Black cumin *Nigella sativa* to the herb extract of Korean mint *Agastache rugosa* is 1:1 to 1:4 and 4:1.
6. The plant composition according to claim 1, **characteristic in that** the weight ratio of the seed extract of Black cumin *Nigella sativa* to the cone extract of Hop *Humulus lupulus* is 1:1 to 1:8 and 8:1.
30
7. The plant composition according to claim 1, **characteristic in that** it further contains typical excipients, bulking agents, thickeners, antioxidants, vitamins, and emulsifiers.
35
8. The plant composition according to claims 1-6, **characteristic in that** it contains a combination of plant extracts demonstrating synergistic

5 effects, given the pharmaceutical form of cr
gels, emulsions, lozenges, powders, drops, syrups, and aerosols.

9. The application of the plant composition according to claims 1-7,
10 **characteristic in that** the plant composition is used to develop products
intended for the treatment of inflammations, allergies and/or asthma in
the pharmaceutical form of creams, ointments, capsules, gels,
emulsions, lozenges, powders, drops, syrups, and aerosols

10. The product containing plant compositions, **characteristic in that** it
contains 1-100% of the composition according to any of claims 1-8.

15 11. Product according to claim 9, **characteristic in that** it is classified in the
following group: dietary supplement, food for special medical purposes,
cosmetic, medical product, medicinal product

12. Product according to claim 11, **characteristic in that** it is classified in the
20 following group: creams, ointments, capsules, gels, emulsions, lozenges,
powders, drops, syrups and aerosols

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30

5

Fig. 1.

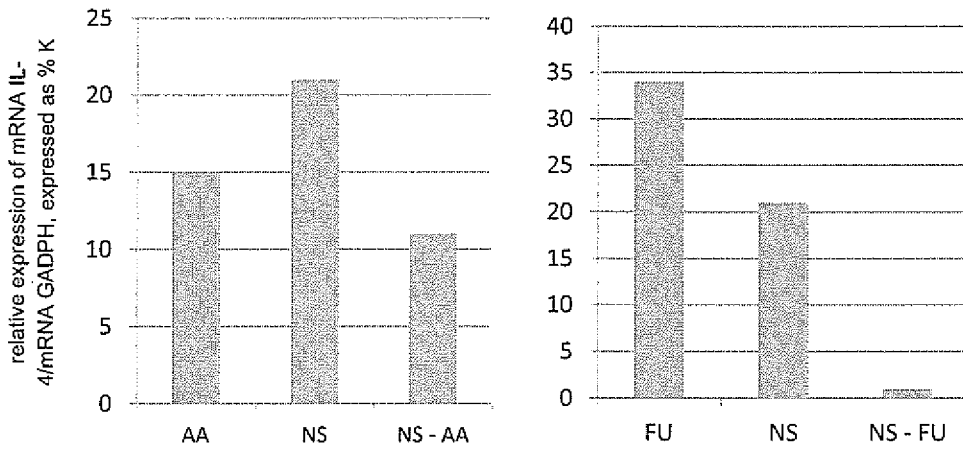
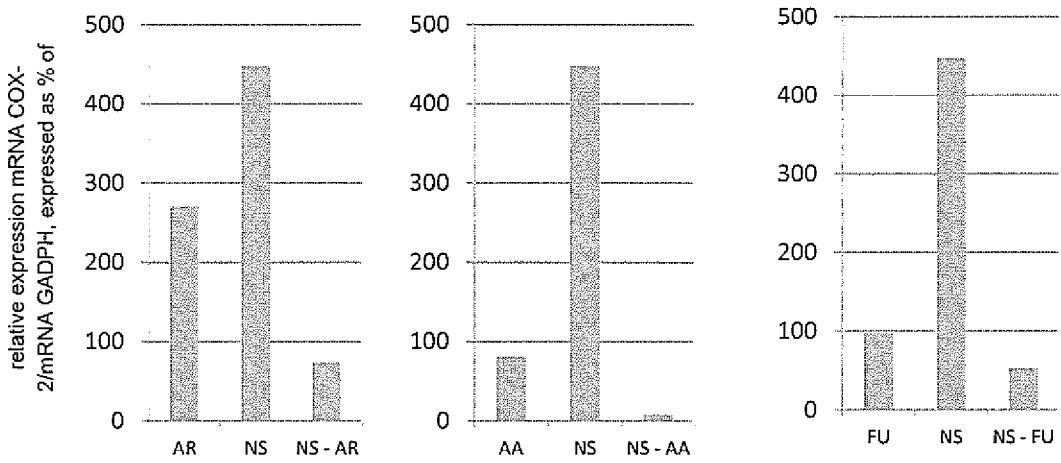


Fig. 2.

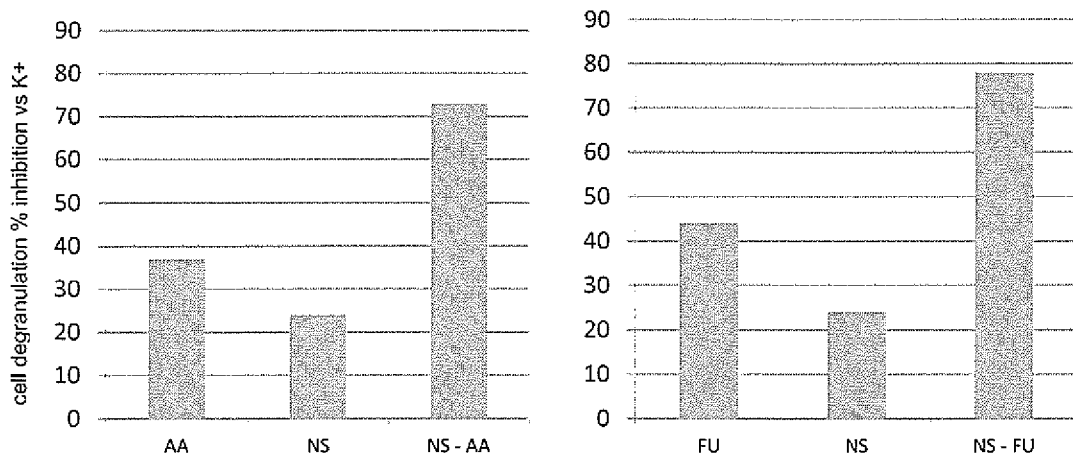


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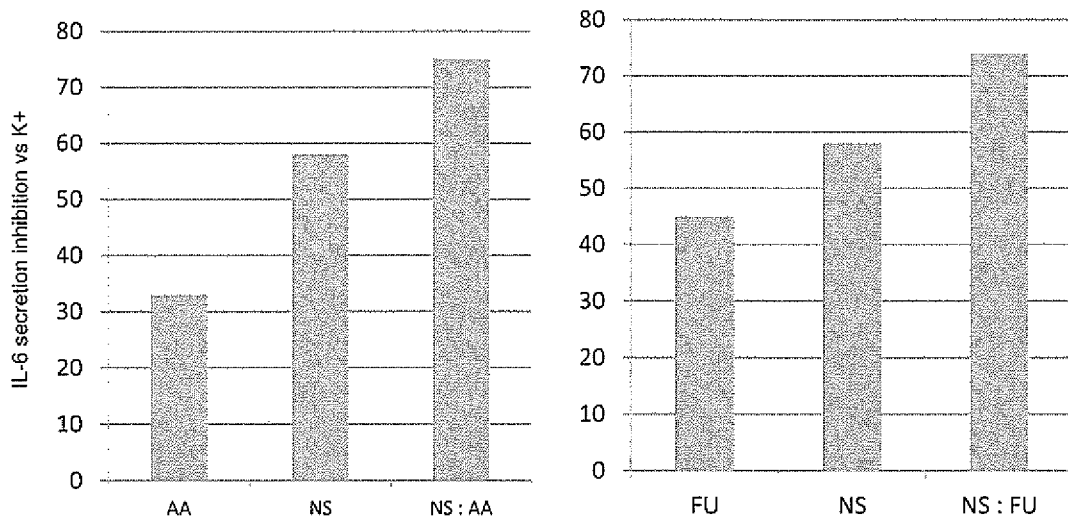
5

Fig. 3.



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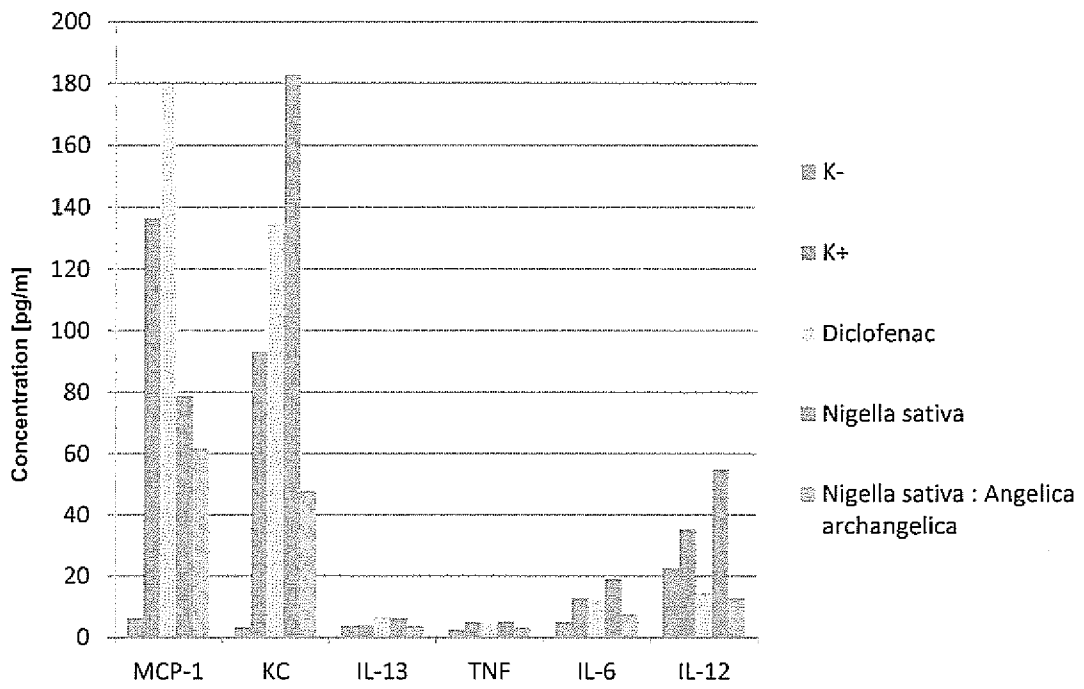
Fig. 4.



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Fig. 5.



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INTERNATIONAL SEARCH REPORT

International application No
PCT/PL2016/000048

A. CLASSIFICATION OF SUBJECT MATTER
 INV. A61K36/185 A61K36/232 A61K36/532 A61K36/71 A61K36/73
 A61P29/00 A61P37/08 A61P11/06
 ADD.
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 201528 Thomson Scientific, London, GB; AN 2015-16939C XP002760162, -& HU 1 300 497 A2 (KUTI J) 2 March 2015 (2015-03-02) abstract	1-12
X	----- WO 00/51580 A2 (AL JASSIM RAWAA [AU]; AL KAISI BAN [US]; JAMES DAVID [QA]; NASSIEF NID) 8 September 2000 (2000-09-08) abstract ----- -/--	1-12

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 27 July 2016	Date of mailing of the international search report 09/08/2016
-------------------------------------------------------------------------------	----------------------------------------------------------------------

Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Pilling, Stephen
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/PL2016/000048

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/PL2016/000048

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/059926 A1 (SKIN MATRIX LTD [IL]; DORON SHIRLI [IL]) 10 May 2012 (2012-05-10) abstract page 5, line 32 - page 6, line 5 -----	1-12
X	EP 1 029 531 A2 (SQUIBB BRISTOL MYERS CO [US]) 23 August 2000 (2000-08-23) abstract -----	1-12
X	US 2006/003030 A1 (CHUN-YING LIN [TW] ET AL) 5 January 2006 (2006-01-05) abstract paragraph [0006] -----	1-12
X	WO 2005/053720 A1 (INDENA SPA [IT]; BOMBARDELLI EZIO [IT]) 16 June 2005 (2005-06-16) abstract -----	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/PL2016/000048

Patent document cited in search report	Publication date	Publication date	Patent family member(s)	Publication date
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			TW I300716 B	11-09-2008
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			SI 1713490 T1	31-08-2007
			US 2007071839 A1	29-03-2007
			WO 2005053720 A1	16-06-2005

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-12

A plant composition with anti-inflammatory, anti-allergic, anti-asthmatic and/or antibacterial properties which exert synergistic effects and comprise at least two extracts from the following plant material: the seeds of Black cumin *Nigella sativa* seeds, the root of Garden angelica *Angelica archangelica*, the herb of Meadowsweet *Filipendula ulmaria*, the herb of Korean mint *Agastache rugosa*, and the cone of Hop *Humulus lupulus*.

1.1. claims: 3(completely); 1, 2, 7-12(partially)

plant composition comprising seed extract of Black cumin *Nigella sativa* and the root extract of Garden angelica *Angelica archangelica*.

1.2. claims: 4(completely); 1, 2, 7-12(partially)

plant composition comprising seed extract of Black cumin *Nigella sativa* and the herb extract of Meadowsweet *Filipendula ulmaria*.

1.3. claims: 5(completely); 1, 2, 7-12(partially)

plant composition comprising seed extract of Black cumin *Nigella sativa* and the herb extract of Korean mint *Agastache rugosa*.

1.4. claims: 6(completely); 1, 2, 7-12(partially)

plant composition comprising seed extract of Black cumin *Nigella sativa* and the cone extract of Hop *Humulus lupulus*.
