

A PLANT EXTRACT COMPRISING STATINS AND PREPARATION TECHNIQUES AND USES THEREOF

Technical Field

The present invention generally relates to plant
5 extracts containing statins. The present invention also
relates to a method of obtaining such an extract and its
use.

Background

10 Herbal supplements are well known and have been used
to supplement traditional dietary and medical regimens.
Typically such supplements are in the form of extracts
which are believed to provide health and medicinal
benefits including the prevention and treatment of
15 disease.

Many plants which are used in the manufacture of
extracts are known to synthesize chemicals that are useful
in the maintenance of health in humans. However, despite
the increase in the use of such extracts the understanding
20 of plant-drug interactions is still in its infancy. This
lack of understanding of such interactions can lead to
adverse reactions which can be life-threatening or, in a
worst case scenario, fatal.

Furthermore, the precise composition of such extracts
25 has not been fully defined and as such many plant extracts
may contain compounds that can adversely affect drug
kinetics in a subject.

For example, a popular plant extract is red yeast
rice. This extract has many uses including food coloring
30 and as an ingredient in rice wine. More recently red
yeast rice extract has been used to lower lipids and
cholesterol levels. However, the known extracts of red
yeast rice contain significant amounts of contaminants.

Accordingly, the removal of one or more of these contaminants would be advantageous in increasing both consumer confidence as to the precise contents of the extract and providing regulatory authorities with accurate data regarding the composition of the extract.

There is a need to provide a plant extract having a reduced amount of contaminants that overcomes, or at least ameliorates, one or more of the disadvantages described above.

There is a need to provide a plant extract having a reduced amount of contaminants having an adverse effect on pharmacokinetics of a drug in a subject.

Summary

According to a first aspect, there is provided a plant extract comprising one or more statins and being substantially free of polar compounds.

According to a second aspect, there is provided a plant extract comprising one or more statins and being substantially free of polar compounds that affect pharmacokinetics of a drug in a subject.

According to a third aspect, there is provided a method of preparing an extract comprising the step of removing polar compounds from a plant material wherein said plant material contains one or more statins.

According to a fourth aspect, there is provided a method of preparing a plant extract comprising the step of removing one or more polar compounds that affect pharmacokinetics of a drug in a subject from said plant extract.

Advantageously, the removal of one or more polar compounds from the plant extract substantially reduces adverse or unwanted drug-herb interactions.

It is a further advantage of the method of the disclosure to provide a consistent effective amount of one or more statins in the plant extract.

According to a fifth aspect, there is provided a
5 composition comprising a plant extract according to the first aspect or the second aspect, together with a pharmaceutically acceptable carrier.

According to a sixth aspect, there is provided a dosage form comprising a plant extract according to the
10 first aspect or the second aspect, together with a pharmaceutically acceptable carrier.

According to a seventh aspect, there is provided use of a plant extract according to the first aspect or the second aspect in the manufacture of a medicament for the
15 treatment or prevention of hyperlipidemia.

According to an eighth aspect, there is provided use of a plant extract according to the first aspect or the second aspect in the manufacture of a medicament for the reduction of cholesterol in a subject.

According to a ninth aspect, there is provided a
20 packaged dosage form comprising a plant extract according to the first aspect or the second aspect together with instructions for use.

According to a tenth aspect, there is provided use of
25 a plant extract according to the first aspect or the second aspect in the manufacture of a medicament for the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in a subject.

According to an eleventh aspect, there is provided a
30 method of preparing a plant extract comprising, in sequence, the steps of:

removing one or more toxins from a plant material;
removing one or more polar compounds from the plant material;

forming an extract from the plant material.

Definitions

5 The following are some definitions that may be helpful in understanding the description of the present invention. These are intended as general definitions and should in no way limit the scope of the present invention to those terms alone, but are put forth for a better understanding of the following description.

10 Unless the context requires otherwise or specifically stated to the contrary, integers, steps, or elements of the invention recited herein as singular integers, steps or elements clearly encompass both singular and plural forms of the recited integers, steps or elements.

15 Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers, but not the exclusion of any other step or element or integer or group
20 of elements or integers. Thus, in the context of this specification, the term "comprising" means "including principally, but not necessarily solely".

Those skilled in the art will appreciate that the
25 invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and
30 compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The term "pharmacokinetics" is to be interpreted broadly to include the action of administered drugs within

the human or animal body. In particular this term refers to drug absorption, distribution, metabolism, and excretion; onset of action; duration of effect; biotransformation; effects and routes of excretion of the metabolites of the drug.

The terms "polar compound", "polar compounds" and grammatical variations thereof, in the context of this specification, refers to compounds, typically organic compounds, in the plant extract which, as a whole, have a non-zero dipole moment.

The term "naturally occurring" in the context of this specification, refer to compounds, such as statins, which occur naturally in a plant material.

The term "plant material" is to be construed broadly to include all members of the Plantae kingdom, Fungi kingdom and algae. The plant material may comprise a solid mass or an extract solution of the plant material.

In the context of this disclosure the term "administering" and variations of that term including "administer" and "administration", includes contacting, applying, delivering or providing a compound or composition of the invention to an organism, or a surface by any appropriate means.

In the context of this disclosure, the term "treatment", refers to any and all uses which remedy a disease state or symptoms, prevent the establishment of disease, or otherwise prevent, hinder, retard, or reverse the progression of disease or other undesirable symptoms in any way whatsoever.

In the context of this specification the terms "therapeutically effective amount" includes a sufficient but non-toxic amount of a compound or composition of the disclosure to provide the desired therapeutic effect. The exact amount required will vary from subject to subject

depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered, the mode of administration, and so forth.

5 Thus, it is not possible to specify an exact "effective amount". However, for any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

In the context of this specification, the term
10 "subject" includes humans and individuals of any species of social, economic or research importance including but not limited to members of the genus ovine, bovine, equine, porcine, feline, canine, primates (including human and non-human primates), rodents, murine, caprine, leporine,
15 and avian.

"Dosage form" as used herein refers to physically discrete units suited as unitary dosages for the individual to be treated; each unit containing a predetermined quantity of plant extract is calculated to
20 produce the desired therapeutic effect in association with the required pharmaceutical carrier. The plant extract may be formulated for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in an acceptable dosage unit. In the
25 case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The language "pharmaceutically acceptable carrier" is
30 intended to include solvents, dispersion media, coatings, anti-bacterial and anti-fungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional

media or agent is incompatible with the compound, use thereof in the therapeutic compositions and methods of treatment and prophylaxis is contemplated

5 The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Alternatively, the composition may still contain from 1-10% of Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

10 As used herein, the term "about", in the context of concentrations of components of the formulations, typically means $\pm 5\%$ of the stated value, more typically $\pm 4\%$ of the stated value, more typically $\pm 3\%$ of the stated value, more typically, $\pm 2\%$ of the stated value, 15 even more typically $\pm 1\%$ of the stated value, and even more typically $\pm 0.5\%$ of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for 20 convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within 25 that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for 30 example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Certain embodiments may also be described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure

also form part of the disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material
5 is specifically recited herein.

Disclosure of Optional Embodiments

Exemplary, non-limiting embodiments of a plant extract comprising one or more statins, will now be
10 disclosed.

The plant extract is substantially free of polar compounds that affect pharmacokinetics of a drug in a subject.

In one embodiment the one or more statins are
15 naturally occurring statins and may be selected from the group consisting of lovastatin, simvastatin, pravastatin and mevastatin.

Preferably, the one or more lovastatins are selected from one or more monacolins. In a preferred embodiment the one
20 or more monacolins are selected from the group consisting of monacolin K, monacolin J, monacolin L and monacolin M. Preferably, the one or more monacolins are selected from monacolin K and monacolin J. In a most preferred embodiment the monacolin is monacolin K.

25
In another embodiment the monacolin K is present in the plant extract in an amount in the range selected from the group consisting of about 0.01-500mg; about 0.1-400mg; about 0.2-300mg; about 0.2-200mg; about 0.2-100mg; about
30 0.2-50mg; about 0.2-25mg; about 0.2-10mg; about 0.2-5mg and about 0.2-2mg per gram of plant extract. Preferably, the monacolin K is present in the plant extract in an amount of from 0.2mg-1mg per gram of extract.

In another embodiment, the plant extract is a product of microbial fermentation. Preferably, the microorganism used in the microbial fermentation is *Monascus purpureus*.

5 Alternatively, the microorganism used in the microbial fermentation is selected from *Monascus ruber* and *Monascus pilosus*.

In one embodiment the plant extract is extracted from a plant belonging to the family Poaceae.

10 In a preferred embodiment the plant is a cereal selected from the group consisting of maize, rice, wheat, barley, sorghum, millet, oat, rye, triticales and buckwheat.

Alternatively, the plant is selected from the family Fabaceae. In a preferred embodiment the plant is a bean selected from the genus *Phaseolus*. Preferably, the bean may be selected from the group consisting of *P. vulgaris*, *P. filiformis*, *P. coccineus*, *P. lunatus*, *P. maculatus* and *P. acutifolius*.

20 In a further preferred embodiment, the cereal is rice. Preferably, the rice is red yeast rice.

In another embodiment, the plant extract is extracted from a fungi. Preferably, the fungi is a *pleurotus* fungi. Preferably, the *pleurotus* fungi is *Pleurotus ostreatus*.

25 In one embodiment, at least 50% of the polar compounds are removed from said extract. Preferably, at least 60%, or at least 70%, or at least 80%, or at least 90% of the polar compounds are removed from said extract. Most preferably, at least 95% of the polar compounds are removed from said extract. Alternatively, the plant
30 extract may have as much as 0.26g of polar compounds removed per gram of plant extract.

In one embodiment, the plant extract has less than 30% by weight polar compounds, preferably less than 25% by

weight polar compounds, preferably less than 20% by weight
polar compounds, preferably less than 15% by weight polar
compounds, preferably less than 10% by weight polar
compounds, preferably less than 5% by weight polar
5 compounds, preferably less than 4% by weight polar
compounds, preferably less than 3% by weight polar
compounds, preferably less than 2% by weight polar
compounds, preferably less than 1% by weight polar
compounds, preferably less than 0.5% by weight polar
10 compounds.

There is also provided a method of preparing an
extract comprising the step of removing polar compounds
from a plant material wherein said plant material contains
one or more statins.

15 There is also provided a method of preparing an
extract comprising the step of removing one or more polar
compounds that affect pharmacokinetics of a drug in a
subject from a plant material containing one or more
statins.

20 In one embodiment the plant material is solid plant
matter.

In another embodiment, the size of the plant material
is selected from the range consisting of about 0.1cm-1cm;
about 0.2-0.9cm and 0.3-0.9cm. Preferably, the size of
25 the plant material is 0.3-0.9cm.

In one embodiment the plant material may be ground
prior to preparing the extract.

Preferably the particle size of the ground plant
material is selected from the range consisting of about
30 0.1mm-2mm; about 0.2mm-1mm; about 0.3mm-1mm; about 0.4mm-
1mm and about 0.5-1mm. Preferably, the particle size of
the ground plant material is 0.5-1mm.

In an optional embodiment, the ground plant material
may be sonicated prior to preparation of the extract.

In one embodiment, the method further comprises the step of removing one or more toxins, including citrinin and tannins, from the plant material prior to the step of removing said one or more polar compounds.

5 In one embodiment, the one or more toxins are removed by exposing the plant material to a solvent selected to dissolve said one or more toxins. In one embodiment, the solvent for dissolving said one or more toxins is slightly acidic, that is the pH of the solvent for dissolving said
10 one or more toxins is between about 5 to less than 7, more preferably about 6 to less than 7, yet more preferably about 6.5 to less than 7. Preferably, the solvent comprises a salt such as an organic salt of an alkaline metal. Exemplary salts may be selected from the group
15 consisting of sodium phosphate, sodium acetate and sodium bicarbonate. Furthermore, an organic acid may also be present in said solvent for dissolving said one or more toxins.

The organic acid is preferably a carboxylic acid.
20 Most preferably the carboxylic acid is acetic acid.

Suitable concentrations of the salt solution are selected from the group consisting of 0.05M, 0.1M, 0.15M and 0.2M. Preferably, the salt solution comprises 0.1M sodium acetate. The acetic acid is preferably a 0.5%
25 solution.

In a preferred embodiment the amount of salt solution used is in the range selected from the group consisting of 0.5ml/g; 1ml/g; 1.5ml/g; 2ml/g; 2.5ml/g; 3ml/g; 3.5ml/g; 4ml/g; 4.5ml/g; 5ml/g; 5.5ml/g; 6ml/g; 6.5ml/g; 7ml/g;
30 7.5ml/g; 8ml/g; 8.5ml/g; 9ml/g; 9.5ml/g and 10ml/g of plant material. Preferably, the amount of salt solution used is 4ml/g of plant material.

In one embodiment the step of exposing the plant material to a solution comprising one or more of a salt

and an organic acid is undertaken for about 8-36 hours at a temperature of 20-60°C. Most preferably, the plant material is exposed for a period selected from the group consisting of about 8-34 hours, about 10-32 hours, about 12-30 hours, about 14-28 hours, about 16-26 hours, about 18-26 hours, about 20-26 hours and about 22-26 hours. In a most preferred embodiment the plant material is exposed to the solution comprising one or more of a salt and an organic acid for 12 hours.

10 In another embodiment the plant material is exposed to the solution comprising one or more of a salt and an organic acid at a temperature of 20-60°C. Preferably the plant material is exposed at a temperature selected from the group consisting of 21-55°C, 22-50°C, 23-45°C, 24-40°C 15 25-35°C, 25-30°C. In a most preferred embodiment the plant material is exposed to the solution comprising one or more of a salt and an organic acid at a temperature of 23 °C for 12 hours.

Preferably, the extract has less than 500µg, more 20 preferably, less than 450µg, more preferably less than 400µg, more preferably less than 350, more preferably less than more preferably less than 300µg, more preferably less than 250µg more preferably less than 200µg, more preferably less than 150µg, more preferably less than 25 100µg of citrinin per gram of red yeast rice extract. In a most preferred embodiment the extract has a level of citrinin between 332µg and 133µg of citrinin per gram of the red yeast rice extract.

In one embodiment, the plant material, once the one 30 or more toxins have been removed, is exposed to an aqueous solution for about 8-36 hours at a temperature of 20-60°C to remove one or more polar compounds. Most preferably, the plant material is exposed to the aqueous solution for

a period selected from the group consisting of about 8-34 hours, about 10-32 hours, about 12-30 hours, about 14-28 hours, about 16-26 hours, about 18-26 hours, about 20-26 hours and about 22-26 hours. In a most preferred embodiment the plant material is exposed to the aqueous solution for 12 hours.

In another embodiment the plant material is exposed to an aqueous solution at a temperature of 20-60°C. Preferably the plant material is exposed to the aqueous solution at a temperature selected from the group consisting of 21-55°C, 22-50°C, 23-45°C, 24-40°C, 25-35°C, 25-30°C. In a most preferred embodiment the plant material is exposed to the aqueous solution at a temperature of 23°C for 12 hours.

In a preferred embodiment the amount of aqueous solution used is in the range selected from the group consisting of 0.5ml/g; 1ml/g; 1.5ml/g; 2ml/g; 2.5ml/g; 3ml/g; 3.5ml/g; 4ml/g; 4.5ml/g; 5ml/g; 5.5ml/g; 6ml/g; 6.5ml/g; 7ml/g; 7.5ml/g; 8ml/g; 8.5ml/g; 9ml/g; 9.5ml/g and 10ml/g of plant material. Preferably, the amount of aqueous solution used is 4ml/g of plant material.

In a most preferred embodiment, the aqueous solution is water.

Advantageously, this step not only removes the polar compounds but also removes any residual salt solution present in the plant material.

Without wishing to be bound by any particular theory it is believed that the one or more polar compounds present in the plant extract interfere with the pharmacokinetics of administered drugs by inhibiting certain enzymes in a subject. It is believed that these enzymes include, but are not limited to, cytochrome P450, including subtypes 1A2, 2C9 and 3A4; and p-glycoprotein. P-glycoprotein is an ATP-dependent efflux pump with broad

substrate specificity. P-glycoprotein transports various substrates across the cell membrane including drugs such as colchicine, tacrolimus and quinidine; chemotherapeutic agents such as etoposide, doxorubicin, and vinblastine; 5 lipids, steroids, xenobiotics, peptides, bilirubin, cardiac glycosides like digoxin, immunosuppressive agents, glucocorticoids like dexamethasone and HIV-type 1 antiretroviral therapy agents like protease inhibitors and non-nucleoside reverse transcriptase inhibitors. Thus, 10 removal of the polar compounds reduces the incidence of adverse drug-herb interactions.

Accordingly, in a preferred embodiment, the one or more polar compounds affect the pharmacokinetics of an administered drug by interacting with cytochrome P450 and 15 p-glycoprotein.

Without wishing to be bound by any particular theory, it is believed that the polar compounds affect the pharmacokinetics of an administered drug by binding to cytochrome P450 and/or p-glycoprotein.

20 In another embodiment, the method further comprises the step of forming a liquid extract from said solid plant material.

Preferably the plant material, once the one or more polar compounds have been removed, is exposed to an 25 organic solution for about 8-36 hours at a temperature of 20-50°C. Most preferably, the plant material is exposed to the organic solution for a period selected from the group consisting of about 8-34 hours, about 10-32 hours, about 12-30 hours, about 14-28 hours, about 16-26 hours, 30 about 18-26 hours, about 20-26 hours and about 22-26 hours. In a most preferred embodiment the plant material is exposed to the organic solution for 12 hours.

In another embodiment the plant material is exposed to the organic solution at a temperature of 20-50°C. Preferably the plant material is exposed to the organic solution at a temperature selected from the group consisting of 21-50°C, 22-45°C, 23-40°C, 24-35°C, 25-30°C. In a most preferred embodiment the plant material is exposed to the organic solution at a temperature of 25 °C for 12 hours.

In a preferred embodiment the amount of organic solution used is in the range selected from the group consisting of 0.5ml/g; 1ml/g; 1.5ml/g; 2ml/g; 2.5ml/g; 3ml/g; 3.5ml/g; 4ml/g; 4.5ml/g; 5ml/g; 5.5ml/g; 6ml/g; 6.5ml/g; 7ml/g; 7.5ml/g; 8ml/g; 8.5ml/g; 9ml/g; 9.5ml/g and 10ml/g of plant material. Preferably, the amount of organic solution used is 4ml/g of plant material.

In one embodiment the organic solution is selected from the group consisting of alcohols, ethers, haloforms, ketones and alkylene glycol. Preferably the organic solution comprises an organic compound that is miscible with water. The organic solution may comprise at least 50% organic compound in water. Preferably, the organic solution comprises at least 70% organic compound in water. In a preferred embodiment, the organic solution is an alcohol. Most preferably the alcohol is 70% ethanol.

In a further preferred embodiment, the method comprises the step of separating the plant material from the liquid extract and evaporating the solvent of the liquid extract to form a dried plant extract.

Preferably, the extraction solution is evaporated using a rotary evaporator.

Agitation may be preferably employed to increase the efficiency of the extraction method. Agitation may be performed at one of more of the steps of the extraction

method. Preferably, the agitation is provided by sonication.

In another embodiment there is provided a composition comprising a plant extract according to the disclosure,
5 together with a pharmaceutically acceptable carrier.

In yet another embodiment there is provided a dosage form comprising a plant extract according to the disclosure, together with a pharmaceutically acceptable carrier.

10 In accordance with the present disclosure, the plant extract may be administered alone. Alternatively, the extract may be administered as a pharmaceutical, nutraceutical, veterinarial, agricultural, or industrial formulation which comprises at least one plant extract
15 according to the invention.

The plant extract may be used in combination with other known treatments, including antifungal treatments, antibiotics, chemotherapeutic agents, etc. Suitable agents are listed, for example, in the Merck Index, An
20 *Encyclopaedia of Chemicals, Drugs and Biologicals*, 12th Ed., 1996, the entire contents of which are incorporated herein by reference.

Combinations of active agents, including plant extracts of the invention, may be synergistic.

25 Convenient modes of administration include oral administration, inhalation, transdermal application, topical creams or gels or powders, or rectal administration. Preferably, the extract is administered orally.

30 Depending on the route of administration, the formulation and/or extract may be coated with a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the therapeutic activity of the compound. Such coatings, for

example enteric coatings, are well known to those of skill in the art.

Dispersions of the plant extract may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, pharmaceutical preparations may contain a preservative to prevent the growth of microorganisms.

In one embodiment, the plant extract may be administered orally, for example, with an inert diluent or an assimilable edible carrier. The plant extract and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into an individual's diet. For oral therapeutic administration, the plant extract may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Suitably, such compositions and preparations may contain at least 1% by weight of the plant extract. Alternatively, the compositions and preparations of the plant extract may, of course, be varied and, for example, may conveniently range from about 2% to about 90%, about 5% to about 80%, about 10% to about 75%, about 15% to about 65%; about 20% to about 60%, about 25% to about 50%, about 30% to about 45%, or about 35% to about 45%, of the weight of the dosage unit. The amount of plant extract in therapeutically useful compositions is such that a suitable dosage will be obtained.

Supplementary active compounds may also be incorporated into the compositions according to the present invention.

In one embodiment, the carrier may be an orally administrable carrier.

Another form of a pharmaceutical composition is a dosage form formulated as enterically coated granules, tablets or capsules suitable for oral administration.

Also included in the scope of this invention are
5 delayed release formulations.

Tablets, troches, pills, capsules and the like can also contain the following: a binder such as gum gragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such
10 as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a
15 capsule, it can contain, in addition to materials of the above type, a liquid carrier. Various other materials can be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules can be coated with shellac, sugar or both. A
20 syrup or elixir can contain the analogue, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and
25 substantially non-toxic in the amounts employed. In addition, the analogue can be incorporated into sustained-release preparations and formulations.

Preferably, the pharmaceutical composition may further include a suitable buffer to minimise acid
30 hydrolysis. Suitable buffer agent agents are well known to those skilled in the art and include, but are not limited to, phosphates, citrates, carbonates and mixtures thereof.

Single or multiple administrations of the pharmaceutical compositions according to the invention may

be carried out. One skilled in the art would be able, by routine experimentation, to determine effective, non-toxic dosage levels of the plant extract and/or composition and an administration pattern.

5 Further, it will be apparent to one of ordinary skill in the art that the optimal course of treatment, such as the number of doses of the plant extract or composition given per day for a defined number of days, can be ascertained using conventional course of treatment
10 determination tests.

 Generally, an effective dosage of the plant extract per 24 hours may be in the range of about 0.0001 mg to about 1000 mg per kg body weight; suitably, about 0.001 mg to about 750 mg per kg body weight; about 0.01 mg to about
15 500 mg per kg body weight; about 0.1 mg to about 500 mg per kg body weight; about 0.1 mg to about 250 mg per kg body weight; or about 1.0 mg to about 250 mg per kg body weight. More suitably, an effective dosage per 24 hours may be in the range of about 1.0 mg to about 200 mg per kg
20 body weight; about 1.0 mg to about 100 mg per kg body weight; about 1.0 mg to about 50 mg per kg body weight; about 1.0 mg to about 25 mg per kg body weight; about 5.0 mg to about 50 mg per kg body weight; about 5.0 mg to about 20 mg per kg body weight; or about 5.0 mg to about
25 15 mg per kg body weight.

 There is provided the use of the plant extract according to the disclosure in the manufacture of a medicament for the treatment or prevention of hyperlipidemia.

30 There is provided the use of the plant extract according to the disclosure in the manufacture of a medicament for the reduction of cholesterol in a subject.

There is provided the use of the plant extract according to the disclosure in the manufacture of a medicament for the reduction of cholesterol in a subject.

5 There is provided the use of the plant extract according to the disclosure in the manufacture of a medicament for the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in a subject.

10 There is also provided a packaged dosage form comprising a plant extract according to the disclosure together with instructions for use.

Brief Description of Drawings

15 The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

20 Fig. 1a shows a HPLC profile of a red yeast rice extract before removal of the polar compounds. Fig. 1b is a HPLC profile of the dried red yeast rice extract, after removal of the polar compounds, obtained in accordance with the method of the disclosure;

25 Fig. 2. shows the results of a cytochrome P450 inhibition study using a red yeast rice extract;

Fig. 3. shows the results of the activity of polar compounds on P-glycoprotein with a negative control (NaVO_4);

30 Fig. 4. shows the results of a P-gp activity assay using red yeast rice extract;

Fig. 5 shows the results of a CaCO-2 absorption study with verapamil using red yeast rice extract, and

Fig. 6. shows the results of an animal absorption study using verapamil using red yeast rice extract.

Examples

5 Non-limiting examples of the invention, including the best mode, and a comparative example will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

10

Example 1

Fermentation of red yeast rice (RYR)

Preparation method:

1. Rice (*Oryza sativa* var. Japonica) was soaked in water
15 (4ml/g) for 1 hr at 23°C .
2. The rice was then kept in an oven at 100°C for 2 hours.
3. 5% (w/w) of *M. purpureus* was then added.
4. The rice was then incubated at 24-30°C for 7-14 days with physical stirring every 2 days.

20

Example 2

Extraction method

1. Whole red yeast rice (RYR) was exposed at 23°C to
25 0.1M sodium acetate (4ml/g of red yeast rice) for 12 hrs. This step was used to remove citrinin and other toxins from the RYR. The salt solution was then removed by filtration on filter paper or cheesecloth
2. The RYR extract was then exposed to water (4ml/g of
30 red yeast rice) at 23°C for 12 hours. This step removes the polar compounds and any residual salt solution in the RYR preparation from step 1 above. The salt solution was then removed by filtration on filter paper or cheesecloth.

3. Finally, the last extraction step used 70% ethanol (4ml/g of red yeast rice) at 23°C for 12 hrs to produce an enriched monacolin K RYR extract.

5 Details of final extraction

The citrinin level was 0.3-0.7µg/g of red yeast rice extract.

The percentage of the polar compounds removed by the
10 extraction method is approximately 94.5%. A high performance liquid chromatography (HPLC) chromatograph of the dried extract is shown in Figure 1. The conditions of the HPLC are: 3µm pore size column, two solvents, solvent A .01% formic acid & solvent B 100% acetonitrile; a flow
15 rate of .7 ml/min; gradient flow such that at 0 minutes solvent A is 97%, and at 50 minutes solvent B is 100%. Peaks falling within the area from 0 minutes to 10 minutes would indicate the presence of polar compounds in the dried extract.

20 As can be seen from the HPLC chromatograph in Figure 1a, the RYR material prior to treatment with water contains polar fractions as shown in the area indicated by 165896201.

Figure 1b demonstrates that the RYR after treatment
25 with water has a substantially reduced polar fraction shown by the area indicated by 9534468 (shown as a red line on Figure 1b). The absence of peaks in this area indicates the removal of polar compounds from the R material. This indicates that approximately 95% of the
30 polar compounds have been removed from the RYR material after treatment with water.

Monacolin K (lovastatin) level was 0.5-2mg/g of red yeast rice extract.

Example 3

Pharmacokinetic validation on RYR extract

5 Cytochrome (CYP) P-450 inhibition study

CYP1A2A, CYP2C9 and CYP3A4 activity assays (CYP1A2, CYP2C9 and CYP3A4 P450-Glo™, Promega) employing luminogenic P450 probe substrates that are derivatives of beetle luciferin, a substrate for luciferase enzymes were
10 used. The derivatives are not substrates for luciferase but are converted by CYP to luciferin, which in turn reacts with luciferase to produce a measurable amount of light that is directly proportional to the P450 activity.

The CYP membrane was treated with 20µl of Luciferin-free water (untreated control), naringenin (CYP inhibitor, negative control) or the RYR water extract at different concentrations (1.25mg/ml, 2.5mg/ml and 5mg/ml, drug treated) in a white opaque 96-well plate (Costar). The plate was pre-incubated at 37°C for 10 min and then the
15 reactions were started by addition of NADPH regeneration solution and incubated at 37°C for 20 min. The reactions were then stopped, and detection of luminescence was initiated by adding an ATP Detection Reagent to all wells and the luminescence of all samples was measured by an
20 Infinite F200 plate reader (Tecan).

The luminescence signal observed in the CYP activity assay was directly proportional to the enzyme activity. The net signals from untreated CYP reactions represent total CYP activity. 100µg/ml of naringenin (a CYP
30 inhibitor) was added as negative control for the experiment. Changes from the average net signal of untreated CYP reactions for reactions with the test compound (RYR water extract) demonstrate the modulation of CYP activity by this compound. The luminescence signal

for the testing compound is higher than the untreated samples demonstrating that this compound can enhance CYP activity; the luminescence signal lower than the untreated samples implying this compound could inhibit the activity of CYP. The results (Figure 2) demonstrate that the RYR water extract induces inhibition of CYP1A2, CYP2C9 and CYP3A4. The EYS-NYP product, which is the RYR extract obtained after removal of the polar compounds, demonstrates very low inhibition of CYP activity.

P-glycoprotein (P-gp) activity study

An enzyme kit, Pgp-Glo™ Assay System (Promega, USA) was used to analysis the influence of RYR water extracts on P-gp activity. This assay detects the effects of compounds on recombinant human P-gp in a cell membrane fraction and relies on the ATP dependence of the light-generating reaction of firefly luciferase. ATP is first incubated with P-gp; then the P-gp ATPase reaction is stopped, and the remaining unmetabolized ATP is detected as a luciferase-generated luminescent signal. P-gp-dependent decreases in luminescence reflect ATP consumption by P-gp; thus the greater the decrease in signal, the higher the P-gp activity. Accordingly, samples containing compounds that stimulate the P-gp ATPase will have significantly lower signals than untreated samples. Preparations of P-gp, the reaction mixture and ATP detection reagent were followed according to the manufacturer's instructions. 20µl of RYR water extract was prepared at different concentrations (1.25, 2.5 and 5mg/ml), verapamil (positive control, 0.5mM) and Na₃VO₄ (a P-gp inhibitor, 0.25mM) were added into a white opaque 96-well plate (with clear flat bottom, Costar Inc., NY) respectively. P-gp was then added into wells containing the testing compounds and incubated at 37°C for 5 min.

The reactions were initiated by addition of Mg-ATP solution, the 96-well plate was incubated at 37°C for 40min. The reactions were then stopped, and detection of luminescence was initiated by adding the ATP Detection
5 Reagent to all wells and measuring the resulting luminescence on an Infinite F200 plate reader (Tecan, Austria).

In the P-gp activity assay, the effect of RYR water
10 extract on Pgp ATPase activity was examined by comparing the untreated samples (basal) and samples treated with RYR water extract to a Na_3VO_4 (sodium orthovanadate)-treated control (see Figure 3). Na_3VO_4 is a selective inhibitor of P-gp, and samples treated with Na_3VO_4 have no P-gp ATPase
15 activity. In the absence of Na_3VO_4 , basal and drug-stimulated P-gp ATPase activities can be detected. ATP consumption in the presence of Na_3VO_4 is attributed to minor non-P-gp ATPase activities present in the membrane preparation. The differences in luminescent signal
20 between Na_3VO_4 -treated samples and untreated samples represents the basal P-gp ATPase activity; while the difference in the luminescent signal between Na_3VO_4 -treated samples and samples treated with the test compound (RYR water extract) represents P-gp ATPase activity in the
25 presence of the test compound.

As shown in Figure 4, addition of RYR water extract significantly enhanced P-gp activity and the activation was dose-dependent. These results suggested that RYR water extract contains a P-gp substrate, when it was
30 incubated with the P-gp membrane, ATP was consumed by the P-gp, and a strong change in luminescence when comparing with the Na_3VO_4 -treated samples was observed. The EYS-NYP product shows relatively low activation of P-gp activity, this implies that the compounds in RYR which could

influence P-gp activity were removed effectively by the method.

CaCo-2 absorption assay

5 Flasks which were 90-100% confluent were harvested with trypsin/EDTA, neutralized with serum-containing medium, and centrifuged. The cell pellet was resuspended in a serum-free medium consisting of a Basal Seeding medium (BD Biosciences) and Mito⁺ Serum Extender (BD
10 Biosciences) and seeded onto 6-well Cell Culture Inserts (Corning, NY) at 6×10^5 cells/cm². The inserts contain a polyester 0.4 μ m microporous membrane with collagen type I. The seeding medium was replaced 24 hours after cell seeding with Enterocytes Differentiation medium (BD
15 Biosciences) in experiments, which required use of differentiation medium; the medium was replaced every 48 hours thereafter and the cells were maintained at 37°C, 95% relative humidity, and 5% CO₂. After three days incubation in the Enterocytes Differentiation medium the
20 CaCo-2 monolayers were ready for permeability studies.

Physiologically and morphologically well-developed CaCo-2 cell monolayers with transepithelial electrical resistance (TEER) values greater than 300 Ω cm² were used for the studies.

25 The transport medium used for these studies was modified Han's buffer containing 10 mM HEPES. The pH of both the apical and basolateral compartments was 7.5. Prior to all experiments, each monolayer was washed twice with buffer and TEER was measured to ensure the integrity
30 of the monolayers. The apical to basolateral (A to B) transport of verapamil was measured in the absence and presence of the test compound. The concentration of verapamil used was 100 μ M, which was much below its K_m value of ~60 μ M. The concentration of test compounds was chosen

to be 10 μ M in this assay. The studies were initiated by adding an appropriate volume of buffer containing digoxin to either the apical (A to B transport) or basolateral (B to A transport) side of the monolayer. The volumes of the apical and basolateral compartments were 1.6 and 2.8ml, respectively, and the test compound (as an inhibitor) was added to both sides of the monolayer at a concentration of 10 μ M. The monolayers were then incubated for 3 hours at 37°C. Samples are taken from the basolateral compartment at 0 min, 10 min, 30 min, 60 min, 90 min, 120 min and 180 min; and from the apical compartment at 0 min respectively, during the incubation period and analyzed for concentrations of verapamil by HPLC. The A to B permeability coefficient (P_{eff}) of verapamil was calculated in the presence and absence of the test compound.

After investigation on the effects of RYR water extract on enzyme level (P-gp and CYP assay), CaCo-2 absorption assay was employed to analyze the effect of RYR water extract in cell level. *In vitro* studies with CaCo-2 cell monolayers are valuable tool for predicting human *in vivo* intestinal permeability. In the experiments, 50 μ l of 1xPBS (control) or RYR water extract at various concentrations (20mg/ml and 50mg/ml, drug treated) were added into the apical compartment of the tissue culture plates with verapamil at 100 μ g/ml. The concentrations of the samples taken from the basolateral and apical compartments were calculated from a calibration curve (data not shown) which was constructed by measuring the peak area obtained from injections of verapamil standards (259 μ g/ml, 125 μ g/ml, 30 μ g/ml, 15 μ g/ml, 7.5 μ g/ml and 3 μ g/ml) and the regression analysis linear equation was $y = 27043x - 11153$ ($r^2 = 0.098$). RYR water extract enhance (2-fold) the net absorption of verapamil in the CaCo-2 study. However, the EYS-NYP product (produced in

accordance with the method of the disclosure) shows similar results as the control. This demonstrated that the method was able to remove the polar compounds that effect pharmacokinetics of a drug in a subject in RYR effectively (Figure 5).

Animal Study

Male Sprague-Dawley rats weighting 200-270g were purchased from Animal House of National University of Singapore (NUS, Singapore). Rat care was in accordance with institutional guidelines. The animals were housed in conventional conditions under controlled cycles of darkness/light (12 hr/12 hr) with a regulated temperature (25°C). Eight rats were allocated into two groups (2x4 animals) with four trial runs in each group.

The rats were pretreated with either of 1ml of 100mg/ml of RYR polar extracts (pretreated group) or 1ml of saline (control group) for 30 minutes, and then the animal was given 10mg/kg of verapamil orally. The rats were anesthetized with ketamine & xylazine (both at 100mg/ml). Blood samples were collected from the jugular vein at 0, 30, 60, 120, 240, 360 and 480 minutes after drug administration into a 1.5ml eppendorf tube, and subjected to immediate centrifugation at 13,000rpm for 2 minutes. The clear plasma layers were transferred to a clean tube and then equal volume of acetonitrile was added to remove protein from the samples. After a brief mix with a vortex, the samples were centrifuged at 13,000rpm for 2 minutes; the supernatants were transferred into HPLC vials and then analyzed for their verapamil content by HPLC. The peak area ratio for an unknown sample was converted to concentration by reference to a calibration curve of verapamil constructed with a drug-free pooled rat plasma sample.

Addtion of RYR water extract enhanced absorption of verapamil in the animal model significantly. At the 30 min time point, the level of verapamil in the drug-treated animal showed about a 1.6 fold higher absorption than the control group and the RYR extract which had the polar compounds removed (EYS-NYP sample) (Figure 6).

Summary

The method of the disclosure demonstrates the effective removal of one or more polar compounds which affect the pharmacokinetics of a drug in a subject. In the examples above, it has been demonstrated that the one or more polar compounds significantly increases the uptake of verapamil. Verapamil is an L-type calcium channel blocker of the phenylalkylamine class. It is used in the treatment of hypertension, angina pectoris, cardiac arrhythmia. Verapamil overdose symptoms include: low blood pressure (hypotension); a slow heart rate (bradycardia); an irregular heart rhythm (arrhythmia) and fluid in the lungs.

Accordingly, this unexpected drug-herb interaction is potentially life threatening for subjects taking RYR extract in an attempt to improve cardiovascular health by reducing lipids and cholesterol.

Applications

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Claims

1. A plant extract comprising one or more statins and being substantially free of polar compounds.
2. A plant extract comprising one or more statins and being substantially free of polar compounds that affect pharmacokinetics of a drug in a subject.
3. The plant extract as claimed in claim 1 or claim 2, wherein said one or more statins are naturally occurring statins selected from the group consisting of lovastatin, simvastatin, pravastatin, mevastatin.
4. The plant extract as claimed in any one of the preceding claims, wherein said plant extract is a product of microbial fermentation.
5. The plant extract as claimed in any one of the preceding claims, wherein said plant extract is extracted from a plant belonging to the family Poaceae.
6. The plant extract as claimed in claim 5, wherein the plant is a cereal selected from the group consisting of maize, rice, wheat, barley, sorghum, millet, oat, rye, triticale and buckwheat.
7. The plant extract as claimed in claim 6, wherein the cereal is rice.
8. The plant extract as claimed in claim 7, wherein the rice is red yeast rice.

9. The plant extract as claimed in any one of claims 1 to 4, wherein the plant is selected from the family Fabaceae.
10. The plant extract as claimed in claim 9, wherein the plant is a bean from the genus *Phaseolus*, selected from the group consisting of *P. vulgaris*, *P. filiformis*, *P. coccineus*, *P. lunatus*, *P. maculatus* and *P. acutifolius*.
11. The plant extract as claimed in any one of claims 1 to 4, wherein said plant extract is extracted from a *pleurotus* fungi.
12. The plant extract as claimed in claim 11, wherein said *pleurotus* fungi is *pleurotus ostreatus*.
13. The plant extract as claimed in any one of claims 1-12, wherein the one or more lovastatins are selected from one or more monacolins.
14. The plant extract as claimed in claim 13, wherein the one or more monacolins are selected from the group consisting of monacolin K, monacolin J, monacolin L and monacolin M.
15. A plant extract as claimed in claim 14, wherein the monacolin is monacolin K.
16. A plant extract as claimed in claim 15, wherein the monacolin K is present in the plant extract in an amount of about 0.2mg-1mg per gram of extract.

17. The plant extract as claimed in any one of the preceding claims, wherein at least 80% of the polar compounds are removed from said extract.

18. The plant extract as claimed in claim 17, wherein at least 95% of the polar compounds are removed from said extract.

19. A method of preparing an extract comprising the step of removing polar compounds from a plant material wherein said plant material contains one or more statins.

20. A method of preparing an extract comprising the step of removing one or more polar compounds that affect pharmacokinetics of a drug in a subject from a plant material containing one or more statins.

21. The method as claimed in claim 19 or claim 20, wherein the plant material is solid plant matter.

22. The method as claimed in any one of claims 19-21, further comprising the step of removing one or more toxins from the plant material prior to the step of removing said one or more polar compounds.

23. The method as claimed in claim 22, wherein the one or more toxins are removed by exposing the plant material to a solvent selected to dissolve said one or more toxins.

24. The method as claimed in claim 23, wherein the solvent for dissolving said one or more toxins is slightly acidic.

25. The method as claimed in claim 24, wherein the pH of the solvent for dissolving said one or more toxins is between about 5 to less than 7
26. The method as claimed in claim 25, wherein the solvent comprises a salt of an alkaline metal.
27. The method as claimed in claim 26, wherein the salt is selected from the group consisting of sodium phosphate, sodium acetate and sodium bicarbonate.
28. The method as claimed in any one of claims 23 to 27, wherein an organic acid is also present in said solvent for dissolving said one or more toxins.
29. The method of claim 28, wherein the organic acid is a carboxylic acid.
30. The method as claimed in any one of claims 22 to 29, wherein the toxin removal step comprises the step of removing citrinin.
31. The method as claimed in any one of claims 21 to 30, wherein said method comprises the step of exposing the solid plant material to an aqueous solution to remove said polar compounds.
32. The method as claimed in claim 31, wherein said exposing the solid plant material to an aqueous solution is undertaken for 8-36 hours at a temperature of 20-60°C.
33. The method as claimed in any one of claims 21-32, comprising the step of forming a liquid extract from said solid plant material.

34. The method as claimed in claim 33, wherein the comprising the step of forming the liquid extract comprises the step of exposing the solid plant material to an organic solution.

35. The method as claimed claim 34, wherein the organic solution is selected from the group consisting of alcohols, ethers, haloforms, ketones and alkylene glycol.

36. The method as claimed in claim 34 or claim 35, wherein the organic solution comprises an organic compound that is miscible with water.

37. The method as claimed in any one of claims 34-36, wherein the organic solution comprises at least 50% organic compound in a water solvent.

38. The method as claimed in any one of claims 34-37, wherein the organic solution comprises at least 70% organic compound in a water solvent.

39. The method as claimed in any one of claims 33-38, comprising the step of:

separating said plant material from said liquid extract; and

evaporating the solvent of said liquid extract to form a dried plant extract.

40. The method as claimed in any one of claims 19-39, wherein said one or more statins are naturally occurring statins selected from the group consisting of lovastatin, simvastatin, pravastatin, mevastatin.

41. The method as claimed in any one of claims 19-40, wherein said extract is a product of microbial fermentation.

42. The method as claimed in any one of claims 19-41, wherein said extract is extracted from a plant belonging to the family Poaceae.

43. The method as claimed in claim 42, wherein the plant is a cereal selected from the group consisting of maize, rice, wheat, barley, sorghum, millet, oat, rye, triticale and buckwheat.

44. The method as claimed in claim 43, wherein the cereal is rice.

45. The method as claimed in claim 44, wherein the rice is red yeast rice.

46. The method as claimed in any one of claims 19 to 41, wherein the plant is selected from the family Fabaceae.

47. The plant extract as claimed in claim 46, wherein the plant is a bean from the genus Phaseolus, selected from the group consisting of *P. vulgaris*, *P. filiformis*, *P. coccineus*, *P. lunatus*, *P. maculatus* and *P. acutifolius*.

48. The method as claimed in any one of claims 19 to 41, wherein said extract is extracted from a pleurotus fungi.

49. The method as claimed in claim 48, wherein said pleurotus fungi is *pleurotus ostreatus*.

50. The method as claimed in any one of claims 40-49, wherein the one or more lovastatins are selected from one or more monacolins.

51. The method as claimed in claim 50, wherein the one or more monacolins are selected from the group consisting of monacolin K, monacolin J, monacolin L and monacolin M.

52. The method as claimed in claim 51, wherein the monacolin is monacolin K.

53. A method as claimed in claim 51 or claim 52, wherein the monacolin K is present in the extract in an amount of about 0.2mg-1mg per gram of extract.

54. The method as claimed in any one of claims 19-53, wherein at least 80% of the polar compounds are removed from said extract.

55. The method as claimed in claim 54, wherein at least 95% of the polar compounds are removed from said extract.

56. A composition comprising a plant extract as claimed in any one of claims 1-18, together with a pharmaceutically acceptable carrier.

57. A dosage form comprising a plant extract as claimed in any one of claims 1-18, together with a pharmaceutically acceptable carrier.

58. Use of a plant extract as claimed in any one of claims 1-18 in the manufacture of a medicament for the treatment or prevention of hyperlipidemia.

59. Use of a plant extract as claimed in any one of claims 1-18 in the manufacture of a medicament for the reduction of cholesterol in a subject.

60. Use of a plant extract as claimed in any one of claims 1-18 in the manufacture of a medicament for the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in a subject.

61. A packaged dosage form comprising a plant extract of any one of claims 1-18 together with instructions for use.

62. A dried plant extract comprising one or more statins and being substantially free of polar compounds.

63. A dried plant extract comprising one or more statins and being substantially free of polar compounds that affect pharmacokinetics of a drug in a subject.