Research Article

Formulation from Herbal Extract of *Clitoria ternatea* and its *In-vitro* Evaluation for Antidiabetic Activity.

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ABSTRACT

The demand for Antidiabetic preparations from Natural therapeutics agents is increasing rapidly. *Diabetes mellitus* disease is a Global epidemic as well as a major health issue in Indian society. Management of Diabetes in Indian society is done with several herbal preparations including Syrup, Tablet, Decoctions, etc. The present study evaluates *Clitoria ternatea* whole plant, extract-based formulation as an Antidiabetic green tea. This formulation made using *Clitoria ternatea* leaf extract as Antidiabetic agents and other ingredients. The formulation was evaluated *in-vitro* by performing alpha amylase assay and Glucosidase assay. Other analyses include Organoleptic and Physiochemical characteristics (LOD test, water and alcohol soluble extract value, ash value, pH, and solubility test, etc.) which were also performed. The formulation fulfilled the standards of antidiabetic formulation and it also attained an anti-diabetic effect. Present study showed potential of this preparation as an user friendly Antidiabetic preparation.

Keywords: *Clitoria ternatea*, Alpha-Amylase assay, Antidiabetic formulations, Herbal formulation.

1. INTRODUCTION

The WHO predicts that diabetes mellitus will be the most prevalent non-communicable disease worldwide by 2025, with the greatest number of diabetics in India[1]. Current pharmacotherapeutics and medicines do not show a 100 % reversal of hyperglycaemia and report several side effects. Major reports suggest that Polyherbal preparation is used in the management of diabetes[1,2,3]. Herbal preparations are commonly used for diabetes treatment because of their health benefits[1,4] Because of properties such as antioxidant, anti-inflammatory, anticancer, anti-diabetic, antimicrobial, neuroprotective, and cardiovascular effects[1]. The Herbal extract was prepared from leaves of several plants like *Clitoria ternorea, Cassia auriculata* L., *Mangifera indica*, *Ficus banghalensis*, *Cinnamomum tamala*, *Moringa oleifera* and *Trichosanthes diocia*[5,6]. Those plants are found in the Indian subcontinent and are scientifically proven for their moderate hypoglycaemic potential.

Several home remedies used for the treatment of diabetes include herbal preparations from Bilberry (*Vaccinium mytillus*), Gooseberry, amla (*Emblica officinalis*), Green tea (*Camellia sinensis*), Gurmar (*Gymnema sylvestre*), Ispaghula husk (*Plantago ovata*), Maidenhair tree (*Gingo biloba*), Mango leaves (*Mangifera indica*), Papaya (*Carica papaya*) and sweet potato leaves (*Ipomoea batatas*), Gokarn leaf *Clitoria ternorea* and also reported in literature[7,8].

Several formulations are made into different types like Tablets, Capsules, and Powder. Most of such formulations show antidiabetic activity because they inhibit the activity of Alpha- amylase enzyme and Glucosidase activity due to herbal extract constituent. Those include Alkaloids, flavonoids, and saponins responsible for the antidiabetic potential[9].

α-amylase catalyses breakdown of glycosidic bond found in polysaccharide and subsequently increases blood sugar level. Inhibition of Alpha-amylase and Glucosidase activities keep blood glucose level stable[10]. This finding, reports the phytochemical analysis of plant extract prepared from the *Clitoria ternorea* plant leaves extract. The present work also aimed to prepare a whole plant extract of the above-mentioned plants which was then subjected to formulate a Tea bag with some supplementation of plant material such as *Clitoria ternatea* plant leaves extract and alpha-amylase assay and glucosidase enzyme assay were performed to check the antidiabetic activity[11].

2. MATERIAL AND METHODOLOGY

2.1. Collection and Authentication of Plant material.

Clitoria ternatea plant leaves were collected and authenticated by expert

2.2. Soxhlet Extraction and preparation of the herbal extract.

After the material were collected, they were washed and dried for a week at room temperature. After drying, the plant material was ground separately. 50 g of crude powder was prepared from each *Clitoria ternatea* extract. A solvent extraction performed with hydroalcoholic (70:30, ethanol: water) solvent. The extract was then collected and the solvent was removed by simple evaporation at room temperature. This extract was later used to make tea bags and in in vitro enzyme analysis.

2.3. Preliminary phytochemical screening and pharmacological evaluation of the extract

The extract was evaluated for several characteristics such as colour, odour, test, Ash content, LOD test, solubility, pH and density, etc.

2.3.1. Colour.

Colour of formulation (untreated portion) was observed Under direct sunlight.

2.3.2. Odour and Taste[12].

To test the taste of the medicine very small quantity (8 mg) of it was put on the tongue to evaluate taste also same breath to determine odour.

2.3.3. Total Ash.

Three grams of the herbal formulations had to be weighed and burned at temperatures up to 45°C until the extract was carbonized, then cooled and weighed experiment performed in triplicate[13].

2.3.4. Loss on drying[14].

To carry out LOD test the weight of the empty Petri dish was taken. 2 gm of extract powder was added to it. This petri dish, along with the extract, was kept in a hot air oven at a temperature of 100°C for 1 hrs, and the weight of the petri dish was calculated until it reached the constant value. This value was used for the calculation of herbal formulation loss on drying.

2.3.5. Water soluble extract value

5mg of the powder sample of the herbal extract was taken in a conical flask with 90 ml of distilled water and 10 ml of chloroform and kept on a magnetic stirrer for 6 hrs. It was then placed for filtration for 18 hours using Whatman filter paper grade number-0100 paper and 25 ml of the filtrate was evaporated to dryness in petri dish, dried at 105°C, and weighed. The percentage of water-soluble extractive concerning air-dried material was calculated[13].

2.3.6. Alcohol soluble extractive value

5 mg of the powder sample of herbal extract was taken in a conical flask with 100 of alcohol and kept on a magnetic stirrer for 6 hrs It was then placed for filtration for 18 hrs using Whatman filter paper grade number-0100 paper and this was filtered rapidly taking precaution against loss of ethanol with using Whatman filter paper grade number-0100 paper. 25 ml of the filtrate were evaporated to dryness in a petri dish, dried at 105° C, and weighed. The percentage of alcohol soluble extractives was calculated with reference to air-dried drug[13].

2.3.7. pH

0.5 gm of sample was taken in a beaker and 5 ml of D/W water was added to it and pH of the sample was checked by using a pH meter or pH paper[15].

2.3.8. Bulk density[14]

Examining bulk density and tapered density is important because the density of the powder determines its packaging. Bulk density is determined by the constant mass method using a measuring cylinder. Powder bulk density is the ratio of the mass of the unused powder sample to its volume, including the fraction of void space between particles. It is expressed in gm/ml and is given by

Bulk density (ρB) = M/Vo

Where, M = mass of the powder (weight taken in g)

Vo = Void volume (Untapped Volume in ml)[15]

2.3.9. Tapped Density

Tapped density gives information on the consolidation of a powder. Consolidated powder is likely to have a higher arc strength than less compacted powder and therefore better withstand powder flow. It was calculated by tapping the bulk volume of powder for 15 minutes.

2.4.1 Alpha-Amylase Enzyme Assay[11]

To perform In-Vitro alpha amylase assay 1 mL of alpha-amylase enzyme pre-incubated with 1 ml of herbal formulation at 37°C for 10 min. After incubation, each tube was filled with 1 mL of 1% (v/v) substrate (starch solution) and incubated for 15 min. at 37°C. followed with determination of enzyme activity by measuring absorbance using a spectrophotometer at 546 nm (Bioera). Enzyme activity was considered in the tube without plant extract considered as control.

2.4.2 *In Vitro* Glucosidase Enzyme Assay

In-Vitro α-glucosidase assay was performed using standard method with slight modification. 0.5 ml of phosphate buffer (100 mM, pH = 6. 8) with 0.1 ml alpha-glucosidase (10 U/ml), and 0.2 ml of formulation was incubated 15 min at 37°C Then, 0.2 ml P-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The absorbance was measured at 409 nm using a Spectrophotometer (Bioera) to analysed released of p-Nitrophenol 100% enzyme activity was considered in the tube without plant extract.

2.5. Antimicrobial activity

Antimicrobial activity of plant extract ethanolic and aqueous extract determined by standard disc diffusion method in accordance with Clinical and laboratory standard institute (CLSI, 2015) against test microorganisms.

3. RESULTS

3.1. Sample authentication: Collected plant material authenticated and used for further study.

3.2. Preparation of Formulations and Evaluation for Organ: Formulations were developed by combining whole plant extract from selected plants with an optimized experimental ratio. All the formulations

were evaluated for their, Organoleptic characteristics and Physiochemical characteristics. Organoleptic characteristics represented in (Table No-01) **Physico-chemical Parameter:** The values obtained for Bulk density for formulations are tabulated in Table no.2. The values were found to be in the range from 0.402-0.513 which followed standards of antidiabetic herbal formulations.

Table 1: Study or Evaluation of Organoleptic characteristics of formulation.

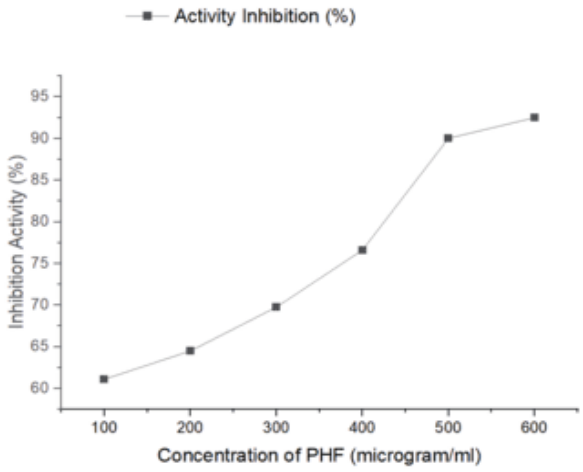
Sr. No.	Parameter	Observation
1	Description	Brown colored powder
2	Colour	Brown
3	Odour	Characteristic
4	Taste	Slightly bitter

Table 2: Study or Evaluation of physico-chemical characteristics of formulation.

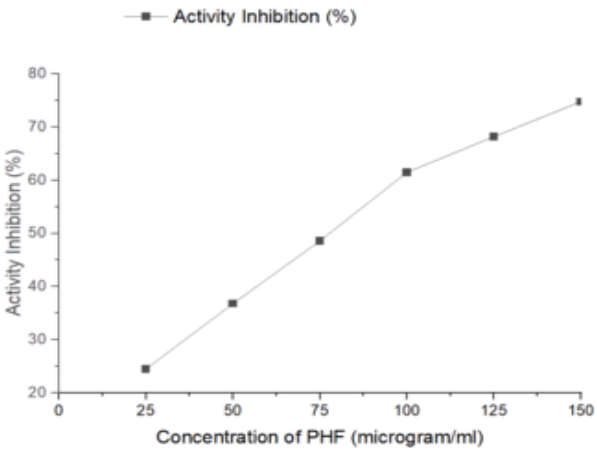
Sr. No.	Parameter	Observation
1	pH	7.2
2	Solubility in Water	17.8 ± 0.2
3	Solubility in Alcohol	11.3 ± 0.4
4	Loss on drying	2.8 ± 0.6
5	Ash content	6.9 ± 0.4
6	Bulked density	0.48 ± 0.2
7	Tapped density	0.53 ± 0.3

3.4. *In Vitro* Alpha-Amylase Enzyme Assay and Glucosidase activity

In-vitro Alpha -amylase and Glucosidase assay predicts significant inhibition of alpha- amylase enzyme as well as Glucosidase enzyme by herbal extract of *Clitoria ternatea* upto 94 % of Alpha amylase activity and 85 % of Glucosidase activity reduces upon addition of Herbal extract when enzyme activity without herbal extract addition considered as 100 %.



Graph 1: a) *In-vitro* Analysis of Alpha amylase assay.



Graph 1: b) *In-vitro* Analysis of Glucosidase assay.

3.5 Antimicrobial activity:

The same herbal extract showed antimicrobial activity against *E. coli* as zone of inhibition another side it showed no antimicrobial effect against *Klebsiella pneumonia* **(Fig-1)**.

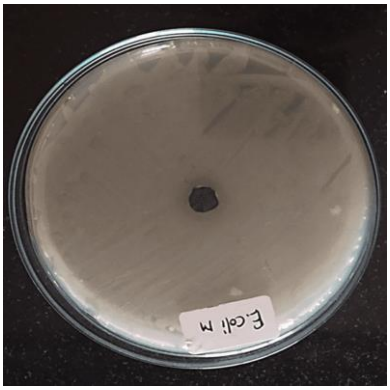


Figure 1: Antimicrobial activity of herbal extract against *E. coli*

4. DISCUSSION

The developed lyherbal extract based tea was standardized for its Organoleptic properties and physio-chemical properties. *In- vitro* anti-diabetic activity was done by using α-amylase inhibition assay method. It possessed significant antidiabetic activity. This research report is supported by several researchers, extracts and formulations of several plants show effects such as reduction of hyperglycemia, reduction of hyperlipidemia and regulation of insulin secretion, which support its antidiabetic effects. The results obtained from the study can be used as a benchmark to set benchmarks for quality control, and it is also recommended that further research be carried out in polyherbal tea bag stability studies, and further testing in Human Volunteers is required. The current report reflects that tea can be served as an ideal tea for people who are diabetic, obese, and have digestion problems. So, we can conclude that this tea can help in maintaining a healthy lifestyle.

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