

PROJECT REPORT

MINOR RESEARCH PROJECT (UGC)

STUDY OF PLANTS IN THE TREATMENT OF DIABETES

DR SUSMITA SAHOO

N. V. PATEL COLLEGE OF PURE & APPLIED SCIENCES

VALLABH VIDYANAGAR

ANAND, GUJARAT

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Introduction

Families Cucurbitaceae, Rutaceae and Fabaceae are highly useful in Diabetes.

Genetic variation within the plant species is possible and suggested in certain plants on the basis of true molecular composition and similarity between the members of individual family would further help identifying the most useful genotype for better management of food cum drug plants.

Diabetes is being considered as one of the major problem causing mortality. It is a disease affecting the body metabolism and therefore a serious matter of concern.

The comprehensive list containing uses of diabetes helps to focus attention on a few families (out of 87) which contain useful plants in diabetes in relative greater frequency than others.

Reported literature shows that plant extracts from the above said families have been used for study.

No literature is there on isolation and characterization of individual chemical components having characteristic property to cure diabetes.

It is therefore quite important to investigate and establish this.

It is therefore aimed at collecting germplasm of the plants from different sources and these would be maintained in different forms like planting them directly on soil, growing them by tissue culture and keeping them in the form of seeds.

Germplasm collection, biochemical characterization of the component present in each plant and identification of the common class of compounds present in plants of said family would help in correlating between varieties of the germplasm collected.

Molecular characterization of the varieties collected would help in breeding of the medicinal plant variety which would be truly helpful in controlling diabetes.

Review of Literature

Medicinal plants are used to treat hypoglycemic or hyperglycemic conditions, are of considerable interest for ethnobotanical community as they are recognized to contain valuable medicinal properties of different parts of the plant have shown varying degree of hypoglycemic and anti hyperglycemic activity. The active principles of many plant species with desired properties are isolated to cure ailments such as diabetes of type 1 and type 2 respectively.

India has about 45,000 plant species and several thousands have been claimed to possess medicinal properties. The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents.

Several species of medicinal plants are used in the treatment of diabetes mellitus, a disease affecting large number of people world wide although traditional plant medicines or herbal formulations might offer a natural key to unlock diabetic complications.

Diabetes mellitus is the major disorder responsible for renal failure, blindness or diabetic cataract, poor metabolic control, increased risk of cardiovascular disease including atherosclerosis and AGE (advanced glycation end) products.

Antioxidants play an important role to protect against damage by reactive oxygen species and their role in diabetes has been evaluated.

Many plant extracts and products were shown to possess significant antioxidant activity.

Materials and Methods

Germplasm collection of medicinal plants used in diabetes has been done. The medicinal plants comprised of the daily used vegetable and pulses which have use in diabetes were taken into consideration.

The plants collected were grown in the medicinal plant garden.

The parts of the medicinal plants were collected and their method of use were taken into consideration.

The biochemical parameters of the plants collected were taken into account.

Germplasm in the form of seeds, tissue culture maintenance of the materials collected from different places and states were done.

Preparation of plant tissue culture media.

Culture medium

A substance used to provide nutrients for growth and multiplication of tissue or microorganisms.

Characteristics of an ideal culture medium: The characteristics of an ideal culture medium are

- i. It must give a rapid growth.**
- ii. It should have a rapid growth.**
- iii. It should be easy to prepare.**
- iv. It should be reasonably cheap.**
- v. It should be easily reproducible.**
- vi. It should make it possible for all the characteristics in which we are interested to be demonstrated.**

Ingredients of an ideal tissue culture medium: An ideal nutrient medium for plant tissue culture contains 5 classes of ingredients.

- 1. Inorganic salt**
- 2. Vitamins**
- 3. Carbon source**
- 4. Growth regulators**

5. Organic supplements

6. Growth regulators

Auxin : Indole 3 acetic acid : Used for callus induction.

Indole 3 butyric acid : Used for rooting shoots, regenerated via organogenesis.

Cytokinin ; Kinetin – Promote cell division, callus induction.

6 – benzylamino purine: Used for callus induction and growth of callus.

Gibberellin: Involved in regenerating cell elongation.

Abscisic acid : Inhibits cell division.

Preparation of plant cell culture media:

The main objective of medium preparation is to culture the cell, tissue or organ in vitro.

Protocol

- 1. Prepare stock solution of major ingredients of MS medium, add vitamins, plant growth regulator and sterile distilled water and MS medium.**
- 2. Sucrose is added.**
- 3. Adjust the pH to 5.5 .**
- 4. Adjust volume with sterile distilled water.**
- 5. Transfer 75 ml batches to 250 ml conical flask, then autoclave.**

Determination of total soluble sugars by ferricyanide method

This method is based on the principle that sugars are oxidized by alkaline potassium ferricyanide and the amount of ferrocyanide produced is then measured either photometrically or volumetrically. The amount of total sugars can also be determined colorimetrically by anthrone method.

1. For extracting the sugars, suspend 1 g of finely powdered oven dried sample in 40 ml of distilled water and heat in boiling water bath for 30 min. centrifuge at 3000 rpm for 20 min. collect the supernatant. again suspend the pellet in 20 ml of water and repeat this extraction step 6-8 times till the supernatant is free of sugars. combine all supernatants.
2. Add 1-2 ml of saturated neutral lead acetate to the collected supernatant and after properly mixing it for 15 min, filter it through whatman no 1 filter paper and make the volume to 250 ml with distilled water. excess of lead acetate is then precipitated out with sodium oxalate.
3. For determination of reducing sugars the above extract is used directly. however for estimating the amount of total sugars take 25 ml of the above extract and hydrolyse it with 5 ml conc hcl for 8 min at 68 degree centigrade. after cooling neutralize the hydrolysate with sodium carbonate and make up the volume to 100 ml with distilled water.
4. To 1 ml of the preparation for reducing sugars and that obtained for total sugars add 0.2 ml of N/12 sulphuric acid and 9 ml of distilled water. mix thoroughly add 0.02 ml sodium tungstate and after shaking the contents allow to stand for 10 mins. centrifuge it at 3000 rpm for 20 min. add 1 ml of ferricyanide solution to 1 ml of cyanide carbonate solution. heat this mixture for 15 min in a boiling water bath and cool immediately under running tap water. now add 5 ml of ferric ammonium sulphate. mix the solution and after 10 min record its od at 690nm .
5. Prepare a reference curve of glucose over a range of 0-100micro gram as standard following the procedure described above.

Estimation of Protein by Lowry's method

It is the most commonly used method for determination of proteins in cell free extracts because of its high sensitivity and quantities as low as 20 micro gram of proteins can be measured. the peptide bonds in polypeptide chain react with copper sulphate in an alkaline medium to give a blue coloured complex. in addition tyrosine and tryptophan residues of proteins cause reduction of the phosphomolybdate and phosphotungstate components of the folin ciocalteau reagent to give

bluish products which contribute towards enhancing the sensitivity of this method. it is however important to remember that several compounds like edta, tris, carbohydrates, ammonium, magnesium and potassium ions thiol reagents, phenols interfere with the colour development and it should be ensured that such substances are not present in sample preparations.

Procedure

1. **Sample extract:** weigh 1 g sample, macerate the sample in pestle mortar in 5 ml of phosphate buffer and transfer the material to centrifuge tubes. centrifuge the homogenate at 8000 rpm for 20 min. collect the supernatant and repeat the extraction 4-5 times. combine the supernatants and make the volume to 50 ml with phosphate buffer.
2. Take 1 ml of the above extract and add 1 ml of 20 % Tca. keep it for half an hour and centrifuge at 8000 rpm for 20 min. wash the pellet with acetone twice and again centrifuge it. discard the supernatant.
3. Dissolve the pellet in 5 ml of 0.1 N Naoh and mix well till it gets dissolved.
4. Take suitable aliquot of above solution and add to it 5 ml of freshly prepared alkaline copper sulphate reagent. mix properly and after 10 min add 0.5 ml of Folin's reagent. mix the contents instantaneously. allow the colour to develop for 30 mins.
5. Record the absorbance at 660 nm after setting the instrument with reagent blank which contains 1 ml of 0.1 N NaOH instead of the sample aliquot.
6. In another set of tubes take suitable aliquots of BSA solution, make the total volume to 1 ml with 0.1 N NaOH and develop the colour as described above. draw a standard curve of absorbance at 660 nm versus microgram of BSA. From this standard curve determine the amount of protein in the sample tube.
7. Calculate the amount of protein per g of the sample.

Results and Discussion

- 1) Bengal gram
- 2) Black gram
- 3) Ground nut

Among pulses belonging to family Fabaceae are useful.

Fruits

- 1) Like cucumber

Vegetable

- 1) Like *Momordica* of family Cucurbitaceae are useful.

Citrus plants

- 1) Like lemon
- 2) and *Murraya koenigii* of family Rutaceae are useful.

Murraya koenigii prevents or postpones the onset of diabetes.

Groundnut oil :

Fatty acid composition.

Total saturates 20.9

Total monounsaturated 49.3

Linoleic N6 -29.9

Total polyunsaturates – 29.9.

It is lower than other oils.

Vitamin E is more in legumes.

Vitamin C is more in Citrus fruits and melon.

Antioxidant beta carotene is present in water melon.

Food value in pulses

- 1) Is proteins 5g and carbohydrates 15g.
- 2) Sprouting enhances the nutritive value of food.
- 3) Especially vitamin C.
- 4) Sprouted legumes are important.

Lemon makes the food acidic and helps in absorption of iron.

Total dietary fibre

1) in Bengal gram: Is 28.3

2) Black gram 20.3

3) Green gram 16.7

4) Lentil 15.8

5) Soyabean 20.3

Insoluble fibre

1) Bengal gram 25.2

2) Black gram 15.4

3) Green gram 6.5

4) Lentil 13.5

5) Soyabean 17.9

Soluble dietary fibre

1) Bengal gram 3.1

2)Black gram 4.9

3)Green gram 1.7

4)Lentil 2.3

5)Soyabean 5.1.

6)In orange it is 1.10.6 and 0.5

7)In bitter gourd it is 4.3,3.2 and 1.1.

Approximate food value

Calories 1586

Carbohydrates 195 gm

Protein 55 gm

Fats 54 gm

And fibre 12 gm.

Fresh lemon with honey 15ml

Sprouted Bengal gram 25 g

Groundnuts 25 g

Orange 100g

Sprouted green gram dal 25g.

Value of Protein in the Germplasm collected:

Protein :

Momordica 0.270

Watermelon -0.80

Snake gourd 1.68

Cucumber 1.980

Groundnut 0.218

Protein content of germplasm of groundnut –

1)0.197

2)0.850

3)0.391

4)0.431

5)0.396

6)0.300

Another set of germplasm of groundnut had protein content-

1) 0.480

2) 0.652

3) 0.520

4) 0.570

5) 0.620

6) 0.425.

Carbohydrate content

- 1) Cucumber 1.535
- 2) Watermelon 1.441
- 3) Snake gourd 1.467.

Protein content

- 1) Snake gourd 0.612 and 1.186.
- 2) Groundnut 1.773 and very high content.

Chlorophyll content

- 1) *Murraya koenigii* 0.698, 0.146 and 0.282.
- 2) *Aegle marmelos* 0.331, 0.140 and 0.312.
- 3) Citrus 0.548, 0.251 and 0.291.
- 4) *Butea monosperma* 0.333, 0.358 and 0.265.

Ash gourd- Benincasa hispida fruit is useful in diabetes. wholesome nutritive and low in calories.

Citrullus lanatus water melon used in china for treatment of diabetes.

Cucumis sativus the fruit is an ingredient in the diet of diabetics.

Momordica charantia whole plant is useful.

Bottle gourd fruit juice useful for excessive thirst.

Butea monosperma bark seed and gum useful in diabetes.

Cajanus cajan hypoglycemic

Bengal gram seeds reduce post prandial glucose in humans.

Soyabean prevents neurological problems in diabetes.

Groundnut checks vascular complications

Lentil is useful in diabetes due to slow release of energy.

Lemon juice retards tendency to diabetes.

Murraya koenigii prevents diabetes due to hereditary factors.

Aegle marmelos is hypoglycemic.

CONCLUSION:

The families Cucurbitaceae, Fabaceae and Rutaceae have maximum use in the treatment of diabetes as evidenced by their biochemical constituents also. They have more of protein content which have use in diabetes.

There is a difference in the germplasm of these families also. So the germplasm having more of protein in it should be taken as a better variety in the treatment of Diabetes.