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Patentee: HERBAL & ANTIOXIDANT DERIVATIVES SRL [IT]

Title: PHYTOCOMPLEX FROM BERGAMOT FRUIT, PROCESS OF
MANUFACTURE AND USE AS DIETARY SUPPLEMENT AND IN THE
PHARMACEUTICAL FIELD

International Filing Date 13/11/2009

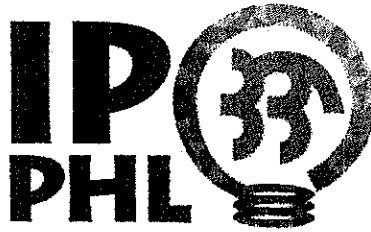
NOTICE OF ISSUANCE OF LETTERS PATENT

This is to inform you that the Letters Patent Certificate for the above-identified invention has been prepared and is now ready for release.

Said Letters Patent Certificate will be released to you or to your authorized representative upon presentation of this letter to this office.

Please be informed that no Letters Patent Certificate shall be released by the Office unless a documentary stamp(s) worth P20.00 is affixed on the face thereof as required under R.A. 7660 date December 23, 1993 and R.A. 9243 dated February 17, 2004, more particularly Title VII, Sections 173 to 201 thereof.

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Patent No. 1-2011-500926

Having complied with the provisions of Republic Act No. 8293 and its regulations, this Office grants **LETTERS PATENT** for an

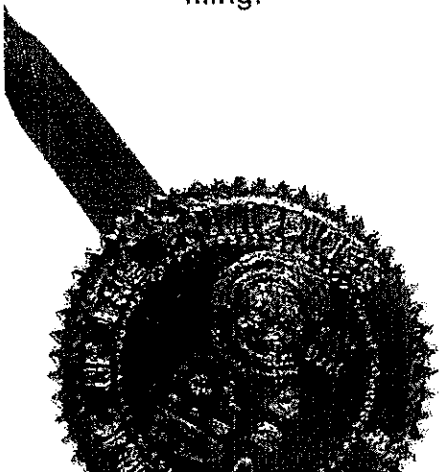
INVENTION

the pertinent data, specification and claim/s of which are hereunto annexed and made part hereof.

Now, therefore, this **LETTERS PATENT** grants unto its owner/s the exclusive right throughout the Philippines to make, use, sell or import the invention and where the invention is or includes a process, including the product obtained directly or indirectly from such process; and,

Unless sooner terminated as provided for by law and the regulations, the term of this **LETTERS PATENT** shall be **TWENTY (20) YEARS** from the date of filing.

IN WITNESS WHEREOF, I have hereunto affixed my hand and the seal of the Intellectual Property Office at Taguig City, Philippines




FRANCIS M. SUGCO

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Bureau of Patents

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[4] Title: PHYTOCOMPLEX FROM BERGAMOT FRUIT,
PROCESS OF MANUFACTURE AND USE AS DIETARY
SUPPLEMENT AND IN THE PHARMACEUTICAL FIELD
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[9] Foreign Application Priority Data: RM2008A000615 17/11/2008 IT
[3] Int. Class⁸: A 61K 36/752, A 61P 3/06, 39/06
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Reference(s) Considered/Cited: NONE

ABSTRACT

The invention relates to a phytoextract obtained from the bergamot fruit albedo (Citrus Bergamia Risso & Poiteau), the production thereof and the use thereof, in particular under the form of dry extract, to be used as normalizer of the metabolic unbalances of lypemic and glycemic type, in the treatment of the cutaneous diseases with inflammatory or degenerative character, as painkilling/anti-inflammatory agent, as antineoplastic agent and dietary supplement.

Technical Field	Biotechnology			
Claim(s)	14	claim/s	Drawing(s)	0 sheet/s
Int. Dep. Claim(s)	0	claim/s	Specification and Claim(s)	11 page(s)
Dep. Claim(s)	0	claim/s	Sequence Listings	0 page(s)
Total of Claim(s)	14	claim/s		

PHYTOCOMPLEX FROM BERGAMOT FRUIT, PROCESS OF MANUFACTURE
AND USE AS DIETARY SUPPLEMENT AND IN THE PHARMACEUTICAL FIELD

DESCRIPTION

The invention relates to a phytocomplex obtained from the bergamot fruit (in particular a dry extract), a process for the production thereof and the use thereof in the pharmaceutical field and as dietary supplement. If administered by oral route, the phytocomplex has the peculiarity of exerting a normalizing and controlling action with respect to the Cholesterol level, Triglycerides and Glycemia in the blood with consequent strong atherogenic action. The effectiveness of such action appears even more interesting as directly proportional to the presence of criticality factors in the human organism linked to hyperglycemia and overweight (metabolic syndrome). Furthermore, in the test animal the phytocomplex exerts an antioxidant/anti-inflammatory action which appears both after oral administration, together with a painkilling effect, and, for topic use, after application in areas wherein cutaneous inflammation has been induced. Finally, from studies performed on tumoral lines in culture, the phytocomplex has shown a consistent activity of inhibiting the cell proliferation.

State of art

Bergamot (Citrus Bergamia Risso & Poiteau) is a citrus fruit grown substantially only in restricted areas of Calabria, three main varieties thereof called Femminello, Fantastico and Castagnaro, respectively, are known.

It is known that bergamot represents, within the Calabrese citrus fruit panorama, a particularly precious niche product. In fact, such citrus fruit develops in a limited strip of Reggio Calabria province territory, going from the Tyrrhenian southern side to the Ionic southern side crossing the area of the Messina strait. Therefore, a strip no longer than 150 Km, extending from the more strictly coast area to the pre-hill area of the hinterland of Reggio Calabria. Up to now bergamot has been used exclusively for the properties of the essential oil, particularly requested in perfumery as endowed with an exclusive fragrance which is still being used as basis for preparing several perfumes by the most famous perfume industries and for producing "Acqua di Colonia". This oil is obtained by peeling from the most external portion (cuticola) of the bergamot peel.

It is also known that bergamot has significant antiseptic properties, so that for several years a bergamot derivative, the Bergamon, has been produced and used as disinfectant in the operating rooms. Furthermore, from the traditional medicine the relaxing properties of the bergamot extracts and the important nutraceutical properties of the same are known. At last, the hydroalcoholic solution of

the bergamot essence is used for preparing some products (one in particular with the name of Bergarytal), which have been proposed as sprays endowed with the skin's decongestive action and particularly effective in keeping away the mosquitos. However, the finding that some components of the bergamot essence, such as the bergaptene, potentially show toxic action has limited the use thereof by requesting procedures for keeping away this compound for the safe use of the essential oil extracts.

The document Gardana Claudio et al "*Evaluation of flavonoids and furanocumarins from Citrus Bergamia (Bergamot) juice and identification of new compounds*" MOLECULES (BASEL, SWITZERLAND) 2008, vol.13, NO. 9, 18 September 2008 (2008-09-18), pages 2220-2228 is directed only to the study of the content of flavonoids and furanocumarins in a sample of bergamot juice.

WO 2008/061536(COSMEDICAL APS [DK] describes, among other things, compositions for treating the eye disorders with compositions containing also bioflavonoids and a method for extracting such bioflavonoids from bergamot. Such method does not use either pectolytic enzymes or a separation with cationic resins and it does not make reference to the furocumarine content of the obtained product.

Mandalari Giuseppina et al "*Enzymatic hydrolysis of flavonoids and pectic oligosaccharides from bergamot (Citrus bergamia Risso) peel*" Journal of Agricultural and Food Chemistry, American Chemical Society, Washington, US vol.54, Nr. 21 1 October 2006 pages 8307 - 8313 describes the extraction of flavonoids and other compounds starting from the peel of the bergamot fruit, peel considered as sub-product of the essential oil industry.

Gattuso Giuseppe et al "*Flavonoid glycosides in bergamot juice (Citrus bergamia Risso)*" Journal of Agricultural and Food Chemistry, vol 54, Nr. 11, May 2006 describes a study developed with the only purpose of differentiating two glycosidates flavonoids contained in a bergamot juice having industrial origin which has been treated with dymethylformamide that is to say a solvent which may not find use in products of medical and/or cosmetic interest.

The authors of the present invention have surprisingly found that, from the bergamot fruit, in particular from the albedo, it is possible to obtain a concentrated phytocomplex showing a great healing action by positively effecting the metabolic activity and by normalizing the lipidic and glycemc levels of the subjects affected by these pathologies. The albedo, in the citrus fruits, is the portion lying between the epidermis including flavedo and pulp and it is formed by cells with tubular structure forming a real net with most part of the tissue volume compressed in the

intercellular space. The phytocomplex obtainable preferably under the form of dry extract, thanks to the particular extraction and purification process, has among the components thereof the drastic reduction (up to nearly the elimination) of the furocoumarines existing in the fruit (Bergaptene and Bergamottine) considered potentially toxic substances, at the same time by keeping and even increasing the described healing action.

Therefore, the object of the present invention is the process claimed by claim 1.

The process will better described hereinafter.

10 **Step a (grinding of partially de-oiled fruit)**

The fruit, without the most external portion (Flavedo) and partially de-oiled for removing the outer cuticle, is minced preferably by means of grinding mill in small strips with size smaller than one cm in length.

Step b (reduction in the pectine content)

15 In the mixture obtained from point 1 enzymes apt to degrade the pectine, pectolytic enzymes) are inoculated and within 20 - 40, preferably 30 minutes, with temperature depending from the used enzyme, a much more fluid product is obtained, therefore better workable.

Step c (Separation of the fibrous portion from the liquid portion):

20 The product of point 2 is reduced to a residual pulp content lower than 0,5% advantageously by means of sequential passage on decanter and centrifuge.

Step d (Inactivation of the pectolytic enzymes):

25 Such result will be obtained after a pastozation of the liquid of point c, the pastozation will have as secondary effect the one of eliminating by evaporation a remarkable portion of residual essential oils.

Step e (Making clear the aqueous solution):

30 It is obtained by means of a ultrafiltration process in a plant equipped with semi-permeable membranes with selectivity equal to 12.000 dalton of molecular weight (particles with PM lower than 12.000 dalton go through the membranes). It is also possible, and forms part of the invention, a variant of the process wherein the making clear action takes place by means of treatment with bentonite.

Step f (adsorption of the limpid solution on columns containing polystyrenic absorbing resins):

35 The separation of the polyphenols from the remaining solution is obtained by entrapping the same in the pores of the polystyrenic absorbing resins having pores with diameter comprised between 100 and 150 Ångstrom (ex. SEPEABEAD SP 207 Mitsubishi). The polyphenols can be extracted, according to a variant of the process

of the invention, also with a mixture of ethyl or methyl alcohol, and ethyl acetate in the ratio 3:1 and recovered by means of subsequent solvent concentration (for example by means of evaporation and subsequent drying).

5 **Step g (recovery of the polyphenolic fraction entrapped in the resins' pores of point f and contemporary lowering of the furocumarine value up to 400 mg/KG of dry product):**

After having performed the outer washing of the resins with hot water at 40 °C, in order to eliminate sugars and acids contained in the solution of step 5, the recovery of the polyphenolic fraction is carried out by opening such organic compounds, 10 characterized by a circular spatial arrangement, thereby assuming a linear configuration, so to be released from the holes wherein they were trapped. Such effect is obtained by rising the PH up to strongly basic values (pH 12- 14). This takes place advantageously by using hydroxides of alkaline metals which also degrade bergaptene and Bergamottine. Equal effect can be obtained if, instead of 15 using the basic solution, ethylic or methylic alcohol is used. In this case the bergaptene and Bergamottine degradation would not be obtained.

Step h (Chemical stabilization of the phytocomplex in aqueous phase):

The polyphenols in the (open) linear form thereof are extremely instable as they tend to be object of oxidative phenomena. It is necessary in very short time to bring 20 them back to the ring-like shape. Such result is obtained by lowering the pH from the value 14 up to strongly acid values (around pH 3). Such effect is obtained by subtracting from the solution cations K+, Na+, etc. This takes place by using strongly cationic resins (ex. Relite CF H+ Mitsubishi). These replace the existing cation with a positive hydrogen (H+). The operation effect is an acidification of the 25 solution up to pH = 3 and a subtraction of cations of the used alkaline hydroxide. The result is a product more valuable under the nutritional aspect.

Step i (Physical stabilization of the product; "Drying"):

It is obtained by means of removing water, advantageously by evaporation in high vacuum, then at temperatures lower than 60°C up to complete drying (that is by 30 obtaining a residual humidity lower than 14%). At such humidity values the proliferation of any type of organic contaminant (yeasts, bacteria, etc.) is inhibited. A dry product is obtained.

It is to be underlined that the extraction and purification process allows reducing in drastic manner the contained furocumarines in (bergaptene and 35 Bergamottine) which represent a problem in the light of their character of potential toxicity; however found at cutaneous level only and in much higher doses than those found in the juice.

The phytocomplex of the invention, in the form of dry extract, appears like a very fine, hydrosoluble powder, with a yellow-brown colour, with characteristic odour and with bitter taste. It can be encapsulated, put into envelopes, mixed with oils for packaging creams etc. and therefore formulated with pharmaceutically compatible excipients, usual in the formulations of dietary supplements.

An additional object of the present invention is a phytocomplex, preferably under the form of dry extract, obtainable from the albedo of the bergamot fruit according to the process of the invention.

The authors of the present invention have further found – and also this forms object of the invention – that the phytocomplex can be used in the clinical practice and/or as dietary supplement since it has an advantageous ratio between nutritive substances (folic acid, vitamins, etc.) and anti-oxidants (flavonoids in particular) which allows it to be used as antidiislipemic and antiaterogeneous agent. Compositions based upon bergamot dry extract as antidiislipemic and,or antiaterogeneous and,or antiinflammatory/painkilling topic and systemic agent and,or antitumoral agent containing the phytocomplex of the present invention, advantageously under the form of dry extract, together with additives and/or vehicles of common use in pharmaceutics, are also object of the present invention.

The compositions can be used both in liquid forms and in other forms, lyophilisate, granulate, powder. It has been demonstrated that the phytocomplex performs its healing action for the skin pathologies with inflammatory character if applied by topic route, anti-inflammatory/painkilling action after systemic administration in the test animal and an anti-proliferative action on tumoral human cells in culture. Advantageously, dosages ranging from 20 and 40, advantageously 30 mg/kg of body weight, are proposed.

Additional objects of the present invention are dietary supplements based upon the phytocomplex of the invention as adjuvant agents in treatments of antidiislipemic and,or antiaterogeneous type and,or for vascular protection.

The phytocomplex of the present invention is a product of natural origin for dietary supplement or pharmaceutical use exerting an action of normalizing the cholesterol level, fats and glycemia in the blood, by means of the combined action of inhibiting the absorption of the cholesterol precursors already at intestinal gastric level, exerted by the polyphenolic fraction contained therein, as well as of inhibiting the activity of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase in the mammals.

Furthermore, upon studying the effects of the phytocomplex according to the invention it was found, in the test animals, an absolute keeping of the main

ematochemical indexes (complete blood count, hepatic transaminase, azotemia and hypercreatininemia) within normality ranges by proving the absence of toxic effects. Such data were confirmed by the absence of steatotic or necrosis phenomena at hepatic and renal level in the treated animals examined post-mortem at microscope.

5 Furthermore, a histopathological analysis of the brain tissue and of the peripheral nerves proved the absence of induced axonopathies or mielinopathies. The study of the phytocomplex toxicity had the following result: "the toxicologic analysis does not show toxicity factors, according to the legal framework in force". In particular, together with the absence of the common citrus fruit contaminants, it was found the

10 absence of pathological values or however values outside the normal reference values as far as the presence of heavy metals, pesticides, PCB, nitrites and nitrates, dyes, moulds is concerned apart from the absence of ocratoxins, bacterial endotoxines, anaerobic germs and moulds. From the examination of organs (liver, kidney) taken from the guinea pigs, no toxic effect for administrations equal to

15 80mg./Kg of body weight/day in the oral administration was noted.

It is to be underlined that the use of hydroxides of alkaline metals, in particular of KoH reduces considerably the quantity of existing furocumarines and the obtained phytocomplex appears much more concentrated as to the functional aspect.

20 The phytocomplex object of the present invention is characterized by the biological singleness of the polyphenolic profile having the main Bioflavonoids, in the following percentages:

Neohesperidin	29.6 % +/- 6.0
Naringine	32.4 % +/- 4.0
Neohesperidin	38.0 % +/- 6.0
Total	100.0%

25 According to the invention, after the step i) of the process, a phytocomplex, under the form of dry extract, is obtained which has a minimum content of neohesperidin, naringine, neohesperidin not lower than 250 gr / kg that is 25%. Furthermore, the furocumarines meant as Bergapten and Bergamottine are present in quantities not higher than 400 mg / kg.

Differently from the greatest part of the extracts of flavonoids on the market, the phytocomplex of the present invention is extremely soluble in addition to alcohol also in water at room temperature (20 °C). The analyses of the dry extract have further shown the equivalence with the substances contained in the bergamot juice.

5 **Examples**

The effects of phytocomplex were tested on 100 patients affected by primitive familiar hypercholesterolaemia with and without associated hypertriglyceridemia. The patients were randomized in equilibrated manner between the two sexes (48 males and 52 females), in an age range comprised between 45 and 70 years old.

10 The patients were classified, with respect to the hematic cholesterol LDL levels (cLDL), according to the risk brackets defined by the National Cholesterol Education Programme (NCEP ATP III) of the NHI (National Health Institute). 500mg/die pills were administered to all subjects for a period of 30 days. The botanic type, used for extracting the phytocomplex, was Citrus Bergamia Risso and Poiteau and fruits

15 coming from cultivar Castagnaro, Femminello and Fantastico were used. The treatment lasted 1 month. The patients (32 out of total) who already were taking at the same time statine or other anti-dislipidemic drugs, were invited to continue the already started therapy. At the end of the treatment, the subjects were being observed for 30 days following the end the phytocomplex assumption. The obtained

20 data show the following results:

- 1) In all subjects, a reduction comprised between 20 and 32% of the plasmatic levels both in the total cholesterol and LDL, with an average increase of 30% in the HDL cholesterol levels was noted.
- 25 2) The subjects affected by familiar hypercholesterolaemia, treated only with diet, who had basal plasmatic levels comprised between 230 and 280 mg/dl of cholesterol showed a reduction in the total cholesterol level of $34\pm 4\%$, of 32 ± 5 in the LDL cholesterol level and an increase in HDL cholesterol level of $28\pm 3\%$.
- 30 3) The subjects affected by familiar hypercholesterolaemia, treated only with diet, who had basal total cholesterol levels comprised between 200 and 230, had a decrease in the total cholesterol plasmatic levels of $28\pm 4\%$, in LDL cholesterol of $22\pm 2\%$ and an increase of $24\pm 5\%$ in the HDL cholesterol levels.
- 35 4) The subjects of the two preceding groups who already were taking statine, had an additional decrease in the total cholesterol plasmatic levels of $20\pm 3\%$, in LDL cholesterol of $20\pm 4\%$ and an increase in HDL cholesterol of $15\pm 3\%$.

- 5) The examined subjects having mixed forms of dislipidemy (hypercholesterolaemia and hypertrygliceridemia) and who were in the range of 40% out of the total (40 out of 100), had an average reduction of $38\pm 6\%$ in the plasmatic levels of triglycerides.
- 5 6) The antidislipidemic effect continued to be quite good on the 60th day after interrupting the ingestion of phytocomplex with total cholesterol values on the average equal to $20\pm 2\%$ of the basal values before the treatment.
- 7) At the end of test, by means of vascular ecodoppler examination the endothelial reactivity was checked, which resulted to have improved on the average by $34\pm 5\%$ in all treated patients, with respect to the control parameters.
- 10 8) No substantial variations in the response with respect to sex and age of the examined subjects were noted. Furthermore, the treatment did not cause side effects or pathological variations in the main organ functionality parameters examined clinically or by means of hematochemical examinations, apart from a fairly good reduction in the pressure and glycemia levels in the subjects with alteration of the glyceemic metabolism (21%) or hypertensive (24% out of total).
- 15 9) An additional effect was studied in test animals (Wistar rat) wherein a painful inflammatory reaction was induced by administration of carragenine in the animal leg. In these animals, both the topical and systemic application of the bergamot phytocomplex induced a reduction in the oedematigeneous inflammatory loco-regional reaction with a reduction in the hyperalgesia induced by carragenine.
- 20 10) At last, the incubation of the invention phytocomplex with human astrocytoma cells in culture reduced the cellular proliferation, by suggesting the potential use in the antineoplastic sense.
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CLAIMS

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PATENT OFFICE
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Republic of the Philippines - [1000]
01 Sep 2014 2:16PM (Asia)

1. A process for the production of a phytoextract from bergamot fruit comprising the following steps
 - 5 a) grinding of the bergamot fruit without its outer cuticle and its flavedo, thereby to obtain a non-degraded mixture,
 - b) inoculation of said mixture with enzymes for degradation of pectin;
 - c) reduction to a value lower 0,5%, of the pulp content of said mixture obtained from step b);
 - 10 d) inactivation of said enzymes introduced in said step b), thereby to obtain a degraded mixture;
 - e) ultrafiltration of said degraded mixture with membranes having a molecular weight cut off of 30000 Dalton thereby to obtain a clarified solution;
 - 15 f) introduction of said clarified solution onto a column containing polystyrene adsorbent resin for adsorption of polyphenols;
 - g) washing of said column having adsorbed polyphenols with water at temperature comprised between 30°-50°C and rising the pH to a value comprised in the interval 12-14, using hydroxides of alkaline metals, thereby obtaining a first eluate;
 - 20 h) passage of said first eluate on a cationic resin and then recovery by lowering the pH to a value lower than 3,0, thereby to obtain a second eluate;
 - i) drying said second eluate up to produce a phytoextract from bergamot fruit in the form of dry extract.
- 25 2. The process according to claim 1, wherein said used bergamot fruit belongs to the varieties Femminello, Fantastico and/or Castagnaro.
3. The process according to claim 1, wherein in said step b) said enzymes are of pectolytic type.
- 30 4. The process according to claim 1, wherein in said step e) said act of making clear the solution takes place through treatment with bentonite or through natural sedimentation.

5. The process according to claim 1, wherein in said step i) a dry extract with values of residual humidity <14%.
6. The process according to claim 1, wherein after said step i), said dry extract is grinded and, or further worked.
- 5 7. Phytocomplex obtainable from the process as claimed in claim 1.
8. Phytocomplex according to claim 1, in the form of dry extract, showing a minimum content of neoeriocitrine, naringine and neoesperidine not lower than 250g/kg.
9. Pharmaceutical compositions with antidyslipemic and hypoglycemic effect, containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
- 10 10. Pharmaceutical compositions with painkilling effect on human being containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
- 15 11. Pharmaceutical compositions for topical application on human being with effect on skin diseases containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
12. Pharmaceutical compositions with antineoplastic effect on human being alone or as coadjuvant of known antineoplastic compounds containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
- 20 13. Pharmaceutical compositions containing as active agent the the phytocomplex according to claim 7.
14. Dietary supplements as adjuvant in the treatment of diseases relating to the glycemic and/or lypemic ematic profile in the treatment of skin disease with inflammatory characters and in the treatment of neoplasies containing the phytocomplex according to claim 7 and additives of common use.
- 25

PHYTOCOMPLEX FROM BERGAMOT FRUIT, PROCESS OF MANUFACTURE
AND USE AS DIETARY SUPPLEMENT AND IN THE PHARMACEUTICAL FIELD

ABSTRACT

5 The invention relates a phytocomplex obtained from the bergamot fruit albedo
(Citrus Bergamia Risso & Poiteau), the production thereof and the use thereof, in
particular under the form of dry extract, to be used as normalizer of the metabolic
unbalances of lypemic and glycemie type, in the treatment of the cutaneous diseases
with inflammatory or degenerative character, as painkilling/anti-inflammatory agent,
as antineoplastic and dietary supplement.

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P2011 0126

March 12, 2015

SOCIETA ITALIANA BREVETTI S.p.A.
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Attention: Stefano Borrini

Re: Philippines Patent Application No. 1/2011/500926
Your Ref: BX2519R/RSB

Gentlemen:

We are pleased to send herewith Letters Patent No. 1/2011/500926 issued on 3 October 2014 in the name of HERBAL & ANTIOXIDANT DERIVATIVES SRL covering the invention entitled: PHYTOCOMPLEX FROM BERGAMOT ...

The patent is valid for twenty (20) years from the date of filing. The patent, however, is subject to cancellation if the patentee fails to pay the annual fees which are required to be paid commencing on the expiration of four years from the date of publication pursuant to Section 44 of RA 8293. It is incumbent upon the patentee to monitor and to pay the annuities when they fall due. The due date of the next annuity payment shall be within three (3) months before May 20, 2015.

The total number of claims is 14.

Please take note also of Section 80 of R.A. 8293 Law which provides that damages cannot be recovered for acts of infringement if the infringer had no prior notice of the patent unless the patentee has given notice thereof by placing on the patented product the words "Philippine Patent" with the number of the patent.

Enclose are Bill Nos. P14585 for US\$100.00 our fee for securing and transmitting the enclosed letters patent, and G14583 for US\$19.52 for reimbursement of the cost of postage stamp.

Very truly yours,

SIGUION REYNA, MONTECILLO
& ONGSIAKO

By:

CELSO L. CRUZ

CLC/esl

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Roma, 11 luglio 2014

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Filippine**

**Domanda di Brevetto per invenzione n. 1-2011-500926
Vs. Rif.: ESTRATTO DA ALBEDO DI BERGAMOTTO
Ns. Rif.: BX2519R/RSE/rmn**

Gentili Signori,

abbiamo ricevuto un comunicato ufficiale per la domanda in oggetto identificata nelle Filippine. La scadenza è il **9 settembre 2014** che non risulta prorogabile.

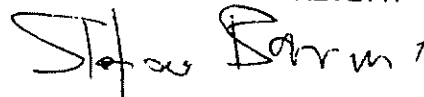
Sostanzialmente, l'Esaminatore locale suggerisce di uniformare nella sostanza le rivendicazioni locali a quelle concesse nella corrispondente domanda di brevetto europeo, apportando tuttavia alcune modifiche formali.

In primo luogo, dovrebbero essere eliminate le dipendenze multiple che si trovano nelle rivendicazioni 4, 5, 6 e 8; riteniamo che sia ipotizzabile mantenere la dipendenza dalla rivendicazione 1. Troverete una bozza di proposta manoscritta in allegato. Se foste viceversa interessati, potremmo aggiungere delle ulteriori rivendicazioni che comporterebbero comunque un aumento di tasse ufficiali.

In relazione alle rivendicazioni 10-15, l'Esaminatore sostiene che tali rivendicazioni siano doppie, nel senso che ci si riferisce tanto ad un eluato quanto ad un fitocomplesso. Di conseguenza suggerisce di dividerle, ciascuna in due. Qualora l'eluato fosse meno importante, si potrebbe semplicemente eliminarlo, lasciando il fitocomplesso senza l'aggiunta di ulteriori rivendicazioni. Vi saremmo grati se poteste fornirci Vostre istruzioni qualora foste interessati, si potrebbe proseguire soltanto per l'eluato.

In attesa di Vostre istruzioni entro il 20 luglio 2014, porgiamo i nostri migliori saluti.

SOCIETÀ ITALIANA BREVETTI



Dott. Stefano Borrini

All.

Società Italiana Brevetti Roma Milano Firenze Verona

società per azioni - capitale versato EUR 400.400,00 - sede legale: Piazza di Pietra, 39 00186 Roma
REA 11013 - Reg. Impr. Rm. / c.f. 00399970581 - IVA/VAT IT 00880811005 - pol. assic. n./insurance # 109K3121 Zurich € 3.500.000,00

Revised claims for nat/reg phase of BW559R

CLAIMS

1. A process for the production of a phytocomplex from bergamot fruit comprising the following steps
- 5 a) grinding of the bergamot fruit without its outer cuticle and its flavedo, thereby to obtain a non-degraded mixture,
- b) inoculation of said mixture with enzymes for degradation of pectin;
- c) reduction to a value lower 0,5%, of the pulp content of said mixture obtained from step b);
- 10 d) inactivation of said enzymes introduced in said step b), thereby to obtain a degraded mixture;
- e) ultrafiltration of said degraded mixture with membranes having a molecular weight cut off of 30000 Dalton thereby to obtain a clarified solution;
- 15 f) introduction of said clarified solution onto a column containing polystyrene adsorbent resin for adsorption of polyphenols;
- g) washing of said column having adsorbed polyphenols with water at temperature comprised between 30°-50°C and rising the pH to a value comprised in the interval 12-14, using hydroxides of alkaline metals,
- 20 thereby obtaining a first eluate;
- h) passage of said first eluate on a cationic resin and then recovery by lowering the pH to a value lower than 3,0, thereby to obtain a second eluate;
- 25 i) drying said second eluate up to produce a phytocomplex from bergamot fruit in the form of dry extract.
2. The process according to claim 1, wherein said used bergamot fruit belongs to the varieties Femminello, Fantastico and/or Castagnaro.
3. The process according to at least one of the preceding claims, wherein in said step b) said enzymes are of pectolytic type.
- 30 4. The process according to ~~at least one of the preceding claims~~, wherein in said step e) said act of making clear the solution takes place through treatment with bentonite or through natural sedimentation.
5. The process according to ~~at least one of the preceding claims~~, wherein in said step i) a dry extract with values of residual humidity <14%.
- 35 6. The process according to ~~at least one of the preceding claims~~, wherein after said step i), said dry extract is grinded and, or further worked.
7. Eluate obtainable from step h) of the process according to claim 1.

8. Phytocomplex obtainable from the process as claimed in ~~at least one of the~~ claims ~~from 1 to 6~~.
9. Phytocomplex according to claim 8, in the form of dry extract, showing a minimum content of neoeriocitrine, naringine and neoesperidine not lower than 250g/kg.
10. Pharmaceutical compositions with antidyslipemic and hypoglycemic effect, containing the eluate according to claim 7 and, or the phytocomplex according to claim 8 and pharmaceutically tolerable additives.
11. Pharmaceutical compositions with painkilling effect on human being containing the eluate according to claim 7 and, or the phytocomplex according to claim 8 and pharmaceutically tolerable additives.
12. Pharmaceutical compositions for topical application on human being with effect on skin diseases containing the eluate according to claim 7 and, or the phytocomplex according to claim 8 and pharmaceutically tolerable additives.
13. Pharmaceutical compositions with antineoplastic effect on human being alone or as coadiuvant of known antineoplastic compounds containing the eluate according to claim 7 and, or the phytocomplex according to claim 8 and pharmaceutically tolerable additives.
14. Pharmaceutical compositions containing as active agent the the eluate according to claim 7 and, or the phytocomplex according to claim 8.
15. Dietary supplements as adjuvant in the treatment of diseases relating to the glycemic and/or lypemic ematic profile in the treatment of skin disease with inflammatory characters and in the treatment of neoplasies containing the eluate according to claim 7 and, or the phytocomplex according to claim 8 and additives of common use.

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Società Italiana Brevetti

Proprietà intellettuale ed industriale
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Roma, 28 luglio 2014

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Herbal & Antioxidant Derivatives S.r.l.
Filippine

Domanda di Brevetto per Invenzione n. 1-2011-500926
Vs. Rif.: ESTRATTO DA ALBEDO DI BERGAMOTTO
Ns. Rif.: BX2519R/RSB/rmn

Egregio Architetto,

in allegato troverà una bozza delle rivendicazioni che Le vorremmo proporre, che tengono conto delle Sue istruzioni del 14 luglio 2014. In mancanza di Sue istruzioni contrarie, inviamo la documentazione al nostro corrispondente filippino entro venerdì prossimo.

Con i nostri migliori saluti.

SOCIETÀ ITALIANA BREVETTI

Dott. Stefano Borrini

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AMENDED CLAIMS marked copy

1. A process for the production of a phytocomplex from bergamot fruit comprising the following steps

- a) grinding of the bergamot fruit without its outer cuticle and its flavedo, thereby to obtain a non-degraded mixture,
- b) inoculation of said mixture with enzymes for degradation of pectin;
- c) reduction to a value lower 0,5%, of the pulp content of said mixture obtained from step b);
- d) inactivation of said enzymes introduced in said step b), thereby to obtain a degraded mixture;
- e) ultrafiltration of said degraded mixture with membranes having a molecular weight cut off of 30000 Dalton thereby to obtain a clarified solution;
- f) introduction of said clarified solution onto a column containing polystyrene adsorbent resin for adsorption of polyphenols;
- g) washing of said column having adsorbed polyphenols with water at temperature comprised between 30°-50°C and rising the pH to a value comprised in the interval 12-14, using hydroxides of alkaline metals, thereby obtaining a first eluate;
- h) passage of said first eluate on a cationic resin and then recovery by lowering the pH to a value lower than 3,0, thereby to obtain a second eluate;
- i) drying said second eluate up to produce a phytocomplex from bergamot fruit in the form of dry extract.

2. The process according to claim 1, wherein said used bergamot fruit belongs to the varieties Femminello, Fantastico and/or Castagnaro.

3. The process according to claim 1, wherein in said step b) said enzymes are of pectolytic type.

4. The process according to claim 1, wherein in said step e) said act of making clear the solution takes place through treatment with bentonite or through natural sedimentation.

5. The process according to claim 1, wherein in said step i) a dry extract with values of residual humidity <14%.

6. The process according to claim 1, wherein after said step i), said dry extract is grinded and, or further worked.

7. Phytocomplex obtainable from the process as claimed in claim 1.

8. Phytocomplex according to claim 1, in the form of dry extract, showing a

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Eliminato: ~~4~~ Eluate obtainable from step h) of the process according to claim 1. ¶

Eliminato: at least one of the

Eliminato: s

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Eliminato: to 6

Eliminato: 8

minimum content of neoeriocitrine, naringine and neoesperidine not lower than 250g/kg.

- 5
9. Pharmaceutical compositions with antidyslipemic and hypoglycemic effect, containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
- 10
10. Pharmaceutical compositions with painkilling effect on human being containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
11. Pharmaceutical compositions for topical application on human being with effect on skin diseases containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
12. Pharmaceutical compositions with antineoplastic effect on human being alone or as coadjuvant of known antineoplastic compounds containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
13. Pharmaceutical compositions containing as active agent the phytocomplex according to claim 7.
14. Dietary supplements as adjuvant in the treatment of diseases relating to the glycemic and/or hyperemic ematic profile in the treatment of skin disease with inflammatory characters and in the treatment of neoplasies containing the phytocomplex according to claim 7 and additives of common use.
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- 25

Eliminato: the eluate according to claim 7 and, or

Eliminato: 8

Eliminato: the eluate according to claim 7 and, or

Eliminato: 8

Eliminato: the eluate according to claim 7 and, or

Eliminato: 8

Eliminato: the eluate according to claim 7 and, or

Eliminato: 8

Eliminato: the eluate according to claim 7 and, or

Eliminato: 8

Eliminato: the eluate according to claim 7 and, or

Eliminato: 8




P20116126

To All To Whom These Presents Shall Come:

This is to certify that the annexed is a true copy from the records of this Office of the original,

Letters Patent No.: 1-2011-500926
Title: PHYTOCOMPLEX FROM BERGAMOT FRUIT, PROCESS OF MANUFACTURE AND USE AS DIETARY SUPPLEMENT AND IN THE PHARMACEUTICAL FIELD
Issued on: October 3, 2014
Inventor(s): Domenico MALARA, et al.
Applicant(s): HERBAL & ANTIOXIDANT DERIVATIVES SRL [POLISTENA RC, IT]

In witness whereof, I have hereunto affixed my hand and the seal of the Intellectual Property Office at Taguig City, Philippines, this 19th day of January 2016


EPIFANIO M. EVASCO
Director, Bureau of Patents

Attested:


ALLAN L. LAFORTEZA
Administrative Officer III

Doc. No.: BOP-015
Series of: 2016
Requested by: **Messrs. Siguion Reyna, et al.**

NOTICE

Under the provision of Section 54 of Republic Act 8293, the term of a patent shall be twenty (20) years from the filing date of the application.

Under the provision of Section 55 of Republic Act 8293, to maintain the patent application or patent, an annual fee shall be paid upon the expiration of four (4) years from the date the application was published pursuant to Section 44 hereof, and on each subsequent anniversary of such date. Payment may be made within three (3) months before the due date. The obligation to pay the annual fees shall terminate should the application be withdrawn, refused, or cancelled.

Under the provision of Section 55.2 of Republic Act 8293, if the annual fee is not paid, the patent application shall be deemed withdrawn or the patent considered as lapsed from the day following the expiration of the period within which the annual fees were due. A notice that the application is deemed withdrawn or the lapse of a patent for non-payment of any annual fee shall be published in the IPO Gazette and the lapse shall be recorded in the register of the office.

Under the provision of Section 55.3 of Republic Act 8293, a grace period of six (6) months shall be granted for the payment of the annual fee, upon payment of the prescribed surcharge for the late payment.

RECORD OF ANNUAL FEE PAYMENTS						
No.	Ann. Year	Date Paid	O.R. No.	Amount Paid	Paid By	Recorded By
1st						
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PHYTOCOMPLEX FROM BERGAMOT FRUIT, PROCESS OF MANUFACTURE
AND USE AS DIETARY SUPPLEMENT AND IN THE PHARMACEUTICAL FIELD

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ABSTRACT

The invention relates a phytocomplex obtained from the bergamot fruit
albedo (Citrus Bergamia Risso & Poiteau), the production thereof and the use
thereof, in particular under the form of dry extract, to be used as normalizer of the
10 metabolic unbalances of lypemic and glycemc type, in the treatment of the
cutaneous diseases with inflammatory or degenerative character, as
painkilling/anti-inflammatory agent, as antineoplastic agent and dietary
supplement.

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the bergamot essence is used for preparing some products (one in particular with the name of Bergarytal), which have been proposed as sprays endowed with the skin's decongestive action and particularly effective in keeping away the mosquitos. However, the finding that some components of the bergamot essence, such as the bergaptene, potentially show toxic action has limited the use thereof by requesting procedures for keeping away this compound for the safe use of the essential oil extracts.

The document Gardana Claudio et al "*Evaluation of flavonoids and furanocumarins from Citrus Bergamia (Bergamot) juice and identification of new compounds*" MOLECULES (BASEL, SWITZERLAND) 2008, vol.13, NO. 9, 18 September 2008 (2008-09-18), pages 2220-2228 is directed only to the study of the content of flavonoids and furanocumarins in a sample of bergamot juice.

WO 2008/061536(COSMEDICAL APS [DK] describes, among other things, compositions for treating the eye disorders with compositions containing also bioflavonoids and a method for extracting such bioflavonoids from bergamot. Such method does not use either pectolytic enzymes or a separation with cationic resins and it does not make reference to the furocumarine content of the obtained product.

Mandalari Giuseppina et al "Enzymatic hydrolysis of flavonoids and pectic oligosaccharides from bergamot (Citrus bergamia Risso) peel" Journal of Agricultural and Food Chemistry, American Chemical Society, Washington, US vol.54, Nr. 21 1 October 2006 pages 8307 - 8313 describes the extraction of flavonoids and other compounds starting from the peel of the bergamot fruit, peel considered as sub-product of the essential oil industry.

Gattuso Giuseppe et al "Flavonoid glycosides in bergamot juice (Citrus bergamia Risso)" Journal of Agricultural and Food Chemistry, vol 54, Nr. 11, May 2006 describes a study developed with the only purpose of differentiating two glycosidates flavonoids contained in a bergamot juice having industrial origin which has been treated with dymethylformamide that is to say a solvent which may not find use in products of medical and/or cosmetic interest.

The authors of the present invention have surprisingly found that, from the bergamot fruit, in particular from the albedo, it is possible to obtain a concentrated phytocomplex showing a great healing action by positively effecting the metabolic activity and by normalizing the lipidic and glycemic levels of the subjects affected by these pathologies. The albedo, in the citrus fruits, is the portion lying between the epidermis including flavedo and pulp and it is formed by cells with tubular structure forming a real net with most part of the tissue volume compressed in the

of the invention, also with a mixture of ethyl or methyl alcohol, and ethyl acetate in the ratio 3:1 and recovered by means of subsequent solvent concentration (for example by means of evaporation and subsequent drying).

Step g (recovery of the polyphenolic fraction entrapped in the resins' pores of point f and contemporary lowering of the furocumarine value up to 400 mg/KG of dry product):

After having performed the outer washing of the resins with hot water at 40 °C, in order to eliminate sugars and acids contained in the solution of step 5, the recovery of the polyphenolic fraction is carried out by opening such organic compounds, characterized by a circular spatial arrangement, thereby assuming a linear configuration, so to be released from the holes wherein they were trapped. Such effect is obtained by rising the PH up to strongly basic values (pH 12- 14). This takes place advantageously by using hydroxides of alkaline metals which also degrade bergaptene and Bergamottine. Equal effect can be obtained if, instead of using the basic solution, ethylic or methylic alcohol is used. In this case the bergaptene and Bergamottine degradation would not be obtained.

Step h (Chemical stabilization of the phytocomplex in aqueous phase):

The polyphenols in the (open) linear form thereof are extremely instable as they tend to be object of oxidative phenomena. It is necessary in very short time to bring them back to the ring-like shape. Such result is obtained by lowering the pH from the value 14 up to strongly acid values (around pH 3). Such effect is obtained by subtracting from the solution cations K⁺, Na⁺, etc. This takes place by using strongly cationic resins (ex. Relite CF H⁺ Mitsubishi). These replace the existing cation with a positive hydrogen (H⁺). The operation effect is an acidification of the solution up to pH = 3 and a subtraction of cations of the used alkaline hydroxide. The result is a product more valuable under the nutritional aspect.

Step i (Physical stabilization of the product; "Drying"):

It is obtained by means of removing water, advantageously by evaporation in high vacuum, then at temperatures lower than 60°C up to complete drying (that is by obtaining a residual humidity lower than 14%). At such humidity values the proliferation of any type of organic contaminant (yeasts, bacteria, etc.) is inhibited. A dry product is obtained.

It is to be underlined that the extraction and purification process allows reducing in drastic manner the contained furocumarines in (bergaptene and Bergamottine) which represent a problem in the light of their character of potential toxicity; however found at cutaneous level only and in much higher doses than those found in the juice.

ematochemical indexes (complete blood count, hepatic transaminase, azotemia and hypercreatininemia) within normality ranges by proving the absence of toxic effects. Such data were confirmed by the absence of steatotic or necrosis phenomena at hepatic and renal level in the treated animals examined post-mortem at microscope. Furthermore, a histopathological analysis of the brain tissue and of the peripheral nerves proved the absence of induced axonopathies or mielinopathies. The study of the phytocomplex toxicity had the following result: "the toxicologic analysis does not show toxicity factors, according to the legal framework in force". In particular, together with the absence of the common citrus fruit contaminants, it was found the absence of pathological values or however values outside the normal reference values as far as the presence of heavy metals, pesticides, PCB, nitrites and nitrates, dyes, moulds is concerned apart from the absence of ocratoxins, bacterial endotoxines, anaerobic germs and moulds. From the examination of organs (liver, kidney) taken from the guinea pigs, no toxic effect for administrations equal to 80mg./Kg of body weight/day in the oral administration was noted.

It is to be underlined that the use of hydroxides of alkaline metals, in particular of KOH reduces considerably the quantity of existing furocoumarines and the obtained phytocomplex appears much more concentrated as to the functional aspect.

The phytocomplex object of the present invention is characterized by the biological singleness of the polyphenolic profile having the main Bioflavonoids, in the following percentages:

Neohesperidin	29.6 % +/- 6.0
Naringine	32.4 % +/- 4.0
Neohesperidin	38.0 % +/- 6.0
Total	100.0%

According to the invention, after the step i) of the process, a phytocomplex, under the form of dry extract, is obtained which has a minimum content of neohesperidin, naringine, neohesperidin not lower than 250 gr / kg that is 25%. Furthermore, the furocoumarines meant as Bergapten and Bergamottin are present in quantities not higher than 400 mg / kg.

- 5) The examined subjects having mixed forms of dislipidemy (hypercholesterolaemia and hypertrygliceridemia) and who were in the range of 40% out of the total (40 out of 100), had an average reduction of $38 \pm 6\%$ in the plasmatic levels of triglycerides.
- 5 6) The antidislipidemic effect continued to be quite good on the 60th day after interrupting the ingestion of phytocomplex with total cholesterol values on the average equal to $20 \pm 2\%$ of the basal values before the treatment.
- 10 7) At the end of test, by means of vascular ecodoppler examination the endothelial reactivity was checked, which resulted to have improved on the average by $34 \pm 5\%$ in all treated patients, with respect to the control parameters.
- 15 8) No substantial variations in the response with respect to sex and age of the examined subjects were noted. Furthermore, the treatment did not cause side effects or pathological variations in the main organ functionality parameters examined clinically or by means of hematochemical examinations, apart from a fairly good reduction in the pressure and glycemia levels in the subjects with alteration of the glycemc metabolism (21%) or hypertensive (24% out of total).
- 20 9) An additional effect was studied in test animals (Wistar rat) wherein a painful inflammatory reaction was induced by administration of carragenine in the animal leg. In these animals, both the topical and systemic application of the bergamot phytocomplex induced a reduction in the oedematigeneous inflammatory loco-regional reaction with a reduction in the hyperalgesia induced by carragenine.
- 25 10) At last, the incubation of the invention phytocomplex with human astrocytoma cells in culture reduced the cellular proliferation, by suggesting the potential use in the antineoplastic sense.

5. The process according to claim 1, wherein in said step i) a dry extract with values of residual humidity <14%.
6. The process according to claim 1, wherein after said step i), said dry extract is grinded and, or further worked.
- 5 7. Phytocomplex obtainable from the process as claimed in claim 1.
8. Phytocomplex according to claim 1, in the form of dry extract, showing a minimum content of neoeriocitrine, naringine and neoesperidine not lower than 250g/kg.
9. Pharmaceutical compositions with antidyslipemic and hypoglycemic effect, containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
- 10 10. Pharmaceutical compositions with painkilling effect on human being containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
- 15 11. Pharmaceutical compositions for topical application on human being with effect on skin diseases containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
12. Pharmaceutical compositions with antineoplastic effect on human being alone or as coadjuvant of known antineoplastic compounds containing the phytocomplex according to claim 7 and pharmaceutically tolerable additives.
- 20 13. Pharmaceutical compositions containing as active agent the the phytocomplex according to claim 7.
14. Dietary supplements as adjuvant in the treatment of diseases relating to the glycemic and/or lypemic ematic profile in the treatment of skin disease with inflammatory characters and in the treatment of neoplasies containing the phytocomplex according to claim 7 and additives of common use.
- 25



Roma, 25 marzo 2016

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Filippine
Domanda di Brevetto per invenzione n. 1-2011-500926
Rilascio n. 1/2011/500926
corrispondente alla domanda italiana n. RM2008A000615
Vs. Rif.: ESTRATTO DA ALBEDO DI BERGAMOTTO
Ns. Rif.: BX2519R/RSB/rma

Trasmissione attestato di rilascio

Titolare:	Herbal & Antioxidant Derivatives S.r.l.		
Titolo:	PHYTOCOMPLEX FROM BERGAMOT FRUIT, PROCESS OF MANUFACTURE AND USE AS DIETARY SUPPLEMENT AND IN THE PHARMACEUTICAL FIELD		
Nazione:	Filippine		
Protezione:	Brevetto per invenzione		
Priorità:	17 novembre 2008	RM2008A000615	Italia
Deposito:	13 novembre 2009	1-2011-500926	
Pubblicazione domanda:	20 maggio 2010	WO2010/055490	
Rilascio:	03 ottobre 2014	1/2011/500926	
Durata:	fino al 13 novembre 2029		
Tasse di mantenimento:	annuali il 20 maggio; prossima tassa 20 maggio 2016		

Abbiamo il piacere di trasmetterVi in allegato l'attestato ufficiale di rilascio del brevetto sopra indicato.

Ci permettiamo inoltre di allegare alla presente la nostra nota di debito per le voci in essa specificate.

Con i nostri migliori saluti.

SOCIETÀ ITALIANA BREVETTI

Reparto Brevetti
(rm/ma)

All.