

FORM 2
THE PATENTS ACT 1970
(39 of 1970)
&
THE PATENT RULES, 2003
COMPLETE SPECIFICATION
(See section 10 and rule 13)

1. TITLE OF THE INVENTION: -

HERBAL ANTIDIABETIC COMPOSITION OF EXTRACT OF ALLIUM HYPHOSIFOLIA

2. Applicant(s)

NAME	NATIONALITY	ADDRESS
UTTARANCHAL UNIVERSITY	INDIAN	ARCADIA GRANT, P.O. CHANDANWARI, PREMNAGAR, DEHRADUN - 248007, UTTARAKHAND, INDIA
ANURADHA JOSHI	INDIAN	ARCADIA GRANT, P.O. CHANDANWARI, PREMNAGAR, DEHRADUN - 248007, UTTARAKHAND, INDIA

3. PREAMBLE OF THE DESCRIPTION

The following specification particularly describes the invention and the manner in which it is to be performed

Title of The Invention

**HERBAL ANTIDIABETIC COMPOSITION OF EXTRACT OF ALLIUM
HYPERSISTUM**

Field of the Invention

5 This invention relates to herbal antidiabetic composition of extract of ALLIUM
hypersistum.

Background of the Invention

Diabetes mellitus is a complex metabolic hormonal disorder associated with
hyperglycemia. It is resulted from either insulin insufficiency or deficient and
10 scientific reports revealed that diabetes cannot be cured completely. Type-1
and type-II are two types of diabetes. Type-1 is developed due to genetically
disorder while type-2 is caused mainly due to life style of people. About 10%
of the population all over the world are suffering from Diabetes mellitus and it
is a chief health problem to urban population.

15 In this invention extract of selected nepali jimbu plany i.e. Allium hypersistum
have been used for controlling and treating type-II diabetes and found effective
in mice experiment.

EP3847897A1 Composition for making a tea beverage or herbal and vegetable
broths- the present reference relates to an infusion product for making a
20 beverage, more specifically to a plant-based composition for making a
beverage, and to a herbal and/or vegetable composition or bouquet garni. The

plants are fruits, herbs, medicinal plants, tea, vegetables and/or spices. In particular, the present invention relates to a fiber-web, preferably a tea bag, made from said fruits, herbs, medicinal plants, tea, vegetable and/or spices

Research Gap: In this patent use of selected plant for making herbal tea but
5 in our invention involves for anti-diabetic purpose.

US8993008B1 Herbal composition for treating diabetes discloses the herbal composition for treating diabetes is a mixture of medicinal herbs, including gymnema (*Gymnema sylvestre*), bilberry (*Vaccinium myrtillus*), Asian ginseng (*Panax ginseng*), fenugreek (*Trigonella foenum-graecum*), marshmallow
10 (*Althaea officinalis*), "true cinnamon" (also known as "Ceylon cinnamon" or "Sri Lanka cinnamon") (*Cinnamomum verum*), bitter melon (also known as "bitter gourd") (*Momordica charantia*), autumn crocus (also known as "meadow saffron") (*Colchicum autumnale*), bay laurel (*Laurus nobilis*), colocynth (*Citrullus colocynthis*) and prickly pear (also known as "cactus pear") (*Opuntia*
15 *ficus-indica*). Preferably, the herbal components of the above composition are dried, ground and packaged in a teabag or the like, allowing the composition to be delivered to the patient as an aqueous extract, similar to a conventional herbal tea. Preferably, about 5 grams of the composition are contained in the teabag, which is steeped in about 150 mL of boiling water for between about
20 three minutes and about five minutes.

Research Gap: In this they used extract and parts of gymnema (*Gymnema sylvestre*), bilberry (*Vaccinium myrtillus*), Asian ginseng (*Panax ginseng*), fenugreek (*Trigonella foenum-graecum*), marshmallow (*Althaea officinalis*), for antidiabetic formulation but they have not taken extract of *Allium hypsistum* for antidiabetic purpose

None of the prior art indicate above either alone or in combination with one another disclose what the present invention has disclosed. Present invention involves explore of extracts of various parts of *Allium hypsistum* (commonly known as Nepalese Jimbu) for antidiabetic purpose. *Allium hypsistum* is a Nepalese species of wild onion in the Amaryllis family. This nepali jimbu is found in hill areas, at high altitutte region, mainly himalayn region of India and Nepal.

SUMMARY OF THE INVENTION

This summary is provided to introduce a selection of concepts, in a simplified format, that are further described in the detailed description of the invention.

This summary is neither intended to identify key or essential inventive concepts of the invention and nor is it intended for determining the scope of the invention. Present invention involves explore of extracts of various parts of *Allium hypsistum* (commonly known as Nepalese Jimbu) for antidiabetic purpose. *Allium hypsistum* is a Nepalese species of wild onion in the Amaryllis family.

This nepali jimbu is found in hill areas, at high altitutte region, mainly himalayn region of India and Nepal.

Allium hypsistum is a herb which is used as spice and local medicine in rural villages and towns of Nepal and India. As a spice, it is used to flavor lentils, 5 vegetables, salads, and pickles whereas medical uses of Allium hypsistum involves antiallergic, common cold and some wound healing purposes.

Researcher has done work on extracts of Allium hypsistum for antimicrobial, anti-cancer purposes but no work done reported on alcoholic extract extraction from leaves or flowers or roots and use for antidiabetic purpose.

10 In this present inevtion Allium hypsistum, Allium przewalskianum and Allium wallichii are chosen for antidiabetic activity in streptozotocin induced diabetic mice. Streptozotocin is an alkylating antineoplastic agent which selectively destructs insuling producing beta cells of the pancrease in mammals. As a result, blood glucose level is raised causing chronic hyperglycaemia,

15 Leaves, stem and root samples were collected from Nepal region and after airdrying extracts were taken by solvent extraction technique in different polar, non-polar and semi polar solvents like aqueous extract, alcoholic extract, n-hexane extract and other extracts.

Different Extracts of of Allium hypsistum for tested for antidiabetic and 20 antioxidant activities in streptozotocin induced diabetic mice. Streptozotocin is an alkylating antineoplastic agent which selectively destructs insuling producing beta cells of the pancrease in mammals. As a result, blood glucose

level is raised causing chronic hyperglycemia. Alcoholic extract of this plant has shown a much better impact on selected mice as antidiabetic agent. Which is comparably as good as synthetic antidiabetic agent.

Disclosed herein A method of isolation of extract of *Allium hypsistum* for herbal composition of snit-diabetic comprising the steps of: Collecting plant parts like flower, leaves stem roots; Taking Aerial parts of the plants and washed with distilled water; and Materials are first chopped into small pieces and air-dried in shade for 15 days at room temperature (28°C to 30°C); and the dried materials of *Allium hypsistum* are ground to get a powder which was sieved with a mesh screen. After that, extraction of the powdered materials was carried out separately by the soxhlet method using ethanol, n-hexane, and distilled water; Drying fully the tinctures dried and free from solvents by evaporating at 40°C and kept in separate containers with proper labels; and are stored in the refrigerator at 4°C until further use.

To further clarify advantages and features of the present invention, a more particular description of the invention will be rendered by reference to specific embodiments thereof, which is illustrated in the appended drawings. It is appreciated that these drawings depict only typical embodiments of the invention and are therefore not to be considered limiting of its scope. The invention will be described and explained with additional specificity and detail with the accompanying drawings.

DETAILED DESCRIPTION OF THE INVENTION

The detailed description of various exemplary embodiments of the disclosure is described herein with reference to the accompanying drawings. It should be noted that the embodiments are described herein in such details as to clearly
5 communicate the disclosure. However, the amount of details provided herein is not intended to limit the anticipated variations of embodiments; on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the scope of the present disclosure as defined by the appended claims.

10 It is also to be understood that various arrangements may be devised that, although not explicitly described or shown herein, embody the principles of the present disclosure. Moreover, all statements herein reciting principles, aspects, and embodiments of the present disclosure, as well as specific examples, are intended to encompass equivalents thereof.

15 The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of example embodiments. As used herein, the singular forms “a,” “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms “comprises,” “comprising,” “includes”
20 and/or “including,” when used herein, specify the presence of stated features, integers, steps, operations, elements and/or components, but do not preclude

the presence or addition of one or more other features, integers, steps, operations, elements, components and/or groups thereof.

It should also be noted that in some alternative implementations, the functions/acts noted may occur out of the order noted in the figures. For example, two figures shown in succession may, in fact, be executed concurrently or may sometimes be executed in the reverse order, depending upon the functionality/acts involved.

In addition, the descriptions of "first", "second", "third", and the like in the present invention are used for the purpose of description only, and are not to be construed as indicating or implying their relative importance or implicitly indicating the number of technical features indicated. Thus, features defining "first" and "second" may include at least one of the features, either explicitly or implicitly.

Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which example embodiments belong. It will be further understood that terms, e.g., those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art and will not be interpreted in an idealized or overly formal sense unless expressly so defined herein.

These and other advantages of the present subject matter would be described in greater detail with reference to the following figures. It should be noted that the description merely illustrates the principles of the present subject matter. It will thus be appreciated that those skilled in the art will be able to devise various
5 arrangements that, although not explicitly described herein, embody the principles of the present subject matter and are included within its scope.

Material and Methods

Plant Extracts

Allium hypsistum plant parts like flower, leaves stem roots were collected from
10 Mustang district and Gorkha district of Nepal. The plant was taxonomically identified with flora of Nepal provided by the Department of Plant Resources, National Herbarium and Plant Laboratories, Govt. of Nepal. Aerial parts of the plants were taken and washed with distilled water. Materials were first chopped into small pieces and air-dried in shade for 15 days at room temperature (28°C
15 to 30°C) The dried materials of *Allium hypsistum* were ground to get a powder which was sieved with a mesh screen. After that, extraction of the powdered materials was carried out separately by the soxhlet method using ethanol, n-hexane, and distilled water. Then, tinctures were fully dried and free from solvents by evaporating at 40°C and kept in separate containers with proper
20 labels. They were stored in the refrigerator at 4°C until further use.

Animals

Healthy and adult albino mice weighing 29 to 38 g and age of 8 to 10 weeks were taken for the study. They were fed with normal diet and kept in standard condition at room temperature of $25\pm 1^{\circ}\text{C}$ following 12 hours light and 12 hour
5 dark cycle. Animal caring and handling were carried out throughout the experiment according to guide for the care and use of laboratory animals (13).

Grouping and dosing of animals

Total 72 albino mice were grouped into twelve groups including six mice in each group. They were categorized as; one normal control, one diabetic control, one
10 diabetic reference and nine test groups. After overnight fasting, freshly prepared 150mg/kg streptozocin (STZ) in 0.1M citrate buffer (pH 4.5) dose were administered via intraperitoneal route to all mice except normal control group. After three days (72 hours), the mice were evaluated for diabetes mellitus by estimating blood glucose level piercing lateral tail vein using
15 Glucometer (Dr. Morepen, India).

Normal control group was designed as negative control (no diabetes) and given distilled water; the next diabetic control group was a positive control (diabetic induced) and normal diet; the third group served as diabetic reference i.e. diabetic induced and administered orally with 500mg/kg dose of reference drug
20 metformin; the remaining diabetic induced groups (group 4th to 12th) were orally

administered with 500mg/kg dose of plant extracts- ethanol, water and hexane extracts of selected plant; *A. hypsistum*, The blood glucose level were estimated after overnight fasting, at 72hours (baseline) after STZ administered and after 7th and 14th day of baseline

5 **Statistics**

The obtained data were analyzed statistically in Microsoft Excel (windows 10) and SPSS (version 25). The result of experiment was expressed as mean \pm SD.

Results and Discussion

10 **Change in body weight of diabetic mice**

Loss of body weight and increase in blood glucose level indicate induction of diabetes which was seen in this study. Diabetes was induced by streptozotocin (STZ) in albino mice. From Table1, the diabetic control (DC) group showed reduction in body weight of albino mice after 72hrs (baseline day) of STZ administration and gradually reduced the body weight from baseline average 31.93 \pm 1.04g to 30.75 \pm 0.78g at 7th and 28.85 \pm 1.46g at 14th day. In contrast, the normal control (NC) group showed slight increase in body weight of mice from baseline average 36.15 \pm 0.92g to 37.52 \pm 0.77g at 7th day and 37.52 \pm 0.77g at 14th day which group did not receive the STZ. The average body weight of all group mice was found to be decreased significantly after 72hrs of STZ

administration. Then, repeated daily exposure of metformin (reference drug) dose showed increment in body weight at 7th and 14th days gradually. Among all plant extracts, repeated daily dose exposure of ethanol extracts of *A. hypsistum* (DHe) showed remarkable increment in the body weight from
5 baseline average 30.82 ± 0.17 g to 33.43 ± 0.23 g at 7th and 35.08 ± 0.47 g at 14th days. Moreover, percent increase in body weight was 8.47% at 7th day and 13.82% at 14th day for ethanol extract of *A. hypsistum* which are more weight reduction than showed by that of reference **metformin**. Similarly, water and hexane extract of *A. hypsistum* were found to be effective gaining the weight
10 of exposed diabetic mice. Comparatively, extracts of *A. hypsistum* showed more effective in weight reduction of diabetic mice than *other extracts*.

Alcoholic extract was found more potent in weight reduction of diabetic mice than water extracts and hexane extract.

Streptozotocin induced diabetic mice are extensively used laboratory animal
15 models to evaluate the antidiabetic properties of herbs or plants. Glibenclamide or metformin is often used standard antidiabetic drug to compare antidiabetic activity of medicinal plants. Body weight loss after STZ administration is a major symptom of diabetic mammals which could be due to insufficient supply of energy fuel to the body resulting excessive burning of fat and tissue protein for
20 energy. In present invention, body weight of mice was reduced after STZ

administration which was then improved after repeated daily dose of selected plant extracts and metformin.

Table1: Effects of repeated daily dose of extract on body weight of diabetic albino mice.

Group	Average Bogy weight (g)				% improved body wt from baseline	
	Before induction of DM	After 72hrs (Baseline)	7 th day	14 th day	7 th day	14 th day
NC	35.37±0.82	36.15±0.9 2	37.52±0.7 7	38.43±1.0 6	3.78	6.31
DC	33.72±0.49	31.93±1.0 4	30.75±0.7 8	28.85±1.4 6	-3.70	-9.65
DR	34.31±0.96	32.41±0.9 6	34.43±0.9 7	36.64±1.1 6	6.22	13.06
DHe	32.92±0.17	30.82±0.1 7	33.43±0.2 3	35.08±0.4 7	8.47	13.82
DHw	33.54±0.54	32.38±0.5 4	33.87±0.6 9	35.11±1.0 5	4.60	8.44
DHh	31.78±0.51	32.91±1.5 6	33.84±1.5 5	34.66±1.8 1	2.80	5.31

5 Values are mean ± SD; n=6 for each group. **Abbreviation:** NC, Normal control- group without diabetes; DC, Diabetic control-group with diabetes; DR: Diabetic Reference-Diabetic group exposure with reference drug (metformin); **DHe**, Diabetic group exposure with ethanol extract of *A. hypsistum*; **DHw**, Diabetic group exposure with water extract of *A. hypsistum*; **DHh**, Diabetic group exposure with hexane extract of *A.*

10 *hypsistum*

Anti-diabetic activities of selected plant extracts

The effect of plant extracts in STZ induced diabetic mice is shown in Table 2. The fasting glucose levels in the diabetic mice were 293.33-345.83 mg/dl. In the study, NC group was considered as normal control mice since they were not exposed with STZ and were non-diabetic group. The fasting glucose levels were markedly dropped gradually at 7th and 14th day with exposure of repeated daily dose of plant extracts. Comparing with reference drug group (DR), **ethanol extract of *A.hypsistum*** showed closed percent reduction in blood glucose level than showed by other plant extracts. DHe (i,e ethanolic extract) has shown antidiabetic property comparable to standard reference.

Table 2: Anti-hyperglycemic activity of repeated daily doses of the plant extracts in diabetic mice.

Fasting blood glucose level (mg/dl)				% Reduction	
Groups	Baseline	7th Day	14th day	7th day	14th day
NC	85.66 ± 5.16	83.16 ± 5.34	84.83 ± 5.03	2.91	0.97
DC	297.66 ± 12.65	311.5 ± 12.46	317.83 ± 9.41	-4.64	-6.77
DR	325.30 ± 12.20	168.66 ± 9.83	140.5 ± 9.20	48.3	56.70
DHe	323.5 ± 9.97	163.66 ± 13.63	142.10 ± 12.70	49.39	56.07

DHw	293.33 ± 9.66	249.16 ±11.40	236.5 ± 9.83	15.05	19.37
DHh	281 ± 13.79	230.83 ±10.18	208.66 ± 14.55	17.85	25.74

Values are mean ± SD; n=6 for each group.

Examples:

A method of isolation of extract of *Allium hypsistum* for herbal composition of snit-diabetic comprising the steps of: Collecting plant parts like flower, leaves
5 stem roots; Taking Aerial parts of the plants and washed with distilled water; and Materials are first chopped into small pieces and air-dried in shade for 15 days at room temperature (28°C to 30°C); and the dried materials of *Allium hypsistum* are ground to get a powder which was sieved with a mesh screen. After that, extraction of the powdered materials was carried out separately by
10 the soxhlet method using ethanol, n-hexane, and distilled water; Drying fully the tinctures dried and free from solvents by evaporating at 40°C and kept in separate containers with proper labels; and are stored in the refrigerator at 4°C until further use.

We Claim:

1. A method of isolation of extract of *Allium hypsistum* for herbal composition of snit-diabetic comprising the steps of:

Collecting plant parts like flower, leaves stem roots;

5 Taking Aerial parts of the plants and washed with distilled water; and

Materials are first chopped into small pieces and air-dried in shade for 15 days at room temperature (28°C to 30°C); and the dried materials of *Allium hypsistum* are ground to get a powder which was sieved with a mesh screen.

10 After that, extraction of the powdered materials was carried out separately by the soxhlet method using ethanol, n-hexane, and distilled water;

Drying fully the tinctures dried and free from solvents by evaporating at 40°C and kept in separate containers with proper labels; and are stored in the refrigerator at 4°C until further use.

15 2. The method as claimed in claim 1, wherein the effect of plant extracts in STZ induced diabetic mice and the fasting glucose levels in the diabetic mice were 293.33-345.83 mg/dl.


3. The method as claimed in claim 1, wherein the NC group was considered as normal control mice since they were not exposed with STZ and are non-diabetic group.

4. The method as claimed in claim 1, wherein the fasting glucose levels are markedly dropped gradually at 7th and 14th day with exposure of repeated daily dose of plant extracts.

5. The method as claimed in claim 1, wherein Comparing with reference drug group (DR), ethanol extract of *A.hypsistum* showed closed percent reduction in blood glucose level than showed by other plant extracts. DHe (i,e ethanolic extract) has shown antidiabetic property comparable to standard reference.

Dated this March 30, 2022

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(Ashish Sharma)
Authorized Agent for the Applicant
Indian Patent Agent Regn No. IN/PA-3021

ABSTRACT

HERBAL ANTIDIABETIC COMPOSITION OF EXTRACT OF ALLIUM HYPHYSISTUM

A method of isolation of extract of Allium hypsistum for herbal composition of
5 snit-diabetic comprising the steps of: Collecting plant parts like flower, leaves
stem roots; Taking Aerial parts of the plants and washed with distilled water;
and Materials are first chopped into small pieces and air-dried in shade for 15
days at room temperature (28°C to 30°C); and the dried materials of Allium
hypsistum are ground to get a powder which was sieved with a mesh screen.
10 After that, extraction of the powdered materials was carried out separately by
the soxhlet method using ethanol, n-hexane, and distilled water; Drying fully
the tinctures dried and free from solvents by evaporating at 40°C and kept in
separate containers with proper labels; and are stored in the refrigerator at 4°C
until further use.

15