EXTRACTION, ISOLATION AND EVALUATION OF ANTI-DIABETIC ACTIVITY OF *CLITORIA TERNATEA* Linn.

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Abstract:
Clitoria ternatea, often known as Butterfly pea, is a member of the Fabaceae family. From *Clitoria ternatea Linn*, several secondary metabolites, such as triterpenoids, flavonol glycosides, anthocyanins, and steroids, were identified. This work aims to assess the preliminary phytochemical screening and anti-diabetic potential of the *Clitoria Ternatea* bark extract in albino wistar rat models. The extract was concentrated after distillation. In alloxan-induced diabetic rats, the extract was administered orally once at doses of 200 and 400 mg/kg to test for anti-diabetic efficacy. At several time points, including acute and sub-acute, the blood glucose level was measured, and histological analyses were carried out to assess whether cells were regenerating. In both the acute phase (after 5 hours) and the subacute study (after 1 day), dosages of 200 and 400 mg/kg of the extract effectively reduced blood glucose levels in diabetic rats. The histopathological investigation revealed that both extract dosages resulted in greater regeneration of cells.

Keywords: *Clitoria Ternatea*, Bark, Extract, Antidiabetic, Glibenclamide, Alloxan, etc.

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Introduction

Hyperglycemia, glycosuria, polyuria, and polydipsia caused by insulin resistance [1] and insulin insufficiency [2] are the hallmarks of diabetes mellitus, a prevalent metabolic condition. According to recent estimates, the number of persons with diabetes worldwide increased from 171 million in 2000 to 366 million by 2030 [1]. Oral hypoglycemic medications like sulphonyl urea are used to treat diabetes mellitus. Alpha glucosidase inhibitors, biguanides, and meglitinides [3]. As they are believed to be non-toxic by nature, many diabetic patients have utilized traditional medications such herbal drugs in their main form or their extracts [4]. In order to assess the *Clitoria Ternatea* bark extract's preliminary phytochemical screening and anti-diabetic activity in albino wistar rat models, the current study was launched.

Clitoria ternatea, sometimes known as butterfly pea, is a member of the Fabaceae family. From *Clitoria ternatea Linn*, a variety of secondary metabolites, including triterpenoids, flavonol glycosides, anthocyanins, and steroids, were identified. Chronic bronchitis, dropsy, goitre, leprosy, mucous disorders, sight weakening, skin illnesses, sore throats, and tumors are all commonly treated with *C. ternatea*. It was traditionally used as an aphrodisiac, to control menstrual flow, and to treat sexual disorders such gonorrhea and infertility [5]. Its extracts have a variety of pharmacological effects, including anti-microbial, anti-pyretic, anti-inflammatory, analgesic, diuretic, local anesthetic, anti-diabetic, insecticidal, and blood platelet aggregation inhibition [6, 7]. They can also be used to relax vascular smooth muscle. This factor led us to choose the plants *Clitoria ternatea* Linn for the treatment of diabetes mellitus.

The anti-diabetic medicine Glibenclamide (INN), also marketed under the name glyburide (USAN), belongs to the sulfonylurea drug class, which is closely connected to the sulfa drug family. For the evaluation of herbal extracts in preclinical research, it serves as the standard control medication. In order to compare the antidiabetic activity of *Clitoria Ternatea* bark extract, we employed Glibenclamide (10 mg/kg) as the standard anti-diabetic medication. In order to test the postulated idea, the phytochemical components of the plant's bark were extracted, along with their potential antidiabetic effects on blood sugar and other biochemical indicators.

Material and Method

Plant Material

Collection and authentication

Plant was collected from the Nagpur region, Maharashtra, India. The plant materials were validated by the taxonomist from Department of Botany, RTMNU, Nagpur. It was then cleaned and dried at normal temperature away from direct sunlight. An herbarium sheet was prepared and authentication of plant was done by the taxonomist from Department of Botany, RTMNU, Nagpur after confirmation of identification features. Plant was authenticated as *Clitoria Ternatea* . Herbarium sample was kept for reference.

Processing of plant material

The dried bark was coarsely sized and reduced in mixer grinder. The particle size difference of crude drug increases the extraction time and fine particles might form bed and increase the extraction time, so the powdered material was sieved through 60-120 mesh to remove fines and larger particles and the powder was used for further evaluation.

Standardization of Bark

The physical confirmation of crude drug contains the confirmation of identity, quality, and purity. Purity contains the absence of any unnecessary content whether inorganic or organic and quality means required concentration of the active constituents in crude drug which makes it as important drug. Various standardization parameters were evaluated to obtain the quality and purity of *Clitoria Ternatea*, *i.e.*, ash value, acid-insoluble and water soluble as well as sulphated ash value, alcohol and water-soluble extractive value, determination of loss on drying and foreign organic matter were performed as per the methods described in I.P. 1996.

Determination of ash value

Ash value is important in identification of quality & purity of *Clitoria Ternatea* bark. It mainly consists of different inorganic variables and radicals like phosphates, carbonates, silica as well as calcium, magnesium, sodium and potassium silicates, calcium oxalate and carbonate, silica are present as inorganic variables which changes 'Total Ash Value'. These variables can be removed by concentrated acid treatment, known as acid insoluble ash.

Total Ash Value

Method

2 gm of completely dried powder of *Clitoria Ternatea* bark was weighed and added in pre weighed silica crucible and burned at room temperature not more than 450º until it become

free from carbon. It was determined by cooling the silica dish in desiccator. This process was performed again and again till it gives fixed weight. Total ash percentage was determined by consideration with weight of initial powder of Clitoria Ternatea bark.

% Total ash value = Wt. of total ash ÷ Wt. of crude drug taken × 100

Water soluble Ash
Method
Clitoria Ternatea bark powder was taken and 2gm powder was added in previously weighed crucible of silica and it was then kept at high temperature not more than 450º until it become free from carbon. It was determined by cooling the silica crucible in desiccators and weighed. The same process was repeated until constant weight was identified. The ash thus obtained was further boiled in 25ml of water for about 5 minutes; ash insoluble in water was collected by filtration in silica plate and washed. The content was burned for few minutes at high temperature but not more than 450º. From the total ash weight of insoluble matter was deducted to get weight of water-soluble ash. Percentage was determined by considering the initial weight of crude drug.

% Water soluble ash value = Wt. of total ash - Wt. of water insoluble ash ÷ Wt. of crude drug taken × 100

Acid-insoluble Ash
Method
2gm of dried *Clitoria Ternatea* bark powder was added in pre weighed crucible of silica and burned at high temperature, less than 450º until free from carbon. It was determined by cooling the silica dish in desiccators and weighed. The same process was repeated till constant weight was obtained. The ash obtained was mixed in 25ml 2M HCL and boiled for 5 minutes. Then insoluble content was added in a silica gel crucible. Again, hot water was added and filtered, then burned and cooled in a desiccator, weight was taken. The percentage was determined by considering initial weight of *Clitoria Ternatea* bark.

% Acid insoluble ash value= Wt. of acid insoluble ash ÷ Wt. of crude drug taken × 100

Sulphated ash value
Method
2 gm of powdered *Clitoria Ternatea* bark was added in previously weighed crucible. It was heated at high temperature not more than 450º until it became free from carbon. It was determined by cooling the silica crucible in desiccators and weighed. The same process was repeated till constant weight was identified. The ash obtained was mixed with 1ml of H_2SO_4, heated until release of white coloured fumes finished. Further ignited at 800ºC±25ºC till all black particles get disappeared. The heating was done away from direct air. The silica crucible was cooled. Again, few drops of H_2SO_4 were added and ignited. This process was done repeatedly to get constant weight.

Water-soluble extractive value
Method
5 gm *Clitoria Ternatea* bark powder was weighed was added in closed flask and kept for maceration in chloroform water(100 ml) for one day with frequent shaking for first 6 hours, then for 18 hours kept aside and filter. In pre-weighed silica dish, 25ml of filtrate was taken and evaporated to dryness. The percentage was determined by considering initial weight of Clitoria Ternatea bark powder.

Calculations
If 25ml of aqueous filtrate gives X g of residue,
Then 100ml of filtrate will give 4X g of residue,
So 5gm of powdered *Clitoria Ternatea* contains 4X g of water-soluble residue
Therefore, percentage of water-soluble extractive value will be 80X.

Alcohol-soluble extractive value
Method
Accurately weight 5gm of powdered *Clitoria Ternatea* bark was mixed with 95% ethanol (100) in a closed vessel. It was macerated for 24 hours with occasional shaking for initial six hours. Kept aside for 18 hours and filtered carefully to avoid evaporation of ethanol. Filtrate (25ml) was evaporated in pre weighed porcelain dish; weight was calculated. The percentage of alcohol soluble extractive value was determined by considering initial weight of powdered *Clitoria Ternatea* bark.

Calculations
25 ml of alcohol filtrate possess about A g of residue,
So, 100 ml of filtrate contains 4A gm of residue.
So, 5gm of powdered Clitoria Ternatea contains 4A gm of residue. And percentage of extractive value will be 80A gm of alcohol (90%) soluble residue.

Foreign organic matter determination

The foreign matter in crude drugs mainly contains either parts of the plant or product. Sometimes it might also contain any organism. It also indicates any mineral content which is not the medicinal plant materials like stones, soil and dust, etc. Enough crude drug was spread on a thin layer of clean paper. With help of magnifying lens or by visual inspection, the content present other than the crude drug was collected and weighed.

Determination of Loss on Drying or Moisture content

The shallow glass-stoppered weighing bottle was dried and weighed. 2gm crude drug was added in the bottle and closed, the weight was taken and crude drug was spread evenly to a height not more than 10mm. Then the bottle was kept in the oven for drying keeping open without the stopper. The sample was dried to constant weight. It was cooled to normal temperature in desiccator again weighed loss on drying was calculated in percent w/w (Indian Pharmacopoeia 1996).

$$\% \text{ Loss on Drying} = \frac{\text{Loss in weight of the sample}}{\text{weight of the sample}} \times 100$$

Extraction Method

The solid extraction of drug represents a solid from solid separation. The liquid-liquid extraction is one, in which any of the two immiscible liquids are used for the extraction (Solvent extraction). Extraction process comes to halt when the distribution of the extractive substance between miscella and drug residue reaches the value 'K' i.e., when the concentration gradient between miscella and drug residue has become zero.

K = Concentration of extracted substances in the miscella / Concentration of extractive substance in the drug residue

Hot continuous extraction- Soxhlation

Soxhlet extractor is the simplest way for preparation of extracts of crude drugs. Pure solvent is used in this technique. The crude drug used for extraction is kept in a 'thimble' made of cloth or cellulose in middle portion of the Soxhlet apparatus. Siphon tube and a side arm both are connected to a lower portion. The solvent used for extraction is kept in the lower portion and a condenser is connected above the middle compartment. The solvent is added in round bottom flask and heated to boil to form vapours. The vapours travel through the side arm into the reflux condenser. The vapour cools there and falls onto the thimble containing the crude drug kept for extraction. The hot solvent passes through the crude drug and extraction takes place. The extract gets deposited in the lower portion of middle compartment. As the height of extract reached to the top of the siphon tube, the extract gets deposited in the middle portion passes through it and goes into the lower container i.e., round bottom flask. The same process was repeated till complete extraction of crude drug takes place. In this technique of extraction, the extract gets collected in the lower RBF, gradually becomes concentrated. The soxhlet extraction process is very helpful for the total extraction of crude drug with a specific solvent. Different solvents with increasing polarity can be used for the continuous total extraction, e.g., benzene, hexane, pet. ether, chloroform, methanol, ethanol, water. The crude drug was dried when it was subjected for extraction using another solvent. The previous solvent should be removed completely and powder should be dried totally. It prevents the mixing of the previous solvent into another solvent.

Extraction Procedure

The dried powder of bark used for extraction procedure was sieved through 60-120 mesh to separate fine and course powder. This course powder (1000gm) was utilized for further extraction. Bark powder was exhaustively defatted using petroleum ether (60-80 ºC) (SG-PE) and extracted successively with chloroform (SG-CH), ethyl acetate, (SG-AC) and ethanol (BP-ET) using Soxhlet apparatus. The confirmation of complete extraction was done by taking a drop of extract from exit of side tube on TLC, drying and exposing to iodine vapors. If extraction is completed then it shows absence of colored spot-on TLC plate. After the complete extraction, solvents were evaporated on rotary evaporator and solvents were removed. The extracts thus obtained with different solvents were measured and the extracts were stored in desiccator. The percentage yield was calculated for all solvent extracts.

Preliminary phytochemical screening of different extracts of *Clitoria Ternatea* [8, 9]

The qualitative phytochemical evaluation of the chloroform, ethyl acetate and ethanolic extracts of Clitoria Ternatea were performed to detect different chemical constituents. The different reagents and tests were used to detect various secondary metabolites.

Tests for Carbohydrates

General test for Carbohydrates (Molisch's test)

Few drops of α-naphthol dissolved in alcohol were added in 2-3ml of extract, mixed well and conc.

H_2SO_4 was added from side wall of test tube. Violet colored ring formation at junction of two liquids shows that carbohydrates are present in the extract.

Benedict's test

Clitoria Ternatea extract 2ml mixed with benedict's reagent (2ml) and mixed well. The mixture was kept in boiling H_2O bath for 4-5 minutes, mixture in test tube forms red, yellow, or green color depends upon the quantity of reducing sugar present in extract.

Fehling's test

Equal quantity of Fehling's A and Fehling's B solutions was mixed. In mixture of Fehling's solution 2ml of *Clitoria Ternatea* extract was mixed, Shaked well. The complete mixture was boiled in water bath for 4-5min. and color of formed precipitate was determined. It gives initially yellow and then brick red precipitate if reducing sugar present.

Barfoed's test

Barfoed's reagent and Clitoria Ternatea extract was mixed in equal quantity and boiled in water bath for 1-2minutes. Red coloured precipitate indicates availability of reducing sugars.

Testes for Non-Reducing Sugars

The *Clitoria Ternatea* extract do not show positive tests for response to Benedict's and Fehling's test.

Tests for Proteins

Test Solution Preparation: *Clitoria Ternatea extract* was dissolved in water to prepare clear solution.

Biuret Test (General Test)

To 5ml test solution, 4% NaOH (5ml) and 1% $CuSO_4$ (Few drops) was mixed. Pink/violet color formation indicates presence of proteins.

Million's Test

The test solution of *Clitoria Ternatea extract* was mixed with double quantity of Million's reagent, presence of proteins gives formation of white colored precipitate.

Tests for Amino Acids

General test of amino acid - Ninhydrin test

To equal quantity of *Clitoria Ternatea extract* same quantity of drops Ninhydrin reagent (5%) was kept in water bath for some time and formation of purple or bluish color was observed.

Test for Tryptophan

Few drops glycol-oxalic acid and conc. H_2SO_4 were added in 3ml of the *Clitoria Ternatea extract*. The reddish violet ring formation takes place at the junction of the two layers.

Test for Tyrosine

Equal volume of *Clitoria Ternatea extract* and million's reagent mixed and boiled. Formation of dark red color indicates presence or absence of amino acids.

Tests for Steroids

Salkowski reaction

Equal volume of *Clitoria Ternatea extract*, conc. H_2SO_4 and chloroform were mixed well. Red color appears to layer of chloroform, acid layer gives greenish yellow colored fluorescence for presence of steroids.

Liberman's test

Equal quantity of *Clitoria Ternatea extract* and acetic anhydride was mixed. Heated and cooled and few drops conc. H_2SO_4 was mixed and formation of blue color indicates availability of steroids.

Libermann-Burchard Test

5ml *Clitoria Ternatea extracts* when mixed with $CHCL_3$. Acetic anhydride (2-3ml) was added and conc. H_2SO_4 (2-3 drops) were added from side wall and formation of initially red, then blue lastly green color indicates steroids are present.

Tests for Glycosides

Preparation of Test solution: *Clitoria Ternatea extract* was dissolved in hydro-alcoholic solution in alcohol.

Cardiac Glycoside test

Baljit Test

Addition of Sodium Picrate to test solution gives formation of yellow to orange color due to presence of cardiac glycoside.

For Cardenoloids-Legal's test

Alcoholic or Hydro-alcoholic test solution, pyridine (1ml) and Na-nitroprusside solution (1ml) was added, formation of pink to reed color indicates presence of cardenolides.

Test for Deoxysugars (Killer Killani Test)

1ml of GAA, 2ml Test Solution and a drop of $FeCL_3$ (5%) were mixed and conc. H_2SO_4 was added from sides of test tube. Formation of bluish green color to upper layer and reddish-brown color formation at the junction of two liquids shows that Deoxy sugars are present.

Liberman's Test (For bufadienolides)

Equal quantity of extract and acetic anhydride was mixed and heated and cooled. When few drops of concentrated H_2SO_4 were added presence of bufadienolides gives formation of blue color.

Test for anthraquinone glycosides

Borntrager's test for O-Anthraquinones

The powdered *Clitoria Ternatea was* mixed with 10% Sulphuric acid (5ml). Boiled and filtered. Filtrate was mixed with benzene and shaken for few minutes. Isolation of benzene layer was done and mixed with 10% NH_3. Both layers were separated. Rosey pink color appears to

ammoniacal layer indicates presence of O-anthraquinone glycosides.

Modified Borntrager's test (O-anthraquinone glycoside)

C-anthraquinone glycosides cannot get hydrolyzed easily it requires oxidative hydrolysis. Hydrolysis of crude drug was performed using acid and $FeCl_3$. The powder of *Clitoria Ternatea was* mixed with dilute HCL and 5% $FeCl_3$ solution (5ml), boiled, filtered, cooled then shaken with organic solvent like chloroform or benzene. The organic solvent was separated, further mixed with ammonia solution (10%) and kept aside. The ammoniacal layer shows reddish pink color due to presence of C-anthraquinones.

Tests for Saponin Glycosides

Hemolytic Test

One drop of blood was kept on glass slide and a drop of *Clitoria Ternatea extract* was added in it. Occurrence of hemolysis was observed form the presence of saponins.

Foam Test

Powder of *Clitoria Ternatea crude* drug was mixed with water and shaken vigorously. Formation of stable foam for minimum of 30 min indicate presence of saponin glycosides.

Cyanogenic glycoside Test

Sodium Picrate test

10% picric acid was soaked in a filter paper strip and dried and then 10% sodium carbonate was soaked in it and again dried. The powder of *Clitoria Ternatea was* kept in flask and small quantity of water is added, mixed and closed. The filter paper strip soaked in picric acid and sodium carbonate was placed in the slit in cork above the powder mixture. The color of filter paper turned brick red or maroon due to presence of Cyanogenic glycosides in the crude drug.

Flavonoids Test

Shinoda Test

In the *Clitoria Ternatea extract*, addition of conc. HCL and 0.5gm Mg-turnings was done. Pink color formation indicates that flavonoids are present. Lead acetate solution was added to *Clitoria Ternatea extract*. Yellow colored precipitate formation indicates that flavonoids are present. When sodium hydroxide was added in increasing amount in above residue it gives yellow coloration, and it decolorizes on addition of any acid.

Alkaloids Test

The *Clitoria Ternatea extract* was boiled in alcohol and filtered. The filtrate was used to perform different chemical tests for identification of alkaloids.

Dragandorf's Test: To the 2-3 l filtrate, Dragandorf's reagent (1ml) was mixed. Orange brown precipitate formation indicates that alkaloids are present.

Mayer's Test: Few drops of Mayer's reagent were mixed with few ml of filtrate. Formation of buff colored precipitate shows that alkaloids are present.

Hager's Test: Alcoholic extract was mixed with half volume of Hager's reagent; formation of orange brown precipitate shows presence of alkaloids.

Wagner's Test: Wagner's reagent was mixed in 2-3ml filtrate. Presence of alkaloids can be confirmed by appearance of brownish red colored precipitate.

Tannins and phenolic compound test

$FeCl_3$ Test: *Clitoria Ternatea extract* was mixed with 5% $FeCl_3$ solution (1ml). Tannins indicate development of greenish black coloration.

Lead acetate solution test: *Clitoria Ternatea (*5ml) was mixed with 10% aq. Lead solution (1ml). Presence of tannins gives formation of yellow colored precipitate.

Potassium dichromate Test: *Clitoria Ternatea extract* (5ml) when mixed with 10% aq. Potassium dichromate solution (1ml), yellowish brown precipitate indicates formation of tannins.

Bromine water test: When freshly prepared bromine water was mixed in 5 ml of *Clitoria Ternatea extract*. Discoloration of bromine water shows presence of tannins.

Dilute of $KMnO_4$ Test: Few ml of *Clitoria Ternatea* extract was mixed with diluted $KMnO_4$ solution. Presence of tannins gives discoloration of $KMnO_4$.

Test for mucilage

The *Clitoria Ternatea* extract was mixed to few ml of ruthenium red solution in alcohol. Pink color formation indicates that mucilage was present.

Test for Gums

The *Clitoria Ternatea* extract was hydrolyzed by addition of HCl. The tests for presence of reducing sugar i.e., Benedict's or Fehling's test were performed. Formation of red color indicates presence of gums.

Development of TLC fingerprints profile of the extracts

All the extracts of selected plant material were subjected to TLC studies using various solvent systems to determine the presence of various phytoconstituents. The Rf values of observed compounds were noted for all extracts. The characteristic fingerprint of the various chemical

constituents in each extract under UV light and after derivatization with suitable reagents was recorded. Preliminary phytochemical screening revealed the presence of carbohydrate, proteins flavonoids, alkaloids, fixed oils, steroid and saponins. Compounds of varying polarity in the extracts well separated using various solvent systems on TLC. Rf value of the separated compounds were recorded and compared with reference standard (The Indian Pharmacopoeia, 2006)

Determination of total phenolic content of different extracts

Procedure

4 ml of Folin Ciocalteu reagent was mixed with 1 ml of extract solution, this solution mixture was kept on standing for 5 min and then 5 ml of sodium carbonate was added to it. The absorbance of reaction mixture was measured against blank (without extract) at 765 nm using UV-Visible spectrophotometer. Gallic acid was used as standard for determination of total polyphenol content of extract. The calibration curve was drawn using various concentrations of gallic acid (50, 100, 150, 200, 250 µg/ml). The total polyphenol content was expressed as gallic acid equivalent in mg/g of the extract and was calculated by using following equation obtained from standard gallic acid graph (r2 = 0.9964) (Swamy, et al, 2012).

Blank solution: blank solution was prepared by the 0.4 ml Folin-ciocalteu reagent, 4 ml of 7% sodium carbonate and volume was made up to 10 ml with dil. water in 10 ml volumetric flask.

Formula: Absorbance (y) = mx + c

Absorbance (y) = 0.009 [Gallic acid (µg)] X + 0.071

Determination of total flavonoids content of CT extracts

Procedure

1 ml of extract solution was mixed with 4 ml of distilled water & 0.3 ml of $NaNO_2$. After 5 min 0.3 ml of $AlCl_3$ & 2 ml of NaOH was added, at last total volume was made up to 10 ml with distilled water. The solution was mixed well and absorbance of the solution was measured at 510 nm against prepared blank (without extract). Rutin was used as standard for determination of total flavonoid content of extracts. The calibration curve was drawn using various concentrations of rutin (100, 200, 300, 400, 500 µg/ml) (Table 4.5). The total flavonoid content was expressed as rutin equivalent in mg/g of the extract & was calculated by using following equation obtained from standard rutin graph (r2 = 0.999). (Ekramul Haque, et al, 2011).

Absorbance (y) = mx + c

Absorbance (y) = 0.003[Rutin (µg)] x + 0.011

Hypoglycemic Activity

Induction of diabetes

Each animal's weight-based dose of alloxan monohydrate was weighed separately before being solubilized with 0.2 ml of saline right before injection. By administering it intraperitoneally at a dose of 100 mg/kg body weight, diabetes was produced. To get over the early hypoglycemia phase, 5% dextrose solution was also administered in a feeding bottle for a day after the animals had received alloxan for 1 hour. After 48 hours of observation, blood glucose levels in the animals were assessed. One group functioned as the control and was given a vehicle by itself. For an experimental investigation, the diabetic rats (glucose level >150 mg/dl) were separated and put into various groups [10,11].

Experimental design

Acute treatment and subacute treatment

Six groups of animals containing six rats in each group were divided as follows.

Group I: Normal control.

Group II: Diabetic control (Normal saline).

Group III: Glibenclamide10mg/kg/day p.o. (Std)

The fasting BGLs of all the rats were measured at regular intervals during the study period in groups IV and V after receiving oral doses of 200 mg/kg and 400 mg/kg/day of Clitoria Ternatea bark extract, respectively. In an acute investigation, the BGLs were checked at 0, 1, 3, 5, and 7 hours after a single dosage of the extract was administered, and at 1, 3, 5, and 14 days after prolonged treatments. The tail-cutting and retro-orbital puncture methods were used to monitor the BGLs in the blood of the diabetic rats. By using the GOD-POD technique, the glucose levels will be assessed [12-14].

Histopathological Study

Each animal's entire pancreas was removed when it was sacrificed, collected in 10% formalin solution, and cut into 5-mm-thick sections for histological analysis using hematoxylin and eosin (H & E) staining [15,16].

Statistical analysis

All the experimental outcomes were expressed as mean SD, and they were all subjected to a one-way ANOVA analysis before being subjected to "Dunnett's Test."

Result and Discussion

Table: Physico-chemical parameters of bark of Clitoria Ternatea

Sr.no.	Standardization parameters	Results
1	% Foreign Organic Matter (w/w)	<2
2	% Total Ash (w/w)	8.65
3	% Acid Insoluble Ash (w/w)	0.26
4	% Water Soluble Ash (w/w)	1.74
5	Sulphated Ash Value (%)	0.985
6	Moisture Content (w/w)	1.367
7	% Extractive Values (w/w)	1.24
8	Alcohol Soluble	24.86%
9	Water Soluble	41.71%

Percentage yield of different extracts of Clitoria Ternatea

Table: % yield of different extracts of Clitoria Ternatea

Sr. No.	Solvent Extract	Colour	Consistency	% Yield (%w/w)
1.	Chloroform	Yellowish brown	Sticky	10.31
2.	Ethyl acetate	Brown	Sticky	16.04
3.	Ethanol (95%)	Dark brown	Sticky	37.02

Preliminary Phyto-chemical screening

Table: Preliminary Phytochemical screening of Clitoria Ternatea extract

Sr. No	Name of test	Chloroform extract	Ethyl acetate extract	Ethanolic extract
1	Test for Carbohydrates	-	-	+
2	Test for Proteins	-	-	+
3	Test for Phenolics	+	+	+
4	Flavonoids Test	+	+	+
5	Test for Glycosides	+	+	+
5a	Cyanogenic Glycosides	-	-	-
5b	Test for Anthraquinone Glycosides	-	-	+
5c	Saponin Glycosides	+	+	+
5d	Cardiac Glycosides	-	-	-
6	Alkaloids Test	-	+	+
7	Test for Tannins	+	+	+
8	Test for Coumarins	-	-	+
9	Test for Saponins	+	+	+
10	Test for Steroids	-	-	+

In above table + indicates presence & - indicates absence of the phytochemical constituents which were screened using various identification tests. After subjecting to phytochemical screening, it was found that flavonoids and alkaloids were present in all the three extracts. Whereas tannins and phenolic compounds were present in ethanolic extract. The ethanolic extract contains alkaloids, flavonoids, tannins, and phenolic compounds i.e., maximum phytoconstituents compared to other extracts.

TLC fingerprint profiles of the extracts

Ethanolic extract **Ethyl acetate extract** **Chloroform extract**

Figure: TLC fingerprinting of various extracts

Table: Thin layer chromatography (TLC) fingerprinting of various extracts

Sr. No.	Extract	Solvent System	Rf value	Derivatizing agent
1.	Ethanol	Methanol: Chloroform (1:5)	0.25,0.66, 0.75, 0.58	Sulphuric acid
		Toluene: Chloroform: Acetone (1:3.1:4.3)	0.34, 0.41, 0.75, 0.80	
2.	Ethyl acetate	Methanol: Chloroform (1:5)	0.25,0.58	
		Toluene: Chloroform: Acetone (1:3.1:4.3)	0.33, 0.58	
3.	Chloroform	Methanol: Chloroform (1:5)	0.20	
		Toluene: Chloroform: Acetone (1:3.1:4.3)	0.26, 0.41	

Total Polyphenolic content

Table: Calibration Curve of Gallic Acid

Sr. No.	Concentration (µg/ml)	Abs. at 765nm
1	0	0
2	10	0.185±0.001
3	20	0.375±0.009
4	30	0.542±0.011
5	40	0.706±0.012
6	50	0.914±0.012

Values are expressed as mean ± SEM (n = 3)

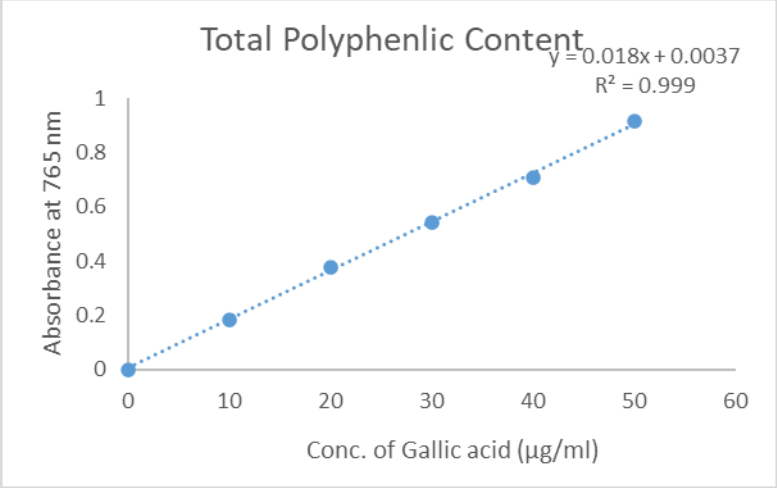


Fig. Calibration Curve of Gallic acid

Table: Total Polyphenolic content of *Clitoria Ternatea* bark extracts

Sr. No.	Extracts	Concentration (µg/ml)	Abs at 765nm	TPC (mg/g)
1	Ethanol	100	0.276±0.015	151.89±0.39
2	Ethyl acetate	100	0.117±0.012	60.11±1.03
3	Chloroform	100	0.080±0.001	42.08±0.83

Values are expressed as mean ± SEM (n = 3)

After subjecting extracts, which showed positive tests for polyphenols, to the total polyphenolic content, the ethanolic & ethyl acetate extracts of Clitoria Ternatea showed maximum content of polyphenols i.e., 54.10 & 49.36 mg/g of SGE. Chloroform extract contains 36.60 mg/g of polyphenols.

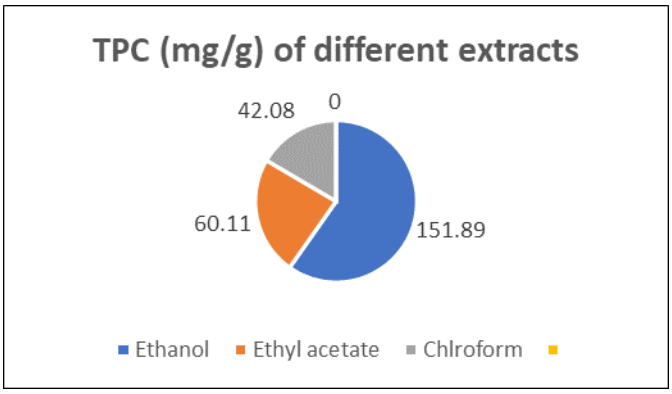


Fig. Total Polyphenolic content of *Clitoria Ternatea* bark extracts

Total Flavonoid content

Table: Calibration Curve of Rutin

Sr. No.	Concentration (µg/ml)	Abs. at 510nm
1	0	0
2	10	0.126±0.008
3	20	0.247±0.012
4	30	0.368±0.011
5	40	0.497±0.011
6	50	0.633±0.011

Values are expressed as mean ± SEM (n = 3)

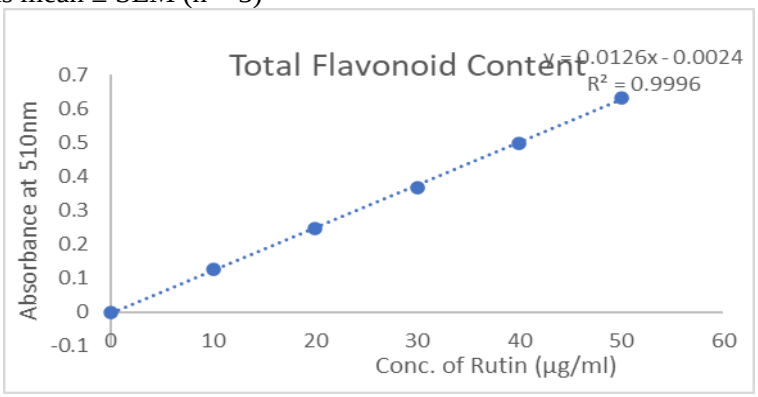


Fig. Calibration Curve of Rutin

Table: Total flavonoid content of *Clitoria Ternatea bark* extracts

Sr. No.	Extracts	Concentration (µg/ml)	Abs. at 510nm	TFC (mg/g)
1	Ethanol	100	0.103±0.008	81.16±0.09
2	Ethyl acetate	100	0.051±0.009	42.16±0.09
3	Chloroform	100	0.025±0.008	20.55±0.01

Values are expressed as mean ± SEM (n = 3)

Ethanolic extracts contains 5.14 mg/g of RE flavonoids, more amount compared to ethyl acetate and chloroform extracts.

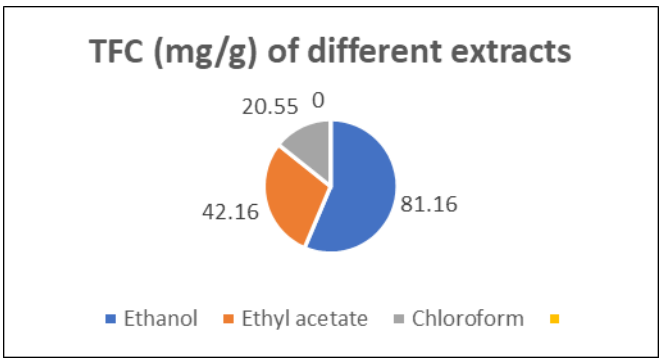


Fig. Total flavonoid content of *Clitoria Ternatea* bark extracts

Hypoglycemic Activity

The graph below illustrates the hypoglycemic impact of *Clitoria Ternatea* bark extract on diabetic rats' fasting blood sugar levels. After a single administration of the extract, blood sugar levels at 200 mg/kg and 400 mg/kg levels significantly decreased after 5 hours and 3 hours, respectively. At the conclusion of the sub-acute investigation, the extract at a dose of 400 mg/kg demonstrated a significant ($p < 0.001$) decrease in blood glucose comparable to that of the group treated with Glibenclamide (10 mg/kg).

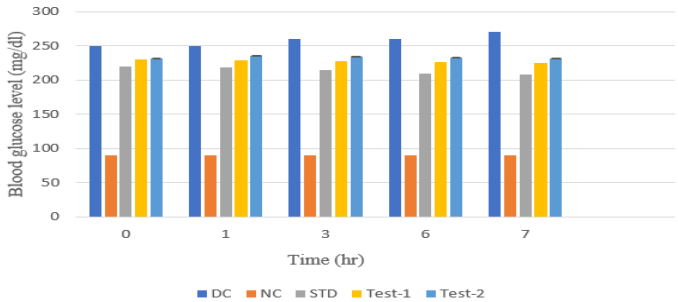
Table: Effect of *Clitoria Ternatea* bark extract on alloxan-induced diabetic rats after single dose

Group	Blood glucose level (mg/dl)				
	0 hr Mean ± SD	1 hr Mean ± SD	3 hr Mean ± SD	5 hr Mean ± SD	7 hr Mean ± SD
I (Normal control)	90.01 ± 6.898	90.01 ± 6.898	90.01 ± 6.898	89.51 ± 6.892	89.34 ± 7.440
II (Diabetic control)	240.9 ± 7.464	244.1 ± 7.326	244.1 ± 7.326	246.6 ± 6.719	250.1 ± 6.798
III (Standard)	216.9 ± 7.275	212.8 ± 7.803	201.6 ± 7.474**	185.6 ± 7.835***	175.8 ± 8.091***
IV (Test-I)	229.4 ± 11.96	227.4 ± 11.43	221.1 ± 11.85	212.8 ± 11.85*	207.1 ± 11.25**
V (Test II)	231.3 ± 9.011	230.0 ± 7.842	216.8 ± 9.938*	205.6 ± 8.919***	193.4 ± 8.712***

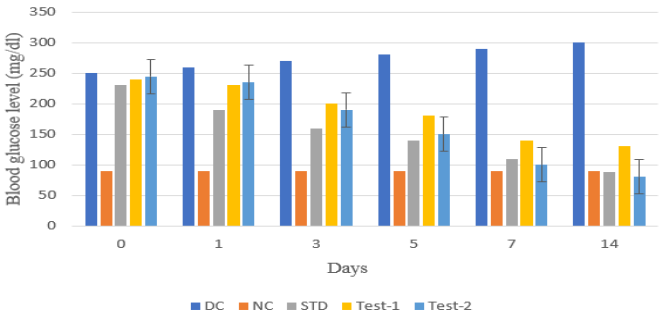
Values are mean ± SD; N=6. *P value 0.01, **p 0.02, ***p < 0.001 vs. Diabetic control.

Table: Effect of *Clitoria Ternatea* bark extract on alloxan-induced diabetic rats after sub-acute treatment

Group	Blood glucose level (mg/dl)					
	0 day Mean ± SD	1 day Mean ± SD	3 day Mean ± SD	5 day Mean ± SD	7 day Mean ± SD	14 day Mean ± SD
I (Normal control)	90.01±6.898	89.72±7.012	89.01±6.898	89.01±6.330	89.01±6.898	89.51±6.649
II (Diabetic control)	240.9±7.464	245.7±7.015	255.9±6.464	269.4±5.889	269.4±6.889	312.9±5.302
III (Standard)	216.81±7.275***	193.91±7.737*** 1	169.31±6.891***	141.31±6.891***	141.31±7.891*** 1	118.81±5.447***
IV (Test-I)	229.4±11.96	217.1±11.53**	204.1±11.50***	183.1±12.98***	162.6±12.05***	134.3±11.99***
V (Test II)	231.21±9.011	211.41±8.687**	180.81±8.999***	144.71±8.502***	97.84±7.495***	67.01±13.461***



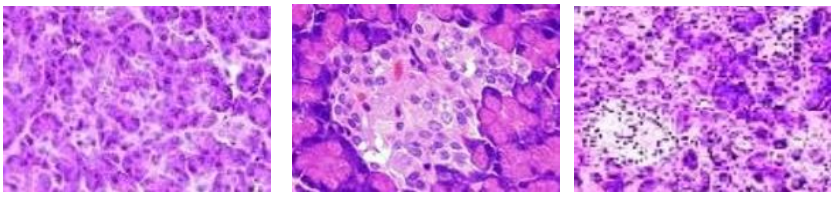
Acute Study



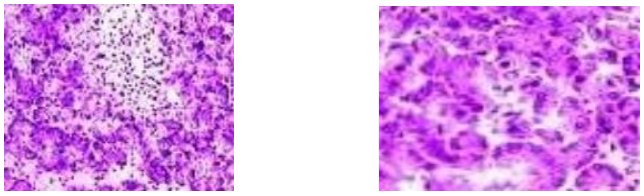
Sub-acute Study

Fig. Hypoglycemic activity of all groups of diabetic rats (acute and sub-acute treatment). The values are mean ± SD, n=6.

According to the histopathology studies, the fundamental reason for the extracts' antidiabetic effect may be the regeneration of beta cells after they have been destroyed by alloxan. The findings demonstrated that, when compared to the diabetic control group, test-1 and test-2 treated groups showed greater cell regeneration. The outcomes are displayed below.



Normal control **Diabetic control** **Standard**



Test-1 **Test-2**

Fig. Regeneration of β-cells in standard and test groups after sub-acute treatment

Discussion and Conclusion

Within the next 25 years, diabetes mellitus, a common heterogeneous metabolic syndrome that affects a large portion of the global population, is expected to overtake AIDS as one of the leading causes of death and disability. To track the glycaemic control mechanism, measurements of blood glucose, urine sugar, and body weight have been used. The climbing, scrambling, or trailing herb *Clitoria Ternatea*, sometimes known as the butterfly pea, has a short lifespan and a robust woody rootstock. From the woody base, it annually generates new, thin annual stems that can reach a length of 3 meters. At the nodes, the stems do not root. For use as food, medicine, and dye, the plant is gathered from the wild. It has also been grown for a very long time as a source of medicine and dye, as an ornamental for its colorful flower display, and to improve the soil in plantations. In rats with diabetes induced by alloxan, *Clitoria Ternatea*, which contains a variety of phytochemicals, possesses anti-diabetic properties. Glibenclamide is an anti-diabetic pharmaceutical that belongs to the sulfonylurea drug class, which is closely connected to the sulfa drug family. It is used as the reference medication to compare the antidiabetic activity of various extracts of medicinal plants in preclinical research to evaluate the effectiveness of herbal extracts for the treatment of diabetes. After a single dose, ethanolic Clitoria Ternatea bark extract doses of 200 mg/kg and 400 mg/kg significantly decreased blood sugar levels in the acute and repeated dose studies, respectively. The regeneration of cells following their destruction by alloxan may be the main reason for the extracts' antidiabetic effect, according to the histopathological studies. Beta cell regeneration was seen in all test groups and the standard group, but it was better in tests 1 and 2 when compared to the diabetic control group, i.e., equal to that of the standard drug treated group. There is no cell destruction in the normal control group, and complete cell damage was seen in the diabetic control group. In comparison to the

diabetic control group, the results demonstrated that the test-2 (400 mg/kg) group and the group receiving standard medication treatment saw better cell regeneration. The discussion leads to the conclusion that the Clitoria Ternatea bark extract (200 mg/kg and 400 mg/kg) significantly reduced alloxan-induced hyperglycaemia. These extracts also improved pancreatic stem cell regeneration, suggesting that they may be useful in the treatment of diabetes. To identify and isolate the precise phytoconstituents responsible for the antidiabetic activity and mechanism of action, more research is required.

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