

# Pharmacological geroprotective composition and method of obtaining thereof

## Abstract

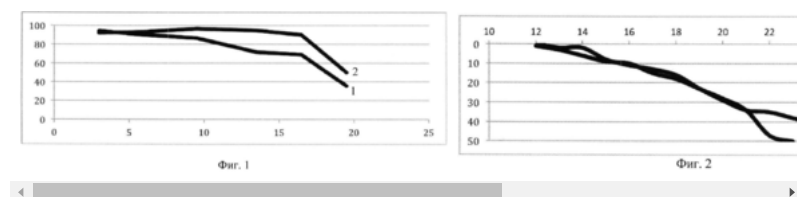
FIELD: chemistry.

SUBSTANCE: invention represents a pharmacological geroprotective composition, which includes a polyphenol component, vitamins and microelements, humic acids, containing polyphenol components, vitamin C, vitamin A, iron (II) chloride and selenium (IV) dioxide, with the composition components being in a specified ratio in wt %.

EFFECT: increased life expectancy and retardation of tumour development.

2 cl, 3 dwg, 2 tbl, 4 ex

## Images (3)



RU2522547C1

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Application RU2012147985/15A events 

**2012-11-12** Application filed by Федеральное государственное бюджетное учреждение "Научно-исследовательский институт онкологии имени Н.Н. Петрова" Министерства здравоохранения Российской Федерации, Общество с ограниченной ответственностью "Нобель"

**2012-11-12** Priority to RU2012147985/15A

**2014-07-20** Application granted

**2014-07-20** Publication of RU2522547C1

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## Claims (2)

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translated from Russian

1. Pharmacological geroprotective composition comprising a polyphenolic component, vitamins and minerals, characterized in that the composition contains humic acids containing polyphenolic components, vitamin C, vitamin A, iron (II) chloride and selenium dioxide (IV) in the following ratio of components, wt %:  
humic acids containing polyphenolic components 0.45-0.55 vitamin c 0.009-0.011 vitamin A 0.0045-0.0055 iron (II) chloride 0.02-0.025 selenium dioxide (IV) 0.0012-0.0015 rest water
2. A method of obtaining a pharmacological geroprotective composition according to claim 1, comprising treating an aqueous solution of the starting humic substances with metal compounds under ultrasonic radiation, characterized in that the aqueous solution of the starting humic acids containing polyphenolic components is treated with iron (II) chloride and selenium dioxide (IV) with ultrasonic radiation with a power of 0.1-2 W / cm<sup>3</sup>, with a frequency of 15 kHz for 5 minutes, then with vitamin A with ultrasonic radiation with a power of 0.1-2 W / cm<sup>3</sup>, with a frequency of 45 kHz for 2 minutes, and then vitamin C with ultrasound radiation with a power of 0.1-2 W / cm<sup>3</sup>, with a frequency of 35 kHz for 0.5 minutes.

## Description

translated from Russian

The invention relates to medicine, namely to the pharmaceutical industry, and relates to geroprotective compositions based on polyphenolic components, vitamins and minerals and methods for its preparation.

Epidemiological data and experimental results indicate that a variety of polyphenolic components (flavonoids) play a large role in the prevention of age-related diseases such as cancer and cardiovascular diseases (Graf B.A., Milbury P.E., Blumberg JB Flavonols, flavones, flavanones, and human health: epidemiological evidents // J.Med. Food. - 2005. - Fall; V.8, N 3. - P.281-290).

There is evidence that a high level of flavonoids is directly associated with a reduced risk of developing myocardial infarction (Hernandez-Diaz S., Martinez-Losa E., Fernandez-Jame E., Serrano-Martinaz M., Martinez-Gonzalez M. Dietary folate and the risk of nonfatal myocardial infarction // Epidemiology. - 2002. - V.13. - P.700-706) and other cardiovascular diseases (Kondo K., Matsumoto A., Kurata N., Tanahashi H., Koda H., Amachi T., Itakura H. Inhibition of oxidation of low density lipoprotein with red wine // Lancet. - 1994. - V.344. - P.1152).

In addition, it was found that resveratrol (a flavonoid found in red wine) has an anti-carcinogenic effect: it inhibits the growth of skin tumors in mice (Jang M., Cai L., Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM Cancer chemopreventive activity of resveratrol, a natural product derived from grapes // Science. - 1997. - V.275. - P.218-220).

There is evidence that other polyphenolic components (catechins contained in green tea) not only reduce lipids in the body and inhibit the development of cardiovascular diseases (Arts IC, Hollman P.Ch., Feskens EJM, De Mesquita HBB, Kromhout D. Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease; the Zutphen elderly study // *Am. J. Clin. Nutr.* - 001. - V.74. - P.227-232), but also capable reduce the risk of cancer (Zheng W., Doyle TJ, Kushi LH, Sellers TA, Hong CP, Folsom AR Tea consumption and cancer incidence in a prospective cohort study of postmenopausal women // *Am. J. Epidemiol.* - 1996. - V.144. - P.175-182).

In medicine, the described components are mainly used in plant extracts.

Known hepatoprotective agent based on artichoke polyphenols (RU 2281112, publ. 08/10/2006), antioxidant agents based on chaga polyphenols (RU 2339390, publ. 08/10/2006) and psyllium herbs (RU 2390347, publ. 05/27/2010), drug for the treatment of precancerous lesions of the skin (RU 2366447, 11/27/2007), improving dietary supplement based on grape seed extract (RU 2350125, publ. 03/27/2009), etc.

Known pharmaceutical compositions based on polyphenols, further comprising ascorbic acid (RU 2229303, publ. 05/27/2004; RU 2301666, publ. 06/27/2007), which has a pronounced antioxidant effect. Also known are compositions comprising retinol (beta-carotene - vitamin A-retinol), which is involved in the antioxidant defense of the body (RU 2350125, publ. 03/27/2009).

In addition, there are polyphenol-containing compositions for the prevention of iron-deficiency anemia based on iron compounds (RU 2005130673, publ. 10.04.2007) and anti-aging agents containing selenium (RU 2433819, publ. 20.11.2012). It is known that a deficiency of selenium can affect the development of cardiovascular diseases, atherosclerosis (Alissa E.M. et al., 2003), cancer and immunity disorders (Seiler WO, 2001), and the use of its drugs significantly reduces the risk of lung cancer (Reid M.E., 2002).

Known pharmaceutical composition based on polyphenols that blocks the proliferation of cancer cells and metastasis used in the treatment of cancer (RU 2301666, publ. 27.06.2007). The composition of the composition includes at least one polyphenolic compound selected from the group consisting of galate epigallocatechin, galate epicatechin, epigallocatechin, epicatechin and catechin. Polyphenolic compounds contained in green tea extract are used. Compositions also include ascorbic acid, lysine, proline, salts of selenium, copper, magnesium. This composition is accepted by the authors as a prototype.

The technical result of the invention is the creation of a stable pharmacological geroprotective composition based on the polyphenol component of humic substances, containing simultaneously poorly soluble inorganic compounds of selenium and iron, and hydrophobic organic substances (vitamin A).

The specified technical result is achieved by the fact that the pharmacological geroprotective composition according to the invention contains humic acids containing polyphenolic components, vitamin C, vitamin A, iron (II) chloride and selenium dioxide (IV) in the following ratio, wt. %:

humic acids containing polyphenolic components 0.45-0.55 vitamin c 0.009-0.011 vitamin A 0.0045-0.0055 iron (II) chloride 0.02-0.025 selenium dioxide (IV) 0.0012-0.0015 rest water

The proposed composition differs from the prototype in that humic acids obtained from wood lignin are used as polyphenol sources (PA 2012 7059). This feedstock is characterized by high production efficiency and high reproducibility of the chemical composition.

One of the most pronounced effects of the use of humic acids is the enhancement of the general immune response due to the presence of polyphenolic groups in their composition (Filov V.A., Berkovich A.M. Humic substances: possibilities for using their biological effects. Veterinary medicine. 2007. No. 8. S.14-17).

It is known that vitamin A (its precursor is beta-carotene and retinol proper) is a strong antioxidant and is involved in the metabolic processes of the body.

It should be noted that the study of the geroprotective effect in mice when animals injected with only one drug, in particular beta-carotene, did not have a significant effect on life expectancy (Massie HR, Ferreira JR Jr., DeWolfe LK Effect of dietary beta-carotene on the survival of young and old mice // *Gerontology.* - 1986. - V.32, N.4. - P.189-195), however, in experiments when the so-called dietary antioxidant mixtures consisting of beta-carotene, alpha-tocopherol, ascorbic acid, rutin, selenium and zinc - in hybrid mice when administered with food, starting from two months of age – the effect was statistically significant compared with control mice – an increase in pancreas was observed (Bezlepkin V.G. et al., 1996).

Thus, studies evaluating the geroprotective properties of beta-carotene in mice suggest that beta-carotene exerts a certain increase in other active active additives or dietary components that have geroprotective properties.

There is also evidence of the ability of vitamin A and beta-carotene to increase the survival of CBA mice after gamma irradiation if drugs were administered 2 days after irradiation (Seifter E., Rettura G., Padawer J. et al. Morbidity and mortality reduction by supplemental vitamin A or beta-carotene in CBA mice given total-body gamma-radiation // *J. Natl. Cancer Inst.* - 1984. - V.73, N.5. - P.1167-1177).

These results confirm the presence of antioxidant properties in vitamin A and beta-carotene, and also indicate the ability to influence immune processes.

It is known that selenium is necessary for some biochemical mechanisms in the body and a decrease in its blood level can lead to the development of diseases such as cancer, cardiovascular diseases, as well as impaired immunity (Seiler WO Clinical pictures of malnutrition in ill elderly subjects // *Nutrition.* - 2001. - V.17. - P.496-498).

Also, some animal studies have shown that selenium deficiency leads to deaths due to acute cardiovascular failure, as well as impaired lymphocyte proliferation (Alissa E.M., Bahjri SM, Ferns GA The controversy surrounding selenium and cardiovascular disease: a review of the evidence // *Med. Sci. Monit.* - 2003. - V.9. - P. RA9-RA18). There are epidemiological data (although in the authors' opinion it is still insufficient for a complete conclusion about the role of selenium), indicating that the lack of selenium plays an important role in the etiology of cardiovascular diseases, as well as in the development of atherosclerosis (Alissa E.M. et al., 2003).

A randomized clinical study of the effect of selenium showed a statistically significant reduction in the risk of developing lung cancer (Reid M.E., Duffield-Lillico AJ, Garland L., Turnbull BW, Clark LC, Marshall JR Selenium supplementation and lung cancer incidence: an update of the nutritional prevention of cancer trial // *Cancer Epidemiol Biomarkers Prev.* - 2002. - V.11. - P.1285-1291).

It is known that iron belongs to the main trace elements necessary to maintain the vital functions of the human body.

There is epidemiological evidence that, with age (when assessing the age level over 70 years), a decrease in the iron content in the body was observed in both men and women, which led to an increased risk of mortality, mainly from cardiovascular diseases (Corti M.S., et al., 1997).

It is also known that hypomicroelementoses that occur in old age (including a decrease in the level of iron in the body) cause the development of immunosuppression, and also increase the likelihood of neoplasms, in particular multiple myelomas (Kudrin A.V., Skalny A.V., etc. Immunopharmacology of microelements. M: publishing house KMK. 2000. 537 S.).

There is evidence of the interaction of food polyphenols and iron - as it turned out, polyphenols inhibit the absorption of heme iron mainly due to a decrease in the basolateral yield of ions (Qianyi M. Et al., 2010).

The studies conducted allowed us to create a pharmacological geroprotective composition based on humic acids containing a polyphenolic component, vitamin C, vitamin A, iron (II) chloride, and selenium dioxide (IV), taking into account the properties and characteristics of its constituents described above.

The proposed composition simultaneously contains sparingly soluble inorganic compounds of iron and selenium, as well as hydrophobic organic substances (vitamin A).

To obtain the stability of the proposed composition, a method for its preparation by ultrasonic treatment of its constituent components is proposed.

A known method of obtaining an antitumor agent (RU 2102083, publ. 20.01.98), in which ultrasonic treatment is used as pre-treatment of medical lignin, which is part of the tool, and the ultrasonic treatment is carried out at a radiation power of  $40 \pm 0.5$  W, the oscillation frequency  $22 \pm 0.5$  kHz for 20-25 minutes.

A known method of producing an anti-cancer agent based on the coordination compound of divalent platinum and humic substances (RU 2368379, publ. 09/27/2009). According to this invention, an aqueous solution containing humic substances is treated with a platinum coordination compound under the influence of ultrasonic radiation in the frequency range from 18 to 66 kHz with a power of from  $0.5$  to  $5$  W / cm<sup>3</sup>. When the radiation power of  $5$  W / cm<sup>3</sup> with a frequency of 22 kHz, the treatment is carried out for 5-20 minutes. The method provides a more homogeneous and more stable during storage of the product. This method is selected as a prototype.

However, all the described methods are not applicable to obtain a stable composition containing simultaneously poorly soluble inorganic compounds and hydrophobic organic substances (vitamin A).

The specified technical result is also achieved by the fact that in the method for producing the proposed pharmacological geroprotective composition, comprising treating an aqueous solution of the starting humic substances with metal compounds under ultrasonic radiation, according to the invention, the aqueous solution of the starting humic acids containing polyphenolic components is treated with iron (II) chloride and selenium dioxide (IV) with ultrasonic radiation with a power of  $0.1-2$  W / cm<sup>3</sup>, a frequency of 15 kHz for 5 minutes, then vitamin A with ultrasonic radiation with a power of  $0.1-2$  W / cm<sup>3</sup>, a frequency of 45 kHz for 2 minutes, and then vitamin C with ultrasound radiation with a power of  $0.1-2$  W / cm<sup>3</sup>, a frequency of 35 kHz for 0.5 minutes.

The invention is illustrated in the drawings, where:

figure 1 presents the dependence, built in the coordinates: X - the life of the animals, in months, Y - the number of mice with regular astral cycles, % of the total number; curve 2 reflects the results for the group receiving the geroprotective agent, and curve 1 reflects the results for the control group;

figure 2 presents the dependence, built in the coordinates: X - the life of the animals, in months, Y - the number of individuals of dead mice, in pieces; curve 2 reflects the results for the group receiving the geroprotective agent, and curve 1 reflects the results for the control group;

figure 3 presents a diagram in which the value along the Y axis reflects the number of tumors in animals at the end of the experiment; column 1 shows the results for the control group, column 2 shows the results for the group of animals treated with geroprotective agent, the upper part of the columns shows the number of breast tumors, and the lower one shows tumors of other locations.

The achievement of the technical result of the invention is confirmed by the following examples.

Example 1. Obtaining geroprotective funds containing polyphenols, vitamins and mineral components during a single treatment with ultrasound.

Humic acids, 40% represented by polyphenolic components, in an amount of 5 g were dissolved in 1000 ml of distilled water with periodic stirring and constant heating to 50 ° C for 0.5 hours. Next, iron (II) chloride was added to the solution in an amount of 225 mg, selenium (IV) dioxide in an amount of 1.4 mg, vitamin C (L-ascorbic acid) in an amount of 100 mg and vitamin A (retinol oil solution) containing 50 mg active substance, after which the solution was treated with ultrasound as described in the prototype (RU 2368379): at a radiation power of 5000 W with a frequency of 22 kHz for 10 minutes.

Subsequently, the chemical composition and stability of the obtained geroprotective agent were investigated (example 3).

Example 2. Obtaining geroprotective funds containing polyphenols, vitamins and mineral components by triple ultrasonic treatment.

Humic acids, 40% represented by polyphenolic components, in an amount of 5 g were dissolved in 1000 ml of distilled water with periodic stirring and constant heating to 50 ° C for 0.5 hours. Next, iron (II) chloride in an amount of 225 mg and selenium (IV) dioxide in an amount of 1.4 mg were added to the solution, after which the solution was treated with ultrasound at a frequency of 15 kHz at a radiation power of 2000 W for 5 minutes. After that, vitamin A (retinol oil solution) was introduced into the solution, containing 50 mg of the active substance under conditions of simultaneous sonication with a frequency of 45 kHz at a radiation power of 1000 W for 2 minutes, and then vitamin C (L-ascorbic acid) was introduced in an amount 100 mg and continued processing at a frequency of 35 kHz with a radiation power of 2000 W for 0.5 minutes.

Further investigated the chemical composition and stability of the obtained geroprotective funds (example 3), as well as its specific effect (example 4).

Example 3. Studies of the chemical composition and stability of the obtained geroprotective funds.

In the initial humic substances by the method of mass spectrometry of ion-cyclotron resonance with Fourier transform (ICR FP MS), the following oligo- and polyphenolic components were identified:

3-Benzyloxy-4,5-dihydroxy-benzoic acid;

5- (furan-2-carbonyloxy) -2-methyl-benzofuran-3-carboxylic acid;

2,6-dimethyl-benzo (1,2-b, 4,5-b ') difuran-3,7-dicarboxylic acid;

5- (furan-2-carbonyloxy) -2-methyl-benzofuran-3-carboxylic acid;

Ramnetin;

Methyl ((4-methyl-6-oxo-6-benzo (s) chromen-3-yl) oxy) acetic acid;

Sulokhrin;

2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyldibenzofuran-1,3 (2H, 9bH) -dione;

Methyl 5-hydroxy-7,8-dimethoxy-1,3-dioxo-1,3,10,11-tetrahydrobenzo [5,6] cislocta [1,2-c] furan-4-carboxylate;

(1-methoxy carbonylmethoxy-6-oxo-6h-benzo (c) chromen-3-yloxy) -acetic acid;

Atranorin;

Phenylpropanoid-substituted epicatechins, etc.

Obtained in examples 1 and 2 geroprotective compositions were characterized by the following composition (table 1).

Table 1 Indicator The composition of example 1 The composition according to example 2 Humic acids containing polyphenolic components 0.48% 0.47% 0.2% 0.2%  
Vitamin C 0.009% 0.008% Vitamin A 0.004% 0.005%

Iron 0.01% 0.009% Selenium 0.0001% 0.0001%

Solutions of the obtained geroprotective compositions were placed under conditions of accelerated storage at a temperature of 40 ° C to establish their stability. The results of controlling the pH and appearance of the solutions at different periods of the study are presented below (Table 2).

table 2 Term The composition of example 1 The composition according to example 2 Sediment pH Sediment pH Day 0 No 7.0 No 7.2 Day 46 Yes 6.2 No 7.0 Day 91 Yes 5.9 No 7.1 Day 137 Yes 5.2 No 6.7 Day 183 Yes 4.8 Yes 6.1

It was revealed that the geroprotective composition according to example 2 is characterized by higher stability.

Example 4. The study of the specific action of geroprotective composition.

The geroprotective composition obtained in example 2 was tested in experiments in vivo to establish its toxicity and effect on life expectancy.

When diluting the preparation for soldering, the animals used tap water (2.5 ml of the initial solution of the preparation was added to 250 ml of water).

The experiment used 100 outbred mice - SHR females of 3 months of age (50 experimental and 50 control) with an average weight of 27 grams with fixed birth dates. The experimental mice constantly received a geroprotective composition at a dose of 15 mg / kg daily with drinking water. Control mice received tap water.

Throughout the experiment, no significant effect of geroprotective composition on the general condition of animals, the dynamics of body weight and feed intake was revealed. The data obtained indicate that the drug has no toxic effect in the studied dose.

Starting from 13.5 months of age, control mice showed age-related disorders of estrous function, expressed in a decrease in the relative frequency of regular cycles (Figure 1). At the same time, in experimental mice treated with geroprotective composition, no such changes were noted. The revealed differences (a decrease in the frequency of regular cycles in the control group compared with the experimental group by 20–25%) in 13.5 and 16.5-month-old mice were statistically significant. Thus, geroprotective agent contributes to the normalization of the astral cycle.

Over the entire period of observation in the control and experimental groups, 50 animals fell (100% of the initial amount). The average life expectancy of mice was: in the control, 578.0 ± 13.85 days; in the experimental group - 621.0 ± 17.21 days (compared with the control p <0.05) (Figure 2). Thus, geroprotective composition significantly increases the life expectancy of mice.

Tumors in the control group were detected in 33 mice, and in the experiment - in 19 mice. The main tumors in animals of both groups were neoplasms of the mammary glands. In the control, they were found in 23 mice, in the experimental group - in 13 animals (p <0.05) (Figure 3). All tumors were single, with the exception of two mice in the control group, which had two neoplasms of the mammary gland. The first tumor in the experimental mouse was detected somewhat later than in the control, at the age of 338 days. The average latent period for the detection of breast tumors was 461 ± 21.97 days in the control, and 510 ± 27.15 days in the experimental group. Thus, in mice treated with the geroprotective composition, breast neoplasms were detected later and less often than in the control.

The proposed pharmacological geroprotective composition has stability due to the method of its preparation and can be used as a means of increasing the life expectancy and slowing down the development of the tumor.

Patent Citations (5)

Publication number	Priority date	Publication date	Assignee	Title
<a href="#">WO1992021368A1</a> *	1991-06-06	1992-12-10	Life Sciences' Technologies, Inc.	Composition and method for disease treatment
<a href="#">RU2301666C2</a> *	2002-01-11	2007-06-27	Маттиас Пат	Polyphenol-base pharmaceutical composition possessing nutrient properties and its using in cancer treatment
<a href="#">CN101298563A</a> *	2008-06-13	2008-11-05	天津市瑞丰原生物修复技术有限公司	Soil organism modifying agent for heavy soda alkaline land afforestation and plating method
<a href="#">RU2365377C1</a> *	2008-03-27	2009-08-27	Общество с ограниченной ответственностью "Лигфарм"	Method of biologically active substance olipifat manufacture
<a href="#">RU2449554C2</a> *	2006-02-01	2012-05-10	Нестек С.А.	Food systems and longevity enhancement methods
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Similar Documents

Publication	Publication Date	Title
<a href="#">Borowska et al.</a>	2016	Chokeberries (Aronia melanocarpa) and their products as a possible means for the prevention and treatment of noncommunicable diseases and unfavorable health effects due to exposure to xenobiotics
<a href="#">CA2649477C</a>	2016-04-19	Composition and method for promoting internal health and external appearance
<a href="#">Cásedas et al.</a>	2017	Anthocyanin profile, antioxidant activity and enzyme inhibiting properties of blueberry and cranberry juices: A comparative study
<a href="#">Haratifar et al.</a>	2014	Bioefficacy of tea catechins encapsulated in casein micelles tested on a normal mouse cell line (4D/WT) and its cancerous counterpart (D/v-src) before and after in vitro digestion
<a href="#">Bhardwaj et al.</a>	2014	Bioactive compounds and medicinal properties of fruit juices
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<a href="#">KR101186898B1</a>	2012-10-02	Antioxidative composition
<a href="#">WO2011146635A1</a>	2011-11-24	Malignant neoplasm treatment protocol
<a href="#">Xiang et al.</a>	2022	The bioactivity and applications of pomegranate peel extract: A review
<a href="#">Zhu et al.</a>	2015	Recent advance on the antitumor and antioxidant activity of grape seed extracts

ES2397085T3	2013-03-04	Polyphenol-Coxib procedures and combinations
Alappat et al.	2015	Anticancer and antioxidant properties of flavored green tea extracts
RU2522547C1	2014-07-20	Pharmacological geroprotective composition and method of obtaining thereof
Rani et al.	2014	Phytochemical, pharmacological and beneficial effects of green tea
Parnham et al.	2019	Mild plant and dietary immunomodulators
JPH06199690A	1994-07-19	Agent for promoting cerebral metabolism and improving cerebral function
Karthishwaran et al.	2012	Assessment of the antioxidant potential of Pergularia daemia (Forsk.) extract in vitro and in vivo experiments on hamster buccal pouch carcinogenesis
TWI731064B	2021-06-21	Composition for Tie2 activation
RU2619207C1	2017-05-12	Biologically active food supplement with cancer-preventive action
WO2014174703A1	2014-10-30	Angiogenesis inhibitor comprising ethanol extract of saw palmetto fruit or red pigment, cosmetic, medicine, crystallized red pigment, composition, food and methods for producing same
Ngai et al.	2019	In vitro antioxidant activity of dichloromethane: methanolic leaf and stem extracts of Papea capensis
RU2328294C1	2008-07-10	Medicinal agent for cancer prevention
Rashid et al.	2014	Kenyan purple tea anthocyanins ability to cross the blood brain barrier reinforcing brain antioxidant capacity in mice
KR101990054B1	2019-06-17	Anti-cancer Composition Comprising Lawsone
RU2453148C1	2012-06-20	Functional food product with antitoxic properties

### Priority And Related Applications

Priority Applications (1)

Application	Priority date	Filing date	Title
RU2012147985/15A	2012-11-12	2012-11-12	Pharmacological geroprotective composition and method of obtaining thereof

Applications Claiming Priority (1)

Application	Filing date	Title
RU2012147985/15A	2012-11-12	Pharmacological geroprotective composition and method of obtaining thereof

Concepts

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Name	Image	Sections	Count	Query match
■ mixture		title,claims,abstract,description	43	0.000
■ geroprotective		title,claims,abstract,description	32	0.000
■ pharmacologic effect		title,claims,abstract,description	9	0.000
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■ Vitamin C		claims,abstract,description	9	0.000

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