DESCRIPTION

Title of the Invention: COMPOSITION FOR ENHANCING EXPRESSION OF AANAT

TECHNICAL FIELD

[0001]

The present invention relates to a composition for enhancing expression of AANAT (arylalkylamine *N*-acetyltransferase), which is a melatonin-synthesizing enzyme.

BACKGROUND ART

[0002]

Tongkat Ali (*Eurycoma longifolia*) is a flowering plant belonging to the family Simaroubaceae. It is indigenous to Indochina (Cambodia, Laos, Malaysia, Myanmar, Thailand, and Vietnam) and Indonesia (Borneo Island and Sumatra Island). In folk medicine in the Southeast Asian region, root of Tongkat Ali has been used for the treatment of malaria, dysentery, glandular swelling, persistent fever, aches, and sexual insufficiency. Today, it is commonly used as a supplement, or a food or drink additive.

[0003]

Insufficient sleep is a serious problem prevalent in today's 24-hour society, and about one-third of the world's population suffers from sleep disorders. Insufficient sleep is prevalent among various age groups, and has been a major problem in public health. As a result of several decades of research, insufficient sleep and sleep disorders have been widely known to cause profound and widespread effects on human health. For example, they are known to be possible causes of increased incidences of cardiovascular morbidity, increased chances of diabetes mellitus, obesity, derailment of cognitive functions, vehicular accidents, and increased accidents at

workplaces, and the like.

[0004]

Recent studies have shown that in C57BL/6 mice, Tongkat Ali has a unique effect that intensifies sleep during the resting period while increasing wakefulness during the active period (Non-Patent Document 1). Further, a Tongkat Ali extract has been shown to be useful as an agent for decreasing the core body temperature (see Patent Document 1).

However, not all of the components contained in Tongkat Ali have so far been identified. Furthermore, it has not been clarified what component contained in Tongkat Ali is involved in sleep, nor what mechanism is responsible for the improvement in sleep.

PRIOR ART DOCUMENTS

[Patent Document]

[0005]

[Patent Document 1] WO 2020/040216

[Non-Patent Documents]

[0006]

[Non-Patent Document 1] Eur J Neurosci., 2023;58:4298-4309

[Non-Patent Document 2] Molecules, 2021;26:5939-5950

[Non-Patent Document 3] Chem.Lett., 1990:749-752

[Non-Patent Document 4] American Journal of Applied Sciences, 2011;8(12):1268-1271

[Non-Patent Document 5] Plant Polyphenols, 1992:73-95

[Non-Patent Document 6] Chem. Pharm. Bull, 1983;31(9):2993-2997

[Non-Patent Document 7] J. Org. Chem., 1980;45(15):3132-3135

[Non-Patent Document 8] An. Acad. Bras. Cienc., 2002;74(3):415-424

[Non-Patent Document 9] Australian Journal of Chemistry, 1966;19(3):455-481

[Non-Patent Document 10] Tetrahedron Lett., 1991;32:1803-1804

[Non-Patent Document 11] Chemical and Pharmaceutical Bulletin, 1992;40:1053-1055

[Non-Patent Document 12] Lloydia, 1972;35:177-180

[Non-Patent Document 13] Journal of natural medicines, 2019;73:124-130

[Non-Patent Document 14] Planta Med., 1987;53(2):140-143

[Non-Patent Document 15] Chem. Lett, 1990;5:749

[Non-Patent Document 16] Phytochemistry, 1980;19(2):313

[Non-Patent Document 17] J. Nat. Prod, 1991;54(5):1360-1367

[Non-Patent Document 18] Int. J. Pharmacogn, Phytochem. Res, 2017;9(10):1288-1292

[Non-Patent Document 19] J. Agric. Food Chem, 2012;60(44):11044−11049

[Non-Patent Document 20] Biotechnol, 2009;19(11):1319–1327

[Non-Patent Document 21] Studies on the anti-tumor constituents of fruits of Ailanthus altissima (Mill) Swingle, Journal of Yangzhou University, 2010;4:39-41

[Non-Patent Document 22] Molecules, 2022;27(22):7742

SUMMARY OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0007]

An object of the present invention is to identify a compound that is contained in Tongkat Ali and is involved in sleep, and to identify a mechanism by which the compound enables the improvement of sleep, thereby providing a novel composition for promoting sleep onset.

MEANS FOR SOLVING THE PROBLEMS

[0008]

As a result of intensive study, the present inventors discovered that an extract obtained from Tongkat Ali with an organic solvent contains three kinds of quassinoid

compounds. Each of the three kinds of quassinoid compounds was found to enhance expression of AANAT, which is a melatonin-synthesizing enzyme, thereby completing the present invention.

[0009]

Specifically, the present invention is as follows.

[1] A composition for enhancing expression of AANAT, the composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof.

[2] The composition for enhancing expression of AANAT according to [1], wherein the quassinoid compound is at least one compound selected from the group consisting of eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone.

[3] The composition for enhancing expression of AANAT according to [1] or [2], wherein the quassinoid compound is eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone.

[4] The composition for enhancing expression of AANAT according to any one of [1] to [3], wherein the quassinoid compound is a compound originally contained in an extract from root of Tongkat Ali.

[5] The composition for enhancing expression of AANAT according to any one of [1] to [4], further comprising at least one compound selected from the group consisting of (+)-syringaresinol, bourjotinolone A, eurylene, canthin-6-one, 9-methoxycanthin-6-one, vanillin, 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one, and 5-(hydroxymethyl)furfural.

[6] A composition for enhancing expression of AANAT, the composition comprising an extract from root of Tongkat Ali.

[7] A sleep-inducing agent comprising the composition for enhancing expression of AANAT according to any one of [1] to [6].

[8] A pharmaceutical comprising the composition for enhancing expression of AANAT according to any one of [1] to [6], or the sleep-inducing agent according to

[7].

[9] A food or drink comprising the composition for enhancing expression of AANAT according to any one of [1] to [6], or the sleep-inducing agent according to [7].

EFFECT OF THE INVENTION

[0010]

Identification of compounds contained in Tongkat Ali led to the discovery that quassinoid compounds enhance expression of AANAT, which is a melatonin-synthesizing enzyme. By this, a composition for enhancing expression of AANAT, the composition comprising a quassinoid compound as an effective component, could be provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011]

Fig. 1 shows (a) the results of HPLC analysis, and (b) the results of HRMS spectral analysis, of three kinds of quassinoid compounds (Compounds 1 to 3).

Fig. 2 shows (a) a ^{1}H NMR spectrum, and (b) a ^{13}C NMR spectrum, of Compound 1.

Fig. 3 shows ^{1}H NMR spectra and ^{13}C NMR spectra of Compounds 2 and 3.

Fig. 4 shows the result on enhancement of expression of the AANAT gene by a Tongkat Ali extract.

Fig. 5 shows the results on enhancement of expression of the AANAT gene by eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone.

MODE FOR CARRYING OUT THE INVENTION

[0012]

<Composition for Enhancing Expression of AANAT>

A first mode of the present invention is a composition for enhancing expression of AANAT, the composition comprising a quassinoid compound or a

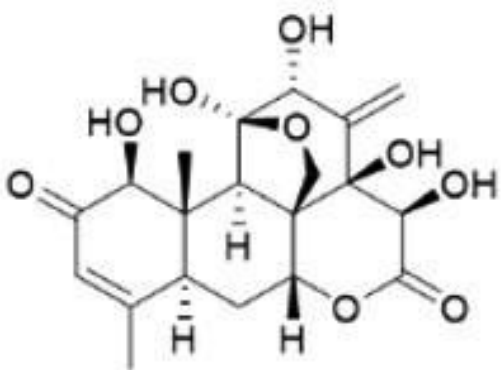
pharmaceutically acceptable salt thereof; or a composition for enhancing expression of AANAT, the composition comprising an extract from root of Tongkat Ali.

[0013]

The quassinoid compound is not limited as long as it enhances expression of AANAT. The quassinoid compound may be, for example, at least one compound selected from the group consisting of eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone. The at least one compound may be only one compound or only two compounds arbitrarily selected from eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone, or may contain all of eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone. The quassinoid compound is not limited to eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone, and may also be another kind of quassinoid compound originally contained in an extract from root of Tongkat Ali. A quassinoid compound obtained by chemical synthesis may also be used as an effective component according to the present invention.

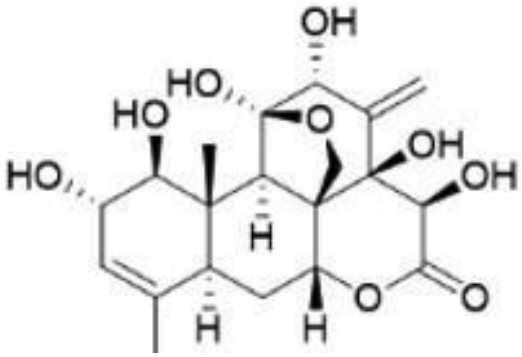
[0014]

Eurycomanone is the compound represented by the following formula.



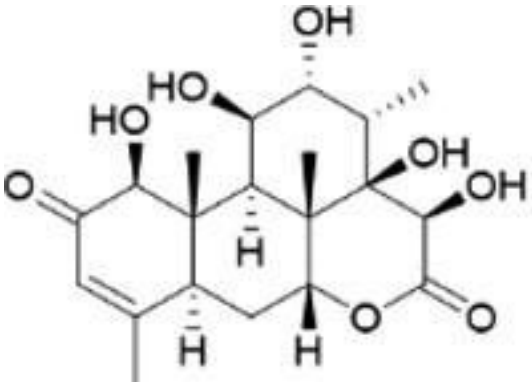
[0015]

Eurycomanol is the compound represented by the following formula.



[0016]

14,15β-Dihydroxyklaineanone is the compound represented by the following formula.



[0017]

The quassinoid compound may be a compound obtained by extraction and/or isolation from Tongkat Ali, may be a synthetic compound, or may be a commercially available compound. The quassinoid compound may be included in the form of a Tongkat Ali extract.

[0018]

Examples of the pharmaceutically acceptable salt of the quassinoid compound include salts with an inorganic base or an organic base, and salts with a basic amino acid.

Examples of the inorganic base include salts of an alkali metal such as sodium or potassium; salts of an alkaline earth metal such as calcium or magnesium; ammonium salts; and aluminum salts.

Examples of the organic base include primary amines such as ethanolamine; secondary amines such as diethylamine, diethanolamine, dicyclohexylamine, and *N*,*N*'-dibenzylethylenediamine; and tertiary amines such as trimethylamine, triethylamine, triethanolamine, pyridine, and picoline.

Examples of the basic amino acid include lysine, arginine, and ornithine.

[0019]

The quassinoid compound may be, but does not necessarily need to be, for example, a compound derived from an extract from root, bark, stem, leaf, or the like of Tongkat Ali. It is preferred to use a compound derived from an extract from root of Tongkat Ali. The type of the Tongkat Ali is not limited, and may be any of the yellow, red, black, and other types. A mixture of a plurality of types of Tongkat Ali may also be used.

[0020]

The extract from Tongkat Ali may be obtained by a known method.

A step of washing, drying, or freeze-drying of Tongkat Ali in the state of a collected plant, a step of grinding to obtain a homogeneous sample, or the like may be carried out as a pretreatment step for the extraction.

Examples of the extraction method include the water extraction method, the solvent extraction method, steam extraction method, subcritical water extraction method, and the supercritical carbon dioxide extraction method.

The water extraction method is a method in which an object is immersed in water at normal temperature or water at high temperature to perform extraction. The extraction may instead be carried out using a liquid prepared by adding a small amount of an organic solvent such as ethanol to water.

The solvent extraction method is a method in which a polar or nonpolar organic solvent is used to perform extraction from an object, wherein an aqueous solution of the organic solvent may also be used. Examples of the polar organic

solvent include alcohols (methanol, ethanol, propanol, butanol, and the like), acetone, and ethyl acetate. Examples of the nonpolar organic solvent include *n*-hexane, chloroform, and toluene.

[0021]

The method of isolating the quassinoid compound from the Tongkat Ali extract is not limited, and examples of the method include the following.

For example, an ethyl acetate-soluble fraction is obtained by extraction of components from Tongkat Ali by the solvent extraction method, and the resulting fraction is applied to a silica flash column (FlashPure ID HP 20-μm particles, 12 g), followed by performing elution with EtOAc: MeOH (9:1) and separation by semi-preparative HPLC (GL Sciences Inc.; 20 × 250 mm; particle size, 5 μm; UV detector, 205 nm; flow rate, 8 mL/min) using an ACN/H_2O gradient (20:80 to 100:0) as the mobile phase. By this, 14,15β-dihydroxyklaineanone can be obtained (21.3 mg, t_R = 30 min).

In another example, an ethyl acetate-soluble fraction is obtained by extraction of components from Tongkat Ali by the solvent extraction method, and the resulting fraction is applied to a silica flash column (FlashPure ID HP 20-μm particles, 12 g), followed by performing elution with EtOAc: MeOH (4:1) and separation by semi-preparative HPLC (GL Sciences Inc.; 20 × 250 mm; particle size, 5 μm; UV detector, 205 nm; flow rate, 8 mL/min) using an ACN/H_2O gradient (20:80 to 100:0) as the mobile phase. By this, eurycomanone (1.1 mg, t_R = 38 min) and eurycomanol (2.2 mg, t_R = 41.6 min) can be obtained.

[0022]

The composition for enhancing expression of AANAT according to the present embodiment may contain components other than quassinoid compounds. For example, the composition may further contain at least one compound selected from the group consisting of (+)-syringaresinol, bourjotinolone A, eurylene, canthin-

6-one, 9-methoxycanthin-6-one, vanillin, 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one, and 5-(hydroxymethyl)furfural, which are components contained in extracts of Tongkat Ali.

[0023]

The composition for enhancing expression of AANAT according to the present embodiment may contain an extract from root of Tongkat Ali.

As described above, extracts from root of Tongkat Ali typically contain quassinoid compounds and the like. Examples of the quassinoid compounds contained in extracts from root of Tongkat Ali include eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone.

[0024]

The composition for enhancing expression of AANAT according to the present embodiment may also contain an arbitrary component other than quassinoid compounds and components contained in extracts from Tongkat Ali, as long as the enhancement of expression of AANAT is not inhibited.

For example, the composition may contain an additive acceptable for pharmaceuticals and foods. Examples of such an additive include, but are not limited to, the following:

sugars such as lactose, glucose, and sucrose; starches such as corn starch and potato starch; celluloses and derivatives thereof such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; cyclodextrin, dextrin, and the like; tragacanth powder; malt; gelatin; talc; stearic acid; magnesium stearate; and calcium sulfate;

vegetable oils such as corn oil, cottonseed oil, and olive oil; polyols such as propylene glycol, polypropylene glycol, glycerin, sorbitol, mannitol, and polyethylene glycol; phosphate buffers; cocoa butter; emulsifiers; vaseline, paraffin, beeswax, and emulsion bases; and other non-toxic compatible substances used in

pharmaceutical formulations and/or food formulations; and

wetting agents and lubricants, such as magnesium stearate; coloring agents, flavoring agents, perfumes, emulsifiers, excipients, tableting agents, stabilizers, antioxidants, and preservatives.

[0025]

The enhancement of expression of AANAT can be judged based on, for example, an increase in the expression level, preferably a significant increase in the gene expression level, of the AANAT gene compared to a control (a sample treated with neither a quassinoid compound nor a Tongkat Ali extract). Alternatively, for example, the enhancement of expression may be judged based on a not less than 1.1-fold, not less than 1.2-fold, not less than 1.3-fold, not less than 1.4-fold, or not less than 1.5-fold increase in the expression level of the AANAT gene as compared to a control.

[0026]

The composition of the present embodiment may be used either for a therapeutic purpose or for a non-therapeutic purpose. “Non-therapeutic purpose” is a concept that does not include medical activity, in other words, a concept that does not include the activity of treatment for a human body. Examples of the non-therapeutic purpose include activities for the purpose of promoting health.

[0027]

The composition for enhancing expression of AANAT according to the present embodiment may be administered not only to humans, but also to pets (pet animals), domestic animals, and the like. The composition may be administered to mammals in general for therapeutic or non-therapeutic purposes. Specifically, the composition is applicable not only to humans, but also to dogs, cats, mice, rats, rabbits, cows, horses, monkeys, and the like.

[0028]

In cases where the composition of the present embodiment is used for a “non-therapeutic purpose”, the composition may be administered to a healthy individual. The “healthy individual” means a subject who has no sleep disorder, but has a sleep-related complaint such as difficulty falling asleep. In cases where the composition of the present invention is used for a non-therapeutic purpose, the composition may be used to facilitate falling asleep of a healthy individual, and to relieve a sleep-related complaint of the individual.

[0029]

In cases where the composition of the present embodiment is used for a “therapeutic purpose”, the composition may be administered to a subject having a sleep disorder such as sleep-onset insomnia. In cases where the composition of the present invention is used for a therapeutic purpose, the sleep-onset insomnia in the subject can be treated therewith.

[0030]

The content of the quassinoid compound and the Tongkat Ali extract as effective components in the composition for enhancing expression of AANAT according to the present embodiment, and the dose of the composition for enhancing expression of AANAT, may be arbitrarily set, and may be increased or decreased as appropriate when desired, as long as the effect of the present invention can be produced in the subject to whom the composition is administered.

[0031]

Another aspect of the present invention is use of a quassinoid compound or a pharmaceutically acceptable salt thereof in the production of a composition for enhancing expression of AANAT.

Another aspect of the present invention is use of a quassinoid compound or a pharmaceutically acceptable salt thereof for enhancing expression of AANAT.

Another aspect of the present invention is a quassinoid compound or a

pharmaceutically acceptable salt thereof for use in enhancement of expression of AANAT.

Another aspect of the present invention is a method of enhancing expression of AANAT, the method including administering a quassinoid compound or a pharmaceutically acceptable salt thereof to a subject in need thereof.

[0032]

<Sleep-Inducing Agent>

Another mode of the present invention is a sleep-inducing agent comprising the composition for enhancing expression of AANAT.

[0033]

AANAT is a melatonin-synthesizing enzyme. It is therefore considered that melatonin synthesis is promoted by enhancement of expression of AANAT. Melatonin, also called as “sleep hormone” is known to play a role in decreasing the pulse rate, the body temperature, the blood pressure, and the like, and informing that the body is ready for sleep, to allow sleep onset. Thus, melatonin has a function to switch between wakefulness and sleep such that natural sleep is induced. Therefore, the composition for enhancing expression of AANAT enhances the AANAT expression to promote the induction of natural sleep (promote sleep onset) in the subject.

[0034]

The sleep-inducing agent of the present embodiment may be used either for a therapeutic purpose or for a non-therapeutic purpose. “Non-therapeutic purpose” is a concept that does not include medical activity, in other words, a concept that does not include the activity of treatment for a human body. Examples of the non-therapeutic purpose include activities for the purpose of promoting health.

[0035]

The content of the quassinoid compound and the Tongkat Ali extract as

effective components in the composition for enhancing expression of AANAT contained in the sleep-inducing agent according to the present embodiment, and the dose of the sleep-inducing agent, may be arbitrarily set, and may be increased or decreased as appropriate when desired, as long as the effect of the present invention can be produced in the subject to whom the sleep-inducing agent is administered.

[0036]

In cases where the sleep-inducing agent of the present embodiment is used for a “non-therapeutic purpose”, the agent may be administered to a healthy individual. The “healthy individual” means a subject who has no sleep disorder, but has a sleep-related complaint such as difficulty falling asleep. In cases where the composition of the present invention is used for a non-therapeutic purpose, the composition may be used to facilitate falling asleep of a healthy individual, and to relieve a sleep-related complaint of the individual.

[0037]

In cases where the sleep-inducing agent of the present embodiment is used for a “therapeutic purpose”, the agent may be administered to a subject having a sleep disorder such as sleep-onset insomnia. In cases where the composition of the present invention is used for a therapeutic purpose, the sleep-onset insomnia in the subject can be treated therewith.

[0038]

Another aspect of the present invention is use of a composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof in the production of a sleep-inducing agent.

Another aspect of the present invention is use of a composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof in induction of sleep.

Another aspect of the present invention is a composition comprising a

quassinoid compound or a pharmaceutically acceptable salt thereof for use in induction of sleep.

Another aspect of the present invention is a method of inducing sleep, the method including administering a composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof to a subject in need thereof.

[0039]

<Pharmaceutical>

Another mode of the present invention is a pharmaceutical comprising the composition for enhancing expression of AANAT or the sleep-inducing agent.

[0040]

Examples of the dosage form of the pharmaceutical of the present mode include, but are not limited to, capsules, soft capsules, powders, granules, tablets, solutions, syrups, suspensions, emulsions, inhalants, external preparations, ointments, and creams. The administration route is not limited, and may be either oral administration or parenteral administration.

[0041]

The pharmaceutical of the present mode may contain a pharmacologically acceptable carrier. Examples of the pharmacologically acceptable carrier include excipients, binders, disintegrants, and lubricants that may be generally used, and also include various carriers, stabilizers, surfactants, plasticizers, solubilizers, reducing agents, buffers, sweetening agents, bases, adsorbents, corrigents, suspending agents, antioxidants, brightening agents, coating agents, coatings, wetting agents, wetting control agents, fillers, antifoaming agents, refrigerants, coloring agents, flavoring agents, perfumes, sugar-coating agents, isotonic agents, softeners, emulsifiers, thickening agents, foaming agents, pH-adjusting agents, diluents, dispersants, disintegration aids, disintegration retardants, aromatic agents, moisture-preventing agents, antiseptics, preservatives, dissolution agents, dissolution aids, solvents,

fluidizing agents, antistatic agents, bulking agents, humectants, and moisturizing agents.

[0042]

The pharmaceutical of the present mode may contain another effective component, when desired.

[0043]

The content of the composition for enhancing expression of AANAT or the sleep-inducing agent in the pharmaceutical of the present mode is not limited as long as the content is effective for the production of the pharmaceutical effect. Although the content varies depending on the dosage form, it may be generally set within the range of, for example, about 0.01% by mass to about 99.9% by mass such that a desired dose can be achieved. The amount of the composition for enhancing expression of AANAT or the sleep-inducing agent relative to the total amount of the pharmaceutical may be 1 ng/mL to 1 mg/mL, or may be, for example, 1 ng/mg to 1 mg/mg.

[0044]

As long as the quassinoid compound and the Tongkat Ali extract as effective components produce their effects and are effective for treatment and/or prevention of a particular disease (for example, a sleep disorder such as sleep-onset insomnia), there is no limitation regarding the subject to whom the pharmaceutical is administered, the dose, the timing of administration, the frequency of administration, the administration period, and the like, and the above conditions may be appropriately controlled depending on the age, sex, body weight, and symptoms of the subject, the therapeutic effect, the area of the treatment site, the dosage form, the mode of administration, and the like. The subject to whom the pharmaceutical is administered may be, for example, a subject having a disease that can be treated and/or prevented by the effects of the quassinoid compound and the Tongkat Ali

extract. In cases of a human (adult) with a body weight of about 60 kg, the dose may be set appropriately within the range of, for example, 0.001 mg to 10,000 mg per day in terms of the amount of the quassinoid compound and/or the Tongkat Ali extract. The timing of administration may be before, between, or after meals, or before bedtime. The above dose of the pharmaceutical may be administered once per day, or dividedly in a plurality of times per day. The administration period may be, for example, 1 day to 1 year, or the administration may be continued for a period longer than this.

[0045]

By the use of the pharmaceutical of the present mode, falling asleep can be better achieved, and natural induction of sleep can be promoted (sleep onset can be promoted). More specifically, sleep satisfaction can be improved for people who have sleep-related complaints such as difficulty falling asleep, but whose complaints are not as serious as those requiring dosing of a sleeping pill and/or a sleeping inducer.

[0046]

The sleep-onset-promoting effect can be evaluated by measuring an electroencephalogram, an electromyogram, an electrocardiogram, body temperature, blood pressure, actions (locomotion), and the like of an animal, and examining the amount of sleep of the animal. Specifically, a polysomnogram is prepared based on measurement data such as an electroencephalogram from the animal. Whether the animal has fallen asleep can be judged on the basis of the presence or absence of actions (locomotion), amplitude of the electroencephalogram (δ wave), θ wave component ratio [θ / (δ + θ)] of the electroencephalogram, and the like for each epoch (for example, 4 to 60 seconds) of the polysomnogram. The judgment of wakefulness or sleep based on the polysomnogram can be performed automatically with, for example, “SleepSign (registered trademark)”, a program for analysis and

research of sleep manufactured by Kissei Comtec Co., Ltd.

[0047]

The pharmaceutical of the present mode contains the composition for enhancing expression of AANAT or the sleep-inducing agent, and especially has an action that shortens the sleep latency. The sleep latency means the time required in order to fall asleep from the wakeful state, and is an indicator of easiness to fall asleep. The pharmaceutical of the present mode can be used for treatment of a sleep disorder whose chief complaint is sleep-onset insomnia.

[0048]

Another aspect of the present invention is use of a composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof, or a sleep-inducing agent comprising the composition, in the production of a pharmaceutical for treatment of sleep-onset insomnia.

Another aspect of the present invention is a composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof, or a sleep-inducing agent comprising the composition, for use in treatment of sleep-onset insomnia.

Another aspect of the present invention is a method of treatment of sleep-onset insomnia, the method including administering a composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof to a subject with sleep-onset insomnia.

[0049]

<Food or Beverage>

Another mode of the present invention is a food or drink comprising the composition for enhancing expression of AANAT or the sleep-inducing agent.

[0050]

Examples of the food or drink include, but are not limited to, ordinary foods

and drinks (such as dietary supplements, health supplements, and nutritional adjusted foods), foods with health claims (that is, foods with nutrient function claims, foods for specified health uses, and foods with function claims), supplements, and food additives. Examples of the food or drink also include those fed to animals, such as livestock feed and pet food.

[0051]

Examples of the drink include tea drinks, coffee drinks, milk drinks, fruit juice drinks, carbonated drinks, alcoholic drinks, soft drinks, and soups.

[0052]

Regarding the form of the food, the food may be in the form of a liquid, paste, solid, or the like. Examples of the food include breads, noodles, jelly-like foods, various snacks, baked confectionery, cakes, chocolates, chewing gums, candies, tablets, capsules, dairy products, frozen foods, convenience foods, supplements, other processed foods, seasonings, and materials thereof.

[0053]

Examples of the supplements may include solid supplements such as capsules, soft capsules, powders, granules, and tablets; and liquid supplements such as solutions, syrups, suspensions, and emulsions.

[0054]

The amount of the composition for enhancing expression of AANAT or the sleep-inducing agent added to the food or drink is not limited as long as its effect can be produced.

[0055]

In addition, the food or drink according to the present embodiment may contain components that are normally added during the production of foods and drinks, and examples of such components include proteins, carbohydrates, fats, nutrients, seasonings, flavoring agents, and additives. Examples of the

carbohydrates include ordinary sugars, such as monosaccharides, for example, glucose and fructose; disaccharides, for example, maltose, sucrose, and oligosaccharides; and polysaccharides, for example, dextrin and cyclodextrin; and also include sugar alcohols, such as xylitol, sorbitol, and erythritol.

[0056]

As long as the food or drink according to the present embodiment can produce the effect of the quassinoid compound and the Tongkat Ali extract, there is no particular limitation regarding the subject who takes the food or drink, the amount of the food or drink to be taken, the timing when the food or drink is to be taken, the frequency at which the food or drink is to be taken, and the like. For example, the subject who takes the food or drink is typically a healthy individual, but may also be an individual without a sleep disorder. Regarding the amount of the food or drink to be taken, in cases of a human (adult) with a body weight of about 60 kg, the amount may be set appropriately within the range of 0.001 mg to 10,000 mg per day in terms of the amount of the quassinoid compound and/or the Tongkat Ali extract. The timing when the food or drink is to be taken may be, for example, before, between, or after meals, or before bedtime. The food or drink in the above-described amount may be taken once per day, or may be taken dividedly in a plurality of times per day.

[0057]

Although the food or drink according to the present embodiment may be taken by any subject including healthy individuals and unhealthy individuals, the food or drink is used for non-therapeutic purposes. By the intake of the food or drink, falling asleep can be better achieved, and natural induction of sleep can be promoted (sleep onset can be promoted). More specifically, sleep satisfaction can be improved for people who have sleep-related complaints such as difficulty falling asleep, but whose complaints are not as serious as those requiring dosing of a

sleeping pill and/or a sleeping inducer.

[0058]

Another aspect of the present invention is use of a composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof, or a sleep-inducing agent comprising the composition, in the production of a food or drink for promoting natural induction of sleep.

Another aspect of the present invention is use of a composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof in promoting natural induction of sleep.

Another aspect of the present invention is a method of promoting natural induction of sleep, the method including administering a composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof to a healthy individual in need thereof.

EXAMPLES

[0059]

The invention is described below more specifically based on Examples, but the present invention is not limited to the following Examples.

[0060]

<Materials>

Air-dried powder of root of Tongkat Ali was provided by Phytes Biotek Sdn Bhd (Malaysia).

[0061]

<TLC Analysis>

Thin-layer chromatography (TLC) analysis was performed using precoated silica gel 60 GF254 (20 × 20 cm, 0.2-mm thickness, Merck, Darmstadt, Germany) or a precoated RP-C18 F254 plate (5 × 7.5 cm, 0.2-mm thickness, Merck, Darmstadt, Germany). After allowing development using an appropriate solvent system, the

plate was subjected to coloring reaction, and the resulting chromatogram was measured with UV lights at 254 nm and 366 nm. Spots were visualized by spraying with vanillin/H_2SO_4 reagent before heating at 110°C for 5 minutes.

[0062]

<Isolation of Products>

Preparative high-performance liquid chromatography (preparative HPLC) was performed using a high-pressure gradient system BUCHI Pure C-850 (Switzerland) equipped with UV and ELSD detector and LaboACE LC-5060 Series (Japan Analytical Industry Co., Ltd).

[0063]

<HPLC Analysis>

High-performance liquid chromatography (HPLC) was performed using a high-pressure Agilent 1260 Infinity II LC system (Agilent Technologies, Inc.) equipped with UV and ELSD detectors.

[0064]

<Specific Rotation Analysis>

The specific rotation $[\alpha]_D$ was recorded with a polarimeter P-2200 (JASCO Corporation). The specific rotation of the isolated compound, based on the equation $[\alpha] = (100\alpha) / (lc)$, was measured as a numerical value with no unit. The unit of the concentration c is g/100 mL, and the unit of the path length l is decimeter.

[0065]

<High-Resolution Mass Spectrometry (HR-MS) Analysis>

High-resolution electrospray ionization time-of-flight mass spectrometry (HRESITOFMS) was performed using the 6220 TOF LC/MS system (Agilent Technologies, Inc.), and recorded in the positive ion mode.

[0066]

<Nuclear Magnetic Resonance (NMR) Analysis>

^{1}H NMR, ^{13}C NMR, and 2D NMR (COSY, HMQC, and HMBC) analyses were performed using purified compounds dissolved in chloroform-d_1, methanol-d_4, and pyridine-d_5. All NMR measurement spectra were obtained with JNM-ECS400 (400 MHz, ^{1}H) (JEOL Ltd.) or a DRX-600 spectrometer (600 MHz,^{1}H) (Bruker Daltonics, USA). Chemical shifts (ppm) were referenced to the solvent peak of chloroform-d_1 (δ_H 7.26/δ_C 77.0), methanol-d_4 (δ_H 3.31/δ_C 49.0), or pyridine-d_5 (δ_H 7.22/δ_C 123.9) as an internal standard.

[0067]

<Cell Culture>

Human neuroblastoma SH-SY5Y (ATCC, Manassas, VA, USA) was cultured in Dulbecco's Modified Eagle's Medium (high glucose) (Wako, Osaka, Japan). The medium was supplemented with 10% heat-inactivated fetal bovine serum (FBS, Corning, CA, USA) and 100 U/mL penicillin-streptomycin antibiotic (Wako, Osaka, Japan). The cells were cultured in a humidified 5% CO_2 incubator at 37°C. In order to estimate the AANAT gene expression by real-time qPCR, SH-SY5Y cells (1.0×10^5 cells/mL) were cultured for 24 hours, and then treated with Tongkat Ali or a compound for 24 hours.

[0068]

<RNA Extraction and Real-Time qPCR>

Total RNA was prepared from the cells using the PureLink RNA Mini kit (Invitrogen, MA, USA) according to the manufacturer's instructions. From the total RNA extracted, cDNA was synthesized using ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO, Osaka, Japan). Real-time quantitative PCR was performed using the Agilent AriaMX Real-Time PCR system (Agilent Technologies, CA, USA). THUNDERBIRD SYBR qPCRMix (TOYOBO, Osaka, Japan) was used for the real-time qPCR reaction. Regarding the reaction conditions for the real-time qPCR, the reaction was carried out by initial denaturation at 95°C for 60

seconds followed by 40 cycles of amplification of the cDNA sample (95°C for 15 seconds and 60°C for 60 seconds). Regarding the primers, TGCCAGTGAGTTTCGCTGCCTC (SEQ ID NO:1) and GTCAGGAAGTGCCGGATCTCAT (SEQ ID NO:2) were used for the amplification of AANAT, and GTCTCCTCTGACTTCAACAGCG (SEQ ID NO:3) and ACCACCCTGTTGCTGTAGCCAA (SEQ ID NO:4) were used for the amplification of GAPDH. The expression level of the AANAT gene was analyzed using the AriaMx Real-Time PCR software in the ΔΔCt analysis mode.

[0069]

<Example 1: Identification of Compounds Contained in Tongkat Ali>

[Reagents]

Organic solvents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Silica gel (75 to 120 mesh) and RP-C18 silica (38 to 63 μm) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). For fractionation and purification, the Buchi Reveleris (registered trademark) preparative system equipped with NP-silica gel, a RP-C18 flash column, or a Develosil ODS-UG-5 column (5 μm, 20 × 250 mm, Nomura Chemical Co., Ltd., Japan) was used.

[0070]

[Extraction and Isolation]

One hundred five grams of the provided air-dried powder of root of Tongkat Ali was fractionated with solvents of different polarity, to obtain fractions soluble in *n*-hexane, methylene chloride (DCM), and ethyl acetate (EtOAc). Their weights were as follows: 302 mg, 1.811 g, 1.843 g, and 100 g. The *n*-hexane-soluble fraction was subjected to chromatography using a Buchi Reveleris (registered trademark) Prep system equipped with NP-silica gel with a UV-ELSD detector, and using an *n*-hexane gradient mobile phase. The mobile phase was *n*-hexane: EtOAc (100:0 to 0:100). The fraction eluted with *n*-hexane: EtOAc (40:60) was collected

as pure Compound 8 (3.2 mg). The fraction eluted with *n*-hexane: EtOAc (70:30) was subjected to chromatography using a Buchi Reveleris (registered trademark) Prep system equipped with a preparative Inertsil ODS-3 column (5 μm, 20 × 250 mm, G. L. Science Corporation, Japan). By elution using H_2O: MeOH (60:40 to 0:100), Compound 9 (20 mg), Compound 7 (1 mg), Compound 6 (1 mg), and Compound 5 (1.1 mg) were eluted with H_2O: MeOH (50:50), H_2O: MeOH (27:73), H_2O: MeOH (8:92), and H_2O: MeOH (2:98), respectively. From the DCM-soluble fraction, 11 fractions (D1 to D11) were obtained using a Buchi Reveleris (registered trademark) Prep system equipped with an RP-C_{18} silica set with a UV-ELSD detector, and using the gradient mobile phase of water: MeOH (95:5 to 0:100). Fractions D1 and D2 were shown to be pure compounds, and these were designated Compound 10 (20 mg) and Compound 11 (10.8 mg), respectively. Fraction D5 was subjected to chromatography using a Buchi Reveleris (registered trademark) Prep system equipped with an RP-C_{18} silica set with a UV-ELSD detector, and using the gradient mobile phase of water: MeOH (95:5 to 60:40), to obtain Compound 4 (2 mg). The EtOAc-soluble fraction was applied to a silica flash column (FlashPure ID HP 20-μm particles, 12 g), and elution was performed with *n*-hexane: EtOAc (2:1), EtOAc, EtOAc: MeOH (9:1), EtOAc: MeOH (4:1), and MeOH, to obtain five fractions (Fr. 1 to Fr. 5). Fr. 3 (77.3 mg) was separated by semi-preparative HPLC (GL Sciences Inc.; 20 × 250 mm; particle size, 5 μm; UV detector, 205 nm; flow rate, 8 mL/min). Using an ACN/H_2O gradient (20:80 to 100:0) as the mobile phase, Compound 3 (21.3 mg, t_R = 30 min) was obtained. Fr. 4 (70 mg) was separated by semi-preparative HPLC (GL Sciences Inc.; 20 × 250 mm; particle size, 5 μm; UV detector, 205 nm; flow rate, 8 mL/min), to obtain Compound 1 (1.1 mg, t_R = 38 min) and Compound 2 (2.2 mg, t_R = 41.6 min).

[0071]

[HPLC]

HPLC chromatograms of the three quassinoid compounds (Compounds 1 to 3) are shown in Fig. 1(a). The three isolated compounds were further analyzed by HPLC. As shown in Fig. 1(a), three peaks were found at 11.57 min, 12.89 min, and 16.29 min, which corresponded to the following quassinoid compounds: eurycomanone (Compound 1), eurycomanol (Compound 2), and 14,15β-dihydroxyklaineanone (Compound 3), respectively. Each compound had a purity higher than 98%.

[0072]

[HRMS]

Electrospray ionization (ESI) is an ion generation technique used in mass spectrometry (MS). In positive mode ESI^+, corresponding ions such as $[M+H]^+$ and $[M+Na]^+$ are often found. Theoretically, the exact $[M+H]^+$ molecular weights of eurycomanone (Compound 1), eurycomanol (Compound 2), and 14,15β-dihydroxyklaineanone (Compound 3) are m/z 409.1512, 411.1640, and 397.1867, respectively. In high-resolution mass spectrometry (HRMS), a peak at m/z 409.1514 (Δ0.2 mmu) in HR-ESIMS, which corresponds to $[M+H]^+$ of Compound 1, and peaks at m/z 411.1647 (Δ0.7 mmu) and m/z 397.1862 (Δ0.5 mmu), which correspond to $[M+H]^+$ of Compound 2 and Compound 3, were found (Fig. 1(b)). Thus, the HRMS results suggested that the three isolated compounds are eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone.

[0073]

[NMR]

From the ion peak at m/z 409.1514 $[M+H]^+$ found in HR-ESIMS, Compound 1 was obtained as a colorless needle-like product having the molecular formula $C_{20}H_{24}O_9$. The ^1H and ^{13}C NMR spectra of Compound 1 indicated the presence of two methyl groups (δ_H 1.62 and 1.77, δ_C 10.8 and 22.8), two methines (δ_H 3.26, δ_C 42.6) and (δ_H 3.83, δ_C 48.1), methylene (δ_H 2.21 and 2.32, δ_C 26.1), one

oxymethylene (δ_H 4.01 and 4.55, δ_C 68.1), four oxymethines (δ_H 4.53, 4.81, 5.26, 5.67, δ_C 84.9, 81.4, 72.2, 76.2), trisubstituted double bond (δ_H 6.15, δ_C 126.4, 162.9), 1,1-disubstituted double bond (δ_H 5.65, 6.12, δ_C 148.5, 119.7), ketone carbonyl (δ_C 197.9), ester carbonyl (δ_C 174.3), two oxygenated quaternary carbons (δ_C 79.8, 109.9), and two quaternary sp^3 carbons (δ_C 46.3, 53.0), suggesting a C_{20} quassinoid skeleton (Fig. 2 and Table 1). Based on comparison with values described in published literature (Non-Patent Document 2), Compound 1 was identified as eurycomanone, which is a C_{20} quassinoid. The stereochemistry of Compound 1 was also determined based on the later-described measurement of optical rotation. Colorless needle-like compound; $[\alpha]_D^{20}$ = +37.8 (c 0.3, MeOH); ^1H and ^{13}C NMR were as shown in Table 1. HR-ESI-MS: m/z 409.1512 $[M+H]^+$ (calcd. for $C_{20}H_{25}O_9$, 409.1499). NMR data are shown in Table 1.

[0074]

[Table 1]

Table 1: ^1H and ^{13}C NMR spectral data of Compounds 1 to 3.

No.	Compound 1		Compound 2		Compound 3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	84.9 CH	4.53, s	84.1, CH	4.07, d (8.2)	82.6, CH	4.04, s
2	197.9, C	—	73.1, CH	4.66, m	198.2, C	—
3	126.4, CH	6.15, brs	127.4, CH	5.82, brs	124.3, CH	6.09, brs
4	162.9, C	—	135.4, C	—	164.5, C	—
5	42.6, CH	3.26, brd (12.8)	42.0, CH	2.85, brd (12.4)	43.6, CH	2.87, brd (12.8)
6	26.1, CH_2	2.21, ddd (14.8, 12.8, 2.8) 2.32, dt (14.8, 2.8)	26.0, CH_2	1.93, brt (14.4) 2.20, brd (14.4)	26.1, CH_2	2.10, m 2.30, td (3.2, 14.8)
7	72.2, CH	5.26, t (2.8)	72.3, CH	5.19, brs	81.5, CH	4.63, t (3.2)
8	53.0, C	—	53.1, C	—	48.2, C	—
9	48.1, CH	3.83, s	48.3, CH	3.58, s	45.0, CH	2.11, d (3.2)
10	46.3, C	—	42.7, C	—	43.0, C	—
11	109.9, C	—	110.2, C	—	73.6, CH	4.88, brs
12	81.4, CH	4.81, s	81.5, CH	4.83, s	77.0, CH	3.91, t (2.8)
13	148.5, C	—	149.0, C	—	35.8, CH	2.43, dq (2.8, 7.3)
14	79.8, C	—	79.8, C	—	75.8, C	—

15	76.2, CH	5.67, s	76.9, CH	5.55, s	71.3, CH	5.33, s
16	174.3, C	—	174.3, C	—	175.9, C	—
18	22.8, CH_3	1.77, s (1H)	21.7, CH_3	1.64, brs	22.8, CH_3	1.97, s
19	10.8, CH_3	1.62, s (1H)	11.3, CH_3	1.76, s	12.4, CH_3	1.18, s
20	68.1, CH_2	4.01, d (8.8) 4.55, d (8.8)	68.3, CH_2	4.07, d (8.2) 4.58, d (8.2)	17.4, CH_3	1.51, s
21	119.7, CH_2	5.65, d (1.6) 6.12, d (1.6)	119.7, CH_2	5.66, d (1.6) 6.13, d (1.6)	12.5, CH_3	1.22, d (7.2)

δ is shown in ppm, and J is shown in Hz. Duplicate signals are shown without specifying the multiplicity.

Compounds 1 and 2 were measured in C_5D_5N. 400 MHz for ^1H NMR, 100 MHz for ^{13}C NMR.

Compounds 3 was measured in $CDCl_3$. 400 MHz for ^1H NMR, 100 MHz for ^{13}C NMR.

[0075]

The ^1H NMR spectral data of Compound 2 were similar to those of Compound 1. Comparison of chemical shifts reported in published literature (Non-Patent Documents 2 and 3) showed that Compound 1 and Compound 2 were identical to those of eurycomanone and eurycomanol, respectively (Fig. 3). Eurycomanol (Compound 2): colorless needle-like compound; $[\alpha]_D^{20}$ = +83.9 (c 0.04, pyridine); ^1H and ^{13}C NMR were as shown in Table 1. HR-ESI-MS: m/z 411.1640 $[M+H]^+$ (calcd. for $C_{20}H_{27}O_9$, 411.1655). NMR data are shown in Table 1.

[0076]

Compound 3 was obtained as a colorless needle-like compound having the molecular formula $C_{20}H_{29}O_8$ as determined from the ion peak at m/z 397.1867 $[M+H]^+$ found in HR-ESIMS. The ^1H NMR spectrum of Compound 3 showed four methyl groups (δ_H 1.18 (3H, s), 1.22 (3H, d, J = 7.2 Hz); 1.51 (3H, s), 1.97 (3H, s)), two oxygenated methylene protons (δ_H 3.91 (1H, t, J = 2.8 Hz) and 4.04 (1H, s)), one oxygenated methine proton (δ_H 5.33 (1H, s)), and a trisubstituted double bond proton (δ_H 6.09 (1H, br s)), suggesting structural similarity to the C_{20} quassinoid

skeleton. Based on comparison with values described in published literature (Non-Patent Document 2), Compound 3 was identified as 14,15β-dihydroxyklaineanone, which is a C_{20} quassinoid (Fig. 3). Compound 3: $[\alpha]_D^{20}$ = +53.2 (c 0.5, MeOH); ^1H and ^{13}C NMR were as shown in Table 1. HR-ESI-MS: m/z 397.1867 $[M+H]^+$ (calcd. for $C_{20}H_{29}O_8$, 397.1862). NMR data are shown in Table 1.

[0077]

[Specific Rotation]

Compounds 1 to 3 were subjected to measurement of the specific rotation. The measurements were as follows: Compound 1, $[\alpha]_D^{20}$ = +37.8 (c 0.3, MeOH); Compound 2, $[\alpha]_D^{20}$ = +83.9 (c 0.04, C_5H_5N); and Compound 3, $[\alpha]_D^{20}$ = +53.2 (c 0.5, MeOH). These results are consistent with values reported in published literature (Non-Patent Documents 2 and 3). From the results on the specific rotation, the stereochemistries of Compounds 1 to 3 were determined as eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone.

[0078]

Regarding Compounds 4 to 11, which are other compounds contained in the Tongkat Ali extract, comparison of their ^1H and ^{13}C NMR data, HRMS data, and specific rotation with known data revealed that a furanoid lignan, a triterpenoid, a squalene-type derivative, two β-carboline alkaloids, and three heterocyclic compounds were (+)-syringaresinol (Compound 4, Non-Patent Documents 4 to 6), bourjotinolone A (Compound 5, Non-Patent Documents 7 to 9), eurylene (Compound 6, Non-Patent Documents 10 and 11), canthin-6-one (Compound 7, Non-Patent Documents 12 to 14), 9-methoxycanthin-6-one (Compound 8, Non-Patent Documents 15 to 17), vanillin (Compound 9, Non-Patent Document 18), 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (Compound 10, Non-Patent Document 19), and 5-(hydroxymethyl)furfural (Compound 11, Non-Patent Documents 20 and 21).

[0079]

<Example 2: Expression Activity of Enzyme Gene Related to Melatonin Biosynthesis in Tongkat Ali Extract>

Melatonin (N-acetyl-5-methoxytryptamine), also called as “sleep hormone,” is known to play a role in decreasing the pulse rate, the body temperature, the blood pressure, and the like, and informing that the body is ready for sleep, to allow sleep onset. Melatonin has a function to switch between wakefulness and sleep such that natural sleep is induced. In addition to the sleep-inducing action, melatonin is known to have an antioxidant action that promotes cellular metabolism, which provides a variety of effects in prevention of diseases and aging. In the pineal body, melatonin is biosynthesized through four enzymatic reactions, which start from tryptophan and proceed to 5-hydroxytryptophan, 5-hydroxytryptamine (serotonin), *N*-acetylserotonin, and then melatonin. Serotonin is acetylated by the first enzyme in the melatonin synthesis, AANAT (arylalkylamine *N*-acetyltransferase), to become *N*-acetylserotonin. By the action of HIOMT (hydroxyindole Omethyltransferase), a methyl group is transferred from *S*-adenosylmethionine to the *N*-acetylserotonin, resulting in synthesis of melatonin (Non-Patent Document 22).

In the present Example, the effect of the Tongkat Ali extract on the melatonin biosynthesis was investigated by investigating the expression level of AANAT, a rate-limiting enzyme in the melatonin biosynthesis. As a result of allowing the Tongkat Ali extract to act on the SH-SY5Y cells, a 1.6-fold increase in the AANAT gene expression was found compared to a control, indicating a significant increase in the expression. The result suggests that a Tongkat Ali extract upregulates the AANAT gene expression to increase the melatonin biosynthesis, thereby causing sleep induction (Fig. 4).

[0080]

<Example 3: AANAT Expression-Inducing Activities of Quassinoid Compounds>

In order to study whether the three quassinoid compounds (Compounds 1 to 3) contained in the Tongkat Ali extract promote the melatonin biosynthesis, the effects of Compounds 1 to 3 on the expression of the AANAT gene were investigated. As a result, eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone were found to significantly increase the expression of the AANAT gene compared to a control (Fig. 5). Therefore, the enhancement of expression of the AANAT gene by the Tongkat Ali extract was found to be due to eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone, which are quassinoid compounds contained in the Tongkat Ali extract. It is thus suggested that eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone upregulate the AANAT gene expression to increase the melatonin biosynthesis, to thereby cause sleep induction.

SEQUENCE LISTING

CLAIMS

[Claim 1] A composition for enhancing expression of AANAT, the composition comprising a quassinoid compound or a pharmaceutically acceptable salt thereof.

[Claim 2] The composition for enhancing expression of AANAT according to claim 1, wherein the quassinoid compound is at least one compound selected from the group consisting of eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone.

[Claim 3] The composition for enhancing expression of AANAT according to claim 1, wherein the quassinoid compound is eurycomanone, eurycomanol, and 14,15β-dihydroxyklaineanone.

[Claim 4] The composition for enhancing expression of AANAT according to claim 1, wherein the quassinoid compound is a compound originally contained in an extract from root of Tongkat Ali.

[Claim 5] The composition for enhancing expression of AANAT according to claim 1, further comprising at least one compound selected from the group consisting of (+)-syringaresinol, bourjotinolone A, eurylene, canthin-6-one, 9-methoxycanthin-6-one, vanillin, 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one, and 5-(hydroxymethyl)furfural.

[Claim 6] A composition for enhancing expression of AANAT, the composition comprising an extract from root of Tongkat Ali.

[Claim 7] A sleep-inducing agent comprising the composition for enhancing expression of AANAT according to any one of claims 1 to 6.

[Claim 8] A pharmaceutical comprising the composition for enhancing expression of AANAT according to any one of claims 1 to 6, or the sleep-inducing agent according to claim 7.

[Claim 9] A food or drink comprising the composition for enhancing expression of AANAT according to any one of claims 1 to 6, or the sleep-inducing agent

according to claim 7.