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Physicochemical, Phytochemical Screening and Evaluation of Antidiabetic Activity of *Ficus dalhousiae* Fruits on Alloxan Induced Diabetic Rats

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ABSTRACT

The aim of the present study was to evaluate Physicochemical, Phytochemical Screening of Antidiabetic Activity of *Ficus dalhousiae* Fruits in Alloxan Induced Diabetic Rats. A plant material like "*Ficus dalhousiae*" member of the genus Moraceae, and physico chemical standards used for ash values. In statistical analysis, all data were represented in mean ± SEM (Standard error mean) values expressed as mean ± SEM of six samples data were analysed by one-way ANOVA followed by Dunnett's test. Effect of extracts of *Ficus dalhousiae* fruit on blood parameters against Alloxan induced Diabetic rats. The Physicochemical standards of the fruit of *Ficus dalhousiae* have been carried out. The result shows that total Ash value was 6.7% w/w, water in soluble Ash 2.5% w/w and acid insoluble ash 0.7 w/w. Moisture content was 14.74% w/w. The water-soluble extractive values were 1.2%w/w, alcohol soluble extractive, Values 1.6% w/w and chloroform soluble extractive values were 4.6% w/v. Foaming index was 100., Fat content was 120mg, resin content was 250 mg, chloroform extract yield Was 4.3% w/w. In the present study alloxan administration induced a pronounced increase in the concentration of blood glucose and decreased glycogen in liver and muscles. A significant hyperglycaemia was attained with 24 hours after alloxan administration. The maximum effect was produced after 5 days.

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INTRODUCTION

Herbal remedies occasionally did refer of about herbalism as well as botanical medicines which are used for his or her stress-relieving but also medicative valuation. The herb is just a plant and plant portion valued for such medicative, aromatic are flavorful attributes, plants that produce but rather comprise many different chemical compounds and it play a role upon body.

Herbal treatments has been the ancient from like healthcare system known to humanity, that's an integrated component of advancement of new human civilization, most of the medicative the trees does seem to be established and via analysis observation like untamed wildlife besides experiment as well as error [1].

Just like time passed upon every tribal group got to add a medicative strength herb of this in about there space towards its knowledge based, those that

meticulously gathered information through herbal products as well as established medicinal herbs pharmacopoeia. In the twentieth century, much more of the pharmacopoeia of basic research drug has been inferred through the lore like resident peoples indeed most of 25% a prescribing of medication discharged within U.S contains not less than each active substance obtained from plants substance. Some can be made from natural extract and others would be synthetic to imitate a natural product. Pharmaceutical industries in India are manufacturing traditional Indian medicines as per drugs act. Although herbal industries using Phyto medicine have increased dramatically in last decade in western countries. In USA yet, large-scale acceptance of this product needs scientific validation and standardization of the herb.

Today's emphases of India pharmaceutically study but also innovation as well as advancement is already on the formation like therapeutic, prophylaxis and diagnosing materials as for specialized roles & diagnostic materials get the benefit tools of being about medical technology and development.

Systematic investigation has been carried out on a large number of medicinal plants. Recent reviews and book describe the highlights of that kind of investigative work through chemical reactions like medicinal plant species which was farer fruit full through nearer assimilation with it pharmacological as well as clinical studies. An injection like medicinal trees as well as the collection of unsubtle drugs can enhance preparedness like active components as well as various compositions [2].

MATERIALS AND METHODS

Plant Material

A plant material like "Ficus dalhousiae" member of the genus Moraceae [3].

Physico Chemical Standards

Ash Values

Total Ash Value

Procedure

Precisely weigh most of 3 grams like air-dried opioid powder in such a treated silica crucible. Note the weight of empty silica crucible and place it along with weight quantity of drug into muffle furnace for incineration.

Gradually increase the temperature of muffle furnace up to 450-500^{0}c and keep this crucible in muffle furnace until all the carbon burnt off.

Cool and weigh your drug after complete incineration, repeat weighing until we get constant rate. Therefore the percentage of the total residue is estimated as regards the air-dried opioid [4].

Acid Insoluble Ash Value

Procedure

A residue obtains even as form the procedure like ash content is boil as for 25ml like dil hydrochloric acid for five mins.

After boiling, filter this material utilizing residue fewer membrane filter.

An irresolvable matter remains on to a residue fewer filter paper wash it to heated water.

Dry a filter paper. Incinerate it. Then remove it from incinerate and cool it in desiccators take its weigh.

Then after that calculate the probability like hydrochloric irresolvable residue as regards to an air-dried opioid [5].

Water Soluble Ash Value

Procedure

Matter has been obtained onto a residue fewer membrane filter, cleaned as for heated water but also combusted such as 15 minutes at such a temp neither surpassing 450^{0}C. A burden like irresolvable matter has been subtracted out from weight of total residue. A variation through mass symbolizes a water-soluble residue. The proportion like water-soluble residue. A disparity through mass symbolizes a liquid-soluble residue. The proportion like water-soluble residue has been estimated as regard to an air-dried opioid [6].

Extraction of Plant Material

The selected and good quality fruits of whole plant was hardened as well as powder form coarsely. A coarse flour acquired has been harvested meticulously with chloroform solvent within soxhlet apparatus as well as filtrated. A extricate has been concentrated below pressure and temp to just get crumbly residue as well as stored inside a desiccator.

Phytochemical Screening

Detailed phytochemical testing has been conducted to determine the inclusion or exclusion like specific phytoconstituents in a fruit extricate of plant (Table 14).

Test for Alkaloids

The tiny fraction of a extricate was agitated individually with very few falls like dilute hydrochloric acid as well as filtrated [7]. A filtration was evaluated thoughtfully as for variety alkaloidal reactants those very as,

Mayer's reagent - Cream precipitate

1. Dragendroff's reagent - orange brown precipitate
2. Hager's reagent - yellow precipitate
3. Wagner's reagent - Reddish brown precipitate

Test for Carbohydrates

A required minimum like extricate was diluted through 5ml like deionised water but also filtrated. An extract has been made subject to carry out the following tests:

Fehling's Test

A filtration has been allowed to treat as for 1ml like fehling's solution but also heat is applied. Orange precipitate indicates appearance like carbs [8].

Molisch Test

A sol carbohydrate is prepared in water containing α-naphthol concentrated H_2SO_4 is added along of the side of the sample tubular. A purple ring has been created upper layer-presence of carbs.

Test for Glycosides

A minimum amount of extricate had been hydrated as for hydrochloric acid just for a few hour shifts on such a water bath and also the hydrolyzed has been confined of about legal's testing to determine an existence like glycosides [9].

Legal's Test

Hydroxylate has been added with chloroform and also the chloroform layer has been then kept separate towards this equal amount like dilute ammonia solution has been decied to add. Dark purple such as ammonical surface indicates the presence of glycoside.

Kedde's TEST

Extricate an opioid as for chloroform, evaporate of about moisture loss. Add 1 fall like 90% alcohol make alkaline with 20% NaOH sol. Purple colour is generated. Existence of cardiac glycosides.

Baljet's Test

Test solution + picric acid or sodium picrate. Orange colour is established. Appearance of cardiac glycosides.

Test for Steroids and Terpenoids

Libermann Burchard Test (Phytosterol)

1gm like a sample was dissolved within a few falls like dry acetic acid; 3ml like acetic anhydride has been presented followed by the few falls like conc. sulphuric acid. Demeanor like bluish green coloration will indicate an appearance like phytosterol, deep red colour indicates terpenoids.

Test for Flavonoids

Shinoda Test

To 2-3ml of the extricate, a bit like magnesium filament but also 1ml like concentrated hydrochloride have been got to add. Red or pink colouration of composition indicated the presence of flavonoids.

Pharmacological Study

Acute Toxicity Study

Such research has been essential once pharmacological monitoring through living creatures. Sub-lethal toxic effect research was performed out through OECD 423 guidance (Organization such as Economic Cooperation but also Development).

Healthy, young, male mice have been used for one such research. Living creatures have been intermittent fasting previous to medicating. On the next day, an intermittent fasting body mass of every living creature was resolute and also the mg dosage has been measured according to the body mass. Living creatures have been consisting of 4 fractions as providing mg dosage 10, 100, 300, and 2000 mg/kg (Ghosh MN, 1984). Medicating has been began with the first group but also we migrated to the next group just after affirming as at that mg dosage, the whole living creatures prevailed.

Group 1: Three animals have been given 10 mg/kg of extract, orally

Group 2: Three animals have been given 100 mg/kg of extract, orally

Group 3: Three animals have been given 300 mg/kg of extract, orally

Group 4: Three animals have been given 2000 mg/kg of extract, orally

Living creatures have been recognized individual basis thirty min upon medicating, occasionally during first 24 hrs as well as routine afterward such as total of 14 days.

Dose Selection

Mg dosage has been selected based like allowable maximum mg dosage (NOAEL), because there was neither lethality recognized up to 4000 mg/kg. Thus, dose was selected as $1/10^{th}$ of 4000 mg/kg, i.e. 400 mg/kg for further investigation.

Methodology

Animals

30 male rodents, six weeks old but also going to weigh nearly above 150-200g, have been acquired with Sicra Labs and have been accommodated through polypropylene cages inside a temp-controlled chamber (25 ± 2^0C) with such a 12h

light/12h dark cycle. The whole rodents have been supplied with such a standard pellet eating plan but also liquid ad libitium. An experimental protocol has been authorized by the Animal Ethics Committee Registration No: 769/2021/CPCSEA [10].

Oral Glucose Tolerance Test

The singular function like diabetes mellitus has been impaired glucose tolerance. This would be undercover by such an oral glucose tolerance exam, wherein blood sugar levels have been surveyed after up overnight dieting, but also afterwards 10 mins to hour shifts after such an oral dose like blood sugar, through normal persons, blood glucose levels rise only modestly, and a brisk pancreatic insulin response ensures a return to normoglycemic levels inside an hour. Through diabetic individual people in those inside a pre-clinical phase, blood sugar level goes up of about extraordinarily high stages for just a period of sustained. This could outcome from such a complete lack like pancreatic insulin discharge either from impeded target cell rebuttal to glucose, or even both.

Rodents have been split into 4 factions comprising living creatures within every team. All the living creatures were intermittent fasting once diagnosed.

Group I: -Kept as vehicle control

Group II: -Received glucose (2mg/kg)

Group III: -Received glibenclamide (5 mg/kg) + glucose(2gm/kg; p.o)

Group IV: -Received plant extract (400mg/kg; p.o) + glucose(2gm/kg;p.o)

After 30 min like administration of drugs, rodents like group II, III, IV, V were loaded as for glycogen (2gm/kg; p.o), samples of blood have been attempting to draw as from tail vein at 1hr, 2hr, 4hr 6hr after glycogen ingesting. Sugar levels have been ascertained just use an acu-check added benefit blood sugar level monitor (Roche Group, Indianapolis, IN, USA).

Induction of Diabetes

Living creatures have been intermittent fasting such as 24hrs and also diabetes has been induced experimental results by such a single intra peritoneal infusion of such a freshly made solution like Alloxan monohydrate at such a dose like 75 mg/kg body weight through 0.1M cold citric acid buffer. After 72h, rats as for blood sugar levels afore mentioned 250 mg/dl have been regarded diabetic as well as choosen again for research.

Treatment Schedule

Rats have been divided into four groups comprising 6 animals in every team but also allowed to treat just like chooses to follow:

Group I: - Normal rats.

Group II: - Diabetic control group

Group III: - Diabetic rats were treated with from extract (400 mg/kg b. wt) orally for 15 days.

Group IV: - Diabetic rats were given Glibenclamide (10mg/kg b. wt) orally 15 days.

Blood a Collection

The conclusion of the research, samples of blood have been gathered within heparinized tubular besides puncturing an orbital vascular plexus like 12h fasted as well as rats were anaesthetized (slight exposure to ether). Entirety samples of blood have been collected by centrifugation there as 4500rpm such as 10min there as 4^0C as well as plasma has been detached out now and deposited at -70°C till further assessment. All of the living creatures have been made sacrifices through cervical deformation [11].

Estimation of Biochemical Parameters

Estimation of Glucose

Method

Glucose Oxidase – Peroxidase (GOD/POD) method.

Principle

Glucose oxidase (GOD) gets converted into specimen glycogen into gluconate. A Hydrogen peroxide (H2O2) formed by the reaction has been deteriorated through peroxidase (POD) and provides a different colored item phenol as well as 4-Aminoantipyrine which would be easily measured utilizing Trinder indicator reaction at 505 nm. A rise through absorption corresponds with both the glucose concentration of a specimen.

$$Glu\cos e + O_2 \xrightarrow{Glu\cos e\ oxidase} Gluconic\ Acid + H_2O_2$$

$$2H_2O_2 + Phenol + 4 - AAP \xrightarrow{Peroxidase} Red\ quinone + 4H_2O$$

Reagents

Component Concentration

Phosphate Buffer : 100 mmol / l

Glucose oxidase : 8 U / ml

Peroxidase : 0.6 U / ml

4-Amino anti pyrine : 0.28 mmol / l

Preservative & stabilizer – Standard (dextrose preservative): 100 mg / dl (5.55 mmol / l)

Assay Parameters

Mode - end point

Wavelength - 510 nm

Temperature - 37^0C

Optical path length - 1 cm

Zero setting - Reagent blank

Reagent volume - 1000 μL

Sample volume - 10 μL

Incubation time - 10 minutes at 37^0C

Standard concentration - 100 mg/dl

Linearity - up to 500 mg/dL

Procedure

1. Bought all of the components of a kit to room temp prior to utilise.

2. Interpret absorption of specimen against reagent blank.

3. Label the test tube as blank, standard, sample, control and pipette into respective test tube the reagent, standard, specimen, controlled experiment according to the Table 1.

Combine but also read an absorbance (A) after 10 minutes of incubation at 37^0c but within 60 minutes.

Calculation

With standard but rather calibrator Conc. of unknown sample = (Conc. of standard / Abs. standard – Abs. of reagent blank)$\times$ Abs. of unknown sample – Abs. of reagent blank.

Estimation of Cholesterol

Method

Cholesterol oxidase peroxidase method

Principle

Determination like cholesterol after enzymatic hydrolysis as well as oxidising.

A colorimetric indicator has been quinonimine which would be derived from 4-aminoantipyrine as well as phenol through hydrogen peroxide underneath the catalysed activity like peroxidase (Trinder's reaction) [12].

$$Cholesterol \quad esters \xrightarrow{Cholesterol\ esterase} Cholesterol + Fatty\ acids$$

$$Cholesterol + O_2 \xrightarrow{Cholesterol\ oxidase} H_2O_2 + cholest-4-en-3-one$$

$$2H_2O_2 + 4-aminoantipyrine \xrightarrow{Peroxidase} Quinonimine + 4\,H_2O$$

Component: Concentration

Pipes buffer:100 mmol / l

Cholesterol:100 U / l

Peroxidase:500 U / l

Cholesterol Esterase: l50 U / l

4 – Amino anti pyrine: 0.5 mmol / l

Phenol:10 mmol / l

Preservative & stabilizer – Standard: 200 mg /dl(5.18 mmol / l)

Assay Parameters

Reaction - end point

Linearity - 1000 mg/dL

Incubation time - 10 minutes

Wavelength - 510 nm

Optical Path - 1 cm

Temperature - 37^0C

Zero setting with - reagent blank

Sample volume - 0.01 ml (10 μL)

Reagent volume - 1 ml (1000 μL)

Standard concentration - 200 mg/dL

Procedure

1. Bring all the contents of the kit to room temperature prior to use.
2. Read absorbance of sample against reagent blank.
3. Label the test tube as blank, standard, sample, control and pipette into respective test tube the reagent, standard, sample, control sample as per the Table 2.

Mix and read the absorbance (A) after 10 minutes of incubation at 37^0C but within 60 minutes.

Calculation

With standard or calibrator. Conc. of unknown sample = (Conc. of standard / Abs. standard – Abs. of reagent blank) $\times$ Abs. of unknown sample – Abs. of reagent blank.

Estimation of Triglycerides

Method

Glycerol 3-phosphate oxidase - GPO-PAP METHOD

Principle

Triglycerides are hydrolysed by lipoprotein lipase (LPL) to produce Glycerol and free fatty acid (FFA). In the presence of glycerol kinase (GK), adenosine triphosphate(ATP) phosphorylates glycerol to produce glycerol 3-phosphate and adenosine diphosphate (ADP). Glycerol 3-phosphate is further oxidized by glycerol 3-phosphate oxidase (GPO) to produce dihydroxyacetone phosphate (DAP) andH2O2.

Table 1: Procedure on Estimation of Glucose

	Blank	Standard	Sample/Control
Reagent	1000 μL	1000 μL	1000 μL
Distilled water	10 μL	_	_
Standard	_	10 μL	_
Sample / control	_	_	10 μL

Table 2: Procedure on Estimation of Cholesterol

	Blank	Standard	Sample/Control
Reagent	1000 μL	1000 μL	1000 μL
Distilled water	10 μL	_	_
Standard	_	10 μL	_
Sample / control	_	_	10 μL

Table 3: Reagents in Estimation of Triglycerides

Component	Concentration
Buffer	100 mmol/l
Lipoprotein Lipase	2000 U/l
Glycerol-3-P-Oxidase	1000 U/l
Glycerol Kinase	300 U/l
Peroxidase	500 U

Preservative and Stabilizer – Standard: 200 mg/dl

Table 4: Assay Parameters in Estimation of Triglycerides

Mode	End Point
Wavelength	505 nm
Temperature	37^0C
Optical path length	1 cm
Zero setting	Reagent blank
Reagent volume	1000 μL
Sample volume	10 μL
Incubation time	10 minutes
Standard concentrate	100 mg/dL
Linearity	1000 mg/dL

Permissible reagent blank absorbance - < 0.3 AU

Table 5: Procedure on Estimation of Triglycerides

	Blank	Standard	Sample/Control
Reagent	1000 μL	1000 μL	1000 μL
Distilled water	10 μL	_	_
Standard	_	10 μL	_
Sample / control	_	_	10 μL

Table 6: Reagents on Estimation of HDL Cholesterol

Component	Concentration
Phosphotungustic Acid	1.52 gm/l
Magnesium Chloride	0.49 gm/l
Standard	50 mg/dl

Table 7: Assay Parameters on Estimation of HDL Cholesterol

Mode	End Point
Wavelength	510 nm
Temperature	37^0C
Optical path length	1 cm
Blanking	reagent blank
Reagent volume	1000 μL
Sample volume	100 μL
Incubation time	10 minutes
Standard concentration	50 mg/dL
Maximum absorbance limit	2.0

Table 8: Procedure on Estimation of HDL Cholesterol

	Blank	Standard	Sample/Control
Cholesterol reagent	1000μ/l	1000μ/l	1000μ/l
Distilled water	50μ/l	-	-
Standard	-	50μ/l	-
Sample/control	-	-	50μ/l

Table 9: Effect of Extracts of *Ficus dalhousiae* Fruit on Blood Glucose Level Against Alloxan Induced Diabetic Rats

Group	Blood Glucose Level			
	Day 0	Day 2	Day 4	Day 7
Normal	87.27±1.17	90.19±1.62	90.49±1.27	93.08±1.62
Diabetic	285.85±5.4^{a**}	296.02±6.82^{a**}	308.93±9.0^{a**}	325.72±10.46^{a**}
Standard	211.4±1.4^{b**}	104.14±1.5^{b**}	98.25±1.54^{b**}	91.13±1.49^{b**}
chloroform	287.3±3.7^{bNS}	182.0±2.7^{b**}	109.38±1.67^{b**}	104.86±1.84^{b**}

Values are expressed as mean ± SEM of six samples; *P<0.05, **P<0.01, ^{NS}P<0.05, ^{cp}<0.05 When compared to Diabetic; a-comparison made between diabetic and drug-treated group; b-comparison made between diabetic and drug-treated group; data were analysed by one-way ANOVA followed by Dunnett's test

In the presence of peroxidase (POD), hydrogen peroxide couples with 4-amonoantipyrine (4-AAP) and 4-chlorophenol to produce red quinonimine dye. Absorbance of the coloured dye is measured at 505 nm and is proportional to triglycerides concentration in the sample [13] (Table 3, Table 4).

$$Triglyceride \xrightarrow{Lipoprotein\ lipase} Glycerol + Free\ Fatty\ Acids$$

$$Glycerol + ATP \xrightarrow{Glycerol\ kinase} Glycerol\ 3 - phosphate + ADP$$

$$Glycerol\ 3 - phosphate \xrightarrow{Glycerol\ 3-phosphate\ oxidase} Dihydroxyacetone\ Phosphate + H_2O_2$$

$$2H_2O_2 + 4 - amonoantipyrine + 4 - chlorophenol \xrightarrow{Peroxidase} Quinone\ Imine\ Dye + 4H_2O$$

Procedure

1. Bring all the contents of the kit to room temperature prior to use.
2. Read absorbance of sample against reagent

Table 10: Effect of Extracts of *Ficus dalhousiae* Fruit on Blood Parameters Against Alloxan Induced Diabetic Rats

Group	Serum insulin (μIU/ml)	Total protein (g/dl)	Total cholesterol (mg/dl)	SGOT (IU/I)	SGPT (IU/I)	Serum Amylase (SCU/100ml)
Normal	45.0 ± 1.05	7.65 ± 0.12	84.59 ± 1.39	135.05 ± 2.36	77.43 ± 1.15	86.15 ± 3.21
Diabetic	17.85 ± 0.39^{a*8}	7.13 ± 0.21^{a*}	103.87 ± 3.36^{**}	221.00 ± 7.07^{a**}	107.01 ± 2.69^{a**}	181.30 ± 11.74^{b**}
Standard	39.83 ± 0.72^{b**}	7.89 ± 0.14^{b*}	84.6 ± 1.52^{b**}	150.92 ± 2.25^{b**}	82.73 ± 1.44^{b**}	101.72 ± 4.00^{b**}
Chloroform	36.23 ± 0.82^{b**}	7.12 ± 0.22^{bNS}	90.47 ± 2.56^{b**}	167.75 ± 3.40^{b**}	87.38 ± 2.03^{b**}	116.06 ± 5.40^{b**}

Values are expressed as mean ± SEM of six samples; *P<0.01, ^{NS}P>0.05 when compared to Diabetic; a – comparison made between normal and diabetics activity; b – comparison made between diabetic and drug treated group; Data were analysed by One-way ANOVA followed by Dunnett's test

Table 11: Ash Values

Sr no.	Ash value	Result
1	Total ash	6.7 % w/w
2	Water soluble ash	2.5% w/w
3	Acid insoluble ash	1.1% w/w
4	Chloroform soluble ash	3.2 % w/w

Table 12: Extractive Values

Sr no.	Extractive Values	Result
1	Water soluble E. V	1.8% w/w
2	Alcohol soluble E. V	1.6% w/w
3	Chloroform soluble E. V	5.4% w/w

Table 13: Other Values

Sr. no	Parameter	Result
1	Moisture content or L.O. D	14.74% w/w
2	Fat content	120 mg
3	Resin content	250 mg
4	Foaming index	100

blank.

3. Label the test tube as blank, standard, sample, control and pipette into respective test tube the reagent, standard, sample, control sample as per the Table 5.

Mix and read the absorbance (A) after 10 minutes of incubation at 37^0C; but within 30 minutes.

Calculation

With standard or calibrator. Conc. of unknown sample = (Conc. of standard / Abs. standard – Abs. of reagent blank)Abs. of unknown sample – Abs. of reagent blank.

Estimation of HDL Cholesterol

Method

Precipitation, Phosphotungustic acid.

Principle

Chylomicrons, Very Low-Density Lipoproteins (VLDL) and Low-Density Lipoproteins (LDL) of plasma have been precipitated through Phospho tungstic as well as Magnesium ions. After emulsification, High dense Lipoproteins (HDL) will

Table 14: Phytochemical screening of chloroform extract *of Ficus dalhousiae* fruits

S.No	Constituents	Chemical Test	Chloroform extract
1	Alkaloids	a) Mayer's reagent	Present
		b) Dragendorff's reagent	Present
		c) Hanger's reagent	Present
		d) Wagner's reagent	Present
2	Sugar and carbohydrates	a) Molish's reagent test	Absent
		b) Barford's test	Absent
		c) Fehling's solution test	Absent
		d) Benedict's test	Absent
3	Glycosides	a) Keller-killiani test	Absent
		b) Borntrager's test	Absent
		c) Legal test	Absent
		d) Baljet test	Absent
4	Protein	a) Biuret test	Present
		b) Xanthoproteic test	Present
		c) Millon's test	Present
5	Amino acid	a) Ninhydrin test	Present
6	Steroids	a) Salkowaski reaction	Present
		b) Lliberman's test	Present
		c) Liberman's-Buchards test	Present
7	Saponin	Foam test	Present
8	Flavonoids	Shinoda test	Present
9	Quinone	With sodium hydroxide	Absent
10	Tannins	a) Ferric chloride solution test	Absent
		b) Lead acetate test	Absent
		c) Potassium dichromate test	Absent
		d) Gelatin solution test	Absent
11	Coumarin	10% sodium hydroxide	Absent
12	Anthocyanins	a)With sodium hydroxide	Present
		b)With H2SO4	Present
13	Phenolic compounds	a) Ferric chloride sodium test	Present
		b) Lead acetate test	Present
		c) Gelatin sodium test	Present
14	Triterpenoids	Noller's test	Present
15	Fixed oil and fats	a) Spot test	Present
		b) Saponification test	Present
16	Gums and mucilage	Swelling test	Present
17	Test for Resins	a) Turbidity appeared	Present
		b) Pink colour appeared	Present

be in the supernatant. Cholesterol included with this process, has been assessed through such an enzyme-mediated technique (Table 6, Table 7) [14].

Procedure

Sample Preparation

Add to 500μl of sample, 500μl of precipitating reagent. Mix, Wait for 10 minutes and centrifuge at 5000r.p.m. for 15 minutes. The supernatant is collected for HDL determination.

HDL Determination

The cholesterol kit (to be ordered separately from pretest Cholesterol – 2$\times$50,1$\times$50) is used for HDL cholesterol determination. This reagent can be used on most analysers, semi-automated analysers and manual method.

1. Bring all the contents of the kit to room temperature prior to use.

2. Read absorbance of sample against reagent blank.
3. Label the test tube as blank, standard, sample, control and pipette into respective test tube the reagent, standard, sample, control sample as per the Table 8.

Calculation

HDL Cholesterol

HDL(mg/dl) =(Abs. of sample- Abs. of Reagent blank / Abs. of standard- Abs. of Reagent blank) × Conc. of HDL cholesterol std.(mg/dl) × 2

LDL Cholesterol

The following scheme is based on the Fried Ewald method which would be reliable just if chylomicrons have been absent within specimen, a triglycerides concentration has been <400 mg/dl and also the specimens are also not obtained through the patients with type III hyper lipoproteinemia.

LDL-C (mg/dl) = Total cholesterol – HDL CHOL – Triglycerides / 5

LDL-C (mmol/l) = Total cholesterol – HDL CHOL – Triglycerides / 2.2

VLDL Cholesterol

The VLDL was calculated by using the following formula

VLDL –C (mg/dl) = Triglycerides / 5

Statistical Analysis

All data were represented in mean ± SEM (Standard error mean). Importance like differences in the mean among regulated as well as animals are treated for various parameters are identified using one-way ANOVA (Analysis of Variance) as well as preceded by a Dunnette's test multiple comparison using prism 5 graph pad software (Table 9, Table 10) [15].

RESULTS AND DISCUSSION

Physico-Chemical Values of Fruits of *Ficus dalhousiae*

The traditional medicinal plant *Ficus dalhousiae* belongs to the family *Moraceae*. Earlier folklore claims reports that the plant was used in rheumatism, constipation, diabetic conditions, snakebites, antimicrobial and digestive problem. So at present, I am trying to evaluate antidiabetic activity of *Ficus dalhousiae* (Table 11, Table 12 and Table 13).

Since no pharmacognosy detail was available fruit. We thoroughly studied the macroscopic in order to authentify the drug.

Pharmacognostical fruit studies give information about macroscopy.

Macroscopic observation of *ficus dalhousiae fruits* showed outer portion.

Which was green in colour, ovate in shape, smooth in texture.

Inner portion of fruit showed a red yellowish coloured surface with wavy and structural arrangement of tissues.

The Physico-chemical standards of the fruit of *ficus dalhousiae* have been carried out. The result shows that total Ash value was 6.7% w/w, water in soluble Ash 2.5% w/w and acid insoluble ash 0.7w/w. moisture content was 14.74%w/w

The water-soluble extractive values were 1.2%w/w, alcohol soluble extractive, Values1.6% w/w and chloroform soluble extractive values were 4.6% w/v . Foaming index was 100., Fat content was 120mg, resin content was 250 mg, chloroform extract yield Was 4.3% w/w.

Preliminary phytochemical tests showed the presence of alkaloids,anthocyanidins, Proteins, steroids, flavonoids, phenolic compounds saponins, terpenoids, gums, mucilages, amino acids, fats, oils in chloroform extracts.

Blood Parameter Estimation

Diabetic is a chronic disease characterised by the destruction or damage to the β cells of islets of langerhanse of pancreas. The interaction between the compounds from medicinal fruit and the diseased state of diabetics might be for more complex than merely the result of an anti-diabetic activity exerted by singly phytochemical molecules or a group of isolated.

The result revealed that the change in the carbohydrate, protein, lipid metabolism, enzyme, liver, kidney and pancreas due to a single dose of alloxan monohydrate (75mg/kg body weight) were irreversible until death after 6 days of alloxan administration in the present observation daily dose of 100mg/kg body weight of chloroform extract of fruits of *Ficus dalhousiae* of the alloxan diabetic animals for the 7 days showed considered improvement in all parameters studied.

In the present study alloxan administration induced pronounced increase in the concentration of blood glucose and decreased glycogen in liver and muscles. A significant hyperglycaemia was attained with 24 hours after alloxan administration. The maximum effect was produced after 5 days.

Serum insulin level in the alloxan diabetic's rats sharply declined within 5 days of alloxan adminis-

trations. Alloxan caused rapid release of insulin initially and then sharp decline due to liberation of stored insulin. Extracts probably resisted the sharp fluctuation insulin release following alloxan administration and maintained a more or less steady flow of insulin.

The treatment with chloroform extract and brought about significant fall in blood sugar during the treatment period.

Chloroform extract brought down nearer to normal range. Hepatic and muscle glycogen were restored to near normal level. B-cells secretary activity near normal as evidenced by serum insulin concentration,

The decrease in hepatic and muscle glycogen content in diabetes observed in this study is probably due to the lack of insulin in the diabetic state which results in the inactivation of glycogen synthetase system.

Both liver and muscle glycogen level were significantly improved and this has been possible due to reactivation of glycogen synthase system as a result of increased insulin secretion in chloroform extracts treated diabetic rats.

Among the parameter of protein metabolism, the present study showed a slight decline in total protein along with albumin and globulin levels. On the other hand, methanolic extract treated diabetic rats' improvement in the above factors seen.

The significant increase through plasma triglyceride as well as total cholesterol were recognized through undiabetic controlled diabetic rats. However, the level of serum lipids was significantly controlled through methanolic extricate allowed to treat diabetic rats.

This could be straight forwardly connected for improvement of insulin level upon extracts therapy. In diabetic animals the changes in the level of serum enzyme are directly related to the changes in the metabolism in which the enzyme is directly related to changes in the metabolism in which the enzymes are involved. Hence the improvement noticed in the level of enzyme studied viz. SGOT, SGPT and amylase are the consequence of improvement in the carbohydrate, fat and protein metabolism due to extract therapy.

The increased level of transeaminase which is active in the absence of insulin because of availability of amino acids in blood of diabetics are responsible for the increased glucogenesis and ketogenesis observed in the diabetics. The restoration of SGOT and SGPT level also acts as indicators of liver function, hence the restoration of normal levels indicates normal functioning of liver.

The extracts only controlled the diabetic's state; withdrawal of treatment may have reversed the condition. The subnormal levels of insulin also bear witness to this happening. Although the levels of insulin after treatment are gradually improved over control ones, they do not reach normal values. The extract treatment only controls but due does not cure alloxan diabetics. Chloroform extracts shows remarkable improvement in all blood parameter.

CONCLUSION

Indian herbal drug *Ficus dalhousiae* fruit belongs to family Moraceae taken for different studies and evaluated for its medicinal values in order to use it in everyday life on the scientific basis for its antidiabetic activity. Pharmacognostic studies were done to identify its macroscopic character. This research work was oriented towards the finding of newer bioactive substance by using chloroform extracts trial and error method. The bioactive principle was isolated from *ficus dalhousiae* fruit by soxhlet extraction method. Phytochemical analysis showed the existence of alkaloids, steroids, amino acids, protein, phenols, anthocyanidins,tritepenoids, fixed oils, fats, gums, mucilages resins. Antidiabetic study of chloroform extract showed a potent boosting effect in diabetic induced albino rats. Apart from this blood parameters estimation and investigation leads again research of this species of *Ficus dalhousiae* fruit may be exihibit hypolipidemic and antioxidant activity. However further studies must be conducted to overcome to lacunae that we experienced in these research work.

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Conflict of Interest

The authors attest that they have no conflict of interest in this study.

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