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Frank MADURA, *et al.* Ashitaba for Longevity

<https://news.yahoo.com/fountain-youth-study-finds-anti-ageing-compound-plant-160025864.html>

Fountain of youth? Study finds new anti-ageing compound in plant

In Japan, the slightly bitter leaves of the Ashitaba plant have long been considered healthy, and a new study has found the traditional belief may have good scientific grounds.

A natural substance in the plant appears to induce a key process that helps remove the "cellular garbage" that can build up as cells age and cause a range of diseases and disorders.

"It is always nice to find a scientific rationale for traditional medical folk tales," said Frank Madeo, a professor at the University of Graz's Institute of Molecular Biosciences, in Austria.

Madeo, who helped lead the research, said the substance known as 4,4'-dimethoxychalcone or DMC, which occurs naturally in Ashitaba plants, induces a process called autophagy.

"This is a cleansing and recycling process," he told AFP. It removes "superfluous material, especially cellular garbage like aggregated proteins."

That "cleaning" process is key to sustained good health as the body ages. When cells fail to promptly and efficiently remove damaged parts, they can build up and that can lead to diseases including cancer.

There are already a handful of compounds known to scientists that work to stimulate the cleaning process. Fasting also appears to naturally encourage cells to undertake spring cleaning.

But in a bid to expand the field of compounds able to protect cells and turn back the hands of time, the team of researchers turned to a class of substances called flavonoids.

Many flavonoids have already been shown to have a range of beneficial effects, ranging from anti-inflammatory properties to protecting against brain degeneration and cancer.

The team reasoned that they might find flavonoids that could also help prevent destructive ageing in cells.

They screened 180 compounds representing various subcategories of flavonoids, looking for candidates that might have the natural ability to "counteract age-related cell demise."

- Cell-protective capacity -

After initial screening, they settled on DMC and started by testing how the substance affected yeast cells.

They discovered it was indeed helping to protect the yeast cells from the effects of ageing, and that the substance performed as well or even better than some existing compounds prized for their cell-protective capacity like resveratrol, which occurs in grape skin, among other places.

The team then tested DMC's effect on cells in both worms and fruit flies -- common test subjects in medical research.

"Remarkably, chronic DMC treatment... prolonged the median lifespan of both model organisms by approximately 20 percent," the study published Wednesday in the Nature Communications journal says.

Additional tests showed the compound helped protect cells in mice hearts through the autophagy process, and even protected against a kind of liver damage caused by ethanol intoxication.

The team also tested DMC's effect on several types of human cells and found that there too the substance worked to slow ageing.

"The experiments indicate that the effects of DMC might be transferable to humans, although we have to be cautious and wait for real clinical trials," said Madeo.

The research is still in the early stages and Madeo said next steps will include testing whether the positive effects of DMC in mice hearts extend more broadly to protect mice against ageing and age-related diseases.

"Eventually, clinical trials on humans are needed," he added.

<https://en.wikipedia.org/wiki/Ashitaba>

Ashitaba





Angelica keiskei, commonly known under the Japanese name of ashitaba (アシタバ or 明日葉), literally "tomorrow's leaf", is a species of flowering plant in the carrot family. It is native to Japan, where it is found on the Pacific Coast.[1] It is endemic to the area of the Bōsō Peninsula, Miura Peninsula, Izu Peninsula, and the Izu Islands. It has been widely cultivated outside its natural range.

Scientific classification edit

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Apiales

Family: Apiaceae

Genus: *Angelica*

Species: *A. keiskei*

Binomial name

Angelica keiskei

Description

It is a perennial, with a typical growth height of 50–120 cm. Like most other members of the carrot family, it produces large umbels of white flowers and has dissected leaves.

Angelica keiskei closely resembles *Angelica japonica*, but can be distinguished by its blooming period, which lasts from May to October, whereas *A. japonica*'s blooming period lasts only between May and July. Another indicator is the characteristic color of its sap.[2]

Taxonomy

This species is named in honor of Keisuke Ito, a Japanese physician and biologist. A named cultivar of this species, "Koidzumi", refers to botanist Gen'ichi Koizumi. The Japanese name of *Angelica keiskei*, "ashitaba", stems from the above-average regenerative capabilities it exhibits after injury.

Cultivation

Many Japanese plant ashitaba in herb gardens, flower pots, and backyards. This is due to the modest conditions for cultivation and fast rate of growth. This is a cold hardy plant, with optimal temperatures ranging between 12 and 22 °C. Harvesting a leaf at the break of day often results in a new sprout growing overnight, being visible the following morning.

Uses

As food

The main use of their stipes, leaves, and taproots is in regional cuisine, where they are prepared as soba, tempura, shōchū, tea, ice cream, pasta, etc. The 'Mikura-jima' variety might excel in this regard as it is reputed to be less bitter than others.[3]

As medicine

A. keiskei has been claimed to exhibit cytotoxic, antidiabetic, antioxidative, anti-inflammatory, antihypertensive, and antimicrobial properties via in vitro studies, but the efficacy of these qualities have not been confirmed in vivo.[4]

Historical use

Traditionally, it is seen as a major contributor to the supposedly healthier, extended lives of the local residents, possibly due to the chalconoids that are unique to this species of *Angelica*. At one point in Edo period, the haulm's yellow sap was effectively used in the external treatment of smallpox, which prompted Kaibara Ekken to describe the herb in his *Yamato honzō* (大和本草), under the name of *ashitagusa* (鹹草), as "a powerful tonic drug." In folk medicine, it is claimed to be diuretic, tonic, to improve digestion, and when applied topically, to speed wound healing and prevent infection. Also, its nutritive qualities are said to be the factor behind the internal exiles and their families' never waning stamina in the face of their arduous compulsory labor.

For similar reasons, it very widely serves as pasture for cattle, reckoned to improve the quality of milk, as well as the yield and to maintain cattle health at the same time. Most of these claims have yet to be proven in trials, while studies have substantiated the presence of furocoumarins in several of these plants' components. Furanocoumarin is known to increase skin sensitivity to sunlight and may cause dermatitis.

Claims of vitamin B12 source

Although it is often suggested that *A. keiskei* is a vegetable source of vitamin B12 (cobalamin), recently published, peer-reviewed scientific investigations of pharmacology and phytochemical constituents of interest report nothing that substantiates this claim.[5][6] Traditional methods for measuring vitamin B12 in foods are compromised by contaminants (e.g. soil, bacteria, etc.) that contain detectable concentrations of inactive B12 analogs, which may explain the origin of this belief.[7] More recent studies reveal certain mushrooms and algae as the only naturally occurring sources of B12 outside of the animal kingdom.[8] Of these, only *Chlorella* has demonstrated the ability to reduce methyl malonic acid (MMA) levels (a product of B12 deficiency) in human subjects.[9]

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<https://pfaf.org/user/Plant.aspx?LatinName=Angelica+keiskei>

Angelica keiskei





Summary

Physical Characteristics

Angelica keiskei is a PERENNIAL growing to 1.2 m (4ft).

It is not frost tender. It is in flower from June to October, and the seeds ripen from July to November. The species is hermaphrodite (has both male and female organs) and is pollinated by Insects. The plant is self-fertile.

Suitable for: light (sandy), medium (loamy) and heavy (clay) soils. Suitable pH: acid, neutral and basic (alkaline) soils. It can grow in semi-shade (light woodland) or no shade. It prefers moist soil. The plant can tolerate maritime exposure.

Synonyms : *Archangelica keiskei*. Miq.

Habitats : Cultivated Beds;

Edible Parts: Leaves; Root; Stem.

Leaves - raw or cooked[105, 116, 177]. Root - cooked[105]. It is often pickled[177]. The root is short and thick[275].

Medicinal Uses: Plants For A Future can not take any responsibility for any adverse effects from the use of plants. Always seek advice from a professional before using a plant medicinally.

Other Uses: None known

Cultivation details: We have very little information on this species and do not know how hardy it will be in Britain, though judging by its native range it should succeed outdoors at least in the milder parts of this country. The following notes are based on the general needs of the genus. Requires a deep moist fertile soil in dappled shade or full sun[200]. Plants are reliably perennial if they are prevented from setting seed[200].

Propagation

Seed - best sown in a cold frame as soon as it is ripe since the seed only has a short viability[200]. Seed can also be sown in the spring, though germination rates will be lower. It requires light for germination[200]. When large enough to handle, prick the seedlings out into individual pots and grow them on in a cold frame for their first winter, planting them out into their permanent positions in the spring. The seed can also be sown in situ as soon as it is ripe.



Patents

Method for rapidly extracting chalcone from fresh ashitaba CN103664562

The invention discloses a method for rapidly extracting chalcone from fresh ashitaba and belongs to the technical field of separation and purification of natural products. The method comprises the following steps: crushing a proper amount of fresh ashitaba, and homogenizing to obtain homogenate; adding composite enzyme into the homogenate, sufficiently stirring, filtering and collecting a filtrate 1; adding a tetrachloroaluminic acid (1-ethyl-3-methyl) imidazole solution into the filtrate 1, extracting for 1-2h at room temperature, filtering and collecting a filtrate 2; performing chromatographic separation on the filtrate 2 by using a polyamide chromatography column, eluting with ethanol, and drying at low pressure to obtain the chalcone. According to the invention, composite enzyme and the non-volatile tetrachloroaluminic acid (1-ethyl-3-methyl) imidazole solution are selected to replace the conventional volatile organic solvents, so that harms of the volatile organic solvents are eliminated to the maximum extent and safety risks are eliminated; the pure chalcone prepared by the method has the purity of more than 96% and the yield of more than 93%.

The invention discloses a method for extracting chalcone from fresh leaves of the future, and belongs to the technical field of separation and purification of natural products. The method of the invention comprises: pulverizing and homogenizing an appropriate amount of fresh Asuka leaves to obtain a homogenate; adding a complex enzyme to the homogenate and stirring well, collecting the filtrate by filtration to obtain a filtrate; adding tetrachloroaluminate to the filtrate one -ethyl-3-methyl)imidazole solution, extraction at room temperature for 1 to 2 h, filtrate collection and filtration to obtain filtrate 2; filtrate 2 was chromatographed on a polyamide column, eluted with ethanol, and dried under low pressure. Ketone. The invention selects the compound enzyme and the non-volatile tetrachloroaluminum acid (1-ethyl-3-methyl)imidazole solution to effectively replace the use of the traditional volatile organic solvent, thereby completely eliminating the harm of the volatile organic solvent and eliminating the A security risk. The pure chalcone prepared by the method has a purity of 96% or more and a yield of 93% or more.

Technical field

The invention belongs to the technical field of separation and purification of natural products, and particularly relates to a method for purifying and rapidly extracting chalcone from fresh leaves of the future.

Background technique

Chalcone refers to a class of natural compounds containing 1,3-diphenylpropenone structure, which are abundant in the roots, leaves and skin of various medicinal plants such as licorice and safflower. The structure is flexible, can be combined with a variety of receptors, and exhibits various biological activities. It has been reported to have anti-tumor, anti-parasitic, anti-HIV, anti-bacterial, anti-inflammatory, anti-platelet aggregation and the like.

Angelica keiskei Koidzumi, also known as Aspergillus, Minguecao, Bazhangcao, etc., is native to Japan's Hachijojima, Miura and other peninsulas. It is a kind of sylvestris herb with anti-aging, blood sugar lowering and blood pressure lowering. , anti-tumor and other multiple effects. The leaves of tomorrow are mainly composed of flavonoids and coumarin compounds, and the flavonoids are mostly composed of chalcone. As a kind of abundant natural plant resources of natural chalcone, there are reports on the extraction and utilization of chalcone in tomorrow.

At present, the method for extracting chalcone from the leaves of tomorrow is mainly an organic solvent hot dip method, and the extraction solvent is mostly an organic solvent such as methanol, acetone or ethyl acetate, and the crude extract is obtained by multiple extraction of the above solvent. Chromatography purification to obtain chalcone. For example, the patent CN201010299189.0 uses dry leaves as raw materials, and is subjected to multiple extractions of ethanol, ethyl acetate, chloroform-methanol mixture and two column separations to obtain chalcone. This method not only has a large amount of organic solvent, but also has a large amount of organic solvent. The two column separations result in low yield, time consuming and low efficiency. The patent CN201010127425.0 uses dry day leaf powder as raw material, and is extracted by organic solvent such as isoamyl alcohol, acetone, chloroform and diisopropyl ether, and recrystallized. In the production process, flammable and explosive organic solvents such as acetone, chloroform and diisopropyl ether are selected, which not only has large pollution, but also has high safety hazard; patent CN201110218657.1 uses petroleum ether, acetone, ethyl acetate and The extraction of chalcone by inorganic salts such as ammonium sulfate, dipotassium hydrogen phosphate and potassium phosphate is not only long in process, but also has many organic solvents, and the discharge of a large amount of phosphorus-containing wastewater will also cause eutrophication of water bodies; the invention patent CN200910266615.8 directly adopts bright day The leaf is used as raw material, which simplifies the process steps, but the preparation process still uses organic solvents such as ethyl acetate, acetonitrile and dimethyl sulfoxide, and the pollution problem has not been solved.

Summary of the invention

In order to solve the above problems in the prior art, the present invention proposes a method for extracting chalcone from fresh leaves of the present day. The extraction method of the present invention does not use an organic solvent, and the chalcone pure obtained by the method is pure. The product has been proved to have a purity of over 96% and a yield of over 93%.

The technical solution of the present invention includes:

A method of extracting chalcone from fresh leaves of the future, the method comprising the steps of:

- a, the appropriate amount of fresh tomorrow leaves are pulverized and homogenized to obtain a homogenate;
- b. adding the complex enzyme to the above homogenate and stirring well, the weight ratio of the complex enzyme to the fresh tomorrow leaves is 0.1-5:100, the pH is controlled to 5.0-6.0, the

temperature is 35-40 °C, and the hydrolysis is 40. ~60min, the filtrate was collected by filtration to obtain a filtrate;

c, adding tetrachloroaluminate (1-ethyl-3-methyl)imidazole solution to the filtrate one, extracting at room temperature for 1 to 2 h, collecting the filtrate by filtration to obtain a filtrate 2;

d. The filtrate obtained in the step c is subjected to chromatographic separation on a polyamide column, eluted with ethanol, and dried at a low pressure of 55 to 60 ° C to obtain chalcone.

As a preferred embodiment of the present invention, the complex enzyme in the above step b is composed of cellulase, glucanase and phytase, and the mass ratio of the above cellulase, glucanase and phytase is 5~. 8:2~5:1~2.

Further, in the above step c, the volume ratio of the (1-ethyl-3-methyl)imidazole tetrachloroaluminate solution to the filtrate one is 2.5 to 3.5:1.

The mass ratio of the above three components of cellulase, glucanase and phytase is 7:3:2.

In the above step a, water is added for homogenization, and the amount of water added is 3 to 5 times the volume of fresh tomorrow leaves.

In the above step d, the flow rate in the chromatographic separation is controlled at 1.5 to 2.0 mL/min.

The beneficial technical effects brought by the invention:

The invention utilizes fresh Asuka as raw material, effectively degrades cell wall tissue by adding a composite enzyme preparation, greatly reduces the extraction temperature and relatively shortens the extraction time, and the energy saving and emission reduction effect is very significant; since the composite enzyme includes phytase, the effective degradation The composite network structure formed by hemicellulose, phytic acid and calcium-magnesium ions in the cell wall of tomorrow leaves not only improves the extraction rate, but also facilitates the concentration, purification, shortening of the process and improving the production efficiency of chalcone.

The use of a complex enzyme consisting of cellulase, glucanase and phytase eliminates the need for acetone, chloroform, diisopropyl ether, petroleum ether, ethyl acetate, isoamyl alcohol, acetyl cyanide, and dimethyl The multi-stage extraction step of toxic or flammable and explosive organic solvents such as sulfoxides also avoids the problems of eutrophication by phosphorus-containing inorganic salts.

The use of tetrachloroaluminate (1-ethyl-3-methyl)imidazole has a pH value consistent with the optimum pH conditions of the complex enzyme of the present invention (pH 5.0 to 6.0), and can be used in combination with a complex enzyme. The strong polarity of the tetrachloroaluminate (1-ethyl-3-methyl)imidazole solution is closer to the polarity of the chalcone in the leaves of the next day, and the selectivity is better, and the extract is also excluded. More impurity components can significantly increase the extraction rate of chalcone, and facilitate the subsequent separation and purification of chalcone in tomorrow's leaves; in addition, non-volatile tetrachloroaluminate (1-ethyl-3-methyl) As a new type of green extractant, imidazole solution can effectively replace the traditional volatile organic solvent, minimize the harm of volatile organic solvents, eliminate safety hazards, and achieve one-step extraction at room temperature. The energy has greatly shortened the process flow and improved the production efficiency by 2-3 times, achieving the purpose of cleaning and quickly extracting chalcone.

The use of polyamide chromatography column to separate chalcone has higher selectivity, better separation with pre-order process, higher yield, simple operation, low cost, and is more suitable for industrial production.

The pure chalcone prepared by the method of the invention has experimentally confirmed that the purity is above 96%, and the yield is above 93%.

DRAWINGS

The present invention will be further described below in conjunction with the accompanying drawings:

Figure 1 is a graph showing the linear relationship between the absorbance of the present invention and the concentration of the chalcone standard solution (C).

Detailed ways

The invention will be further clearly and completely described below in conjunction with the specific embodiments.

The raw materials and equipment involved in the present invention can be purchased commercially. The cellulase of the present invention is purchased from Weifang Kangdien Biotechnology Co., Ltd., and the enzyme activity is 3,000 U/g; the glucanase is purchased from Qingdao Blue Bio. Group Co., Ltd. has an enzyme activity of 20,000 U/g; phytase was purchased from Weifang Kangdien Biotechnology Co., Ltd., and the enzyme activity was 50,000 U/g.

The complex enzyme of the present invention is composed of a cellulase, a glucanase and a phytase.

The mass ratio of cellulase, glucanase and phytase in the complex enzyme is 5-8:2-5:1~2. For the composition and ratio of the complex enzyme of the present invention, the following comparative experiment is made--composite Enzyme synergistic effect comparison experiment:

- 1)Take fresh Asuka leaves as raw materials, firstly cut, then add 3-5 times of fresh 3-5 times the volume of water, homogenize, and pour the homogenate into the extraction tank;
- 2)The complex enzyme is added to the above homogenate and stirred well, and the weight ratio of the complex enzyme to the fresh Aoba leaves is 0.1-5:100, the pH is controlled to 5.0-6.0, the temperature is 35-40 °C, and the enzymatic hydrolysis is 40-60 min. Filtering and collecting to obtain a filtrate one;
- 3)To the above filtrate one, a solution of (1-ethyl-3-methyl)imidazole tetrachloroaluminate is added, and the volume ratio of the solution of tetrakis(Aethyl-3-methyl)imidazole tetrachloride to the filtrate is 2.5~3.5:1, extracting at room temperature for 1~2h, filtering and collecting to obtain filtrate 2;
- 4)Take the filtrate 2 obtained in step 3), chromatographic separation on the polyamide column, the flow rate is controlled at 1.5-2.0 ml/min; elution with 40%-60% (v/v) ethanol; at 55-60 °C After low pressure drying, chalcone is obtained.

The experimental settings of the present invention are respectively set in the following five groups:

- Group A - only cellulase was added;
 - Group B - only add glucanase;
 - Group C - only phytase was added;
 - Group D - only add cellulase and glucanase;
 - Group E - Cellulase, glucanase and phytase, i.e., the complex enzyme of the present invention, and the mass ratio of each component is from 5 to 8:2 to 5:1 to 2.
- The yield and purity of the chalcone obtained from the A group, the B group, the C group, the D group and the E group were measured, and the results are shown in

Table 1

The results in Table 1 show that the complex enzyme of the present invention has the best effect in both yield and purity, and the synergistic effect is remarkable.

Example 1:

The present invention provides a method for rapidly extracting chalcone from fresh leaves of the future, comprising the steps of:

Step 1: preparing a composite enzyme, weighing according to the mass ratio of cellulase, glucanase and phytase of 8:2:1, and preparing;

Step 2: Weigh 1kg of fresh tomorrow leaves, cut and pulverize first, then add 3 times of water to homogenize; pour the homogenate into the extraction tank;

Step 3: adding 1 g of the compound enzyme prepared in the first step to the extraction tank, stirring uniformly; adjusting the pH to 5.0, enzymatically dissolving for 40 min at 35 ° C; filtering, collecting the filtrate as filtrate one;

Step 4: adding a solution of (1-ethyl-3-methyl)imidazole tetrachloroaluminate (1-ethyl-3-methyl)imidazole solution to the filtrate one, and the volume of the filtrate The ratio is 2.5:1, and the mixture is extracted at room temperature for 1 hour, and the filtrate is collected and filtrated to obtain a filtrate 2;

Step 5: The filtrate was separated from the polyamide column by chromatography, and the flow rate was set to 1.5 ml/min; and eluted with 40% (v/v) ethanol solution to obtain a chalcone ethanol solution;

Step 6: The above chalcone ethanol solution was dried under vacuum at 55 ° C to obtain pure Chalcone from Asarum, a total of 28.29 g.

Example 2:

The present invention provides a method for rapidly extracting chalcone from fresh leaves of the future, comprising the steps of:

Step 1: preparing a composite enzyme, weighing and formulating according to the mass ratio of cellulase, glucanase and phytase of 7:3:2, and standby;

Step 2: Weigh 1kg of fresh tomorrow leaves, cut and pulverize first, then add 3 times of water to homogenize; pour the homogenate into the extraction tank;

Step 3: adding 50 g of the compound enzyme prepared in the first step to the extraction tank, stirring uniformly; adjusting the pH to 6.0, enzymatic hydrolysis at 40 ° C for 60 min; filtering, collecting the filtrate as filtrate one;

Step 4: adding a solution of (1-ethyl-3-methyl)imidazole tetrachloroaluminate (1-ethyl-3-methyl)imidazole solution to the filtrate one, and the volume of the filtrate The ratio is 3.5:1, and the mixture is extracted at room temperature for 2 hours, and the filtrate is collected and filtrated to obtain a filtrate 2;

Step 5: The filtrate is separated from the polyamide column by chromatography, and the flow rate is set to 2.0 ml/min; and eluted with 60% (v/v) ethanol solution to obtain a chalcone ethanol solution;

Step 6: The above chalcone ethanol solution was dried under vacuum at 60 ° C to obtain a pure

chalcone ketone, a total of 29.51 g.

Example 3:

The present invention provides a method for rapidly extracting chalcone from fresh leaves of the future, comprising the steps of:

Step 1: preparing a composite enzyme, weighing and formulating according to the mass ratio of cellulase, glucanase and phytase of 5:5:1.5, and standby;

Step 2: Weigh 1kg of fresh tomorrow leaves, cut and pulverize first, then add 5 times volume of water to homogenize; pour the homogenate into the extraction tank;

Step 3: adding 20 g of the compound enzyme prepared in the first step to the extraction tank, stirring uniformly; adjusting the pH to 6.0, enzymatically dissolving for 50 min at 38 ° C; filtering, collecting the filtrate as filtrate one;

Step 4: adding a solution of (1-ethyl-3-methyl)imidazole tetrachloroaluminate (1-ethyl-3-methyl)imidazole solution to the filtrate one, and the volume of the filtrate The ratio is 3:1, and the mixture is extracted at room temperature for 1.5 hours, and the filtrate is collected and filtrated to obtain a filtrate 2;

Step 5: The filtrate is separated from the polyamide column by chromatography, and the flow rate is set to 1.5 ml/min; and eluted with 50% (v/v) ethanol solution to obtain a chalcone ethanol solution;

Step 6: The above chalcone ethanol solution is dried under vacuum at 60 ° C to obtain a pure chalcone ketone, a total of 29.14 g.

Determination of the yield and purity of chalcone of the invention:

Chalcone yield

The chalcone yield K is calculated as follows:

In the formula, the weight of the chalcone sample (g) was obtained from m-1Kg fresh tomorrow leaves, and the W-fresh asperate leaf chalcone detection content value (30.35 g / 1 Kg fresh tomorrow leaves).

Establishment of the chalcone standard curve

Accurately weigh 1.0g of chalcone standard (Sigma, USA >99% purity), dilute to 10mL volumetric flask with anhydrous methanol; dilute the standard solution with anhydrous methanol to obtain a concentration of 0.0005~ a 0.005 mg/mL chalcone standard solution; the absorbance is measured by an ultraviolet-visible spectrophotometer at 310 nm, and a linear relationship between the absorbance and the concentration of the chalcone standard solution (C) is established, such as As shown in Fig. 1, the regression equation is: $A = 150.22C + 0.0519$, $R^2 = 0.9995$.

Chalcone purity test

The method uses spectrophotometric method to detect the purity of the prepared chalcone sample.

Accurately weigh a certain amount of pure sample of chalcone prepared, dissolve it with anhydrous methanol to obtain a solution of CP concentration; draw 1 mL of the solution, and make up to volume with anhydrous methanol in a 10 mL volumetric flask, then measure at 310 nm. Absorbance, calculated by the following formula:

After calculation and detection, the yields and purity of the present day Chalcone prepared in Examples 1 to 3 of the present invention are shown in Table 2:

Table 2

It can be seen from Table 2 that the yield and purity of the chalcone obtained by the method of the present invention satisfy the requirements of production, and the conditions of each process parameter in the embodiment 2 are optimal.

It should be noted that any equivalents or obvious modifications made by those skilled in the art in the teachings of the present invention are intended to be within the scope of the present invention.

Process for extracting chalcone type ingredients from ashitaba CN102020544

The invention belongs to the technical field of extracting chemical ingredients and specifically relates to a process for extracting chalcone type ingredients from ashitaba, which comprises the following steps: selecting dry stems and leaves of the ashitaba, smashing, carrying out hot dipping extraction with ethanol, merging extraction solution, decompressing, recovering the ethanol till having no alcohol smell and further getting liquid extract; and extracting with ethyl acetate after using water to dissolve the liquid extract, decompressing, recovering the ethyl acetate, getting an extract which is a chalcone crude product, adopting an AB-8 macroporous adsorption resin column to separate and purify the chalcone crude product, collecting eluant, further separating and purifying the eluant via a 100-mesh silica gel chromatography column and getting chalcone. The ethanol is adopted as an extraction solvent, thereby effectively reducing the activity of a chalcone enzyme, avoiding the decomposition of the chalcone during extraction and improving the extraction rate; and the process is simple, and the consumed time is short, thereby providing an industrial way for extraction of chalcone plants.

DESCRIPTION

The invention belongs to the technical field of chemical component extraction, and particularly relates to a method for extracting chalcone components in tomorrow leaves, which is to select dry stems and leaves of tomorrow leaves, pulverize, extract by hot dip with ethanol, combine extracts, and recover by vacuum. Ethanol to a non-alcoholic taste to obtain a flow extract; dissolve the extract in water, extract with ethyl acetate, and recover the ethyl acetate under reduced pressure to obtain an extract, which is a crude product of chalcone, and the crude chalcone is made of AB-8 macropores. The column was adsorbed and purified, and the eluate was collected. The eluate was separated and purified by a 100 mesh silica gel column to obtain chalcone. The invention adopts ethanol as the extraction solvent, can effectively reduce the activity of chalcone enzyme, avoids the decomposition of chalcone during extraction, improves the extraction rate, has simple process and short time, and provides an industrialized route for plant extraction of chalcone.

Technical field

The invention belongs to the technical field of chemical component extraction, and in particular relates to a process for extracting chalcone components in the leaves of tomorrow.

Background technique

Tomorrow leaves are a kind of vegetables native to Hachijojima and Izu seven islands in Japan. The source is the roots, stems and leaves of the genus Asteraceae (Umbelliferae, Latin name Umbelliferae). The Latin name is Angelica Keiskei Koidzumi.

The main components in the leaves of tomorrow are chalcone and coumarin compounds, of which

the content of chalcone yellow compound is the highest. Yellow pigment is a mixture of various water-soluble active ingredients in the leaves of tomorrow, belonging to the effective part of chalcone, soluble in polar solvents such as water and dilute alcohol, insoluble in anhydrous ethanol, acetone, ether and petroleum ether. Good anti-tumor, anti-cancer, anti-ulcer, anti-thrombotic, antihypertensive, anti-allergic, anti-AIDS, anti-dementia, anti-diabetes and many other effects, especially 4-hydroxyderrcin and xanthoangelol .

At present, domestic research on chalcone only stops by using organic chemical synthesis method to prepare chalcone (as an intermediate of flavonoids), and the obtained product has many side reactions, which takes a long time, is cumbersome to operate, and is not easy to post-treat. At the same time, the product is low in purity and has many disadvantages such as enantiomers. However, there are few studies on food and medicine for extracting chalcone active ingredients from tomorrow leaves, and there is no relevant literature after searching.

Summary of the invention

The object of the present invention is to provide a process for extracting chalcone components from the leaves of tomorrow, which can effectively reduce the activity of chalcone enzyme by using ethanol as an extraction solvent, avoid decomposition of chalcone during extraction, and improve chalcone. The extraction rate, as well as the extraction rate of 4-hydroxyderrcin (4-hydroxydisincin, 4HD for short) and xanthoangelol (xanthohumol, abbreviated as XA) in the chalcone.

The technical solution adopted by the invention:

A method for extracting chalcone components from the leaves of tomorrow, is to select dried stems and leaves of the genus Umbelliferae, Angelica Keiskei Koidzumi, and pulverize, and use 9-11 times by weight to 40% to 60% ethanol by weight ratio. The extract is hot-dipped at 65 ° C to 75 ° C for 2 to 4 times, each time for 2 to 3 hours, and the extracts are combined, and the ethanol is recovered under reduced pressure until the alcohol-free taste is obtained to obtain a flow extract; 7 to 9 times by weight is used. The water is dissolved in the extract twice, and the water extract is combined, and then extracted with ethyl acetate for 5 to 7 times. The extract is combined, and the ethyl acetate is recovered under reduced pressure to obtain an extract, which is a crude product of chalcone. AB-8 macroporous adsorption resin column, using distilled water as eluent, resin particle size 30 mesh, flow rate of 15ml/min, separation and purification, collecting eluate, eluate and then separated by 100 mesh silica gel column Purified to obtain chalcone.

The above chalcone was eluted with a gradient of chloroform-methanol (100:1?30:1), and the target 4HD and XA were collected by HPLC, and then recrystallized twice with 50% ethanol to finally obtain 4HD and XA. Pure.

The chloroform-methanol mixture was mixed at a ratio of chloroform to methanol in a volume ratio of chloroform:methanol = 100:1 to 30:1.

The advantages of the invention are:

The reaction conditions are mild and the key process parameters are easy to control.

The use of ethanol in the extraction of chalcone can effectively extract chalcone from tomorrow's leaves. The advantage is that the chalcone enzyme in the plant decomposes the chalcone during the extraction, making it free and reducing its water solubility. Sexually, the use of ethanol can reduce the activity of the enzyme in the enzyme, so that the chalcone is extracted as completely as possible, and the extraction rate is improved.

The separation and purification were carried out on a 100-mesh silica gel column, and the gradient

elution was carried out with a chloroform-methanol mixture eluent. The separation efficiency of the chalcone was good, the product purity was high, and the final product extraction rate and product purity were relatively high.

Recrystallization from 50% ethanol at low temperature (0 ° C), the product obtained is of higher purity, avoiding the transformation of the target product configuration caused by recrystallization of other organic solvents (eg, xanthohumol is prone to thermal isomerism above 50 ° C) The yellow ketone is produced to reduce the target product).

Detailed ways

The invention is further illustrated by the following non-limiting examples.

Embodiment 1

Select 2.0kg of dried stems and leaves of the umbrella plant *Angelica Keiskei* Koidzumi, pulverize, extract twice with 50 times of 50% ethanol and 70 °C hot dip for 2 h, combine the extracts, and recover the ethanol under reduced pressure. To the non-alcoholic extract, the solution was dissolved twice by adding 8 times of water, and the water extract was combined and extracted with ethyl acetate 6 times. The extracts were combined, and the ethyl acetate was evaporated under reduced pressure to obtain an extract. That is to obtain 51.6g crude chalcone, the extract is AB-8 macroporous adsorption resin column, using distilled water as eluent, resin particle size 30 mesh, flow rate is 15ml/min, separation and purification, collecting eluent, washing The mixture was separated and purified by a 100 mesh silica gel column to obtain 43.7 g of a yellow extract.

The extract is reacted with boric acid and the reaction product is bright yellow.

The extract was reacted with hydrochloric acid-magnesium powder and was negative.

From this, it was judged that the obtained extract was chalcone.

The obtained chalcones were eluted with a gradient of chloroform-methanol (90:1). The target 4HD and XA were collected by HPLC, and then recrystallized twice with 50% ethanol to obtain 4HD and XA pure products, respectively. It is 18.44g and 6.34g.

Embodiment 2

Select 3.0kg of dried stems and leaves of the umbrella plant *Angelica Keiskei* Koidzumi, pulverize, and extract it by hot dip at a temperature ratio of 8 times and 45% ethanol at 68 °C for 3 hours, and combine the extracts. The ethanol was recovered under reduced pressure to obtain a stream extract without alcohol odor; the extract was dissolved twice with water in a ratio of 7 times by weight, and the water extract was combined, and then extracted with ethyl acetate 7 times, and the extract was combined and decompressed. The ethyl acetate was recovered to obtain the extract, which was 80.4 g of crude chalcone. The crude chalcone was eluted with AB-8 macroporous resin resin, and distilled water was used as the eluent. The resin particle size was 30 mesh and the flow rate was 15 ml/min. Separation and purification were carried out, and the eluate was collected, and the eluate was separated and purified through a 100 mesh silica gel column to obtain 68.3 g of a yellow extract.

The extract is reacted with boric acid and the reaction product is bright yellow. The extract was reacted with hydrochloric acid-magnesium powder and was negative. From this, it was judged that the obtained extract was chalcone.

The obtained chalcones were eluted with a gradient of chloroform-methanol (60:1). The target 4HD and XA were collected by HPLC, and then recrystallized twice with 50% ethanol to obtain 4HD and XA pure products, respectively. It is 29.53 g and 11.25 g.

Embodiment 3

Select 1.0kg of dried stems and leaves of the genus Umbelliferae, *Angelica Keiskei* Koidzumi, and pulverize them. Dilute and extract 3 times with 7 times of 55% ethanol at 73 °C for 4 hours, and combine the extracts. The ethanol was recovered under reduced pressure until the alcohol-free taste was obtained to obtain a flow extract; the extract was dissolved twice with water in a weight ratio of 9 times, and the aqueous extract was combined, and then extracted with ethyl acetate 5 times, and the extract was combined and decompressed. The ethyl acetate was recovered to obtain the extract, which was 24.8 g of crude chalcone. The crude chalcone was packed with AB-8 macroporous adsorption resin column, distilled water as eluent, resin particle size 30 mesh, flow rate 15 ml/min. Separation and purification were carried out, and the eluate was collected, and the eluate was separated and purified through a 100 mesh silica gel column to obtain a yellow extract (21.3 g). The extract is reacted with boric acid and the reaction product is bright yellow. The extract was reacted with hydrochloric acid-magnesium powder and was negative. From this, it was judged that the obtained extract was chalcone.

The obtained chalcones were eluted with a gradient of chloroform-methanol (40:1). The target 4HD and XA were collected by HPLC, and then recrystallized twice with 50% ethanol to obtain 4HD and XA pure products, respectively. It is 10.26g and 3.57g.

CHALCONE-CONTAINING POWDER COMPOSITION JP4986320

PROBLEM TO BE SOLVED: To provide a method for producing powder from viscous juice of *Ashitaba* (*Angelica keiskei*) or a solvent extract of *Ashitaba* and obtain a powdery composition produced thereby. **SOLUTION:** Viscous juice or solvent extract of *Ashitaba* is mixed with a cyclodextrin, preferably cyclodextrin free from branch, the mixture is dried and the obtained solid is pulverized to obtain a powdery composition containing a chalcone derived from *Ashitaba*. The powdery composition containing the chalcone derived from *Ashitaba* contains the viscous juice component or solvent extract of *Ashitaba* in combination with the cyclodextrin.

DESCRIPTION

[0001]

BACKGROUND OF THE INVENTION 1. Field of the Invention The present invention relates to a method for preparing a tomorrow's leave juice or a solvent extract having a viscous liquid in powder form and to a powder composition obtained by the method.

[0002]

Tomorrow leaves (*Angelica keiskei* Koidz.) Is a plant of the family *Seriaceae* that is native to warm regions such as Hachijojima and Izu Islands and is unique to Japan that is prized as strong herbal, tonic, longevity and longevity medicinal herbs from ancient times. Traditionally, it is known that tomorrow leaves are rich in vitamins, minerals, high-quality protein and dietary fiber, but in recent years also chalcones, which are the main components of tomorrow's leaves juice from medicine, are sterilized It has been found that it exhibits an interesting pharmacological activity such as action (anti-gram positive bacterial activity), antacid action, antiulcer effect, carcinogen promoter inhibitory action and the like.

[0003]

However, since tomorrow's leaves juice has a viscous nature, it is difficult to process into a form easy to take such as powdery, granular or tablet, and for some time it has the above excellent effect There was a need for a method to easily process and commercialize the yellow juice of leaves

tomorrow.

[0004]

SUMMARY OF THE INVENTION An object of the present invention is to solve the above conventional problems.

That is, it is an object of the present invention to provide a method for conveniently processing and preparing the yellow juice of tomorrow's leaves having a viscous liquid form into powder form. It is a further object of the present invention to provide a powder composition of tomorrow leaves of yellow juice obtained by such a method.

[0005]

DISCLOSURE OF THE INVENTION In view of the demands of the above-mentioned industry, the inventors of the present invention conducted research on a method of solidifying tomato leaves of yellow juice day and night, and found that the yellow juice was cyclodextrin, In particular, it was found that by mixing with cyclodextrin without branching, it can be very easily solidified and can be further easily processed into fine powder. That is, according to the above method, the present inventors can dry solidify the leaves of the leaves tomorrow without drying using a special drying method such as lyophilization, spray drying or reduced pressure drying, and can be dried and solidified by a usual drying method It was found that the solid matter can be easily prepared in a fine powder form by grinding according to the usual method, and furthermore, it was confirmed that such a method can be similarly applied to the solvent extract of the leaf tomorrow. Further, the present inventors confirmed that chalcones in yellow juice used as a raw material can be recovered with high yield in the fine powder obtained by the above method. The present invention has been accomplished based on this finding.

[0006]

That is, the present invention is a method for producing a chalcone-containing powder composition derived from tomorrow's leaves as listed below: (1) A tomorrow leaves juice or a solvent extract is mixed with cyclodextrin and dried to obtain a solid A process for producing chalcone-containing powder composition derived from leaves tomorrow including the step of pulverizing the chalcone. (2) The chalcone-containing powder composition according to (1), wherein the cyclodextrin is at least one selected from the group consisting of a-cyclodextrin, Method for manufacturing an article.

[0007]

Furthermore, the present invention is a chalcone-containing powder composition derived from tomorrow's leaves as set forth below: (3) It is derived from tomorrow's leaf containing tomato leaves yellow juice component or extract and cyclodextrin A chalcone containing powder composition. (4) The chalcone-containing powder composition according to (3), wherein the cyclodextrin is at least one selected from the group consisting of a-cyclodextrin without branching, β -cyclodextrin and γ -cyclodextrin object.

[0008]

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The tomato leaf yellow juice or solvent extract solution to which the present invention is directed is represented by the formula (1)

[0010]

4-hydroxyderricin represented by the formula (2)

[0012]

So-called chalcones having a chalcone skeleton such as Xanthoangelol (hereinafter collectively referred to as "chalcone") represented by the following formula.

) As a main component.

[0013]

Therefore, the tomorrow's leave juice or solvent extract used in the present invention is not limited at all depending on the site to be obtained and the method of obtaining as long as it contains the chalcone.

For example, juice or juice collected from tomorrow leaves stalks or rhizomes as tomorrow leaf yellow juice can be used, and if necessary, these are further subjected to filtration treatment and washing treatment to remove contaminants such as dust Or it can be sterilized by heating or filtration treatment or the like. As a solvent extract of tomorrow's leaves, it is preferable to subject the whole grass of tomorrow's leaves or a part thereof (leaves, stems, roots, etc.) to a solvent extracting operation as it is or as a crushed product, or, after drying, pulverized powder It can be prepared by subjecting to solvent extraction operation as a body.

[0014]

Examples of the extraction solvent include water; lower alcohols having 1 to 4 carbon atoms such as methanol, ethanol, propanol and butanol; lower alkyl esters such as ethyl acetate and the like; esters such as ethylene glycol, butylene glycol, propylene glycol, glycerin Glycols; other known organic solvents such as polar solvents such as ethyl ether, acetone, and acetic acid; hydrocarbons such as benzene and hexane; and nonpolar solvents such as ethers such as ether and petroleum ether. These solvents may be used singly or in combination of two or more kinds in any combination. For example, if necessary, an appropriate amount of water can be added to the above-mentioned organic solvent to use it as a water-containing organic solvent. As such a solvent, a mixed solvent of water and alcohol is preferable. In the present invention, water, alcohol (for example, ethanol etc.), or a mixture of water and alcohol is more preferable.

[0015]

As a method for extraction, commonly used methods can be adopted, and widely include extraction methods used for preparation of various preparations such as extract, elixir, infusion / decoction, flow extract and tincture. There is no limitation, for example, a method in which tomorrow leaves whole grass or a part of leaves (as it is or coarse, shredded), or a dry crushed material thereof (powder etc.) is immersed in cold infiltration, digestion etc. A method of extracting with hot stirring and filtration to obtain an extract, or a percolation method and the like. If necessary, the obtained extract can be used as it is after filtration or centrifugation to remove solid matter, or it can be used as a concentrate by distilling off the solvent. After concentration, the concentrated solution may be purified by washing with a non-dissolving solvent, or it may be dissolved or suspended in an appropriate solvent and used. It is also possible to obtain and purify fractions containing chalcones such as 4-hydroxyderricin and Xanthoangelol by using commonly used purification methods such as countercurrent distribution method, liquid chromatography, etc. .

[0016]

To simplify the acquisition, tomato leaf yellow juice is preferably used.

[0017]

There is no particular limitation on the cyclodextrin used as a mixture with the above-mentioned juice of the leaves of tomorrow or a solvent extract solution, and conventionally known cyclodextrin can be broadly cited.

Preferably, it is a cyclodextrin without branching. Although either cyclodextrin having branch or cyclodextrin without branch can be used to solidify and powder the tomato leaves juice or solvent extract, cyclodextrin without branching is used Since it can be prepared as a solid with high dryness, it can be pulverized easily and there is an advantage that it can be prepared into a uniform fine powder which is smooth by pulverization. Furthermore, in the present invention, a-

cyclodextrin, β -cyclodextrin and γ -cyclodextrin containing glucose in a ratio of 6 to 8 molecules can be used. Preferable are β -cyclodextrin and α -cyclodextrin, particularly preferably β -cyclodextrin.

[0018]

The compounding ratio of the juice of the leaves of tomorrow or the solvent extract and the above cyclodextrin is not particularly limited as long as it does not interfere with solidification and powdering of the leaves juice or solvent extract tomorrow, And can be prepared by suitably selecting so that a desired amount of chalcone is contained in the powder composition. For example, cyclodextrin is usually added in a proportion of 30 to 300 parts by weight, preferably 50 to 200 parts by weight, more preferably 60 to 150 parts by weight, still more preferably 70 to 100 parts by weight, based on 100 parts by weight of yellow juice of tomorrow leaves Can be blended.

[0019]

When tomorrow's leaves juice or solvent extract is mixed with cyclodextrin as described above, tomorrow leaves juice or solvent extract which was in a viscous liquid is probably based on the inclusion phenomenon of cyclodextrin, Heat changes and changes in the form of clay (paste). In mixing these two, it is preferable to coexist with water in order to accelerate the inclusion reaction of cyclodextrin and to efficiently proceed the reaction. The mixing ratio of water is not particularly limited, but it can be exemplified in the range of about 5 to 100 parts by weight, preferably 20 to 70 parts by weight, and more preferably 40 to 50 parts by weight with respect to 100 parts by weight of cyclodextrin.

[0020]

The chalcone-containing powder composition derived from the tomorrow's leaf of the present invention can be prepared by drying the clay-like material (paste) and then pulverizing the obtained solid material.

[0021]

The drying method is not particularly limited, and any of cold drying, normal temperature drying and warm drying, natural drying and air drying can be adopted.

Preferably, it is a method of blowing air dry from room temperature to warming. Specifically, there can be mentioned a method in which air drying is carried out under heating at about 40 to 60 ° C., preferably about 50 ° C.

[0022]

The pulverization method is also not particularly limited, and it can be prepared in powder form to fine powder form using a commonly used pulverizer such as a mortar, a mixer, a cutter mill and a hammer mill according to a conventional method.

[0023]

The powder composition of the present invention thus obtained contains as an active ingredient chalcone derived from tomorrow's leaves (for example, 4-hydroxyderricin, Xanthoangelol), itself as a food, quasi-drug or pharmaceutical, Further, it can be effectively used as a raw material (bulk) for preparing these various formulations.

The powder composition of the present invention may be suitably prepared so that the above-mentioned chalcone is contained in a total amount of 2 to 30% by weight, preferably 4 to 10% by weight according to the content ratio in tomorrow leaves of yellow juice Good.

[0024]

The powder composition of the present invention may contain other carriers and additives pharmaceutically or food hygienically acceptable as other ingredients as long as the effect of the

present invention is not hindered. For example, (Eg, starches such as dextrin, saccharides such as lactose etc.), excipients, lubricants, binders, corrigents, odor control agents and sweeteners.
[0025]

EXAMPLES

The present invention will be explained in more detail with reference to the following examples, but the present invention is not limited to these examples at all.

50 g of yellow juice collected by cutting the tomorrow's leaf stem with sterilized boiled water was used as raw material (stock solution), and it was put in a round bottom flask together with 67.1 g of 99.5% ethanol, and it was kept at room temperature for 1 hour Followed by stirring. The resulting mixture was filtered with a Nutsche filter, and the filtrate was concentrated under atmospheric pressure to obtain 47.1 g of a brown oily liquid. 21.1 g of water and 51.5 g of β -cyclodextrin were added to and mixed with 37.4 g of the obtained oily liquid, and sufficiently stirred and mixed until the whole became a paste. The obtained paste-like mixture was spread on a stainless steel vat and dried in an air dryer (50 ° C.) to obtain 58.9 g of a pale yellow brittle solid mass. This solid was pulverized with a mixer to prepare a pale yellow fine powder (33.9 g). The content of 4-hydroxyderricin and Xanthoangelol contained in the obtained fine powder was analyzed by high performance liquid chromatography under the following conditions.

[0026]

Apparatus: Shimadzu LC-6A, Shimadzu CR7A chromatography pack Column: COSMOSIL 5C18-AR (4.6 f × 250 mm) Mobile phase: methanol-water (7: 3) Temperature: 50 ° C Flow rate: 0.9 ml / min Detection: UV 330 nm Sample volume: 2 μ l.

[0027]

As a result, 4-hydroxyderricin was contained at 2.29% and Xanthoangelol was included at 4.60%. These contents correspond to 96.2% and 89.1% of 4-Hydroxyderricin and Xanthoangelol contained in the yellow broth of the ashi leaf used as a raw material, and from this it can be seen that in the powder composition of the present invention , It was confirmed that 4 - Hydroxyderricin and Xanthoangelol in the yellow broth of the leaf tomorrow were recovered with a yield as high as about 90 to 96%.

[0028]

Comparative Example 21.1 g of water and 51.5 g of dextrin (Pain Index No. 3: manufactured by Matsutani Kagaku KK) were added to and mixed with 37.4 g of the oily liquid obtained in the same manner as in Example 1, and the mixture was sufficiently stirred and mixed at room temperature. As a result, unlike the case of Example 1 using β -cyclodextrin, a heterogeneous mixture with low viscosity was obtained. This mixture was spread on a stainless steel vat and dried in an air dryer (50 ° C.), but it turned into a candy shaped brown solid and could not be powdered.

[0029]

According to the present invention, chalcone (4-Hydroxyderricin and Xanthoangelol) having pharmacological activities such as bactericidal action (anti-gram-positive bacterial activity), antacid action, antiulcer effect, It is possible to easily solidify and powderize the tomorrow's leave juice or solvent extract contained therein without any complicated methods such as freeze-drying or spray drying. The powder composition thus obtained is useful not only as a food, a quasi-drug, a medicine per se, but also as a raw material (bulk) used for formulation of these.

FIBROBLAST GROWTH FACTOR-7 (FGF-7) PRODUCTION PROMOTER, INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) PRODUCTION PROMOTER, AND HEPATOCYTE GROWTH FACTOR (HGF) PRODUCTION PROMOTER

PROBLEM TO BE SOLVED: To provide an FGF-7 production promoter, an IGF-1 production promoter, and an HGF production promoter having excellent action and high safety. ;**SOLUTION:** There are provided an FGF-7 production promoter, an IGF-1 production promoter, and an HGF production promoter each of which contains ashitaba (*Angelica keiskei*) and/or luteolin-7-O-glucoside as active ingredients. It is possible to formulate them into dermatological preparations for external use including hair growing agents and hair cosmetics, to formulate them into foods and beverages and medicaments for cosmetological use, and to desirably use them as reagents for researches

Tissue culture rapid propagation technology for ashitaba leaves
CN104145817

The invention discloses a tissue culture rapid propagation technology for ashitaba leaves. The tissue culture rapid propagation technology comprises the following steps: selecting ashitaba leaves; washing with running water; soaking with a 75% alcohol solution; washing with sterile water; sterilizing in 0.1% L of a mercury solution and then washing with sterile water again; dicing; breeding in a callus induction medium, and culturing for 20-30 days so that a callus mass grows on a leaf cut; dicing the callus mass; transferring to a callus proliferation medium, culturing for 20-30 days and proliferating calluses; dicing the proliferated calluses; transferring to a cluster bud induction medium, culturing for 30 days, and differentiating to produce cluster buds; cutting the calluses on the roots of the cluster buds; splitting into single buds; inoculating a root medium with the buds and culturing for 20 days to obtain regeneration plant seedlings; and after the seedlings are domesticated for one week, transplanting into sterilized coconut shell land till the seedlings survive. The tissue culture rapid propagation technology has the beneficial effects that the cost is low and the material source is wide since the ashitaba leaves are used as propagation materials.

The invention discloses a technique for tissue culture and rapid propagation of leaves of tomorrow leaves. The leaves of tomorrow leaves are selected, washed with running water, soaked in 75% alcohol solution, rinsed with sterile water, disinfected in 0.1% mercuric acid solution, and then sterile. Washing with water; dicing; growing callus in the callus induction medium for 20-30 days at the leaf incision; then cutting the callus into pieces and transferring to callus proliferation medium Callus proliferation was carried out for 20-30 days; the proliferated callus was cut into pieces and transferred to the shoot bud induction medium for 30 days to differentiate to produce cluster buds; the callus of the bud base was excised and divided into single pieces. Buds, the shoots were inoculated in rooting medium for 20 days to obtain regenerated plant seedlings; the domesticated seedlings were transplanted into the sterilized coconut shell soil one week later until the seedlings survived. The beneficial effect of the invention is that the leaves of the leaves of the next day are made of propagation materials, the cost is low, and the materials are widely used. Leaf tissue culture and rapid propagation technique of tomorrow

Technical field

The invention belongs to the technical field of plant cultivation, and relates to a technique for tissue culture and rapid propagation of leaves of tomorrow.

Background technique

The use of medicine and food in tomorrow's leaves, especially in the medicinal field, is a new vegetable that is widely recognized as a development potential. China introduced the Japanese

leaves from Japan more than 20 years ago. At present, only a few companies in China have just introduced seeds, and the cultivation area is very small. The raw materials are expensive in the international market and the demand is large. In the production process, the plants of tomorrow's leaves usually enter the breeding period 1-2 years after planting, and die immediately after flowering and fruiting. The flowering period of the leaves of the next day is longer, the fruit set rate is lower, the seed maturity is different at harvesting, and the fruit is double-suspended fruit, which causes the seed germination rate of the leaves of the next day to be extremely low, and it is difficult to cultivate on a large scale. Nowadays, the breeding and cultivation techniques of seedlings of tomorrow's leaves have become a bottleneck restricting the development of tomorrow's leaves and their processed products. However, there are few reports on the breeding and cultivation of tomorrow's leaves, which leads to the price of tomorrow's leaves and their products. No high.

In order to expand the industrial production scale of tomorrow's leaves, there have been some research reports on the tissue culture of tomorrow's leaves. At present, the published research results are divided into three types: First, Li Jia and other studies have found that the petiole and leaves of tomorrow's leaves are not suitable for tissue culture as explants, but the callus induction is carried out by using the bud stem segments. Differentiation and other cultures; the second is Lu Xiuli and other reports on the use of seeds of the next day to obtain sterile seedlings for differentiation and proliferation; third, Guo Zhiyou et al reported the use of petioles and leaves as explants for tissue culture.

Compared with the above three studies, this study is the same as the use of tissue culture to construct the breeding system of tomorrow's leaves, but it is very different from the three. First, compared with Li Jia and other studies, this study broke the conclusion that leaves could not be induced as explants, and the material with bud stems was significantly restricted compared with the leaves selected in this study. Compared with Lu Xiuli and other studies, the material limitation of this study is greatly reduced. Finally, compared with the results of Guo Zhiyou and others, although the results are similar, the results of this study show that MS as a basic medium works better, and Guo Zhi The friend's report believes that the N 6 basal medium is suitable for culture, so the basal medium used for the two is very different.

Summary of the invention

The object of the present invention is to provide a technique for tissue culture and rapid propagation of leaves of the next day, which solves the problem that the germination rate of seeds of the next day leaves is low, the raw materials in the market are insufficient, and the price is expensive.

The technical solution adopted by the present invention is carried out according to the following steps:

- (1) Select the leaves of tomorrow leaves, rinse with running water, and put them on the aseptic table for use;
- (2) Soak in 75% alcohol solution, rinse 3 times with sterile water, sterilize in 0.1% liters of mercury solution, then rinse 5 times with sterile water;
- (3) Dry the leaves of the leaves with sterile filter paper and then cut;
- (4) The leaves were inoculated into callus induction medium and cultured in a 24 ° C incubator at 2000 lx light intensity. The light conditions were 16 hours light per day, 8 hours darkness, and cultured for 20-30 days. Organization group
- (5) The callus pellet was diced and transferred to the callus proliferation medium at a culture temperature of 25 ° C, light conditions of 16 hours light, 8 hours darkness, light intensity 3000 Lx, culture 20-30 days for callus proliferation;
- (6) The proliferated callus was cut into pieces and transferred to a cluster bud induction medium for culture at a temperature of 25 ° C, light conditions of 16 hours of light, 8 hours of darkness, light

intensity of 3000 Lx, and culture for 30 days to differentiate into shoots;

(7) The callus of the bud base was excised, divided into individual buds, and the buds were inoculated into the rooting medium at a culture temperature of 25 ° C, light conditions of 16 hours of light, 8 hours of darkness, light intensity of 3000 Lx, and cultivation for 20 days to obtain regeneration. Plant seedlings;

(8) The robust seedlings were selected in tissue culture flasks and transferred to natural environment for 3 days. The tissue culture bottle caps were opened for 3 days, and the domesticated seedlings were transplanted into the sterilized coconut shell soil after one week of domestication and refining. Watering, maintaining air humidity, gradually open the film after 1 week until the seedlings survive.

Further, the steps 3, 5, and 6 are diced to 1 cm < 2 >.

Further, the callus induction medium is MS+1mg/L6-BA+3mg/LNAA; the callus proliferation medium is MS+6-BA2mg/L+2,4-D1mg/L; The shoot bud induction medium was MS+6-BA1 mg/L+NAA 0.5 mg/L; the rooting medium was 1/2 MS+0.02 mg/L NAA.

The beneficial effect of the invention is that the leaves of the leaves of the next day are used as propagation materials, the cost is low, and the material sources are wide.

Detailed ways

The present invention will be described in detail below in conjunction with specific embodiments.

The invention adopts MS as a basic medium and configures a combination of different plant growth substances:

Callus induction medium: MS + 1 mg / L 6 - BA + 3 mg / L NAA;

Callus proliferation medium: MS+6-BA2mg/L+2,4-D1mg/L;

Cluster bud induction medium: MS+6-BA1mg/L+NAA0.5mg/L;

Rooting induction: 1/2 MS + 0.02 mg / L NAA.

The invention proceeds as follows:

(1)The leaves are explants, the leaves are preferably upper, slightly young leaves, but avoid collecting new leaves, rinse with running water for 15-20min, and put them on the aseptic table for use.

(2)In the inoculation room, the plant material was soaked in a 75% alcohol solution for 30 seconds on the ultra-clean workbench, and then washed out in sterile water for 3 times, dissolved in 0.1% mercuric acid solution for 7 min, and rinsed 7 times with sterile water.

(3)The leaves were blotted with sterile filter paper and cut to a size of 1 cm < 2 >.

(4)The leaves were inoculated into callus induction medium and cultured in a 24 ° C incubator at 2000 lx light intensity. The light conditions were 16 hours light per day, 8 hours dark, and cultured for 20-30 days until the leaf incision grew. The wound tissue group increased.

(5)The callus mass was evenly cut into 1cm < 2 > and transferred to the callus proliferation medium at a culture temperature of about 25 ° C. The light conditions were 16 hours light, 8 hours dark, light intensity 3000 Lx, and culture 20- The 30-day callus can proliferate to about 6 cm < 2 >.

(6)The proliferated callus was cut into a size of 1 cm < 2 >, and transferred to a cluster bud induction medium for culture at a temperature of about 25 ° C, light conditions of 16 hours of light,

8 hours of darkness, light intensity of 3000 Lx, and culture 15 After -20 days, the callus re-differentiated to produce cluster buds, and the shoot buds grew well after 30 days.

(7)The callus of the bud base was excised, divided into individual buds, and the buds were inoculated into the rooting medium at a culture temperature of about 25 ° C. The light conditions were 16 hours light, 8 hours dark, light intensity 3000 Lx, and buds after 20 days of culture. The base is partially rooted and grows well, and the regenerated plant seedlings are obtained.

(8)The robust seedlings were selected in tissue culture flasks and transferred to natural environment for 3 days. The tissue culture bottle caps were opened for 3 days, and the domesticated seedlings were transplanted into the sterilized coconut shell soil after one week of domestication and refining. Pay attention to watering, keep the air humidity, gradually open the film after 1 week, and reduce the watering frequency, the survival rate can reach more than 94%.

The invention constructs a novel breeding system of the future leaves by leaf tissue culture, breaks the production bottleneck caused by the low seed germination rate, and provides conditions for large-scale production.

The above is only a preferred embodiment of the present invention, and is not intended to limit the present invention in any way. Any simple modifications, equivalent changes and modifications made to the above embodiments in accordance with the technical spirit of the present invention belong to the present invention. Within the scope of the inventive solution.

CULTIVATION OF "ASHITABA" **JPS5729218**

The present invention relates to a cultivation method of Escherichia Columbus, which is characterized by sowing seeds between trees having root roots and collecting a large amount of vitamin B group and vitamin E-containing algae by the action of root nodules . The Ashitaba of this invention naturally breeds to the southern part of the Izu Peninsula, Oshima, Hase Island etc. and it is said that it is effective for health when we eat the leaves and stems, and the people of the land used for edible use, It is said that cultivation is very difficult, cultivation has never been tried on the scale of management. Also, the tree having a root knob used in the present invention, such as alder, is known as a land improvement tree for cultivation of nodules, sandy lands, nonfabricated lands and the like as being useful for maintaining and strengthening the ground power, but regarding the formation and composition of the root gang Has not been fully elucidated yet.

According to the present invention, various studies are made on the relation between trees having rootsroot roots and Ashitaba according to the above-mentioned ginseng. When seeded between trees having root roots, when this is grown, the vitamin B group and Vitamin E It was possible to collect rich albatross soil by cultivation, and led to the development of this invention. Regarding the enhancement of the ground force of the root knot, it is thought that it is due to the fixation of nitrogen in the air, but the vitamin 11tt in the root knot has not been investigated at all.

However, the vitamin B group and Pitami / E are contained in a large amount in such stems and leaves of sown or propagated among the roots of roots and have excellent use as raw materials for nutritious processed foods This is EndPage: 1. The analytical test results of leaf stalks of Ashitaba cultivated according to the present invention and leaf stalks of naturally propagated leaf stalk are shown in the following table. Cultivation according to the invention Naturally propagating ones Bitami, yB, 0.32 MISO, 07 "9% vitamin B, 0.9710.10 # fi mini yBs 0.55% Skin 0.054 pts% Vitamin B ,, 0.32 # 0.00 # Chlorophyll 2860.3 # Calcium 1790% 40.1 # Xanthophyll 31.8 "F 9 G

2.07 u - Iron 28.411.961 Magnesium 251 # " ' = 18.84 # Inositol 344 # 37.4 # carotene 8.7 # 0.946 # niamine 4.75 # 0.521 biotin 14.8 pf% 1.7 ipfT. Sodium 66.6% Capillary% 82.111 q Chi pantothenic acid 1.78 # 0.3 # Choline 7015 Folic acid 45 µf th 10.6 µyes phosphorus 14611 f% 70.63 (- 111 potassium 1530 # 783.94 # As such, compared with natural breeding cultivated ones according to the present invention, Vitami 7 B, 4.57 times, B, 9.7 times, B, 10.17 times, B,!

Shows that it contains 032 for natural breeding 0 and vitamin 8 increases 16.8 times. And vitamin B group has excellent effect on fatigue recovery and health promotion, and according to a recent study Vitamin E causes an aging phenomenon as an antioxidant vitamin which suppresses the oxidation of unsaturated fatty acid in the body It has been announced that it is possible to prevent the formation of coalescence of fatty acid overgrowth and protein and to prevent the pigment from depositing on cardiac tissue, nerve, adrenal gland and other cells, and have a remarkable effect on prevention of aging O Next, an embodiment of the present invention will be described. A ridge of about 10 cm in height is formed between alderous trees planted at an interval of about 5 meters, and at the interval of about 30 cm at the end of 2 Fi I sow seeds of Ashesha Ba. About 10 days after sowing the seeds started to germinate. Although Ashitaba is a perennial, - years are growing to about 50 centimeters, the second year is about 100 centimeters, and the priestess grows to about 150 centimeters, but after that it is naturally revealed. Then, when the lower leaf of Ashitaba comes into contact with the ground, we reap this and improve the ventilation. Also from the sun from the east, if you try to hit half a day Xiyang will grow well growing well around the end of August flowers will bloom and you can harvest seed until mid-ninth. As required, the present invention succeeds in cultivation of Ashitaba cultivated conventionally as impossible to cultivate as described above, and by using a tree having a root root, the vitamin B group and vitamin l are abundantly added It is one that succeeds in collecting a new kind of high-nutritious product, Ashiba, contained in it, and the technical and economic effects that made it possible to produce a large quantity are quite large.

CULTIVATION OF ASHITABA JPS59159714

Ashitaba is a part of the Izu Peninsula that is wild in the Izu Peninsula, especially on the Izu Islands, it is used as an ancient meal, and it is also used as an agriculture pasture to promote lactation of milk cows and improving milk quality . In recent years, flavor and richness like Mitsuba and celery combined and vitamins D1, B2, B6, B1. , Vitamin E 1 Lutcolin e glucos'ide, Isoquercitrin. Due to the abundance of nutritional components such as Psoralen, Angelicin, Xanthotoxin germanium, etc., demand for Ashitaba is increasing. Therefore, establishment of an effective cultivation method of Ashitaba at the scale of management was keenly desired. The present invention aims at establishing cultivation method of cultivation of Ashitaba, stabilizing cultivation, enabling production according to market demand, and cultivation method of Ashitaba with abundant component content from nutritional and livestock breeding . The present inventors established a technique relating to the present invention as a result of years of research on cultivation techniques and plant physiology. That is, when cultivating Ashitaba, it is necessary to sow and cultivate Ashitaba during the planting of this tree every 5 to 6 m after examination of the forest of trees belonging to the subgenus Yoshapushi belonging to the birch genus. It is the feature of the above. In the present invention, trees belonging to the subgenus Yashagushi are effectively used as "1st", such as Yashagushi, Okayama Yabushi and Kiln Oysterbush. The purpose and results of blending trees belonging to subgenus Yershushi and Ashitaba are summarized in the following items. In other words, it is possible to cultivate full-year Ashitaba by avoiding direct sunlight by moderately concealing the cultivation surface of Shishitaba by branches and leaves of trees. Due to avoiding direct sunlight, in Ashitaba in July and August of midsummer it is possible to obtain the cultivation yields of habitual. Because it avoided direct sunlight, transpiration of moisture from the cultivation area is suppressed and it is possible to cultivate Ashitaba also in the Shimane gorge

where the rainfall is not enough. Tree belonging to subgenus Yershushi is a root nodule plant that secretes compound nitrogen from the ovine nodule or the root system into the ground. As a result of secretion of compound nitrogen, the nitrogen compound is absorbed by the roots of Ashitaba, resulting in favorable results for growth. The trees belonging to the subgenus Yashapushi showed a 2 to 3 times increase in the production of carbon dioxide compared to other non-nodular plants, so that the carbonation anabolism of Ashitaba was made active and the water retention of the soil accordingly. As a result, to promote the growth of Ashitaba.

The trees belonging to subgenus Yoshabushi are deciduous leaves with a high amount of fallen leaves and high nitrogen content, decomposed on the cultivation floor and reduced to the soil, increase the humus, increase the physical, chemical and microbiological conditions of the soil. As a result of improvement, improve the cultivation of Ashitaba.

Ashitaba is cultivated surrounded by trees belonging to subgenus Yashapushi, this tree has the effect of protecting the growth of Ashitaba as a windbreak forest, especially during the season when wind is strong, the growth of Ashitaba in the Shimane region. To be promoted.

The root system and root collapse of trees belonging to subgenus Yashagushi has a large nitrogen content compared to other plants, and as a result they are reduced into soil and promote the growth of Ashitaba. Ashitaba cultivated with mixed trees belonging to subgenus Yushabushi by a total effect of 0 or more has increased crop yield per unit area and increased nutritional and domestic feeding active ingredients, namely vitamin B 1, B 2, B 6, Luteolin-7-glucoside, I', B12, V, E, chlorophyll, xanthophyll, folic acid, choline, pantothenic acid, biotin, niacin, carotene, inositol, calcium, iron, magnesium, potassium, so-quercitrin, Psoralen, Angelicin, Bergapten. Increased contents of Xanthotoxin, Angelic acid, Behenic acid, germanium compounds, energy, proteins, lipids, carbohydrates, etc. There are two lines of green grasses and spring grasses in Ashitaba, but the present invention can be effectively applied to any of these. As a method of raising seedlings of Ashitaba, a method of seeding in a field that is well-grounded between the forests of the subgenus Yashapushi and growing it for 4 to 5 years as it is or a method of nursing the original size and planting the plant after leaves come out. Any of these methods can be effectively applied to the present invention. For fertilization to Ashitaba, organic matter such as poultry feces and slow release chemical fertilizer etc are effective. As cultivation control of Ashitaba, once seeded, it has a tropism of 3 to 4 years. The growing stocks will bloom in the second year and become major stocks, so there is a way to change this and repeat the sprout from the root again and prolong the harvest. For harvesting, shoots and soft things are cut by hand from the root, and edible. Shipment is done by aligning well with the leaf tip, cutting off the stem, weighing and packaging it. It can also be used as a green leaf or a diver, or it can be used as a powder by drying and crushing.

Next, the present invention will be described by examples.

EXAMPLES AND COMPARATIVE EXAMPLES

Three fields are used, each of which is hereinafter referred to as 1.2. 3, field. As the first field used palm oil shavings and forests of *Pleurotus albopictus*, we used a slope south facing southerly with 3 to 5 'm intervals irregularly planted with Pear and Oysters. The remaining weeds and the other mushrooms were plowed and then partly plowed, and there were transplanted the seedlings of Ashitaba (5 to 6 pieces of trees) and planted them in the B 0 sub interval. When the strain grew with growth and the lower leaves got densely, the lower leaves were discarded and the ventilation was kept well. No fertilizer was used and pesticides were not used at all. Q The 2nd field was a field used as a taro field, mixed with a freshwater fish and a cabbage shark, and used a field planted as a windbreak forest around the larvae did. The size of the field was 6 tn × 22 tn, and there were windproof forests in the circumference. Planting and cultivation of seedlings of Ashitaba conformed to the conditions of the field - field. In the 3 rd field, we used a large well-grounded

field. The size of the field was 18 m × 43 m. Trees were totally around the surroundings. Characteristic values on cultivation in each field are shown in the following Table 1. Table 1 * (1) Exposure coefficients of the ground surface were calculated as follows. That is, the solar illuminance (Lux) of the ground surface in each field was measured. Then, the solar illuminance of the third field without shading was taken as 100, and the converted value was taken as the exposure coefficient. * (2) Wind speed coefficient of the ground surface was calculated as follows. That is, the wind speed (Th / sec) of the ground surface in each field was measured with a Robinson anemometer. Next, the wind speed of the third field without shading was taken as 100, and the converted value was taken as the wind speed coefficient. Next, the results of cultivation of Ashitaba in the first to third field are as shown in Table 2. Table 2 * (3) Yield factor of the field was calculated as follows. In other words, the yield of adults of each plot was measured in increments of 9 for 10 ares. Then, the yield factor of the third field was converted to 100, and the result was taken as the yield coefficient. As shown by the above results, it was observed that the yield of the Erythrabania increased by the method of the present invention. The third field is an example of comparison.

Preparation process of seaweed ashitaba tea CN107279409

The invention discloses a preparation process of seaweed ashitaba tea, belongs to the technical field of tea preparation and mainly solves the technical problem that seaweed is fishy, ashitaba tea is low in chalcone dissolution and nutritional healthcare value is low in the prior art. The preparation process includes steps: selecting and picking; freezing and stem opening; soaking in ice to keep green; dehydrating to fix humidity; spraying enzyme for chain opening; high-temperature deactivation; twisting and shaping; drying and setting; parching to enhance aroma; cooling and aging; blending tea with seaweed; baking for the second time to improve aroma; cooling and large packing; aging and uniformizing aroma; inspecting and calibrating; sub-packaging. The preparation process can modify aroma of ashitaba tea and bring synergistic healthcare effect of the ashitaba tea and the seaweed and has the advantages that the preparation process is high in mechanical level, conducive to large-scale and standard production and especially capable of effectively improving dissolution of chalcone and seaweed polysaccharide which are healthcare ingredients during brewing and drinking of the ashitaba tea; the seaweed ashitaba tea prepared by the process has no stink odor that the seaweed has and is conducive to fully utilizing medicinal active ingredients and improving healthcare effect.

Ashitaba cleaning fluid and preparation method thereof CN106562906

Process for ashitaba liquid and packaging container thereof TW200631507

Preparation process of ashitaba fermented tea CN104171178

Convenient ashitaba food and processing method thereof CN103652722

Ashitaba drink and preparation method thereof CN102715604

PRODUCTION OF PASTE OF ASHITABA (GREEN VEGETABLE)
JPH06319481

PREPARATION OF ASHITABA TEA

JP2896975 / JPH099930 / JP2896974 / JPH01262781 / JP2641895 / JP2006280338 /
CN103564113 / CN103719490 / CN107279408 / CN107279411

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