

TITLE:

Aloe leaf products and their preparation

DESCRIPTION:

The invention relates to medicinal and health food products, including tablets prepared by vacuum freeze-drying of aloe leaves and to methods for the preparation of such products.

Aloe has been extensively used as folk medicine. Reference to aloe powders is also made in the Japanese Pharmacopeia. Certain low-, medium- and high molecular weight compounds have been separated from aloe. For example, it is known that *Aloe arborescens* var. *natalensis* (referred to hereinafter as "Kidachi aloe") contains polysaccharides such as aloetin, alocenin, aloeursin or D-mannose, aloemannan or aloe albonacite; that aloe vera contains polysaccharides; and that Cape aloe contains anthraquinonic ingredients such as aloin or aloe-emodin.

Intensive attempts have been made to process the aloe into medicines. Presently, as an officially allowed health food, food products prepared from Kidachi aloe have been extensively commercialized in Japan. Most of these products, however, are prepared by drying aloe leaves under the sun or by hot air and, at the time of tableting, up to about 10% of an additive such as Avicel micro crystalline cellulose or corn starch, is added as a binder to facilitate tableting. "Avicel" is a Trade Mark.

It is noted that most ingredients isolated and purified from the aloe and found to be medically efficacious to animals by animal experiments, are polysaccharides, glycoproteins or enzymes, all of which may exhibit activity in a stable state only when treated for

purification at lower temperatures. For this reason, it is generally thought to be desirable to apply as little heat as possible for the preparation of a health food or medicine from aloe.

However, since the products now available on the market are prepared by drying aloe leaves under the sun or by circulation of hot air, the polysaccharides, glycoproteins and enzymes in them will have been thermally degraded or oxidized in the course of drying.

Furthermore, the additives for facilitating tableting, such as Avicel or corn starch, are added in an amount of several to tens of percents in the conventional aloe tablet products. Since a small amount of water is added under heating to knead the additives with the dried aloe mass, the resulting product undergoes further thermal degradation by such heating.

The invention provides:

- i) A vacuum freeze-dried mass of homogenised entire leaves of a plant belonging to the genus Aloe of the family Liliaceae.
- ii) A method for producing a vacuum freeze-dried mass of the leaves of a plant belonging to the genus Aloe of the family Liliaceae,, the method comprising homogenising entire leaves of the plant and then vacuum freeze-drying the homogenate.
- iii) A tablet formed from a vacuum freeze-dried mass of homogenised entire leaves of a plant belonging to the genus Aloe of the family Liliaceae.
- iv) A method for producing a tablet of a vacuum freeze-dried mass of the leaves of a plant belonging to the genus Aloe of the family Liliaceae, the method comprising:
 - (a) homogenising entire leaves of the plant,
 - (b) vacuum freeze-drying the homogenate,

- (c) granulating the vacuum freeze-dried mass, and
- (d) compacting the resultant granules into tablets.

The vacuum freeze-dried mass is suitably in the form of granules. The homogenisation may be carried out under cooling. The tablet of the invention may consist solely of the vacuum freeze-dried mass or may include a further nutrient and/or medicinal ingredient, which may have been incorporated in any of steps (b) to (d) of the production method of the tablet.

The dried mass, granule or the tablet according to the invention exhibits the following properties:

- i) it presents a pale green colour;
- ii) it has a grassy smell;
- iii) it has a strongly bitter taste;
- iv) it is soluble in water, while being partially insoluble in methanol, ethanol or acetone;
- v) in protein determination reactions, it is positive both in bicinchoninic acid (BCA) reaction and in Lowry-Folin reaction;
- vi) as to saccharide determination reactions, it is positive in the anthrone sulphuric acid reaction and in the phenol sulphuric acid reaction;
- vii) as to proteolytic enzyme activities, it has activities of carboxypeptidase and trypsin, and it has also protease-inhibitory activity;
- viii) it exhibits positive cell agglutination activity with erythrocytes and weakly positive cell agglutination activity with cancer cells; and
- ix) it exhibits weakly positive blastogenetic activity with lymphocytes.

Each of the dried mass, the granule and the tablet of the plant belonging to the genus Aloe of the family Liliaceae according to the invention contains the medicinal ingredients endogenously contained in the genus Aloe of the family Liliaceae, such as polysaccharides,

glycoproteins and enzymes, in a state substantially free from degradation caused by heating or oxidation. A variety of physiologically active substances, found to be contained in the plant by animal experiments, such as aloin or aloe carboxypeptidase, are also present without undergoing thermal degradation. Hence, the dried mass, granule or tablet according to the invention may be used as medicines, such as laxative, stomachics, anti-gastrointestinal ulcer drug, anti-inflammatory drug, anti-fungal drug, anti-hyperglycemia drug, anti-burn edema drug, or immunomodulator, or as a variety of health foods.

The tablet of the invention may consist of 100% of the abovementioned dried mass, no additive for tableting being necessary, so that a high quality tablet is provided which contains the medicinal ingredients of the plant belonging to the genus Aloe of the family Liliaceae with a high purity.

The tablet of the invention is easy to swallow, even though it has a strong bitter taste and a flavour displayed by the plant when chewed.

In the method for producing the dried mass of the invention, the liquid obtained by crushing the leaves of the plant belonging to the genus Aloe of the family Liliaceae is freeze-dried in vacuum, without resorting to the step of drying the liquid by heating at a temperature higher than the ambient temperature, therefore, the dried mass containing the abovementioned medicinal ingredients in the plant is prepared without degrading those ingredients.

According to the method for preparing the granules of the invention, the dried mass is press moulded into granules, and the granules contain the medicinal ingredients without thermal degradation.

In the method for producing the tablet of the invention, the dried mass is press moulded into granules, and then tableted. Thus, the tablet contains the medicinal ingredients without thermal degradation. Since no additive for facilitating tableting is required, tablets containing the medicinal ingredients in higher percentages are produced. Thus the tablet may contain up to 100 percent of effective ingredients in the dried mass.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Dried Mass

The vacuum freeze-dried mass of a plant belonging to the genus Aloe of the family Liliaceae, such as Kidachi aloe, Aloe vela or Cape aloe, is obtained upon freeze-drying a liquid obtained by crushing the leaves of the plant. The dried mass obtained in this manner is usually in the flocculated state and is easily pulverized, for example, by charging the mass into a bag. The specific gravity of the dried mass is usually from 0.085 to 0.090.

Method for Preparing the Dried Mass

The leaves of the plant are crushed in a homogenizer. Since heat is generated during homogenization, the outer side of the homogenizer is cooled by ice water.

The entire time for freeze-drying the crushed liquid in vacuum is about 30 hours. The vacuum degree, expressed in terms of the pressure of the remaining gas, is less than 0.4 mbar, not more than 0.2 mbar, and not more than 0.1 mbar during the initial ten hours, during intermediate ten hours, and during the final ten hours respectively. The temperature of the crushed and freezed liquid during drying is not higher than minus 20°C, and preferably not higher than minus 40°C during the abovementioned initial and intermediate periods, and gradually raised from minus 40°C to ambient temperature, e.g., plus 25°C, during the period of ten hours from the start until the end of the final drying period. This enables the moisture to be evaporated off to provide a completely dried mass. According to the method of the invention, the temperature at the end of the final drying period is ambient temperature, e.g. from plus 20°C to plus 25°C.

Granule

The particle size of the granule for producing the tablet is preferably in the range from 18 to 20 mesh JIS, and the specific gravity of the granule is preferably in the range from 0.65 to 0.75.

Method for Producing the Granules

The granules are produced by press moulding (or compacting) the dried mass under a pressure of, for example, 150 kgf/cm². Granules having a specific gravity of 0.74 are produced by press moulding under the above pressure. Granulation may be carried out by using a commercially available granulator.

Tablet

The tablet may be in the form of, for example, a cylindrical column with a length of 5 mm and a diameter of 5 mm. Preferably, the tablet weights 130 to 150

mg, and has a specific gravity of 1.3 to 1.5.

The tablet may contain nutrient or medicinal ingredients not originally contained or contained only in a limited quantity in the genus Aloe of the family Liliaceae. For example, the tablet may contain garlic powders or vitamin C.

Method for Producing the Tablets

The tablets may be produced by tableting the granules alone which contain endogenous nutrient and/or medicinal ingredients in the plant of the genus Aloe of family Liliaceae, or a mixture of the granules and the powders or granules of the other nutrient or medicinal ingredients. The tableting may be carried out, for example, at a pressure of 600 kgf/cm², resulting in a specific gravity of about 1.3 when the granules alone are tableted.

EXAMPLE

About 11 kg of fully grown leaves of Kidachi aloe, cultivated for about three to four years, with each leaf weighing about 100 to 120 g, were collected. The leaves were washed thoroughly with a scrubbing brush. Non-leaf parts (thorns and white hard fibres at the leaf roots) were removed by a cutter, and the leaves were washed with distilled water.

The leaves weighed about 9 kg at this time, corresponding to a volume of 9 litres. The leaves were cut into small pieces and charged into a homogenizer to produce a crushed aloe liquid, referred to hereinafter as aloe juice.

Since the capacity of a vacuum freeze-drying tray for KYOWA vacuum freeze-drier RLE 203 is 3 litres, the homogenate weighing 9 kg is divided into three fractions.

The three aliquots of homogenates are frozen at minus 30° to minus 40°C and charged into the freeze-drier. The 9 litres of the aloe juice were dried in about 30 hours by the KYOWA vacuum freeze-drier RLE 203 to produce 360 g of dried powders.

These powders are charged into a dry granulator, such as a roll compactor, for granulation. The volume of the powders at this stage is reduced to about one tenth.

If the granules are not of uniform size, the tableted product tends to fluctuate in quality. In order to prevent this, granulation by the roll compactor may be repeated two or three times.

The granules are tableted immediately on a tablet machine. If tableting is to be achieved without additives, each tablet preferably weighs about 130 to 150 mg.

In this manner, about 350 g of the vacuum freeze-dried powders are produced from the batch of 9 kg of the aloe juice, so that about 2,600 tablets are produced.

The tablets thus prepared from the vacuum freeze-dried aloe powders are in the form of a cylindrical column with a length of 5 mm and a diameter of 5 mm.

The tablets have a fresh pale green colour and a bitter taste similar to that of the fresh aloe leaf. On the other hand, the conventional aloe tablets, produced by drying at a higher temperature, are excessively oxidized, and are of a pale yellow to brownish colour and have a bitter taste somewhat different from the taste of the fresh aloe. This is due to increased oxidized derivatives of aloin, calcium

succinate salt, which is yellowish and is mainly composed of barbaloin, i.e. anthrone C glycoside.

Comparative Test Between Inventive and Conventional Products

The product of the invention and the conventional product (control) were compared with respect to test items (a) to (h), as shown in Table 1.

The tablets produced in the preceding Example were employed as the product of the invention. Commercially available, conventional aloe tablets (prepared by drying aloe leaves under hot air circulation and tableting the resultant dried mass admixed with an additive for facilitating tableting, such as Avicel or corn starch, and a humidifier, such as water or alcohol) were used as the control.

Table 1

Test items	Inventive	Control
(a) Color	Pale green colour	Yellowish green colour
(b) Smell	Strong grassy smell	Grassy smell
(c) Taste	Strong bitter taste similar to that of green raw leaf	Bitter taste
(d) Solubility	Soluble in water, partially insoluble in organic solvent	Same as inventive product
(e) Protein determination reaction		
1) BCA reaction	15.7 mg/ml*	4.1 mg/ml*
2) Lowry-Folin reaction	20.2 mg/ml*	6.5 mg/ml*
(f) Saccharide determination reaction		
1) Anthrone-Sulphuric Acid reaction	13.6 mg/ml*	8.0 mg/ml*
2) Phenol-Sulphuric Acid reaction	48.0 mg/ml*	30.5 mg/ml*
(g) Proteolytic enzyme		
1) Carboxypeptidase	5.4 U/ml*	0.69 U/ml*
2) Trypsin	18.1 µg/ml*	0.5 µg/ml*
3) Protease inhibitory (pepsin inhibitory) activity	42 %	5 %
(h) Erythrocyte agglutination activity, human O-type	16 titer	2 titer

* 1.0 g of tablet was dissolved into 10.0 ml of distilled water and was measured by each reaction, ml represents 1.0 ml of this solution.

The methods for the tests on the items (e) to (h), the results and their significances are summarized as follows:

(e) Protein Determination Reaction

The tablet of the invention and the control tablet are crushed into powders. 1.0 g each of the tablets was dissolved in 10 ml of distilled water, stirred and centrifuged at 12,000 rpm for 30 minutes at 4°C. The total protein in the produced supernatant was determined by the bicinchoninic acid method (BCA method), using a protein assay reagent kit manufactured by PIERCEW, U.S.A. as described by Smith, P.K. et al (Measurement of Protein Using Bicinchoninic Acid. Anal. Biochem. (1985) 150, 76-85), and also by the Lowry-Folin method, as described by Lowry et al. (Protein Measurement with the Folin Phenol Reagent, J. Biol. Chem. (1951) 193, 265-275).

The results of the analyses have revealed that the tablet of the invention contained 15.7 mg of the protein per each 1 ml of a liquid extract, as measured with the BCA method, whereas the protein content of the control product was 4.1 mg.

The protein content of the tablet of the invention, as measured by the Lowry-Folin method, was 20.2 mg, whereas that of the control product was 6.5 mg.

The results show that the tablet of the invention contains natural proteins more abundantly and that the amount of the aloe material per unit weight is higher in the tablet of the invention because of the absence of additives.

(f) Saccharide Determination Reaction

The tablet of the invention and the control tablet are crushed into powders. 1.0 g of each of the

powdered tablets was dissolved in 10 ml of distilled water, stirred and centrifuged at 12,000 rpm for 30 minutes at 4°C. Saccharide determination tests were conducted on the produced supernatants by the anthrone-sulphuric acid reaction with respect to the monosaccharide, as described by R. Drywood (Ind. Eng. Chem., Anal. Ed., 18, 499 (1946)), and also by the phenol-sulphuric acid reaction for oligosaccharides, polysaccharides or glycoprotein, as described by M. Dudols et al. (Anal. Chem., 28, 350 (1956)).

A large amount of hexose, such as glucose, is contained in both products, as indicated by strong anthrone-sulphuric acid reaction with green to blue green colour. The hexose concentration, calculated as glucose, was 13.6 mg/ml and 8.0 mg/ml for the product of the invention and the control, respectively.

Both products present a yellow-brown colour in the phenol-sulphuric acid reaction. The concentration, measured as glucose, was 48.0 mg/ml and 30.5 mg/ml for the product of the invention and the control, respectively.

It is shown that the saccharides originating from the glycoprotein and polysaccharides remain in the tablet of the invention.

(g) Proteolytic Enzyme Activity

Three proteolytic enzymes have been isolated from Kidachi aloe by the present inventors. Since these enzymes are the heat-labile proteins or glycoproteins, it is crucial from the viewpoint of the product quality whether these enzymes remain in fact in the inventive product.

The tablet of the invention and the control tablet

were crushed into powders. 1 g each of the powders was dissolved in 10 ml of distilled water, stirred and centrifuged at 12,000 rpm for 30 minutes at 4°C. The supernatants were subjected to the following tests.

1) Test for Carboxypeptidase Activity

Bradykinin or Z-Phe-Tyr, Z-Gly-Pro-Leu-Gly was incubated with 50 μ l of the supernatants of the inventive and control products at 37°C for one hour at pH 5.0, and the products, amino acids, were subjected to a ninhydrin reaction to measure the enzyme activity.

The reaction with Z-Gly-Pro-Leu-Gly as substrate has revealed that the product of the invention and the control exhibit a carboxypeptidase activity of 5.4 units and 0.69 unit per ml of the supernatant, respectively.

The product of the invention thus exhibits the enzyme activity of carboxypeptides even after tableting. Carboxypeptidase enzyme exhibits anti-inflammatory effects and anti-edematous effects on burns and is capable of effectively decomposing bradykinin, the chemical mediator of inflammation.

2) Trypsin Activity

A proteolytic enzyme similar to trypsin has been separated by the present inventors from Kidachi aloe.

N-Benzoyl-arginine p-nitroanilide or acetyl-arginine p-nitroanilide was incubated with 200 μ l of the product of the invention and the control at 37°C at pH 8.0 for three hours.

The results of the measurement of isolated p-nitroaniline have revealed that the inventive and control products contained p-nitroaniline in amounts of 18.1

µg/ml and 0.5 µg/ml, respectively, thus indicate that trypsin remains in the product of the invention without loss of its activity.

Trypsin exhibits similar reactivity as enterokinase, thrombin, plasmin or kallikrein. It is known that trypsin separated from actinomyces exhibits anti-inflammatory effects when administered orally.

3) Protease Inhibitor

200 µl of the supernatants of the inventive and control products, or distilled water, were added to 1 ml of casein as a substrate, respectively, and the reaction was continued under pH 2.0 at 37°C for three minutes. Then 0.1 ml of the pepsin solution was added to the reaction system and the reaction was further carried out for 30 minutes.

For termination of the reaction, 2 ml of 1.7M perchloric acid solution was added, and the resulting mixture was centrifuged. Absorbance of the supernatants was measured at a wavelength of 280 nm.

With the absorbance of the reaction system, added to by the distilled water, being equal to 100 percent, the absorbance of the inventive product was 142 percent, while that of the control product was 105 percent. The inventive and the control products inhibited the reaction of pepsin with casein as substrate by 42 percent and 5 percent, respectively. Thus, the pepsin inhibitor activity was found to persist in the inventive product.

The presence of the pepsin inhibitor in the inventive product indicates that pepsin in gastric ulcer may be inhibited by oral administration of tablets according to the invention.

(h) Erythrocyte Agglutination

The tablet of the invention and the control tablet were crushed into powders. 1.0 g each of the powders were dissolved in 10 ml of distilled water. After stirring, each of the resulting solutions was centrifuged at 4°C for 30 minutes at 12,000 rpm. The erythrocyte agglutination activity of each of the produced supernatants was examined in accordance with the microtiter method. A buffer solution obtained by mixing phosphoric acid with physiological saline to pH 7.4 was added to 100 µl each of the supernatants for two-stage dilution until 128-fold dilution was reached. To each diluted solution, 100 µl of the 1% human O-type erythrocytes were added, and reaction was carried out at 37°C for one hour to observe the progress of agglutination.

It was found that the agglutination titer of the product of the invention was 16 titer, while that of the control product was 2 titer. The agglutination titer of Concanavalin A, used as control, was 32 titer, at a concentration of 1 mg/ml.

Therefore, the substance termed lectin which produces erythrocyte agglutination as Concanavalin A is contained at a higher concentration in the tablet of the invention.

Lectin has been demonstrated to specifically agglutinate tumour cells and to exhibit cytotoxicity, or as to modulate immune-related cells, such as promotion of blastogenesis or differentiation of lymphocytes.

These results prove that the product of the invention contains a larger quantity of lectin than the control product.



CLAIMS

1. A vacuum freeze-dried mass of homogenised entire leaves of a plant belonging to the genus Aloe of the family Liliaceae.
2. A vacuum freeze-dried mass according to claim 1, which is in the form of granules.
3. A method for producing a vacuum freeze-dried mass of the leaves of a plant belonging to the genus Aloe of the family Liliaceae, the method comprising homogenising entire leaves of the plant and then vacuum freeze-drying the homogenate.
4. A method according to claim 3 wherein the homogenisation is carried out under cooling.
5. A method according to claim 3 or claim 4 further comprising granulating the freeze-dried mass by moulding it under pressure.
6. A tablet formed from a vacuum freeze-dried mass of homogenised entire leaves of a plant belonging to the genus Aloe of the family Liliaceae.
7. A tablet according to claim 6 consisting solely of the vacuum freeze-dried mass.
8. A tablet according to claim 6 further comprising a nutrient and/or medicinal ingredient.
9. A method for producing a tablet of a vacuum freeze-dried mass of the leaves of a plant belonging to the genus Aloe of the family Liliaceae, the method comprising:
 - (a) homogenising entire leaves of the plant,
 - (b) vacuum freeze-drying the homogenate,



- (c) granulating the vacuum freeze-dried mass,
and
- (d) compacting the resultant granules into
tablets.

10. A method according to claim 9 in which a nutrient and/or medicinal ingredient is incorporated in any one of the steps (b) to (d).

11. A method according to claim 9 or claim 10 wherein the homogenisation is carried out under cooling.
