Title: COMPOSITION COMPRISES BAMBOO EXTRACT FOR ANDROGEN AGONIST

Abstract: The present invention relates to a use of bamboo or bamboo extract to prevent or treat symptoms related to decrease of androgen; a composition for androgen agonist comprising bamboo or bamboo extract; a method for preventing or treating symptoms related to decrease of androgen by administering composition for androgen agonist; and a method of preparing composition for androgen agonist. The present composition obtained from natural material can be used as Phyto-androgen for the preventing and treating symptoms of male climacteric without dangerousness according to hormone replacement therapy.
COMPOSITION COMPRISING BAMBOO OR BAMBOO EXTRACT FOR
ANDROGEN AGONIST

TECHNICAL FIELD

The present invention relates to a use of bamboo or bamboo extract to prevent or treat symptoms related to decrease of androgen; a composition for androgen agonist comprising bamboo or bamboo extract; a method for preventing or treating symptoms related to decrease of androgen by administering a therapeutically effective amount of bamboo or bamboo extract to mammal; and a method of preparing a composition for androgen agonist by extracting bamboo with polar solvent or non-polar solvent.

BACKGROUND ART

Androgen known as male hormone is one of steroid hormones, and is known to conduct the physiological function and physiological control function through medium of androgen receptor (AR) which is distributed over various tissues such as reproductive system of seminal glands, testis, etc., central nervous system, cardiovascular system, immune system, digestive system, kidney, lung, etc. [Heinlein CA and Chang C, Endocrine Reviews, 2002, 23(2), 175-200]. In terms of function, androgen is produced in seminal glands, arrives at aimed cells through blood vessel, enters the aimed cells by simple diffusion, and affects the transcription activity of the aimed gene through androgen
receptor which is the transcription factor in the nucleus [Heinlein CA and Chang C, Endocrine Reviews, 2004, 25(2), 276-308]. It is known that androgen receptor is one of steroid hormone receptors such as glucocorticoid receptor, progesterone receptor, estrogen receptor, and mineralcorticoid receptor, and testosterone, cortisol, progesterone, estradiol, aldosterone, etc., and works as counter ligand thereof [Beato M and Klug J, Human Reproduction Update 2000, 6, 225-236]. In the male as well as the female, the climacteric occurs from before and after 50, and the frequency of occurrence is increased in years. After 60, in 30% of the male, the climacteric occurs (Schneider HPG, Annals of New York Academy Science, 2003, 997, 292-306). However, male climacteric symptoms are exhibited very slowly, and thus many men may not feel any. Also, many men feel climacteric symptoms, but think them due to stress or natural change by aging. One cause of male climacteric is decrease of male hormone due to decrepitude of brain and testis in addition to other causes (Schneider HPG, Annals of New York Academy Science, 2003, 997, 292-306). Main symptoms of male climacteric are fatigue, decline of memory, melancholia, decline of muscular strength, increase of body fat, and weakening of bone. Also, the sexual dysfunction, impotence, and decline of sexual desire, etc. are usually accompanied. Moreover, it is known that lack of androgen results in many symptoms such as decline of sexual desire, impotence, decline of muscle, decline of physical strength, increase of body fat, change of hair, decline of bone density, etc.

If androgenic drug is administered to patients showing the above symptoms, it is shown to be effective for reducing of body fat, increase of muscle, increase of bone density, increase of hand-grip strength, improvement of mood, reduction of melancholia, increase
of sexual desire, improvement of the quality of life in AIDS patients, etc. (Bhasin S and Bremner W, Journal of Clinical Endocrinology and Metabolism, 1997, 82, 3-8). Besides, it was reported that androgen inhibits the phosphorylation of tau protein which is the cause of dementia, and thus is applicable for preventing dementia (Papazosomenos Sch, Shanavas A, Proceedings of National Acadademy of Science 2002, 99, 1140-1145).

Bamboo is a species of Gramineae, and 280 kinds of bamboo are known worldwide, and 70 kinds of bamboo grow in nature or are cultivated in South Korea. The 11 representative kinds of bamboo consist of *P. nigra var. henonis*; *P. bambusoides*; *P. pubescence*; *P. nigra*; *P. nigra for. punctata*; *S. borealis var. gracilis*; *S. coreana* Nakai; *S. borealis var. chiisanensis*; *S. borealis*; *S. borealis Makino*; and *P. japonica* etc. Among them, the mainly cultivated kinds are *P. bambusoides*; *P. nigra var. henonis* and *P. pubescence*.

Since the ancient, the bamboo’s bark, branch, leaf, sprout, endoderm as *B. Caulis in Taeniis*, etc. are used as a Chinese medicine material. In particular, *Bambusae Caulis in Taeniis* is middle layer of *Phyllostachys nigra var. henonis* or *Phyllostachys bambusoides Sieb. et Zucc.* whose outer bark is removed from, and is known to have a pharmacological effect for vomiting, removal of phlegm, haemostasis, and comforting embryo. Tabasheer extracted by heating bamboo is reported to be effective for treating palsy and hypertension in Donguibogam, Botanical List, and Encyclopedia of Chinese Medicine, and is reported to be effective for the treatment of hypertension, atherosclerosis, cardiovascular disease, etc. or the prevention of cancer and aging. According to Donguibogam, Botanical List, and Encyclopedia of Chinese Medicine, bamboo is effective
for the treatment of palsy and hypertension, and particularly used for thirst in pneumonia, bronchitis, etc. to alleviate fever, discharge phlegm, and refresh. Recently, it is reported that bamboo is effective for the treatment of hypertension, atherosclerosis, and cardiovascular disease, and is introduced to be good for anticancer and prevention of aging. These functions of bamboo are regarded as closely related to the antioxidant effect. Also, phytochemicals like organic acid, dietary fiber, tannin, and benzo furane, existing in bamboo extract are expected to contribute to the prevention of circulatory system disorders through antioxidant function, thorombolysis, lipid reduction function, etc.

At present, the kinds of bamboo naturally growing in South Korea may be divided into *Phyllostachys*, *Sasan* and *Pseudosasa*. In case of *Phyllostachys*, the leaf sheath is fallen early, the number of stamen is 3, the height is 10-30 cm, the diameter is 3-20 cm, the stem is big, and two buds come out from each joint thereof. In the world, there are 40 kinds of *Phyllostachys*, which are mainly in China and India, and some of which are in Japan, Europe, and North Africa. In South Korea, 6 kinds of bamboo habitant are known: *Phyllostachys pubescenc*, *P. nigra*, *P. nigra var. henonis*, *P. nigra for. punctata*, *P. compressa*, and *P. bambusoides*.

1) *Phyllostachys nigra var. henonis* is perennial evergreen shrub and a mutated species of *Phyllostachys nigra*. The subterranean stem of *Phyllostachys nigra var. henonis* grows sideward from joints, and its height reaches up to 10 m. The bamboo sprout comes out in April and May, and edible, and its color is brown.

2) The stem color of *P. nigra* is green in its first year, but becomes black from the
second year to be completely black. The height of P. nigra is 3-20m, the diameter is 2-5cm, and P. nigra grows forthright. The flower of P. nigra blooms in June and July, and is spike and shaped like an oval having the length of 2.5-3cm, and the color of flower is purplish green. With a period of about 60 years, P. nigra blooms, bears fruits, and dies.

3) The joint of P. bambusoides has two rings. P. bambusoides grows up to the height of 20m and the diameter of 5-10cm. The leaves of P. bambusoides are 5 to 8, and the length of leaf is 10-20cm. There is fluff at the joint of leaf and stem. The sprout of P. bambusoides is eaten early summer. B. caulis in Taeniis is a thin shell like a piece of paper existing in the inner stem of of P. bambusoides, and is used for tooth heat and hematemesis.

4) P. pubescence is called as “juksundae” since juksun (bamboo sprout) coming out in May is favorable to eat, or as “maengjongjuk” which is originated from “Maengjong” who devoted to his parents by serving bamboo sprout in snowy winter. P. pubescence appears to have only one ring on its joint. The fluff at a joint of leaf and stem is fallen, little left. P. pubescence is mainly planted in the southern area.

5) P. nigra for. punctata is a kind of P. nigra. The stem height of P. nigra for. punctata is about 10m. The stem color is varied depending on environment, but the stem generally has black spot on the yellow base. The flower of P. nigra for. punctata blooms in June and July and is panicle, and many small flower ears thereof are compactly hung thereto.

6) P. comprossa is characterized in that the first joint of branch is flatly pressed,
and its seed leaf has fine hairs. The flower of *P.comprossa* is panicle, and several small flower ears thereof are hung thereto.

The leaf sheath of *Sasa* has soft or hard, long hairs. The bag of flower ears of *Sasa* is long, the number of stamen is 3 or 6, the height is 0.3-5m, the diameter is 2-15m, and the size is small. 200 kinds of *Sasa* are distributed over East Asia such as Korea, China, Japan, etc., and some examples thereof are *Sasa coreana* Nakai, *S. coreana*, *S. kurilensis*, *S. quelpaertensi*, *S. borealis*, *S. borealis* var. *chiisanensis*, *S. borealis* var. *gracilis*, etc.

1) *Sasa coreana* Nakai is an endemic species of Korea, distributed over Myeongcheon, Hamkyeongyangbuk-do, and grows at the foot of mountain in a group. The height of *Sasa coreana* Nakai is 30-80cm, and the diameter is 3-8mm. The root stock is short, the branch is divided, and the gap of joint is short. The branches mainly come out of at the height of 5-20cm, and the stem and branches are grooved. The 5 to 8 leaves of *Sasa coreana* Nakai hang at the end of branch, each shaped like a long oval or egg shape. The length of leaf is 3-12cm, and the width is 6-22mm. The font side of leaf does not have any fluff, but the back side of leaf has much fluff, serrate leaf, and 5-6 types of leaf venation. Most of the leave sheaths do not have fluff. The leaf of *Sasa coreana* Nakai is similar to, but smaller than, that of *S. kurilensis*, and the branch of *Sasa coreana* Nakai is denser than that of *S. kurilensis*. It is known that the leaf of *Sasa coreana* Nakai is used for hemostatic, expectorant, and diuretic, particularly nephritis, in the oriental medicine.
2) *Sasa borealis* Makino is perennial evergreen shrub, and grows up to the height of 1-2m. The bract surrounds the stem for 2 or 3 years, and has fluff. The leaves come out of at the end of branch by twos to threes, and the shape of leaf is long oval and lanceolate. The length of leaf is 10-25cm, and the leaf is acuminate or long like a tail. The basipetal and sheath of the back side of leaf have fluff. Serrate like a prickle exists at the edge of leaf. The flower blooms in April every five years, and then dies after blooming. The fruit ripens in May and June.

*Pseudosasa* grows at the foot of mountain of the southern area or plain in group, and is raised for ornament. One branch comes out of each joint every two years. A new sprout thereof comes out wrapped in a shell having tough fluff at the end of subterranean stem. The bark is longer than the gap between joints. The length of leaf is about 30cm. The flower blooms from late spring to summer. *Pseudosasa* consists of two kinds: *P. japonica* and *P. japonica* var. *purpurascens*.

1) *Pseudosasa japonica* mainly grows in the central and southern area of Korea. The height is 2-4m, the diameter is 5-15mm, and 5-6 branches come out of the upper middle part. The leaf is lanceolate and has no fluff. The length of leaf is 10-30 cm, and the width is 1-4 cm. The flower is coniform, and 5 to 10 of small petals thereof come out. The bamboo sprout comes out in May.

2) *Pseudosasa japonica* var. *purpurascens* is a mutated species of *P. japonica*, meaning that the leafstalk and leaf is purple colored, and grows in Cheju Island.
DISCLOSURE OF THE INVENTION

The present inventors searched natural products acting as a transcription activating factor through androgen receptor by observing reporter gene expression through ARE (Androgen Receptor Element) using ARE4-Luc reporter plasmid, to develop androgen agents from natural products, distinguishably from the development strategy in the developed countries. They experimented to develop a material similar to androgen whose activity is proven by using natural product library, and then found out that the bamboo extract shows androgen activity, to complete the present invention.

Thus, an object of the present invention is to provide a new composition for androgen agonist comprising bamboo or bamboo extract.

Another object of the present invention is to provide a use of bamboo or bamboo extract to prevent or treat symptoms related to decrease of androgen.

Another object of the present invention is to provide a method of prevention or treatment of symptoms related to decrease of androgen by administering a therapeutically effective amount of bamboo or bamboo extract to mammal.

Another object of the present invention is to provide a method for preparing a composition for androgen agonist by extracting bamboo with polar solvent or non-polar solvent.

BRIEF DESCRIPTION OF THE DRAWINGS
Fig. 1 is a graph showing the androgen activity of bamboo alcoholized extract.

Fig. 2 is a graph showing the androgen activity of *B. caulis* in Taeniis extract and solvent fraction thereof.

Fig. 3 is a graph showing the androgen activity of *S. coreana* Nakai extract and solvent fraction thereof.

Fig. 4 is a graph showing the androgen activity of *P. nigra* var. *henonis* extract and solvent fraction thereof.

Fig. 5 is a graph showing the androgen activity of *P. japonica* extract and solvent fraction thereof.

Fig. 6 is a graph showing the androgen activity of bamboo hydrothermal extract.

**BEST MODE FOR CARRYING OUT THE INVENTION**

According to the above objects, the present invention provides a composition for androgen agonist comprising bamboo or bamboo extract as effective ingredient.

The present invention also provides a use of bamboo or bamboo extract to prevent or treat symptoms related to decrease of androgen.

The present invention also provides a method of prevention or treatment of symptoms related to decrease of androgen by administering a therapeutically effective amount of bamboo or bamboo extract to mammal.

The present invention also provides a method for preparing a composition for androgen agonist by extracting bamboo with polar solvent or non-polar solvent.
In the present composition, it is preferable to select bamboo from the group comprising *Phyllostachys*, *Sasa*, or *Pseudosasa*; the *Phyllostachys* bamboo is preferably selected from *Phyllostachys nigra* var. *henonis*, *P. nigra*, *P. bambusoides*, *P. pubescence*, *P. nigra* for *Punctata*, or *P. compressa*; the *Sasa* bamboo is preferably selected from *Sasa coreana* Nakai, *S. coreana*, *S. kurilensis*, *S. quelpaertensis*, *S. borealis*, *S. borealis* var. *chiisanensis*, or *S. borealis* var. *gracilis*; the *Pseudosasa* bamboo is preferably selected from *Pseudosasa japonica*, or *Pseudosasa japonica* var. *purpurascens*, and bamboo can be used by root, stem, leaf, or herb.

In the present composition, bamboo can be used by herb, branch, shell, leaf, sprout, root, endodermis, etc., preferably used in the form of powder or extract.

The bamboo extract can be used by extracting bamboo with water, organic solvent, or mixing solvent thereof.

All solvents can be used as the above organic solvent, preferably polar solvent such as water, *C*$_{1-4}$ alcohol, etc., or non-polar solvent such as n-hexane, dichloromethane, etc.

The above non-polar solvent extract of bamboo comprises extract extracted with non-polar solvent selected from n-hexane, dichloromethane, chloroform, or ethylacetate, preferably n-hexane, dichloromethane, and ethylacetate.

The above polar solvent extract of bamboo comprises extract extracted with polar solvent selected from acetone, water, or *C*$_{1-4}$ alcohol such as methanol, ethanol, propanol, butanol, etc.

The present bamboo extract also may be water fraction or n-hexane fraction.
obtained by suspending the above C_{1-4} alcohol extract with water and adding n-hexane thereto; dichloromethane fraction obtained by adding dichloromethane to the above water fraction; ethylacetate fraction obtained by adding ethylacetate to water fraction remaining after separation of the above dichloromethane fraction; n-butanol fraction obtained by adding n-butanol to water fraction remaining after separation of the above ethylacetate fraction; or extract obtained by column chromatography of the above extracts and fractions.

A process for extracting bamboo of the present invention is specifically described in a following example.

* B. caulis in Taeniis is sliced to small pieces, and then water, methanol, or ethanol in the amount of 5 or 25 folds of dry weight of the pieces are added thereto, and extracted under reflux condenser to obtain water extract, methanol extract, or ethanol extract of *B. caulis in Taeniis*. To the above methanol or ethanol extract is added distilled water, and the mixture is suspended and fractioned by adding N-hexane thereto to obtain water soluble fraction and n-hexane soluble fraction. Also, to the water fraction remaining after separation of the n-hexane soluble fraction is added dichloromethane to obtain dichloromethane fraction. Again, to the water fraction remaining after separation of the dichloromethane fraction is added ethylacetate to obtain ethylacetate fraction. Also, to the water fraction remaining after separation of the ethylacetate fraction is added n-butanol to obtain n-butanol fraction. Then, the above extracts and fractions are separated by chromatography to obtain purified extract.

The above extraction may be carried out by conventional methods such as hot
water extraction or sonication. Lyophilized product of the extract can be used for the present composition.

The present composition can be used as androgen agonist and phyto-androgen obtained from natural materials. Thus, the present composition can be used for the treatment and prevention of male climacteric, specifically reduction of body fat, increase of muscle, increase of bone density, increase of hand-grip strength, improvement of mood, reduction of melancholia, increase of sexual desire, or prevention or treatment of dementia.

The composition of the present invention can be prepared according to conventional methods in the pharmaceutical field into conventional pharmaceutical preparations, for example, solution such as drinks, syrup and capsule, by mixing with pharmaceutically acceptable carrier, excipient, etc.; and administered orally or parenterally. Preferably, the composition of the present invention may be orally administered in drink before and/or after the meal for quick effect.

Capsule and solution comprising the composition of the present invention may be used as medicine or health care products. Here, "health care products" mean food products prepared and processed in the form of tablet, capsule, powder, granule, solution, pill, etc., by using material or ingredients having useful function to the human body.

The composition of the present invention is appropriately administered according to the extent of absorption of active ingredients into the body; excretion rate; age, weight, sex, and condition of patient; severity of treated disease, etc. However, generally, in solution, it is preferable to administer the present composition 1~3 times a day, 0.01~500 mg/kg, preferably 0.1~200 mg/kg each to adult. In other preparations, an appropriate
amount based on the above dose for solution can be administered orally.

Hereinafter, the present invention will be described in more detail with reference to the following examples, but the scope of the present invention should not be construed to be limited thereby in any manner.

5

Examples

Example 1. Preparation of *B. caulis in Taeniiis* alcoholized extract

1-1) *B. caulis in Taeniiis* used in the experiment was produced in Korea, and purchased in the Kyung-Dong market. The purchased *B. caulis in Taeniiis* was washed by clean water, and air-dried to be used as sample for extraction. To 1 kg of *B. caulis in Taeniiis* made into small fragments after the drying was added 15L of 70% ethanol in the amount of 15 folds of the dry weight of *B. caulis in Taeniiis*, which was continuously repeatedly extracted three times at a constant interval (every 12h) at 80°C, and then filtered under reduced pressure with filter paper (Watman Co., USA). The filtrate was collected and concentrated under reduced pressure by vacuum rotation at 60°C, and thus extracted residue was dried by lyophilizer to obtain 72g of *B. caulis in Taeniiis* crude extract, which was kept in a freezer of −20°C, and used in the experiment.

1-2) Preparation of *B. caulis in Taeniiis* n-hexane soluble fraction

To 50g of crude extract of *B. caulis in Taeniiis* obtained in the above 1-1) was added 1L of distilled water, and the mixture was suspended and mixed by adding
n-hexane 11, and then repeatedly fractioned three times to obtain 2L of water soluble fraction and 2L of n-hexane soluble fraction. And, the n-hexane soluble fraction was filtered, and dried under reduced pressure to obtain 10.2g of *B. caulis in Taeniis* dried powder of n-hexane soluble fraction, which was used as sample.

1-3) Preparation of *B. caulis in Taeniis* dichloromethane soluble fraction

To 2L of water soluble fraction obtained in the above 1-2) was added 1L of dichloromethane and mixed, and then the mixture was repeatedly fractioned three times to obtain 2L of water soluble fraction and 2L of dichloromethane soluble fraction. Then, the dichloromethane soluble fraction was filtered and dried under reduced pressure to obtain 8.1g of *B. caulis in Taeniis* dried powder of dichloromethane soluble fraction, which was used as sample.

1-4) Preparation of *B. caulis in Taeniis* ethylacetate soluble fraction

To 2L of water soluble fraction obtained in the above 1-3) was added 1L of ethylacetate and mixed, and then the mixture was repeatedly fractioned three times to obtain 2L of water soluble fraction and 2L of ethylacetate soluble fraction. Then, the ethylacetate soluble fraction was filtered and dried under reduced pressure to obtain 5.5g of *B. caulis in Taeniis* dried powder of ethylacetate soluble fraction, which was used as sample.

1-5) Preparation of *B. caulis in Taeniis* n-butanol soluble fraction
To 2L of water soluble fraction obtained in the above 1-4) was added 1L of n-butanol and mixed, and then the mixture was repeatedly fractioned three times to obtain 2L of water soluble fraction and 2L of n-butanol soluble fraction. Then, the n-butanol soluble fraction was filtered and dried under reduced pressure to obtain 7.1g of *B. caulis in Taeniis* dried powder of n-butanol soluble fraction and 13.5g of *B. caulis in Taeniis* dried powder of water soluble fraction.

**Example 2. Preparation of *B. caulis in Taeniis* hydrothermal extract**

2-1) *B. caulis in Taeniis* used in the experiment was produced in Korea and purchased in the Kyung-Dong market. The purchased *B. caulis in Taeniis* was washed by clean water, and air-dried to be used as a sample for extraction. To 2 kg of *B. caulis in Taeniis* made into small fragments after the drying was added 30L of purified water in the amount of 15 folds of the dry weight of *B. caulis in Taeniis*, which was continuously repeatedly extracted two times at a constant interval (every 10h) at 80°C, and then filtered under reduced pressure with filter paper (Watman Co., USA). The filtrate was collected and concentrated under reduced pressure by vacuum rotation at 60°C, and thus extracted residue was dried by lyophilizer to obtain 87g of *B. caulis in Taeniis* crude extract, which was kept in a freezer of −20°C, and used in the experiment.

**Example 3. Preparation of *S. coreana* Nakai alcoholized extract**

3-1) *S. coreana* Nakai used in the experiment was purchased in Daebat Goeul,
Seojeong-ri, Gonyang-myeon, Sacheon-si, Gyeongsangnam-do, Korea. The purchased *S. coreana* Nakai was washed by clean water, and air-dried to be used as a sample for extraction. To 2 kg of *S. coreana* Nakai made into small fragments after the drying was added 30L of 70% ethanol in the amount of 15 folds of the dry weight of *S. coreana* Nakai, which was continuously repeatedly extracted three times at a constant interval (every 12h) at 80°C, and then filtered under reduced pressure with filter paper (Watman Co., USA). The filtrate was collected and concentrated under reduced pressure by vacuum rotation at 60°C, and thus extracted residue was dried by lyophilizer to obtain 71.6 g of *S. coreana* Nakai crude extract, which was kept in a freezer of −20°C, and used in the experiment.

3-2) Preparation of *S. coreana* Nakai solvent fraction

Each solvent fraction was prepared by using 50 g of crude extract obtained in the above 3-1) with same methods of the above 1-2) to 1-5) of Example 1, to obtain solvent fraction as following Table 1.

<table>
<thead>
<tr>
<th>Extract name</th>
<th>Extract yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-2) N-hexane soluble fraction of <em>S. coreana</em> Nakai</td>
<td>2.5 g</td>
</tr>
<tr>
<td>3-3) Dichloromethane soluble fraction of <em>S. coreana</em> Nakai</td>
<td>1.2 g</td>
</tr>
<tr>
<td>3-4) Ethylacetate soluble fraction of <em>S. coreana</em> Nakai</td>
<td>1.4 g</td>
</tr>
<tr>
<td>3-5) N-butanol soluble fraction of <em>S. coreana</em> Nakai</td>
<td>2.4 g</td>
</tr>
<tr>
<td>3-6) Water soluble fraction of <em>S. coreana</em> Nakai</td>
<td>42.5 g</td>
</tr>
</tbody>
</table>
Example 4. Preparation of \textit{S. coreana} Nakai hydrothermal extract

4-1) \textit{S. coreana} Nakai used in the experiment was purchased in Daebat Goeul, Seojcong-ri, Gonyang-myeon, Sacheon-si, Gyeongsangnam-do, Korea. The purchased \textit{S. coreana} Nakai was washed by clean water, and air-dried to be used as a sample for extraction. To 2 kg of \textit{S. coreana} Nakai made into small fragments after the drying was added 30L of purified water in the amount of 15 folds of the dry weight of \textit{S. coreana} Nakai, which was continuously repeatedly extracted two times at a constant interval (every 10h) at 80\degree C, and then filtered under reduced pressure with filter paper (Watman Co., USA). The filtrate was collected and concentrated under reduced pressure by vacuum rotation at 60\degree C, and thus extracted residue was dried by lyophilizer to obtain 89.6 g of \textit{S. coreana} Nakai crude extract, which was kept in a freezer of \textendash20\degree C, and used in the experiment.

Example 5. Preparation of \textit{S. borealis} alcoholized extract

5-1) \textit{S. borealis} used in the experiment was purchased in Daebat Goeul, Seojcong-ri, Gonyang-myeon, Sacheon-si, Gyeongsangnam-do, Korea. The purchased \textit{S. borealis} was washed by clean water, and air-dried to be used as a sample for extraction. To 1 kg of \textit{S. borealis} made into small fragments after the drying was added 15L of 70\% ethanol in the amount of 15 folds of the dry weight of \textit{S. borealis}, which was continuously repeated extracted three times at a constant interval (every 12h) at 80\degree C, and then filtered
under reduced pressure with filter paper (Watman Co. USA). The filtrate was collected and concentrated under reduced pressure by vacuum rotation at 60°C, and thus extracted residue was dried by lyophilizer to obtain 57 g of *S. borealis* crude extract, which was kept in a freezer of -20°C, and used in the experiment.

5-2) Preparation of *S. borealis* solvent fraction

Each solvent fraction was prepared by using 50 g of crude extract obtained in the above 5-1) with same methods of the above 1-2) to 1-5) of Example 1, to obtain solvent fraction as following Table 2.

<table>
<thead>
<tr>
<th>Extract name</th>
<th>Extract yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-2) N-hexane soluble fraction of <em>S. borealis</em></td>
<td>9.5 g</td>
</tr>
<tr>
<td>5-3) Dichloromethane soluble fraction of <em>S. borealis</em></td>
<td>4.1 g</td>
</tr>
<tr>
<td>5-4) Ethylacetate soluble fraction of <em>S. borealis</em></td>
<td>4.8 g</td>
</tr>
<tr>
<td>5-5) N-butanol soluble fraction of <em>S. borealis</em></td>
<td>27.9 g</td>
</tr>
<tr>
<td>5-6) Water soluble fraction of <em>S. borealis</em></td>
<td>27.9 g</td>
</tr>
</tbody>
</table>

Example 6. Preparation of *S. borealis* hydrothermal extract

6-1) *S. borealis* used in the experiment was purchased in Daebat Goeul, Seojeong-ri, Gonyang-myeon, Sacheon-si, Gyeongsangnam-do, Korea. The purchased *S. borealis* was washed by clean water, and air-dried to be used as a sample for extraction.
To 1 kg of *S. borealis* made into small fragments after the drying was added 15L of purified water in the amount of 15 folds of the dry weight of *S. borealis*, which was continuously repeatedly extracted two times at a constant interval (every 10h) at 80 °C, and then filtered under reduced pressure with filter paper (Watman Co. USA). The filtrate was collected and concentrated under reduced pressure by vacuum rotation at 60 °C, and thus extracted residue was dried by lyophilizer to obtain 61.4 g of *S. borealis* crude extract, which was kept in a freezer of −20 °C, and used in the experiment.

**Example 7. Preparation of *P. nigra var. henonis* alcoholized extract**

7-1) *P. nigra var. henonis* used in the experiment was purchased in Daebat Goeul, Scojeong-ri, Gonyang-myeon, Sacheon-si, Gyeongsangnam-do, Korea. The purchased *P. nigra var. henonis* was washed by clean water, and air-dried to be used as a sample for extraction. To 1 kg of *P. nigra var. henonis* made into small fragments after the drying was added 15L of 70% ethanol in the amount of 15 folds of the dry weight of *P. nigra var. henonis*, which was continuously repeatedly extracted three times at a constant interval (every 12h) at 80 °C, and then filtered under reduced pressure with filter paper (Watman Co. USA). The filtrate was collected and concentrated under reduced pressure by vacuum rotation at 60 °C, and thus extracted residue was dried by lyophilizer to obtain 52 g of *P. nigra var. henonis* crude extract, which was kept in a freezer of −20 °C, and used in the experiment.
7-2) Preparation of *P. nigra var. henonis* solvent fraction

Each solvent fraction was prepared by using 50 g of crude extract obtained in the above 7-1) with same methods of the above 1-2) to 1-5) of Example 1, to obtain solvent fraction as following Table 3.

<table>
<thead>
<tr>
<th></th>
<th>Extract name</th>
<th>Extract yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-2</td>
<td>N-hexane soluble fraction of <em>P. nigra var. henonis</em></td>
<td>9.1 g</td>
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<tr>
<td>7-3</td>
<td>Dichloromethane soluble fraction of <em>P. nigra var. henonis</em></td>
<td>4.6 g</td>
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<tr>
<td>7-4</td>
<td>Ethylacetate soluble fraction of <em>P. nigra var. henonis</em></td>
<td>4.3 g</td>
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<tr>
<td>7-5</td>
<td>N-butanol soluble fraction of <em>P. nigra var. henonis</em></td>
<td>7.1 g</td>
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<tr>
<td>7-6</td>
<td>Water soluble fraction of <em>P. nigra var. henonis</em></td>
<td>25.1 g</td>
</tr>
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Example 8. Preparation of *P. nigra var. henonis* hydrothermal extract

8-1) *P. nigra var. henonis* used in the experiment was purchased in Daebat Goeul, Seojeong-ri, Gonyang-myeon, Sacheon-si, Gyeongsangnam-do, Korea. The purchased *P. nigra var. henonis* was washed by clean water, and air-dried to be used as a sample for extraction. To 1 kg of *P. nigra var. henonis* made into small fragments after the drying was added 15L of purified water in the amount of 15 folds of dry weight of *P. nigra var. henonis*, which was continuously extracted two times at a constant interval (every 10h) at 80°C, and then filtered under reduced pressure with filter paper (Watman Co. USA). The
filtrate was collected and concentrated under reduced pressure by vacuum rotation at 60°C, and thus extracted residue was dried by lyophilizer to obtain 57 g of *P. nigra* var. *Henonis* crude extract, which was kept in a freezer of −20°C, and used in the experiment.

**Example 9. Preparation of *P. japonica* alcoholized extract**

9-1) *P. japonica* used in the experiment was purchased in Daebat Goeul, Seojeong-ri, Gonyang-myeon, Sacheon-si, Gyeongsangnam-do, Korea. The purchased *P. japonica* was washed by clean water, and air-dried to be used as a sample for extraction. To 2 kg of *P. japonica* made into small fragments after the drying was added 15 L of 70% ethanol in the amount of 15 folds of the dry weight of *P. japonica*, which was continuously repeatedly extracted three times at a constant interval (every 12 h) at 80°C, and then filtered under reduced pressure with filter paper (Watman Co. USA). The filtrate was collected and concentrated under reduced pressure by vacuum rotation at 60°C, and thus extracted residue was dried by lyophilizer to obtain 73.4 g of *P. japonica* crude extract, which was kept in a freezer of −20°C, and used in the experiment.

9-2) Preparation of *P. japonica* solvent fraction

Each solvent fraction was prepared by using 50 g of crude extract obtained in the above 9-1) with same methods of the above 1-2) to 1-5) of Example 1, to obtain solvent fraction as following Table 4.

Table 4
<table>
<thead>
<tr>
<th>Extract name</th>
<th>Extract yield</th>
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<tr>
<td>9-2) N-hexane soluble fraction of ( P.japonica )</td>
<td>2.7 g</td>
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<td>9-3) Ethylacetate soluble fraction of ( P.japonica )</td>
<td>3.1 g</td>
</tr>
<tr>
<td>9-4) N-butanol soluble fraction of ( P.japonica )</td>
<td>2.5 g</td>
</tr>
<tr>
<td>9-5) Water soluble fraction of ( P.japonica )</td>
<td>41.7 g</td>
</tr>
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</table>

**Example 10. Preparation of \( P.japonica \) hydrothermal extract**

10-1) \( P.japonica \) used in the experiment was purchased in Daebat Goeul, Seojeong-ri, Gonyang-myeon, Sacheon-si, Gyeongsangnam-do, Korea. The purchased \( P.japonica \) was washed by clean water, and air-dried to be used as a sample for extraction. To 1 kg of \( P.japonica \) made into small fragments after the drying was added 15L of purified water in the amount of 15 folds of the dry weight of \( P.japonica \), which was continuously repeatedly extracted two times at a constant interval (every 10h) at 80°C, and then filtered under reduced pressure with filter paper (Watman Co. USA). The filtrate was collected and concentrated under reduced pressure by vacuum rotation at 60°C, and thus extracted residue was dried by lyophilizer to obtain 53.3 g of \( P.japonica \) crude extract, which was kept in a freezer of -20°C, and used in the experiment.

**Experimental example: Test method for the activity analogous to phytoandrogen**

Luciferase plasmid induced by androgen receptor and androgen was introduced
into COS cell (Korean Cell Line Bank) to test whether bamboo extract shows the activity analogous to androgen.

The control group was the cell treated with only medium in plasmid made of ARE. The transcription activity value to all the experimental results represents the value of fold, compared with the control group (AR+ARE).

The reagent related to cell culture such as DMEM and the like was purchased from Gibco, and luciferase assay kit was purchased from Promega for use. All the other reagents were purchased from Sigma. pARE4-Luc plasmid and the other plasmids are provided by Dr. Chang in Rochester University, USA, for the present experiment example.

1) Culture of the cell line

COS cell was cultured under the conditions of 5% CO₂ and 37°C in DMEM containing 10% of fetal bovine serum. The medium was exchanged every 2 or 3 days and changed with new one while treating the reagents.

2) Transient transfection

COS cell was cultured to 70%-80% in 100mm tissue culture plate, and after removing the medium therefrom, the cell was washed by PBS. After separating the cell from culture plate by treating trypsin, the cell was put into the medium containing serum to inactivate the action of trypsin.

For electroporation, the cell pellet was made into the concentration of 5x10⁶ cells/ml, and was centrifuged by cold PBS. The cell pellet was suspended in 400μL of PBS, mixed with prepared hAR and pARE4-Luc plasmid, then put into electroporation cuvette to transfect the plasmid into the cell by electric pulse of 250F, 350V with Gene
Pulser (Bio-Rad), left in CO₂ incubator at 37°C for 10 min, and diluted in DMEM containing 10% of calf serum, to be seeded into 96 well plate. After 24 hours, the medium was changed with new one containing 10% of charcoal-decram stripped calf serum, and the activity of luciferase was determined after 24 hours from the drug treatment.

3) The determination of luciferase activity

The cell transfected with the plasmid was washed with PBS, and was destroyed by adding lysis buffer (125mM Tris pH 7.8, 10mM CDTA, 10mM DTT, 50% glycerol, 5% Triton X-100), to obtain the supernatant, and the amount of protein therein was quantified by Bradford assay. The luciferase activity was determined after adding 100μl assay buffer (20 mM Tricine, 1.07 mM (MgCO₃)₂Mg(OH)₂ · 5H₂O, 2.67 mM MgSO₄, 0.1 mM EDTA, 33.3 mM DTT, 270 μM Coenzyme A(lithium salt), 470 μM Luciferin, 530 μM ATP) to 20μl of cell extract. The luciferase activity was determined after adding 100μl assay buffer (20 mM Tricine, 1.07 mM (MgCO₃)₂Mg(OH)₂ · 5H₂O, 2.67 mM MgSO₄, 0.1 mM EDTA, 33.3 mM DTT, 270 μM Coenzyme A(lithium salt), 470 μM Luciferin, 530 μM ATP) to 20μL of cell extract, and then the light emission was determined for 20 sec with Luminometer (LUMAT LB 9501/16).

Result

1. Androgen activity of bamboo alcoholized extract

The activity analogous to androgen was determined by using the ethanol crude
extract of bamboo prepared in the above Example 1. The results were shown in the Figure 1. As shown in the Figure 1, most bamboo extracts show more androgen activity than the control group not treated at all. The activity analogous to androgen of each bamboo extract compared with the control group was as follows: *P. nigra* (2.45 folds), *P. bambusoides* (2.57 folds), *P. pubescence* (2.26 folds), *P. nigra var. henonis* (3.12 folds), *S. borealis* (2.76 folds), *S. coreana* Nakai (3.23 folds), *P. japonica* (2.98 folds), and *B. caulis* in Taeniis made with inner shells of *P. bambusoides* and *P. nigra var. henonis* (5.29 folds).

2. Androgen activity of *B. caulis* in Taeniis extract and solvent fraction thereof

The activity analogous to androgen of *B. caulis* in Taeniis extract and solvent fraction thereof processed from *Phyllostachys* showing the greatest activity among bamboo extracts was determined. The results were shown in the Figure 2. As shown in the Figure 2, hexane layer (*B. caulis* in Taeniis-hX, 3.37 folds) and dichloromethane layer(*B. caulis* in Taeniis-DC, 4.67 folds) of *B. caulis* in Taeniis show the greatest androgen activity. The activity analogous to androgen of extract and each layer of *B. caulis* in Taeniis compared with the control group was as follows: extract (3.04 folds), ethylacetate layer (*B. caulis* in Taeniis-EA, 1.53 folds), butanol layer (*B. caulis* in Taeniis-BuOH, 3.07 folds) and water layer (*B. caulis* in Taeniis-w, 1.39 folds).

3. Androgen activity of *S. coreana* Nakai extract and solvent fraction thereof.

The activity analogous to androgen of *S. coreana* Nakai extract and solvent fraction thereof prepared from *S. coreana* Nakai belonging to *Sasa* was determined. The
results were shown in the Figure 3. As shown in the Figure 3, activity analogous to androgen of S. coreana Nakai extract was 20.8 folds of that of control. The activity analogous to androgen of each layer of S. coreana Nakai compared with the control group was as follows: Hexane layer (S. coreana Nakai-Hx, 3.92 folds), ethylacetate layer (S. coreana Nakai-EA, 3.22 folds), butanol layer (S. coreana Nakai-BuOH, 1.02 folds) and water layer (1.21 folds).

4. Androgen activity of P. nigra var. henonis extract and solvent fraction thereof.

The activity analogous to androgen of P. nigra var. henonis extract and solvent fraction thereof belonging to Phyllostachys was determined. The results were shown in the Figure 4. As shown in the Figure 4, activity analogous to androgen of P. nigra var. henonis extract showed 2.92 folds of that of control. The activity analogous to androgen of each layer of P. nigra var. henonis compared with the control group was as follows: hexane layer (P. nigra var. henonis-Hx, 3.63 folds), dichloromethane layer (P. nigra var. henonis-DC, 3.21 folds), ethylacetate layer (P. nigra var. henonis-EA, 1.56 folds), butanol layer (P. nigra var. henonis-BuOH, 2.21 folds) and water layer (P. nigra var. henonis-w, 1.30 folds).

5. Androgen activity of P. japonica extract and solvent fraction thereof.

The activity analogous to androgen of P. japonica extract and solvent fraction thereof belonging to Pseudesasa was determined. The results were shown in the Figure 5. As shown in the Figure 5, activity analogous to androgen of P. japonica extract showed
2.86 folds of that of control. The activity analogous to androgen of each layer of *P. japonica* compared with the control group was as follows: hexane layer (*P. japonica*-Hx, 3.77 folds), ethylacetate layer (*P. japonica*-EA, 2.39 folds), butanol layer (*P. japonica*-BuOH, 1.29 folds) and water layer (*P. japonica*-w, 1.71 folds).

6. Androgen activity of bamboo hydrothermal extract.

The activity analogous to androgen was determined by using the bamboo hydrothermal extract prepared in the above Example 2, 4, 6, 8 and 10. The results were shown in the Figure 6. As shown in the Figure 6, most of bamboo hydrothermal extract showed more androgen activity than the control group not treated at all. The activity analogous to androgen of each bamboo hydrothermal extract compared with the control group was as follows: *P. nigra* (1.95 folds), *P. bambusoides* (2.11 folds), *P. pubescence* (1.89 folds), *P. nigra var. henonis* (2.32 folds), *S. borealis* (2.05 folds), *S. coreana* Nakai (2.42 folds), *P. japonica* (2.21 folds), and *B. caulis* in Taenii made with inner shell of *P. bambusoides* and *P. nigra var. henonis* (3.23 folds).

**Formulation Example 1: Preparation of Solution**

*B. caulis* in Taenii ethanol extract of Example 1 20g

Sugar 10g

Isomerized sugar 10g

Smell of lemon proper quantity

Total amount after adding purified water 100ml
The above-mentioned ingredients were mixed according to conventional preparation method for solution, and sterilized to give solution.

**Formulation Example 2: Preparation of Solution**

S. coreana Nakai ethylacetate fraction of Example 2 30g  
Sugar 10g  
Isomerized sugar 10g  
Smell of lemon proper quantity  
Total amount after adding purified water 100ml  
The above-mentioned ingredients were mixed according to conventional preparation method for solution, and sterilized to give solution.

**Formulation Example 3: Preparation of Capsule**

P. nigra var. henonis dichloromethane fraction of Example 4 500mg  
Lactose 50mg  
Starch 50mg  
Talc 2mg  
Magnesium Stearate proper quantity  
The above-mentioned ingredients were mixed, and filled in a gelatin capsule according to conventional preparation method for capsule to give capsule.

**Formulation Example 4: Preparation of Capsule**
\textit{P. japonica} ethanol extract of Example 5 & 500mg \\
Lactose & 50mg \\
Starch & 50mg \\
Talc & 2mg \\
5 & Magnesium Stearate & proper quantity \\

The above-mentioned ingredients were mixed, and filled in a gelatin capsule according to conventional preparation method for capsule to give capsule.

**Formulation Example 5: Preparation of Capsule**

\textit{P. japonica} hydrothermal extract of Example 10 & 10mg \\
Lactose & 50mg \\
Starch & 50mg \\
Talc & 2mg \\
Magnesium Stearate & proper quantity \\

The above-mentioned ingredients were mixed, and filled in a gelatin capsule according to conventional preparation method for capsule to give capsule.

**INDUSTRIAL APPLICABILITY**

The present composition comprising bamboo extract can be used for androgen agonist because androgen activity of the composition is outstanding. Also, the present composition obtained from natural material can be as a medicine or health care product for
the prevention and treatment of male climacteric without dangerousness according to hormone replacement therapy.
CLAIMS

1. A composition for androgen agonist comprising bamboo or bamboo extract as effective ingredient.

2. The composition according to claim 1, wherein the extract is extracted with polar solvent such as water, acetone, or C$_1$-4 alcohol, or mixing solvent thereof.

3. The composition according to claim 1, wherein the extract is extracted with non-polar solvent such as n-hexane, dichloromethane, or ethylacetate.

4. The composition according to any of claims 1 to 3, wherein the bamboo is selected from Phyllostachys, Sasa, or Pseudosasa.

5. The composition according to claim 4, wherein the Phyllostachys bamboo is selected from Phyllostachys nigra var. henonis, P. nigra, P. bambusoides, P. pubescence, P. nigra for. punctata or P. compressa; the Sasa bamboo is selected from Sasa coreana Nakai, S. coreana, S. kurilensis, S. quelpaertensis, S. borealis, S. borealis var. chiisanensis or S. borealis var. gracilis; and the Pseudosasa bamboo is selected from Pseudosasa japonica or Pseudosasa japonica var. purpurascens.

6. The composition according to claim 5, wherein the extract is water fraction or
n-hexane fraction obtained by suspending the above C<sub>1-4</sub> alcohol extract with water, adding n-hexane thereto, and distributing; dichloromethane fraction obtained by adding dichloromethane to the above water fraction, and distributing; ethylacetate fraction obtained by adding ethylacetate to water fraction remaining after separation of the above dichloromethane fraction, and distributing; n-butanol fraction obtained by adding n-butanol to water fraction remaining after separation of the above ethylacetate fraction, and distributing; or extract obtained by column chromatography of the above extracts and fractions.

7. The composition for the prevention and treatment of male climacteric according to any of claims 1 to 3.

8. The composition for reducing of body fat, increase of muscle, increase of bone density, increase of hand-grip strength, improvement of mood, reduction of melancholia, increase of sexual desire, or prevention or treatment of dementia according to claim 7.

9. A use of bamboo or bamboo extract to prevent or treat symptoms related to decrease of androgen.

10. The use according to claim 9, wherein the extract is extracted with polar solvent such as water, acetone, or C<sub>1-4</sub> alcohol, or mixing solvent thereof.
11. The use according to claim 9, wherein the extract is extracted with non-polar solvent such as n-hexane, dichloromethane, or ethylacetate.

12. The use according to any of claims 9 to 11, wherein the bamboo is selected from *Phyllostachys*, *Sasa*, or *Pseudosasa*.

13. The use according to claim 12, wherein the *Phyllostachys* bamboo is selected from *Phyllostachys nigra var. henonis*, *P. nigra*, *P. bambusoides*, *P. pubescence*, *P. nigra for. punctata* or *P. compressa*; the *Sasa* bamboo is selected from *Sasa coreana* Nakai, *S. coreana*, *S. kurilensis*, *S. quelpaertensis*, *S. borealis*, *S. borealis var. chiisanensis* or *S. borealis var. gracilis*; and the *Pseudosasa* bamboo is selected from *Pseudosasa japonica* or *Pseudosasa japonica var. purpurascens*.

14. The use according to claim 13, wherein the extract is water fraction or n-hexane fraction obtained by suspending the above C1-4 alcohol extract with water, adding n-hexane thereto, and distributing; dichloromethane fraction obtained by adding dichloromethane to the above water fraction, and distributing; ethylacetate fraction obtained by adding ethylacetate to water fraction remaining after separation of the above dichloromethane fraction, and distributing; n-butanol fraction obtained by adding n-butanol to water fraction remaining after separation of the above ethylacetate fraction, and distributing; or extract obtained by column chromatography of the above extracts and fractions.
15. The use according to any of claims 9 to 11, wherein the symptom related to decrease of androgen is symptom of male climacteric.

16. The use according to claim 15, the symptom of male climacteric is selected from increase of body fat, decline of muscle, decline of bone density, decline of hand-grip strength, decline of mood, melancholia, decline of sexual desire or dementia.

17. A method of prevention or treatment of symptoms related to decrease of androgen comprising administering a therapeutically effective amount of bamboo or bamboo extract to mammal.

18. The method according to claim 17, wherein the extract is extracted with polar solvent such as water, acetone, or C₂₄ alcohol, or mixing solvent thereof.

19. The method according to claim 17, wherein the extract is extracted with non-polar solvent such as n-hexane, dichloromethane, or ethylacetate.

20. The method according to any of claims 17 to 19, wherein the bamboo is selected from Phyllostachys, Sasa, or Pseudosasa.

21. The method according to claim 20, wherein the Phyllostachys bamboo is selected from Phyllostachys nigra var. henonis, P. nigra, P. bambusoides, P. pubescence, P. nigra
for. punctata or P. compressa; the Sasa bamboo is selected from Sasa coreana Nakai, S. coreana, S. kurilensis, S. quelpaertensis, S. borealis, S. borealis var. chiisanensis or S. borealis var. gracilis; and the Pseudosasa bamboo is selected from Pseudosasa japonica or Pseudosasa japonica var. purpurascens.

22. The method according to claim 21, wherein the extract is water fraction or n-hexane fraction obtained by suspending the above C1-4 alcohol extract with water, adding n-hexane thereto, and distributing; dichloromethane fraction obtained by adding dichloromethane to the above water fraction, and distributing; ethylacetate fraction obtained by adding ethylacetate to water fraction remaining after separation of the above dichloromethane fraction, and distributing; n-butanol fraction obtained by adding n-butanol to water fraction remaining after separation of the above ethylacetate fraction, and distributing; or extract obtained by column chromatography of the above extracts and fractions.

23. The method according to any of claims 17 to 19, wherein the symptom related to decrease of androgen is symptom of male climacteric.

24. The method according to claim 23, the symptom of male climacteric is selected from increase of body fat, decline of muscle, decline of bone density, decline of hand-grip strength, decline of mood, melancholia, decline of sexual desire or dementia.
25. A method for preparing a composition for androgen agonist by extracting bamboo with polar solvent such as water, acetone, or C\textsubscript{1-4} alcohol, or mixing solvent thereof.

26. A method for preparing a composition for androgen agonist by extracting bamboo with non-polar solvent such as n-hexane, dichloromethane, or ethylacetate.

27. The method according to claim 25 or 26, wherein the bamboo is selected from 

\textit{Phyllostachys}, \textit{Sasa}, or \textit{Pseudosasa}.

28. The method according to claim 27, wherein the \textit{Phyllostachys} bamboo is selected from \textit{Phyllostachys nigra} var. \textit{henonis}, \textit{P. nigra}, \textit{P. bambusoides}, \textit{P. pubescence}, \textit{P. nigra for. punctata} or \textit{P. compressa}; the \textit{Sasa} bamboo is selected from \textit{Sasa coreana} Nakai, \textit{S. coreana}, \textit{S. kurilensis}, \textit{S. quelpaertensis}, \textit{S. borealis}, \textit{S. borealis} var. \textit{chiisanensis} or \textit{S. borealis} var. \textit{gracilis}; and the \textit{Pseudosasa} bamboo is selected from \textit{Pseudosasa japonica} or \textit{Pseudosasa japonica} var. \textit{purpurascens}.

29. The method according to claim 25, additionally comprising step that the above C\textsubscript{1-4} alcohol extract is suspended with water, added n-hexane thereto, and distributed to obtain water fraction or n-hexane fraction; step that to the above water fraction is added dichloromethane and distributed to obtain water fraction and dichloromethane fraction; step that to water fraction remaining after separation of the above dichloromethane fraction is added ethylacetate and distributed to obtain water fraction and ethylacetate fraction; step
that to the water fraction remaining after separation of the above ethylacetate fraction is added n-butanol and distributed to obtain water fraction and n-butanol fraction; or step that the above extracts and fractions are separated by column chromatography to obtain extract.
Fig. 1

Bamboo alcoholized extract (50µg/ml)

Fig. 2

Solvent fraction layer of B. caulis in Taenii (50µg/ml)

Fig. 3

Solvent fraction layer of S. coreana Nakai (50µg/ml)
Fig. 4

Solvent fraction layer of *P. nigra* var. *henonis* (50μg/ml)

Fig. 5

Solvent fraction layer of *P. japonica*

Fig. 6

Bamboo hydrothermal extract (50μg/ml)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC\(^2\): A61K 35/78
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC\(^2\): A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPIDOC, TXTE, medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>X</td>
<td>KR 2003/021640 A (KOREA CHUNGA EDUCATIONAL FOUND) 15 March 2003 (15.03.2003) abstract (WPI; Acc.No.: 2003-551584).</td>
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<td>X</td>
<td>CN 1228968 A (UNIV ZHEJIANG AGRICULTURAL) 22 September 1999 (22.09.1999) abstract (EPIDOC).</td>
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[X] Further documents are listed in the continuation of Box C. [See] See patent family annex.

* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claims(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
24 August 2005 (24.08.2005)

Date of mailing of the international search report
15 September 2005 (15.09.05)

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Authorized officer
KRENN M.

Telephone No. +43 /1 /534 24 /435

Form PCT/ISA/210 (second sheet) (January 2004)
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<td>JP 11199502 A (KIKUCHI S) 27 July 1999 (27.07.1999) abstract (WPI; Acc.No.: 1999-496823).</td>
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Continuation of first sheet

Continuation No. II:

Observations where certain claims were found unsearchable

(Continuation of item 2 of first sheet)

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

Claims Nos.: 9-24 because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 9-24 are directed to a therapeutic method of treatment of the human/animal body, a search is commonly carried out. However, only claim 16 refers to a concrete subject matter; thus only for claim 16 a search has been carried out and is based on the alleged effects of the composition. Claims 9-15 and 17-23: see below.

Claims Nos.: 2, 3, 9-15, 17-23 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Characterization of a product or a use claim (claims 2,3,10,11,18,19) by its way of manufacture is not allowed; a reformulation to product-by-process claims (Product obtainable by a process according to claims 1-x) should be contemplated.

The terms "symptoms related to decrease of androgen" and "symptom of male climactic" (claims 9,17 and the dependent claims 10-15 and 18-23) are not sufficiently clear.
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Form PCT/ISA/210 (patent family annex) (July 1998; reprint January 2004)