Phytochemical and *in Vitro* and *in Vivo* Evaluation of Anti Diabetic Activity of *Tecomaria capansis* Extract of Leaves in Wistar Albino Rats

SK. Mallika*, S. Manohar Babu, K. Vadivel, Fathima Asra, Ch. Puja, P. Sabira and B. Prasanna Kumar

Department of Pharmacology, SIMS College of Pharmacy, Mangaldas nagar, Guntur, Andhra Pradesh, India.

Received: 02 Jul 2019 / Accepted: 12 Aug 2019 / Published online: 1 Oct 2019

*Corresponding Author Email: mallikashaik29@gmail.com*

**Abstract**

Diabetes mellitus is a metabolic issue at first recognized by lost glucose homeostasis, because of disturbances of carbohydrate, fat and protein digestion, coming from imperfections in insulin generation, emission, insulin activity. Diabetes comprises in two types; Type I and Type II. Most hypoglycemic plant constituents, such as alkaloids, phenolic acids and amino acids, inhibit insulinase in vitro and are hypoglycemic in vivo in normal rats. The wide kind of chemical classes indicates that a variety of mechanisms might be involved in the lowering of the blood glucose level. Our results shown that oral administration of *Tecomaria capansis* extract has a beneficial effect on the reducing hyperglycaemia, SGOT & SGPT levels, total cholesterol, triglycerides levels, LDL-cholesterol, VLDL-cholesterol and improving the HDL status. This study suggests that the induction of diabetes mellitus may be prevented by flavonoids of plant constituent’s administration. There is a need to continue to explore the mechanisms for anti-diabetes.

**Keywords**

Diabetes mellitus, medicinal plant, antidiabetic activity.

*****

**INTRODUCTION**

Diabetes mellitus is the most common type of metabolic, non-communicable diseases globally. Recent studies states that it is the fourth leading causes of death in the most developed countries [1]. It is characterized by hyperglycemia resulting from defects in either insulin action, insulin secretion, or both. As per World Health Organization (WHO), at least 171 million people (2.8% of worldwide population) have suffered from diabetes alone in year of 2000. It is estimated that by the year 2030, this number of people suffering from diabetes may almost be doubled. The increase in incidence of diabetes in developing countries follows trend of highly urbanization and lifestyle changes, may be because of “Western-style” diet. Diabetes mellitus is classified into two types, insulin-dependent diabetes mellitus (IDDM, Type I) and non-insulin-dependent diabetes mellitus (NIDDM, Type II). Type I diabetes is an autoimmune disease characterized by a local inflammatory reaction in and around islets of pancreas that is followed by selective destruction of insulin-secreting β cells. Type II diabetes is characterized by peripheral insulin resistance and impaired insulin secretion. [2]
Herbal medicine includes preparation of biologically active natural products that consist of herbal material or herbs, some recipes may contain materials such as fungal and bee products, as well as minerals (kaolin, bentonite), insects, shells, ash, and animal parts, and are used for the maintenance of health and management of various diseases [3].

MATERIALS AND METHODS:
Identification and authentication of selected plant species
The leaves of *Tecomaria capansis* (Thumb.) Spach was collected from Guntur district, A. P and it was authenticated by professor Dr. S.M. Khasim, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur. The specimen (No: ANU/00129/2009/AP) was deposited in the department of botany and microbiology for future reference.

Collection of plant material: Fresh plant material was collected in bulk, washed under running tap water to remove adhering material, dried under shade and pulverized in a mechanical grinder. The coarse powder was passed through sieve no. 60”. Care was taken to select healthy plants and normal organs.

Macroscopic study
As per standard procedure matured 25 leaves were taken for the evaluation of morphology of leaves and studied various parameters such as length, width, margin, apex, surface, colour, odour, taste, type, base, midrib and size. By visual method the organoleptic characteristics of leaf was found.

Microscopic study
The required samples of different organs were cut and removed from the plant and fixed in FAA (formalin-5ml+acetic acid-5ml+70%ethyl alcohol-90ml) after 24 hours of fixing the specimen were dehydrated with graded series of tertiary-butyl alcohol. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-69˚C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks [5].

Procedure for staining
The paraffin embedded specimens were section with the help of rotary microtome. The thickness of the section was 10-12 micrometers. De waxing of the secretion was by customary procedure. The section was stained with Toulidine blue as per the method published by O’Brien et al., 1964 since Toulidine blue is a polychromatic stain the staining results were remarkably good and some cyto chemical reactions were also obtained. They rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein
Determinations of different extractive value

**Determination of alcoholic soluble extractive**
Macerate 5g of the air dried drug, coarsely powdered, with 100ml of Alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during six hours and allowing standing for 18hrs. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105˚C to constant weight and weigh. Calculate the % of alcohol soluble extractive with reference to the air-dried drug.

**Determination of water-soluble extractive**
Macerate 5g of the air dried drug, coarsely powdered, with 100ml of chloroform water of the specified in a closed flask for 24 hrs, shaking frequently during 6 hrs and allowing to stand for 18 hrs. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105˚C to constant weight and weigh. Calculate the % of chloroform water soluble extractive with reference to the air-dried drug.

**Determination of ether soluble extractive**
Transfer a suitably weighed quantity (depending on the fixed oil content) of the air dried, crushed drug to an extraction thimble, extract with solvent ether (petroleum ether boiling point 40˚-60˚) in a continuous extraction apparatus (Soxhlet apparatus, Elite, India) for 6 hrs. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105˚C to constant weight. Calculate the % of ether soluble extractive with reference to the air-dried drug.

**Ash Value Determination**

**Determination of total ash**
Incinerate about 2 to 3 gm accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450˚C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450˚C. Calculate the % of ash with reference to the air dried drug.

**Determination of acid insoluble ash**
Boil the ash obtained in total ash for 5 minutes with 25ml of dilute hydrochloric acid, collect the insoluble matter in a Gooch crucible or on a less filter paper, wash with hot water and ignite to constant weight. Calculate the % of acid insoluble ash with reference to the air-dried drug.

---

**Photomicrographs**
Microscopic description of tissue or supplemented with micrographs were necessary photographs of different magnifications were taken with Nikon Labophot2 microscopic unit. For normal observations bright field was used for the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have bire stringent property, under polarized light they appear bright against dark background, magnification of the figures is indicated by the scale bars [6].

**Determination of physicochemical constants [7]**

**Determination of moisture content (loss on drying)**
Place about 10g of drug (without preliminary drying) after accurately weighing (accurately weighed within 0.01g) it in a tarred evaporating dish. For example, for underground or un powdered drug, prepare about 10g of sample by cutting shredding so that the parts are about 3mm in thickness. Seeds and fruits, smaller than 3mm should be cracked. Avoid the use of high-speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tarred evaporating dish dry at 105˚C for 5 hours, and weigh. Continue the drying and weighing corresponds to not more than 0.25 percent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01g difference.

**Determination of foreign matter**
Weigh 100-500g of the drug sample to be examined or the minimum quantity prescribed in the monograph and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of lens (6xs). Separate and weigh it and calculate the percentage present.

%of foreign matter =Amount of foreign matterx100/Amount of drug taken
**Determination of water-soluble ash**
Boil the ash for 5 minutes with 25ml of water, collect insoluble matter in a Gooch crucible, or an ash less filter paper, wash with the hot water, and ignite for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash. And Calculate the % of water-soluble ash with reference the air-dried drug [7].

**Preparation of extracts [8]**
The technique commonly used in the field of phytochemistry are extraction, isolation, and structural elucidation of natural products, as well as chromatographic techniques. The solvent extraction of any botanical materials may yield very less quantity of volatile oils and large yield of nonvolatile components like resins, pigments, waxes and fatty acids.

**Petroleum ether extract**
This extract was prepared by using soxhlet apparatus. About 150gm of dried flower powder was taken in a muslin cloth bag. The purified pet ether was passed through the tube where the powder bag was kept. The pet ether will pass through siphon tube and reach the round bottom flask in which the condenser and reach the tube containing powdered bag and the process is repeated. This is continuing for 24hrs. then the round bottom flask containing extract is transferred to a beaker and is allowed to evaporate in a water bath. This concentrated pet ether extract is used for further studies.

**N-hexane extract**
This extract was prepared by using soxhlet apparatus. About 150gm of dried flower powder was taken in a muslin cloth bag. The purified N-Hexane was passed through the tube where the powder bag was kept. The N-Hexane will pass through siphon tube under the reach the round bottom flask in which porcelain chips are provided. The vapors containing the constituents pass through the condenser and reach the tube containing powder bag and the process is repeated. This is continued for 24hrs. Then the round bottom flask containing flaks is transferred to a beaker and is allowed to evaporate in a water bath. This concentrated N-Hexane extract is used for further studies.

**ETHYL ACETATE EXTRACT**
This extract was prepared by using soxhlet apparatus. About 150gm of dried flower powder was taken in a muslin cloth bag. The purified Ethyl acetate was passed through the tube where the powder bag was kept. The Ethyl acetate will pass through siphon tube and reach the round bottom flask in which porcelain chips are provided. The vapors containing the constituents pass through the condenser and reach the tube containing powder bag and the process is repeated. This is continued for 24hrs. Then the round bottom flask containing extract is transferred to a beaker and is allowed to evaporate in a water bath. This concentrated Ethyl acetate extract is used for further studies.

**Chloroform extract**
This extract was prepared by using soxhlet apparatus. About 150gm of dried flower powder was taken in a muslin cloth bag. The purified chloroform was passed through the tube where the powder bag was kept. The chloroform will pass through siphon tube and reach the round bottom flask in which porcelain chips are provided. The vapors containing the constituents pass through the condenser and reach the tube containing powder bag and the process is repeated. This is continued for 24hrs. Then the round bottom flask containing extract is transferred to a beaker and is allowed to evaporate in a water bath. This concentrated chloroform extract is used for further studies.

**Ethanol extract**
This extract was prepared by using soxhlet apparatus. About 150gm of dried flower powder was taken in a muslin cloth bag. The purified ethanol was passed through the powder bag was kept. The ethanol will pass through siphon tube and reach the round bottom flask in which porcelain chips are provided. The vapors containing the constituents pass through the condenser and reach the tube containing powder bag and the process is repeated. This is continued for 24hrs. Then the round bottom flask containing extract is transferred to a beaker and is allowed to evaporate in a water bath. This concentrated ethanol extract is used for further studies.

**Aqueous extract**
This extract was prepared by using soxhlet apparatus. About 150gm of dried powder was taken in a muslin cloth bag. The purified water passed through the tube where the powder bag was kept. The water will pass through the siphon tube and reach the round bottom flask in which porcelain chips are provided. The vapors containing the constituents pass through the condenser and reach the tube containing powder bag and the process is repeated. This is continued for 24hrs. Then the round bottom flask containing extract is transferred to a beaker and is allowed to evaporate in a water bath. This concentrated aqueous extract is used for further studies.

**Recovery of solvent**
For purification of solvents the distillation apparatus was first set. 40% of solvents were added to the
bottom flask kept in electrical mantle. The respective solvents were allowed to distill, and pure solvents were collected in a vessel from the adapter attached to the condenser.

**Phytochemical screening [9, 10]**
The following chemical tests were performed to identify the photochemical constituents present in chloroform of *Tecomaria capensis*.

**Test for alkaloids**

**Dragendorff’s reagent:**
The alcoholic extract was shaken with dilute HCL to 2-3ml of filtrate add few drops of Dragendorff’s reagent. Orange brown precipitate is formed.

**Mayer’s test:**
The alcoholic extract was shaken with dilute Hcl and filtered. 2-3 ml of the filtrate add Mayer’s reagent. Cream precipitate is formed.

**Hager’s test:**
The alcoholic extract was shaken with dilute Hcl and filtered. To 2-3 ml of filtrate add Hager’s reagent. Yellow precipitate is formed.

**Wagner’s test:**
The alcoholic extract was shaken with dilute Hcl and filtered. And to the 2-3 ml of filtrate add Wagner’s reagent. Reddish brown precipitate is formed.

**Test for flavonoids**

**Schinoda test:**
To the extract add 5ml 95%ethanol few drops of conc.HCL and 0.5g magnesium turnings. Pink color is observed.

**Alkaline test:**
10mg of extract was dissolved in 2 ml of water and treated with 1ml of 10% ammonium hydroxide and observe for coloration. 2 drops of dilute Hcl was added and again observe for discoloration. The formation of intense yellow colour which turns to colorless on addition of dilute acid indicates the presence of flavanoids.

**Test for cardiac glycosides**

**Baljet test:**
A thick section shows yellow to orange colour with sodium picrate.

**Legal’s test:**
To the alcoholic extract add 1 ml of pyridine and 1ml of sodium nitroprusside. Pink to red color appears.

**Liebermann’s test:**

**Test for anthraquinolone glycosides**

**Borntrager’s test:**
To 3ml of extract add dilute sulphuric acid. Boil and filter. To cold filtrate add equal volumes benzene and chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammonial layer turns pink or red.

**Modified brontrager’s test:**
To 5ml extract add 5ml of 5% ferric chloride and 5ml dilute Hcl. Heat for 5mins in boiling water bath. Add equal volume of chloroform or benzene. Shake well. Separate the organic solvent. Add ammonia. Ammonial layer shows pinkish red colour.

**Test for saponin glycosides**

**Foam test:**
Shake the drug extract or dry the powder vigorously with water. Persistent foam is produced.

**Hemolytic test:**
Add drug or dry powder to one drop of blood placed on a glass slide. Hemolytic zone appears.

**Test for tannins and phenolic compounds**

**Lead acetate solution:**
To 2-3ml of alcoholic extract add few drops of lead acetate solution. A white precipitate is formed.

**5% ferric chloride solution:**
To 2-3ml of the alcoholic extract add few drops of 5% ferric chloride solution. A deep blue colour appears.

**Test for steroids**

**Salkowski reaction:**
To 2ml of extract add 2ml chloroform and 2ml conc. Sulphuric acid. Shake well. chloroform layer appears red and acid layer shows greenish yellow fluorescence.

**Liebermann’s test:**

**Test for carbohydrates**

**Molish’s test:**
To 2-3 ml of extract add, few drops of alpha naphthol solution in alcohol, shake and add concentrated sulphuric acid from sides of the test tube. Violet ring is formed at the junction of 2 liquids.

**Fehling’s test:**
About 50gms of extract was hydrolyzed with 10ml of dilute hydrochloric acid and neutralized with alkali. The mixture was heated with 1 ml of Fehling’s solution. A and B and observes for precipitate. Formation of red precipitate indicates the presence of reducing sugars.

**Benedict’s test:**
To 0.5ml of filtrate add 0.5ml of Benedicts reagent. The mixture was heated on water bath for 2 minutes and observe for precipitate. Formation of orange red precipitate indicates the presence of reducing sugars.

**Test for proteins**

**Xanthoprotein test:**
Mix 3ml test solution with 1ml conc. Sulphuric acid. Boil. Precipitate turns to
yellow. Add ammonium hydroxide, precipitate turns orange.

**Biuret test:**
To 3 ml test solution add 4% ammonium hydroxide and few drops of 1% copper sulphate solution. Violet or pink color appears.

**Test for fats and oils**

**Solubility test:**
Oils are soluble in ether, benzene and chloroform, but insoluble in 90% ethanolic and water.

**Sudan red 3 test:**
Place a thin section of drug on glass slide. Add a drop of Sudan red 3 reagent. After 2 minutes wash with 50% alcohol. Mount in glycerin. Observe under microscope. Oil globules appear as red.

**Test for gums**
Test solution is hydrolyzed and treated with Benedict’s reagent. Red colour is developed.

**Pharmacological screening**

**Alpha-amyase inhibition assay** [11, 12, 13]

Importance alpha amylase enzyme in the body in humans, the digestion of starch involves several stages. Initially, partial digestion by the salivary amylase results in the degradation of polymeric substrates into shorter oligomers. Later on in to the gut these are further hydrolyzed by pancreatic alpha amylase into maltose, malto triose and small malto oligosaccharides. The digestive enzyme is responsible for hydrolyzing dietary starch, which breaks down into glucose prior to absorption. Inhibition of alpha amylase can lead to reduction in post prandial hyper glycaemia in diabetic condition. Importance of alpha glucosidase enzyme in the body alpha glucosidase is a membrane bound enzyme located on the epithelium of the small intestine, catalyzing the cleavage of disaccharides to form glucose. Inhibitors can retard the uptake of dietary carbohydrates and suppress post prandial hyperglycemia. Therefore, inhibition of alpha glucosidase could be one of the most effective approaches to control diabetas. Glucosidases are not only essential to carbohydrate digestion, but also vital for the processing of glycoprotein and glycolipids. The enzyme is a target for antiviral agents that interfere with the formation of essential glycoprotein required in viral assembly, secretion and infection. **Glucosidases are also involved in a variety of metabolic disorders and carcinogenesis.**

**MATERIALS AND METHODS**

The selected methanol and petroleum ether extract of *Ipomoea sepiaria* were serially diluted to get a required concentration to perform alpha amylase assay.

**Alpha-amyase inhibition assay principle and procedure** [14, 15]

Alpha-amyase activity was measured in-vitro by hydrolysis of starch in presence of α-amylase enzyme. This process was quantified by using iodine, which gives blue colour with starch. The reduced intensity of blue colour indicated the enzyme-included hydrolysis of starch into monosaccharides. If the substance/extract possesses α-amylase inhibitory activity, the intensity of blue colour will be more. In other words, the intensity of the blue colour in test sample is directly proportional to α-amylase inhibitory activity. Enzyme: [Type VI B: From porcine pancreas, 5,00,000U, [otto-S2366] [15.8U/mg solid at pH 6.9] – stored at 2-8°C Substrate: Starch 1% Positive Control: Acarbose-stored at RT- Glucobay (Bayer pharma, India).

Sodium dihydrogen orthophosphate (NaHat RT)
Disodium hydrogen phosphate (Na Indicator: Iodine solution 1% instrument -UV-visible spectrometer.

**Preparation of working solution:**
Phosphate buffer (40mM, pH 7, 25°C)
Solution A: 6.24 g of NaH₂PO₄ 1L
Solution B: 7.12 g of NaHPO₄ 1L
Enzyme (0.5128U/ml) – 3.246 mg α-amyase-100ml of 40 mM Phosphate Buffer
NaCl solution (0.006M)
Positive control:
Stock 50mg of Acarbose in 50ml of 40m Phosphate buffer
Working stock: Take 25 μl stock, made upto 10ml (2.5 μg/ml) with 40 mM phosphate buffer.

**Procedure:**
α-amylase activity was carried out by starch-iodine method. 10μL of α-amyase solution (0.025mg/mL) was mixed with 390μl of phosphate buffer (0.02M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37°C for 10min, 100μL of starch solution (1%) was added, and the mixture was re-incubated for 1hr. Next, 0.1 ml of 1% iodine solution was added, and after adding 5ml distilled water, the absorbance was taken at 565nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated as (% = (A-C) × 100/(B-C)), where, A=absorbance of sample, B=absorbance of blank (without α-amylase) and C= absorbance of control (without starch).
**Schematic flow chart of α-amylase inhibition assay procedure**

1. **Added 390 μl of 0.02M Phosphate buffer pH 7 / Positive control**
2. **Different concentration of test samples +10μL of α-amylase**
3. **Pre-incubated at 37 °C for 10 min**
4. **Added 10μl of starch**
5. **Re-incubated at 37 °C for 1h**
6. **Added 0.1 ml 1% Iodine solution +5ml of dist. water and measured OD at 565nm**

**Experimental animals**
White male Wister rats weighing about 150-180 gm were used. They were purchased from the Mahaveer enterprises Hyderabad. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5 °C) and normal 12-hr light/dark cycle. Moreover, they had free access to water and were supplied daily with standard diet of known composition ad libitum. All animal procedures were in accordance with the recommendation of the ethical committee guidelines for Care and Use of Animals.

**Induction and treatment of diabetes**
Diabetes was induced by a single injection fasting for at least 16hr, in freshly prepared 1% sodium carboxy methyl cellulose, blood glucose levels were measured 48hr development of diabetes mellitus was proven by sustained hyperglycemia (diabetic rats had glycaemia> 200mg/dL). The diabetes developed rats were selected for the study and treated with the Ethanolic extract of *Tecomaria capensis* 200 mg/Kg per oral (p.o.), Ethanolic extract of 400mg/Kg (p.o.), and Metformin 100 mg/Kg (p.o) for 8 consecutive days.

**Chemical agents:**
- Cleome Gynandra plant : Chittoor district of A.P, India
- Metformin : Natco. Pharma, Hyderabad, India
- Glucose Kit : Excel diagnostics Pvt Ltd. Mumbai
- Lipid profile kit : Excel diagnostics Pvt Ltd. Mumbai
- SGOT : Excel diagnostics Pvt Ltd. Mumbai
- SGPT kit : Excel diagnostics Pvt Ltd. Mumbai
- Estimation of Blood Glucose:
The determination of glucose is one of the most frequently performed tests in a clinical laboratory. The test based on the reducing property of glucose does not measure true glucose, as there is much interference. Subsequently other chemical and enzymatic methods were developed. Enzymatic methods are preferred because of their reliability and safety. This glucose kit is based on Tinder’s method in which Glucose Oxidase and peroxidase enzymes are used along with the chromogenic 4-Aminoantipyrine and phenol. The method is one step, simple and rapid. It does not have any interference due to reducing substances or haemoglobin, etc.

**Principle:**
Glucose is oxidized by the enzyme glucose oxidase (GOD) to give D-gluconic acid and hydrogen peroxide. Hydrogen peroxide in presence of the enzyme peroxidase (POD) oxidized phenol which combines with 4-Aminoantipyrine to produce a red coloured quinoneimine dye. The intensity of the colour developed is proportional to glucose concentration in the sample.
Reagents  | 5×100ml | 2×500ml | 5ltr |
--- | --- | --- | --- |
Enzyme Reagent Buffer | 5 vials | 2 vials | 1 vial |
Solution | 5×100ml | 2×500ml | 5000 ml |
Standard (100mg%) | 3ml | 3ml | 2 × 3 ml |

The reagents are stable at 2-8 °C till the expire date is mentioned on the label.

**Sample:**
Serum/ Heparinized or EDTA plasma / CSF

**Reagent Preparation:**
Dissolve one vial of Enzyme Reagent (I) in one bottle of Buffer solution (2). Mix gently to dissolve. The prepared Working Enzyme Reagent is stable for at least 2 months at 2-8 °C.

**Expected Range:**
Random Glucose : 70-170 mg%
Fasting Glucose : 70-110 mg%
Post Lunch Glucose : up to 170 mg%
CSF Glucose : 50-80 mg%

**Directions for Use on Analyzers:**

**Procedure:**
Pipette into clean, dry tubes labelled Blank (B) standard (S) and test (T) and add the reagents in the following order.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>B</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Enzyme Reagent (ml)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Distilled water (ml)</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard (ml)</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>Serum/Plasma/CSF (ml)</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Mix well and incubate at 37 °C for 10 min or at R.T. for 20min. Measure the absorbance of Test (T) and Standard (S), against Blank (B) on a photo colorimeter with green filter or on a spectrophotometer at 505nm.

**Calculations:**
Glucose in mg% = A of (T) / A of (S) × 100 (std conc)

**Estimation of Triglycerides:**

**Summary and Explanation of test:**
Triglycerides circulate in blood as complexes with protein molecules called lipoproteins. Triglycerides reach maximum level in blood approximately 4 to 6 hours post prandial. Elevated levels of both cholesterol and triglycerides in blood have been identifies as risk factors related to atherosclerotic disease. The levels of cholesterol and triglycerides can vary independently; therefore, evaluation of hyper-lipidemia include determination of both cholesterol and triglyceride.

**Principle:**
Triglycerides are usually assayed either by chemical methods or enzymatic methods. The chemical methods involve solvent extraction; chemical hydrolysis to release glycerol; oxidation of glycerol to form aldehyde which is then quantitated by coupling it with chromogens. These chemical methods are cumbersome, time consuming and are reliable only in expert hands. In contrast the enzymatic methods are simple, and easy to perform.

Triglycerides are hydrolysed by lipase to glycerol and free fatty acids. Glycerol is phosphorylated by ATP in the presence of glycerol kinase (GK) to Glycerol-3-phosphate (G-3-P) which is oxidized by the enzyme Glycerol-3-phosphate oxidase (G-P-O) producing hydrogen peroxide. Hydrogen peroxide so formed reacts with 4-aminantipyrine and ESPAS in the presence of the enzyme peroxidase (POD) to produce a Brown colour complex. The intensity of the colour developed is proportional to the tri-glycerides concentration.
lipase

\[
\text{Triglycerides} + H_2O \rightarrow \text{glycerol} + \text{fatty acids}
\]

Glycerol + ATP

GK

\[
\text{G-3-P} + \text{ADP}
\]

GPO

\[
\text{H}_2\text{O}_2 + \text{dihydroxy acetone phosphate}
\]

\[
\text{H}_2\text{O}_2 + \text{ESPAS} + 4\text{-AAP} \rightarrow \text{Brown colour} + H_2O
\]

Reagents:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>5x10</th>
<th>2x50</th>
<th>2x100</th>
<th>5x100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Enzyme Reagent</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2. Standard (200 mg %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample: Serum / Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expected Range:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum triglycerides: Up to 150 mg%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Directions for use on Analysers:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procedure:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pipette into clean dry tubes labelled blank (B), standard (S), and test (T).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix well and incubate for 10 min at 37 °C. Read absorbance of Standard and Test against Blank on photo colorimeter at 546 nm/green filter.

Calculations:

**Triglycerides conc. in mg %:**

\[A \text{ of } (T) / A \text{ of } (S) \times 200 \text{ (std.conc)}\]

**SI conversion factor:**

mMol/L = mg % \times 0.0113

**Estimation of Cholesterol:**

**Summary and Explanation of test:**

Cholesterol is both coming from food and synthesized by the human body, mainly in hepatic and intestinal cells. Cholesterol is a component of cells and organic membranes. It is a metabolic precursor of bile acids, vitamin D and steroid hormones. Cholesterol, insoluble molecule, circulates associated with lipoproteins (HDL, LDL, and VLDL).

Quantification of total cholesterol allows the detection of hyper-cholesterolemia, isolated of associated with hyper-triglyceridemia. High Cholesterol concentration are associated with a high risk for vascular accident and apparition of atherosclerosis. The LDL/HDL ratio should be taken into consideration for evaluating the risk of developing cardiovascular diseases.

**Principle:**

Enzymatic determination of total cholesterol according to the following reactions.

- Cholesterol ester + H_2O
  - Cholesterol esterase
  - cholesterol + fatty acids
- Cholesterol + O_2
  - Cholesterol oxidase
  - 4-cholesterol-3-one + H_2O
- 2H_2O_2 + phenol + 4-aminoantipyrine peroxidase
  - Quinoneimine + 4 H_2O

**Reagents:**

1. Cholesterol reagent 5x10, 2x50, 2x50, 5x100ml
2. Cholesterol Standard 1 1 1 2x1ml
3. HDL PPT Reagent 5 10 2x10 50ml

The reagents are ready to use and usable to the expiration date when stored at 2-8 °C and protected from light, if contamination is avoided.

**Sample:**

Serum

Heparin or EDTA plasma from fasting patient

**Expected Range:**

Cholesterol Normal: up to 250 mg HDL cholesterol

Male: 30-63 mg%    female: 35-75 mg%

**Directions for use on Analysers:**

- Reaction type: end point with std
- Wavelength: 500 nm (green filter)
- Incubation temp: 37 °C
- Incubation time: 5 min

**Calculations:**

Cholesterol conc. in mg %:

\[A \text{ of } (T) / A \text{ of } (S) \times 200 \text{ (std.conc)}\]

**SI conversion factor:**

mMol/L = mg % \times 0.0113

**Estimation of Cholesterol:**

**Summary and Explanation of test:**

Cholesterol is both coming from food and synthesized by the human body, mainly in hepatic and intestinal cells. Cholesterol is a component of cells and organic membranes. It is a metabolic precursor of bile acids, vitamin D and steroid hormones. Cholesterol, insoluble molecule, circulates associated with lipoproteins (HDL, LDL, and VLDL).

Quantification of total cholesterol allows the detection of hyper-cholesterolemia, isolated of associated with hyper-triglyceridemia. High Cholesterol concentration are associated with a high risk for vascular accident and apparition of atherosclerosis. The LDL/HDL ratio should be taken into consideration for evaluating the risk of developing cardiovascular diseases.

**Principle:**

Enzymatic determination of total cholesterol according to the following reactions.
Total Cholesterol Procedure:
Pipette in a clean dry test tube labelled as blank (B), standard (S), and test (T)

<table>
<thead>
<tr>
<th></th>
<th>Enzyme</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>1ml Reagent</td>
<td>1ml</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.01 ml De-ionized</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>water</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td>0.01 ml</td>
<td>0.01 ml</td>
</tr>
<tr>
<td></td>
<td>Serum/plasma</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix and read the optical density (OD) at 500 nm against blank after 5 min incubation (37 °C). The final colour is stable for at least 1 hr.

Calculation:

Cholesterol conc. in mg % = A of (T) / A of (S) × 200 (std.conc)

HDL cholesterol procedure:

Step 1: Precipitation

<table>
<thead>
<tr>
<th>Sample</th>
<th>B</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>0.01 ml</td>
<td>-</td>
</tr>
<tr>
<td>Serum/plasma</td>
<td>-</td>
<td>-</td>
<td>0.01 ml</td>
</tr>
</tbody>
</table>

Incubate for 5 min at 37 °C. Read the optical density at 500 nm against blank.

Calculation:

HDL conc. = abs of test / abs of standard × 5.

LDL cholesterol procedure:

Below is general example of the direct LDL test procedure for an automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>B</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>0.01 ml</td>
<td>-</td>
</tr>
<tr>
<td>Serum/plasma</td>
<td>-</td>
<td>-</td>
<td>0.01 ml</td>
</tr>
</tbody>
</table>

Mix well and incubate for 10 min at 37 °C. Read absorbance of standard and test against blank on automated analyzer between 660 nm and 546 nm.

Calculation:

LDL conc. = Abs of test / Abs of standard × 5

SGOT (ASAT) Estimation:

Summary:

SGOT is an enzyme found mainly in heart muscle, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of enzyme in blood stream. Elevated levels are found in myocardial infarction, cardiac operations, hepatitis, cirrhosis, acute pancreatitis, acute renal diseases, primary muscle disease. Decreased levels may be found in Pregnancy, Beri-beri an Diabetic ketoacidosis.

Principle:

SGOT converts L-Aspartate and a Ketoglutarate to Oxaloacetate and glutamate. The oxaloacetate formed reacts with 2,4-dinitropheny hydrazine to produce a hydrazine derivative, which in an alkaline medium produces a brown coloured complex whose intensity is measured. The reaction does not obey Beer’s law and hence a calib0ration curve is plotted using a pyruvate standard. The activity of SGOT (ASAT) is read off this calibration curve.

\[
\text{L-Aspartate} + \text{SGOT} \rightarrow \text{oxaloacetate} + \]\[pH 7.4\]
\[
\text{keto glutarate} + \text{L-Glutamate}
\]
oxid acetate + 2, 4, DNPH = alkaline medium 2, 4, Dinitro phenyl hydrazone (brown coloured complex)

Normal Reference Values
Serum: 8-40 units/ml

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents
L1: Substrate reagent 25 ml
L2: DNPH reagent 2×12.5 ml
L3: NaOH reagent (4N) 25 ml
S: Pyruvate standard (2mM) 5 ml

Storage / stability
Contents are stable at 2-8 °C till the expiry mentioned on the labels. NaOH can be stored at R.T. till the expiry mentioned.

Reagent Preparation
All the reagents are ready to use except NaOH reagent (4N) which has to be diluted 1:10 with distilled/ deionized water. Working NaOH reagent: Dilute the NaOH to 250 ml or for every 1.0 ml of NaOH reagent (4N) add 9.0 ml of dist. Water.

Sample Material
Serum free form hemolysis SGOT (ASAT) is reported to be stable in serum for 3 days at 2-8 °C.

Procedure
Wavelength / filter: 505nm (Hg 546 nm) / green
Temperature: 37 °C and R.T.
Light path: 1cm
Assay:
Pipe into clean dry test tubes labelled as blank (B) and test (T):

<table>
<thead>
<tr>
<th>Addition sequence</th>
<th>B (ml)</th>
<th>T (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate reagent (L1)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Incubate for 37 °C for 3 min</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>0.50</td>
</tr>
<tr>
<td>Mix well and incubate at 37 °C for 60 min</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>DNPH reagent (L2)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Mix well and allow to stand at R.T. for 20 min</td>
<td>0.10</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Working NaOH reagent (L3)</td>
<td>5.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Mix well and allow to stand at R.T. for 10min. Measure the absorbances of the test (T) against blank (B) and read the activity of the test form the calibration curve plotted earlier.

SGPT (ALAT) Estimation:

Summary:
SGPT is found in a variety of tissues but is mainly found in the liver. Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic diseases. Slight elevation of the enzymes is also seen in myocardial infarction.

Principle:
It recommended that each laboratory established its own normal range representing its patient population.

Contents
25 ml 75 ml
L1: enzyme reagent 20ml 60 ml
L2: starter reagent 5 ml 15 ml

Storage / stability
Contents are stable at 2-8°C till the expiry mentioned on the label.

Reagent preparation: Reagents are ready to use.

Working Reagent:
For sample start assays a single reagent is required, pour the contents of 1 bottle of L2 (starter reagent) into 1 bottle of L1 (enzyme reagent). This working
reagent is stable for at least 3 weeks when stored at 2-8°C. Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (enzyme reagent) and 1 part of L2 (starter reagent). Alternatively, 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

**Substrate start assay:**

Pipette into a clean dry test tube labelled as test (T):

<table>
<thead>
<tr>
<th>Addition sequence</th>
<th>(T) 25 °C / 30 °C</th>
<th>(T) 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme reagent (L1)</td>
<td>0.8 ml</td>
<td>0.8 ml</td>
</tr>
<tr>
<td>Sample</td>
<td>0.2 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Incubate at the assay temperature for 1 min and add Starter reagent (L2)</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
</tr>
</tbody>
</table>

Mix well and read the initial absorbance A and repeat the absorbance reading after every 1, 2 and 3 min. Calculate the mean absorbance change per min (ΔA/min).

**Sample start Assay:**

Pipette into a clean dry test tube labelled as test (T).

<table>
<thead>
<tr>
<th>Addition sequence</th>
<th>(T) 25 °C / 30 °C</th>
<th>(T) 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent (L1)</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Incubate at the assay temperature for 1 min and add Sample</td>
<td>0.2 ml</td>
<td>0.1 ml</td>
</tr>
</tbody>
</table>

Mix well and read the initial absorbance A after 1 min and repeat the absorbance reading after every 1, 2 and 3 min. Calculate the mean absorbance change per min (ΔA/min).

**Calculations:**

**Substrate / Sample start:**

SGPT (ALAT) activity in U/L 25 °C / 30 °C = A/min × 952
SGPT (ALAT) activity in U/L 37 °C = A/min × 1746.

**Experimental study design:**

The rats were randomly divided into 5 groups (n=6) as follows:

- **Group I:** control animals (sod. Carboxymethyl cellulose – 1%, orally).
- **Group II:** diabetic animals
- **Group III:** ethanolic extract of *Tecomaria capensis* 200 mg/kg
- **Group IV:** ethanolic extract of *Tecomaria capensis* 400 mg/kg
- **Group V:** metformin 100 mg/kg

**Biochemical evaluation:**

Blood samples were collected from the retro orbital puncture of rats on 0 and 8th days of control, diabetic and ethanolic extract of *Tecomaria capensis* treated diabetic rats and determined blood glucose levels, lipid profiles and SGPT were estimated after 8 days treatment.

**Statistical analysis:**

The data are presented as mean ± S.D Statistical comparisons were made by one-way analysis of variance (ANOVA) and followed by Student-Neuman-Keuls test. Data was considered significant when p values were lower than 0.05.

**RESULTS AND DISCUSSION**

The herbal medicines are effective in the treatment of various life-threatening diseases. Very often these drugs are unscientifically exploited and / or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science. The detailed investigation and documentation of plants used in total health traditions and pharmacological evaluation can lead to the development of invaluable plant drugs for many treated drugs.

**Macroscopic Characters**

- **Leaf:** Isobilateral
- **Shape:** Ovate
- **Size- length:** 6 - 7.5 cm
- **Width:** 2.5 – 3.5 cm
- **Texture:** Glabrous
- **Apex:** Acute
- **Margin:** Serrated
- **Base:** Decurrent
- **Petiole:** Expetiolate
- **Surface:** Glossy
- **Colour- outer:** Dark green
- **Inner:** Light green
- **Venation:** Pinnate
- **Odour:** Characteristic

**Microscopic Characters**

Transverse section of leaves of *Tecomaria capensis*
**Type:** Isobilateral, convex type of leaf.

**Upper epidermis:** It is of single layer non-lignified rectangular shape of cells arranged transversally.

**Lower epidermis:** It is of single non-lignified rectangular shape of cells arranged transversally.

**Trichomes:** Both covering as well as glandular trichomes are present. Covering trichomes are present just above the epidermal layer. Glandular trichomes are present in more number in the midrib portion.

**Stomata:** Anamocytic stomata.

**Palisade cells:** Below the upper epidermis and above the lower epidermis palisade cells are longitudinally compactly arranged and they are terminated up to the lamina portion or mesophyll portion.

**Calcium Oxalate Crystals:**
Mesophyll region contains calcium oxalate crystals. Isolate or cluster of prism of calcium oxalate crystals.
Physiochemical constituents:
Determination of physiochemical constituents are performed as per the standard protocol followed in the Ayurvedic pharmacopoeia. The values are tabulated in Table 1 and 2.

**Table 1: Different Extractive Values**

<table>
<thead>
<tr>
<th>Extractive Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol soluble extraction</td>
<td>0.25 gm</td>
</tr>
<tr>
<td>Water soluble extraction</td>
<td>0.36 gm</td>
</tr>
<tr>
<td>Ether soluble extraction</td>
<td>0.04 gm</td>
</tr>
</tbody>
</table>

**Table 2: Different Ash Values of Tecomaria Capensis leaves**

<table>
<thead>
<tr>
<th>Ash values</th>
<th>In gms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash value</td>
<td>0.93</td>
</tr>
<tr>
<td>Acid insoluble ash value (dil. HCl)</td>
<td>0.01</td>
</tr>
<tr>
<td>Sulphate ash value (H₂SO₄)</td>
<td>0.06</td>
</tr>
<tr>
<td>Water soluble ash value (H₂O)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Percentage yield of Tecomaria capensis extracts**

**Table 3: % yield of different leaves extracts of Tecomaria capensis.**

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Solvents</th>
<th>Nature of extracts</th>
<th>Colour</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pet-ether</td>
<td>Semisolid</td>
<td>Dark yellow</td>
<td>0.35</td>
</tr>
<tr>
<td>2</td>
<td>N-Hexane</td>
<td>Semisolid</td>
<td>Dark yellow</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>Semisolid</td>
<td>Dark green</td>
<td>0.20</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl acetate</td>
<td>Semisolid</td>
<td>Dark green</td>
<td>0.70</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol</td>
<td>Semisolid</td>
<td>Dark green</td>
<td>0.52</td>
</tr>
<tr>
<td>6</td>
<td>Aqueous</td>
<td>Semisolid</td>
<td>Dark brown</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**Table 4: Preliminary Phyto Chemical Screening**

<table>
<thead>
<tr>
<th>Phyto constituents</th>
<th>Pet.ether</th>
<th>N-hexane</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>alkaloids</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Saponin glycosides</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Coumarin glycosides</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Tannins</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Steroids &amp; terpenoids</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Protein</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Inulin</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Waxes</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Mucilage</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

++ present, -- absent
Phytochemical studies of *Tecomaria capensis* leaves revealed the presence of cardiac glycosides, saponin glycosides and volatile oils in pet-ether extract. In n-hexane extract cardiac glycosides, steroids, tri-terpenoids and volatile oils are present. In chloroform extract, flavonoids, cardiac glycosides, saponin glycosides, steroid, tri-terpenoids, volatile oils are present. In ethyl acetate extract, flavonoids, cardiac glycosides, coumarin glycosides, saponin glycosides, stereds, terpenoids, carbohydrates, proteins and volatile oils are present. In Ethanolic extract terpenoids, flavonoids, cardiac glycosides, coumarin glycosides, tannins, steroids, terpenoids, protein, inulin, volatile oils and mucilage are present. In water extract, flavonoids, inulin and mucilage are present. In ethanol and ethyl acetate extracts maximum phytochemical constituents have been observed.

**Pharmacological Screening**

**In-vitro alpha-amylase inhibition assay:**

The below table shows the inhibition of α-amylase activity for the methanol and pet ether extracts and are compared with standard Acarbose. All the tested extracts showed dose dependent enzyme inhibition.

The extracts exhibited IC$_{50}$ μg/mL will be considered active in comparison with other tested extracts showed enzyme inhibition activity with IC$_{50}$ 131.33±1.8, 123.59±1.2, 133.81±0.7, 127.62±53, 128.00±0.6, 127.22±1.4, 128.43±0.8, 117.52±1.2, 119.64±1.5, 110.45±1.2, 121.93±0.8, 114.26±1.2 and standard shows 0.32 μg/mL respectively.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (mg/ml)</th>
<th>IC$_{50}$ (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether extract</td>
<td>100</td>
<td>131.33±1.8</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>200</td>
<td>123.59±1.2</td>
</tr>
<tr>
<td>n-hexane extract</td>
<td>100</td>
<td>133.81±0.7</td>
</tr>
<tr>
<td>n-hexane extract</td>
<td>200</td>
<td>127.62±53</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>100</td>
<td>128.00±0.6</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>200</td>
<td>127.22±1.4</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>100</td>
<td>128.43±0.8</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>200</td>
<td>117.52±1.2</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>100</td>
<td>119.64±1.5</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>200</td>
<td>110.45±1.2</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>100</td>
<td>121.93±0.8</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>200</td>
<td>114.26±1.2</td>
</tr>
<tr>
<td>Acarbose</td>
<td>2.5</td>
<td>0.32±0.12</td>
</tr>
</tbody>
</table>

All values reported as Mean ± S.E.M (n=3)

A-amylose activity was measured in-vitro by hydrolysis of starch in presence of α-amylose enzyme. This process was quantified by using iodine, which gives blue colour with starch. The reduced intensity of blue colour indicates the enzyme-induced hydrolysis of starch into monosaccharides. If the substance/extract process α-amylose inhibitory activity, the intensity of blue colour will be more. In other words, the intensity of blue colour in test sample is directly proportional to α-amylose inhibitory activity.

Acarbose shows the best when compared to extracts, among all concentrations of different extracts, the ethanol extract of 200 mg/ml showed the superior anti-diabetic activity when compared to rest of the concentrations of different extracts.

**In-vitro evaluation of antidiabetic activity:**

The effects of Ethanolic extract of *Tecomaria capensis*100mg/kg on blood glucose, SGOT, SGPT levels (0 & 8 days) of control, diabetic and ethanolic extract of treated diabetic rats were summarized in Table 1. Lipid profiles were significantly (p<0.001) reduced the blood glucose and SGOT and SGPT concentration diabetic groups (p< 0.001). The ethanolic extract of *Tecomaria capensis* was significantly reduced, the triglycerides, LDL-cholesterol, and total cholesterol but increased HDL cholesterol levels after treatment. *Tecomaria capensis* occurs throughout the tropic and sub-tropic regions. It contains chemical constituents such as triterpenes, tannins, anthroquinones, flavonoids, saponins, steroids, resins, lectins, glycosides, sugars, phenolic compounds and alkaloids and these are more beneficial in diabetes and its associated complications, holding hope of the new generation anti-hyperglycaemic drug.
SUMMARY AND CONCLUSION

India is one of the largest producers of medicinal herbs in the world. The Indian traditional healthcare system, Ayurveda provides relatively organized database and more exhaustive description of botanical materials, many of which have been used as templates for novel drug development. Nature has provided a complete storehouse of remedies to cure all ailments of mankind by providing us drugs in the form of herbs, plants and algae to cure the incurable diseases without any toxic effect. Research on medicinal plants is an important fact of biochemical research in India because of several reasons. Review of literature was done on herbal plant *Tecomaria capensis* species and its literature was collected and reviewed. Form the review of literature we identified that less biological activity was estimated. So, we have made an attempt for in-vitro evaluation of anti-diabetic activity.

The plant was selected from in and around of Guntur district and it was authenticated by professor Dr.M. Raghu Ram, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur. The leaves were dried and powered. The powdered material was extracted by using non-polar (petroleum ether) to polar (water) solvents. Phytochemical screening revealed that in pet ether extracts glycosides and in ethanol extract, tannins and steroids were presented. At present in-vitro evaluation of anti-diabetic activity on extracts of leaves of *Tecomaria capensis* was performed.

We concluded that the ethanol extracts of leaves of *Tecomaria capensis* have revealed that a significant activity against diabetes. Further-work has to be done for the phyto constituent’s responsible for the activities.

Our results shown that oral administration of *Tecomaria capensis* extract has a beneficial effect on the reducing hyperglycaemia, SGOT & SGPT levels, total cholesterol, triglycerides levels, LDL-cholesterol, VLDL-cholesterol and improving the HDL status. This study suggests that the induction of diabetes mellitus may be prevented by flavonoids of plant constituent’s administration. There is a need to continue to explore the mechanisms for anti-diabetes.

**REFERENCE:**

2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4061597/

**Table 6: Blood glucose levels were estimated in Normal, Diabetic control & Treatment groups.**

<table>
<thead>
<tr>
<th>Groups/Parameters (mg/dl)</th>
<th>Normal</th>
<th>Metformin (25 mg/kg)</th>
<th>CG-I (100 mg/kg)</th>
<th>CG-II (200 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose</td>
<td>83.1±11.2</td>
<td>111.3±19.4</td>
<td>199.6±38.6</td>
<td>169±47.1</td>
</tr>
<tr>
<td>TC</td>
<td>46±2.7</td>
<td>61±5.3</td>
<td>72.3±5.2</td>
<td>63.6±9.2</td>
</tr>
<tr>
<td>TG</td>
<td>54.7±4.3</td>
<td>60.9±4.2</td>
<td>73.8±3.8</td>
<td>66.7±4.7</td>
</tr>
<tr>
<td>HDL</td>
<td>23.6±1.6</td>
<td>17.7±0.3</td>
<td>13±0.4</td>
<td>15.1±0.5</td>
</tr>
<tr>
<td>LDL</td>
<td>11.1±0.5</td>
<td>11.8±0.8</td>
<td>21.0±0.6</td>
<td>11.5±0.6</td>
</tr>
<tr>
<td>VLDL</td>
<td>11.6±1.1</td>
<td>11.27±0.9</td>
<td>15.36±0.51</td>
<td>12.78±5.4</td>
</tr>
<tr>
<td>SGOT</td>
<td>25±3</td>
<td>31.5±4.1</td>
<td>52.6±6.7</td>
<td>41.6±3.7</td>
</tr>
<tr>
<td>SGPT</td>
<td>41.9±9</td>
<td>54.9±3.1</td>
<td>75.9±4.7</td>
<td>60.9±4.7</td>
</tr>
</tbody>
</table>