(54) Title: EXTRAIT DE VEGETAUX D'AGYROLOBIUM ROSEUM POUR LE TRAITEMENT DU DIABETE
(54) Title: ARGYROLOBIUM ROSEUM PLANT EXTRACTS FOR TREATING DIABETES

(57) Abstract:
The present invention relates to a process for isolating plant Argyrobiunm roseum extract that contains flavonoid glycoside, wherein the extract possesses hypoglycaemic activity. The present invention also contemplates a composition containing the extract and a method of treating various hyperglycaemic conditions including non-insulin dependent diabetes mellitus disease condition by administering the extract.
Title: ARGYROLOBIUM ROUSEM PLANT EXTRACTS FOR TREATING DIABETES

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ARGYROLOBIUM ROSEUM PLANT EXTRACTS FOR TREATING DIABETES

TECHNICAL FIELD

The present invention relates to an antidiabetic extract obtained from the plant, Argyrolobium roseum, methods for isolating the extract, and use of the extract for treating patients that have hyperglycaemic-related diseases or conditions.

BACKGROUND ART

Argyrolobium roseum is a herb or shrub found in the tropical and sub-temperate tracts of north-western India. The plant grows, for instance, in the hilly area of Udhampurb district of Jammu and Kashmir, India, where it typically flowers and bears fruit between April and June, (see Chowdhary and Wadhwa, flora of himachal pradesh pp 181-1821; Chapman and Hail, dictionary of natural products, Vol. 7, 414, 1994. Vol. 1, 457, 1994; Index Kewensis, Vol. 1, 184, 1895; Om, wild and cultivated plants of J&K and ladakh, Page 23, 1997).

Descriptions of Argyrolobium plants can be found in FLORA OF WEST PAKISTAN, Page 385, volume 100, by E. Nasir; FLORA OF THE HIMALAYA, Page 94, Plate No. 323, d 460, by Oleg Polunin, Adam Stainton; FLORA OF JAMMU AND PLANTS OF NEIGHBOURHOOD Volume II, 1983, Plate 64, Page-180-1821; FLORA OF LHMACHAL PRADESH, Page 181-182. By H.J. Chowdhary and B.M. Wadhwal; FLORA OF BASHAHR ITMALAYAS, Page 69, 1997, by N.C. Nair; and FLORA OF BRITISH INDIA, Vol. II, Page 63, by Hooker. Other species of Argyrolobium include Argyrolobium megharizum and Argyrolobium flaccidum (DICTIONARY OF NATURAL PRODUCTS, Vol. 1, 457, 1994, By Chapman & Hall). However, there have been no reports that Argyrolobium roseum has any medicinal or biological uses for animals or mammals such as humans. Specifically, there has been no description of the use of this natural product to treat hyperglycaemic conditions.

SUMMARY OF THE INVENTION

The present invention relates to a process for isolating an Argyrobiunm roseum extract that possesses hypoglycemic activity and which contains a flavonoid glycoside compound. The present invention also encompasses a composition comprising the extract suitable for administration to an animal, and also a method of treating various hyperglycaemic conditions such as non-insulin dependent diabetes mellitus (NIDDM), by administering that composition.
One object of the present invention, then, is a method for isolating an extract from a Argyrolobium roseum plant, wherein the extract has hypoglycemic activity and contains a flavnoid glycoside compound. That extract can be formulated, such as into tablet form, into a composition useful for treating hyperglycemia and NIDDM in animals, including humans. Further, the present invention contemplates the admixing of other hypoglycemic agents and additives in a formulation comprising the inventive extract composition.

An object of the present invention, then, is to develop a method of treating hyperglycemia and NIDDM in animals, such as mammals, by administering an extract isolated from Argyrolobium roseum, wherein the extract contains a flavnoid glycoside compound. A human, for example, can be treated with the extract, so long as the extract or formulated composition does not induce any harmful side effects in the animal, mammal or human. Other hypoglycemic agents and additives may be co-administered with the inventive A. roseum extracted composition. Another object of the present invention is to develop a dosage regime for treating hyperglycaemic disease conditions like diabetes.

DETAILED DESCRIPTION

Accordingly, the present invention relates to a method of treating hyperglycemia and NIDDM in animals including humans, by administering an effective amount of extract from plant Argyrolobium roseum containing compound flavnoid glycoside, or by administering the compound flavonoid glycoside per se. The present invention shows that model animals treated with the inventive extract composition overcame hyperglycaemic disease-associated symptoms. Indeed, the present invention uses, for the first time, an Argyrolobium roseum extract to treat hyperglycaemic diseases such as diabetes mellitus, which afflicts all levels of society world-wide. In doing so, the present invention provides a natural, plant-based, non-toxic herbal preparation that can be used to treat diabetes mellitus. The extract can be easily prepared by isolating the particular plant powder using solvents and then vacuum-dried, without losing antidiabetic activity. Furthermore, there was no undesirable side effects in animals that had been treated with up to 2000 mg/kg p.o. doses of the extract. Accordingly, the anti-diabetic activity of the extract can be used to control hyperglycemia and particularly NIDDM.

Pharmaceutically acceptable additives can also be co-administered with the extract. Suitable additives include, but are not limited to a group of nutrients comprising proteins, carbohydrates, sugar, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste, and/or pharmaceutically acceptable carrier, excipient, diluent, solvent and other hypoglycemic agents.
In another embodiment of the present invention, wherein the effective amount of the inventive extract may be a dosage that ranges from about 1 to about 3000-mg/kg p.o./day; wherein the dosage regime may be continued for about 2 to about 4 weeks. Preferably, the effective amount is a dose that ranges between about 200 to about 1000 mg/kg p.o./day over about 2-4 weeks of treatment.

The beneficial effects of the administered extract should last for about 8 hours once administered to the animal or mammal. Indeed, the inventive extract composition can be about 20 times more effective as compared to the whole extract, without inducing undesirable side effects in that animal or mammal.

Thus, the present invention provides a composition useful for treating hyperglycemia and NIDDM in animals including humans, said composition comprising an effective amount of extract from the plant Argyrolobium roseum, containing compound flavnoid glycoside or compound flavnoid glycoside per se, optionally along with additives.

In another embodiment of the present invention, the additives are selected from a group of nutrients comprising proteins, carbohydrates, sugar, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste, and/or pharmaceutically acceptable carrier, excipient, diluent, solvent, and other hypoglycemic agents. Typically, the additives are not detrimental to the beneficial properties of the extract. In yet another embodiment of the present invention, the composition is administered orally as a capsule, tablet, syrup, concentrate, powder, granules, aerosol, or beads.

The present invention also envisions a process for isolating an extract from plant Argyrolobium roseum, which extract contains a flavnoid glycoside compound and has hypoglycemic activity. That method may comprise:

(a) drying the whole plant in powder form,
(b) percolating the powder about 3-5 times with polar or non-polar solvents,
(c) macerating the extract,
(d) obtaining the macerated extract of yield ranging between 7-10%,
(e) drying the extract in vacuum,
(f) isolating the pure compound flavnoid glycoside by chromatography, and,
(g) obtaining the compound flavnoid glycoside in the range of 0.015-0.019%.

In another embodiment of the present invention, wherein the said compound is isolated by column chromatography on silica gel 60-120 microns mesh size.

In still another embodiment of the present invention, wherein macerating the extract with compound selected from a group comprising hexane, ethyl acetate, acetone, chloroform.
In still another embodiment of the present invention, wherein preferred solvents for percolation are alcohols and acetones.

In still another embodiment of the present invention, the flavonoid glycoside itself is purified from the extract and used for preparing a pharmaceutical composition for treating non-insulin dependent diabetes in mammals and human beings.

In still another embodiment of the present invention, wherein said compound flavonoid glycoside having significant hypoglycemic activity

In still another embodiment of the present invention, the compound alcoholic extract having the activity of regeneration of p-cells of pancreas in streptozotocin treated rats.

In still another embodiment of the present invention, the flavonoid glycoside isolated from Argyrolobium roseum can be used at 20 times less dose, i.e. 10 mg as compared to 200mg/kg p. o. dose of alcoholic or acetone fraction of the plant showed significant hypoglycemic activity

In still another embodiment of the present invention, wherein the aqueous extract, alkaloidal and non-alkaloidal fractions do not possess any significant hypoglycemic activity.

The invention is described in the examples given below which are provided by a way of illustrations only and should not be construed to limit the scope of the present invention.

**EXAMPLES**

Example -1

The dose of streptozotocin is used to induce diabetes mimicking to NIDDM, encountered clinically in Majority of patients. The NIDDM diabetic rats when treated with alcoholic extract of the plant Argyrolobium roseum, for 2-3 weeks recovered to normal state, whereas the rats of control NIDDM diabetic group continued to have diabetes and died in due course of time.

Example - 2

1 kg of A. roseum (whole plant) is shade dried and powdered. The powdered plant material was extracted with ethyl alcohol (5 litres) for 20 hours. The alcohol extract was vacuum dried. The yield was 91 gms. The dried alcoholic extract was macerated successively with hexane and chloroform to give 8.2gms. and 39.3 gms. of the extracts respectively. A flavonoid glycoside (230mgs1 was isolated from the chloroform extract by repeated column chromatography over silica gel of mesh size 60-120 micron. The extract (200mg/kg p.o.) showed significant hypoglycemic activity as tested on normal, 18h
fasting, glucose loaded & streptozotocin induced hyperglycaemic rats. The onset of effect within 1/2h and the effect lasted for more than five hours. The fall of blood sugar recorded with above dose was 26mg/dl (n=24) as compared to fall of 17 & 23 mg/dl recorded with tolbutamide, 50mg/kg p.o. (n=9) and glipizide, 0.5mg/kg p.o. (n=8) respectively in 18h fasting rats.

The hypoglycaemic activity was also recorded with plant powder (2.5g/kg p.o.) and flavonoid glycoside isolated from the plant.

Example - 3

1 kg of A. roseum (whole plant) is dried and powdered. The powdered plant material was percolated four times with 95% methyl alcohol (3 lit. each time). The alcohol extract was vacuum dried 85gms. The alcohol extract was macerated successively with hexane and acetone to give 6.7 gms. and 49.6 gms. of the extracts respectively. A flavonoid glycoside (170mgs) was isolated from the acetone extract by repeated column chromatography over silica gel of mesh size 60-120 micron. There was highly significant recovery of rats from non-insulin dependent diabetes mellitus treated with the extract (200mg/kg p.o./day) for 2-3 weeks as per with following parameters: i) Blood glucose level, ii) Effect on body weight, iii) Survival of the animals, iv) Water intake, urine output & presence of glucose in urine, v) General condition of the animals and vi) Visit of ants to the voided urine of the rats.

Example - 4

1 kg of A.roseum (whole plant) was shade dried and powdered. The powdered plant material was percolated four times with 95% acetone (3 lit each time). The acetone extract was vacuum dried. The yield was 87 gms. The alcoholic extract was macerated successively with hexane and ethyl acetate to give 7.2 gms and 42.8gms of the extracts respectively. A flavonoid glycoside (210mgs) was isolated from ethyl acetate extracts by repeated column chromatography over silica gel of 60-120\textsuperscript{\circ} mesh size. There was highly significant recovery of rats form non-insulin dependent diabetes mellitus treated with KA (200mglkg p. o. /day) for 2-3 weeks as per with following parameters: 1) Blood glucose level, ii) Effect on body weight, iii) Survival of the animals, iv) Water intake, urine output & presence of glucose in urine, v) General condition of the animals and vi) Visit of ants to the voided urine of the rats.
CLAIMS

1. use of pharmaceutically effective amount of Argyrolobium roseum extract comprising compound flavonoid glycosidein an animal having hyperglycemia and non-insulin dependent diabetes mellitus.

2. The use of claim 1, wherein said method further comprises co-administering at least one pharmaceutically acceptable additive.

3. The use of claim 1, wherein said animal is a mammal.

4. The use of claim 3, wherein said animal is a human.

5. The use of claim 2, wherein said additive is selected from a group consisting of proteins, carbohydrates, sugar, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste.

6. The use of claim 2, wherein said additive is a pharmaceutically acceptable carrier, excipient, diluent, solvent or other hypoglycemic agent.

7. The use of claim 1, wherein said effective amount is from about 1 to about 3000 mg/kg p.o./day.

8. The use of claim 1, wherein said effective amount is from about 200 to about 1000 mg/kg p.o./day.

9. The use of claim 1, wherein said period of time is from about 2 to about 4 weeks.

10. The method of claim 1, wherein said extract produces no harmful side effect in said animal.

11. A purified composition comprising an effective amount of an Argyrolobium roseum extract, wherein said compound comprises a flavonoid glycoside compound.

12. The composition of claim 11, further comprising at least one additive.

13. The composition of claim 12, wherein said additive is selected from a group consisting of proteins, carbohydrates, sugar, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste.

14. The composition of claim 12, wherein said additive is a pharmaceutically acceptable carrier, excipient, diluent, solvent or other hypoglycemic agent.

15. The composition of claim 11, wherein said composition is formulated as a capsule, tablet, syrup, concentrate, powder, granules, aerosol, or bead.

AMENDED CLAIMS [ARTICLE 34]
16. A process for isolating an flavnoid glycoside compound-containing Argyrolobium roseum extract, comprising (a) drying a Argyrolobium roseum plant into powder form; (b) percolating the powder about 3-5 times with polar or non-polar solvents; (c) macerating the extract; (d) drying the extract in vacuum; and (e) isolating the flavnoid glycoside compound by chromatograph y, wherein said flavnoid glycoside compound has hypoglycemic activity.

17. The process of claim 16, wherein the flavnoid glycoside compound is isolated using a silica gel of 60-120 microns mesh size in a chromatography column.

18. The process of claim 16, wherein macerating the extract is performed in the presence of hexane, ethyl acetate, acetone, or chloroform.

19. The process of claim 16, wherein percolation is performed using an alcohol or an acetone.

AMENDED CLAIMS [ARTICLE 34]