Project Report

"Evaluation of Pharmacognostical, Phytochemical and Antidiabetic Profiles of Indigenous Plant"

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FINAL REPORT

1. **Project title-** Evaluation of pharmacognostical, phytochemical and antidiabetic profiles of indigenous plant.
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4. Other Scientific
   Staff- Dr. Neminath A Rajmane
5. Non scientific
   Staff- Nil
6. Implementing
   Institutions- Appasaheb Birnale college of pharmacy, Sangli.
7. Date of start- 1st Nov. 2008
8. Duration- 2 years
9. Objectives of the proposal-
   - Selection of Plant (*Careya arborea*).
   - Pharmacognostical evaluation of bark of *Careya arborea* Roxb.
   - Extraction of above drugs by different extraction techniques like soxhlet, maceration etc.
   - Phytochemical investigation of the extracts.
   - Evaluation of anti-diabetic activity.
10. Deviation made from original objectives if any, while implementing the project and reasons thereof. _____Nil___

11. Experimental work --

(Part-I 1st progress report)

Pharmacognostical evaluation of *Careya arborea* Roxb. bark

Plant material

The bark of the *C. arborea* were collected from Dajipur jungle (Radhanagari wild life sanctuary), Kolhapur, Maharashtra. The Plant was authenticated by the botanist in the botany department, Willingdon College, Sangli and also by Dr. S. S. Sathe, Vasantdada Patil science college, Tasgaon. The voucher specimen has been preserved in our laboratory for future reference.

Pharmacognostic Studies

Organoleptic evaluation of bark was carried out. Behavioural characters of powdered bark of *C. arborea* were carried out under UV (Short wavelength) and visible light by using different chemical reagents. Microscopic study of bark was carried out with the help of Photo-micrographic equipment (Make- Kyowa-Getner, Model – 11UP with Bio-plus-55 software). Different staining reagents were used to study transverse section as well as microscopic characteristics of powdered bark. Under physical evaluation total ash, sulphated ash, water soluble ash, acid insoluble ash of the bark was calculated as per the procedure. Water soluble and alcohol soluble extractive values were also calculated. Moisture content was determined by loss on drying.

RESULTS

Organoleptic evaluation of bark

Bark was thick, rough, dark grey in colour, showed shallow cracks and exfoliating in narrow flakes. Bark was odourless with astringent taste.

Table 1: Behavioural characters of powdered bark of *Careya arborea*

Sr. No.	Particulars	Under Visible light	U.V. Light Short wavelength
1.	Powder as such	Greyish brown	Brown
2.	Powdered drug + Conc. HCl	Brown	Dark brown
3.	Powdered drug + Conc. H_2SO_4	Brown	Dull green
4.	Powdered drug + Conc. HNO_3	Light brown	Dull green
5.	Powdered drug + G.A.A.	Brown	Dull green
6.	Powdered drug + NaOH(Aq.)	Dark brown	Green
7.	Powdered drug + NaOH (Alc.)	Dark brown	Green

8.	Powdered drug + 10% HCl	Light brown	Green
9.	Powdered drug + 10% H_2SO_4	Brown	Pale green
10.	Powdered drug + 10% HNO_3	Light brown	Pale green
11.	Powdered drug + $FeCl_3$ (Aq.)	Greenish yellow	Dark green
12.	Powdered drug + $FeCl_3$ (Alc.)	Dark brown	Dull green

Microscopic evaluation of bark

Transverse section of bark showed distinct cork, cortex and secondary phloem. The Cork cells were multilayered (8-16), thick walled, rectangular, blackish brown in colour. Cork was followed by 2-3 layers of Phellogen and then by Phelloderm. Cortex was extensive, parenchymatous with rectangular to polygonal cells. Cortical cells were multilayered while some cells were golden yellow in colour with yellowish contents. Secondary phloem was made up of fibres, phloem parenchyma, medullary rays and vessels. The fibres were round in shape. They formed round patch inside the section. Medullary rays were 1-2 seriate. Calcium oxalate crystals were present in, cells of cortex and phloem parenchyma in secondary phloem. Starch grains were very few.

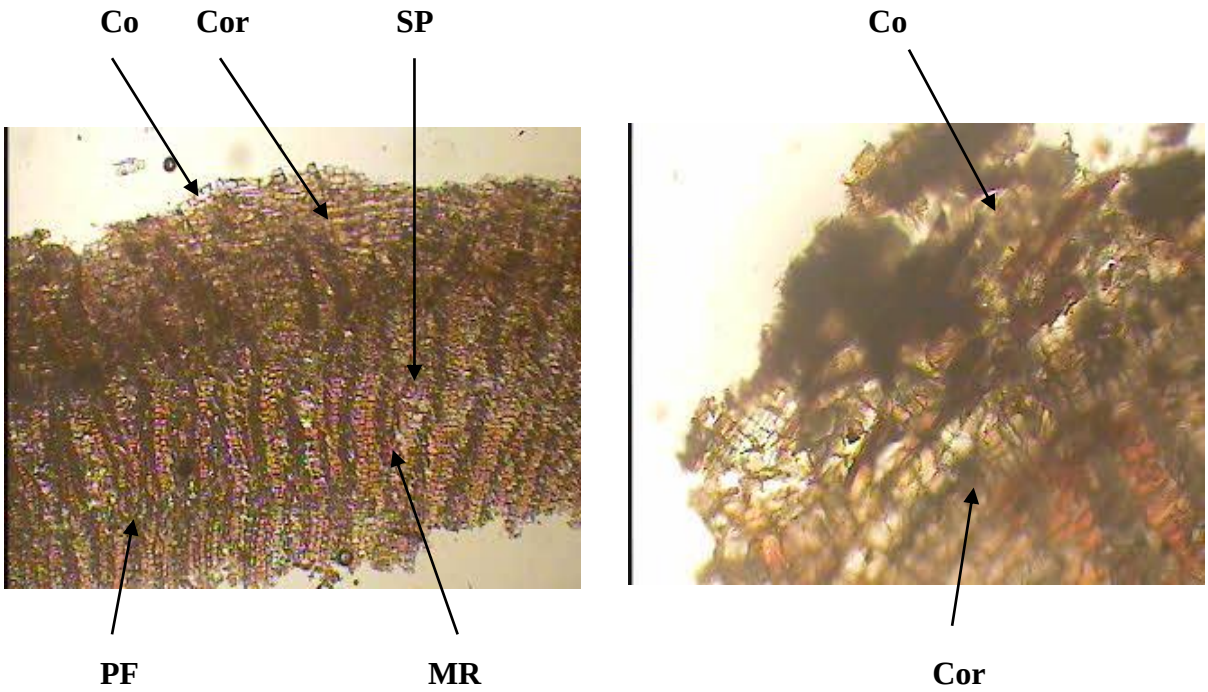
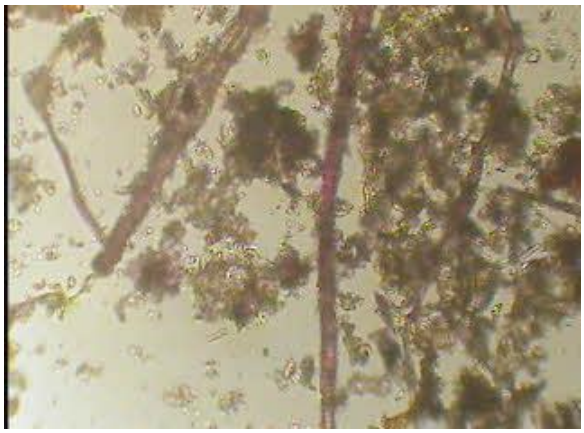


Figure 1: T.S. of *Careya arborea* bark.

Co- Cork, Cor- Cortex, SP- Sec. Phloem, PF- Phloem fibres, MR- Medullary rays.



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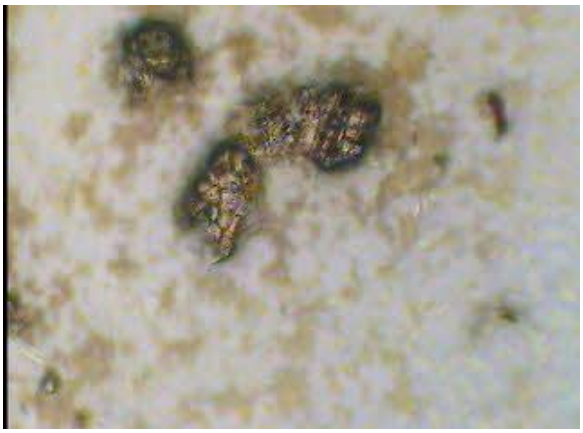

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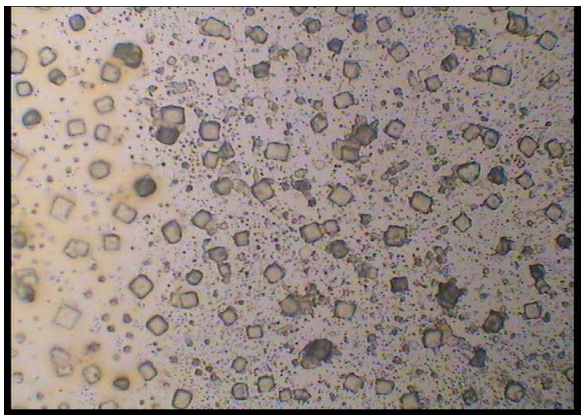

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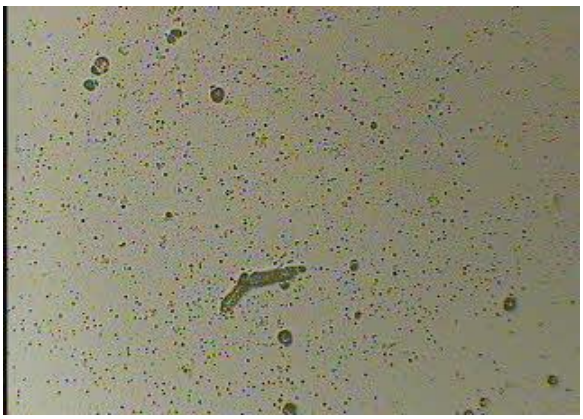

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(e)

Figure 2: Microscopical characteristics of powdered bark of *Careya arborea*
(a) Lignified fibres, (b) Cork cells, (c) Calcium oxalate crystals, (d) Starch grains,
(e) Parenchymatus cells

<u>The Microscopic characteristic of powdered bark showed,</u>

Fibres- the fibres were lignified having very thick wall. They were long, slender and of 38-66 μ in length.

Cork cells- blackish brown thick walled, rectangular to oval shaped cork cells.

Calcium oxalate crystals- the crystals were tetragonal prism type having 10-15 μ in size.

Starch grains - were very few, simple, upto 5 μ in diameter. The parenchymatous cells were rectangular to polygonal in shape.

Determination of physical constants

- **Loss on drying**

Loss on drying is the loss of mass expressed as percent w/w. The test for loss on drying determines both water and volatile matter in the crude drug. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible.

An accurately weighed quantity of about 5 g of powdered drug was taken in a porcelain dish. The porcelain dish kept open in vacuum oven and the sample was kept at a temperature 100^0C. Then it was cooled in a desiccator to room temperature, the procedure was repeated till constant weight is observed. % Loss on drying was calculated using the following formula.

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

- **Ash value**

Ash value is helpful in determining the quality and purity of a crude drug, especially in the powdered form. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.

i. Determination of Total ash value

Accurately weighed 2 g of the powdered drug in a silica crucible, previously ignited and weighed. Incinerated by gradually increasing the heat to temperatures not exceeding 450^0C for 4 h, until free from carbon, crucible was cooled and weighed. Calculated the percentage of ash with reference to air-dried drug using following formula,

$$\%\ \text{Total Ash Value} = \frac{\text{Weight of Total Ash}}{\text{Weight of Crude Drug Taken}} X\,100$$

ii. Sulphated ash value.

Weighed accurately about 2 gms of the powdered drug in a silica crucible. The drug is moistened with Sulphuric acid. Then it was incinerated at 600^0C by using muffle furnace. Crucible was cooled and weighed. Calculated the percentage of ash with reference to air dried drug.

iii. Water soluble ash value

The ash was boiled with 25 ml of water for 10 minutes. Filtered and collected the insoluble matter on filter paper, washed with hot water and ignited in a crucible at a temperature not exceeding 450^0C for 4 h. Cooled in a desiccator and weighed. The difference in weight represented weight of water soluble ash. Calculated the percentage of water soluble ash with reference to the air-dried drug using the following formula,

$$\% \text{ Water soluble Ash value} = \frac{\text{Weight of total ash} - \text{Wt of water soluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

iv. Acid insoluble ash value

Boiled the ash for 10 min with 25 ml of 2 M HCl. Filtered and collected the insoluble matter on filter paper, washed with hot water and ignited in a crucible at a temperature not exceeding 450^0C for 4 hr. cooled in a desiccator and weighed. Calculated the percentage of acid insoluble ash with reference to the air-dried drug using following formula,

$$\% \text{ Acid insoluble Ash value} = \frac{\text{Weight of Acid insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

Extractive values:

- **Determination of Alcohol soluble extractive value**

5 gm powdered drug was macerated with 100 ml of alcohol (90%v/v) in a stoppered flask and kept for 24 hrs with intermittent shaking after every 6 hrs. Rapidly filtered the macerate through filter paper, taking necessary precaution to avoid excess loss of alcohol. Evaporated 25 ml of alcoholic extract to dryness in a tarred dish at $105^0 \pm 1^0$C and weighed it. Calculated the percentage w/w of alcohol soluble extractive with reference to the air-dried drug.

- **Determination of Water soluble extractive value**

  Followed the procedure as above using chloroform water I.P. instead of alcohol

  Results

Evaluation parameters	**Values**
Loss on drying	**14%**
Total ash	**12%**
Sulphated ash	**9.49%**
Water soluble ash	**2%**
Acid insoluble ash	**0.9%**
Water soluble extractive value	**16%**
Alcohol soluble extractive value.	**7.2%**

Phytochemical investigation of *Careya arborea* Roxb. bark

- **Aqueous Extraction**

  About ½ kg of bark powder was subjected to cold maceration with chloroform water I.P. (10%) in a two litres round bottom flask for about 7 days at room temperature. The flask was securely plugged with absorbent cotton and was shaken periodically till complete maceration. After maceration, the mark was pressed in a muslin cloth and the filtrate was concentrated with the help of rotary vacuum evaporator to residue at low temperature.

- **Successive solvent extraction:**

  About 500 gms of fresh air-dried and standardized bark powder of *careya arborea* was extracted with 1000 ml of petroleum ether, benzene, chloroform, acetone and ethanol successively by using soxhlet extractor.

- **Alcoholic Extraction**

  About 500 gms of fresh air-dried and standardised bark powder of *careya arboreya* was extracted with ethanol by using soxhlet extractor.

  The extract was filtered and concentrated with the help of rotary vacuum evaporator. The dried extracts were stored carefully for standardisation, phytochemical investigation and antidiabetic activity.

QUALITATIVE CHEMICAL INVESTIGATION OF EXTRACTS:

Qualitative tests were conducted for all the extracts of bark of *Careya arborea* to identify the various phytoconstituents. The various tests and reagents used are given below and observations are recorded in the Table

1. Tests for Carbohydrates:

Preparation of test solution: The test solution was prepared by dissolving the test extract with water. Then it was hydrolyzed with 1 volume of 2N HCl and subjected to following chemical tests.

Molisch's test (General test):

To 2-3 ml aqueous extract, added few drops of α-naphthol solution in alcohol, shaken and added concentrated H_2SO_4 from sides of the test tube, then observed for violet ring at the junction of two liquids.

For Reducing Sugars:-

a) **Fehling's test**: 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min. observed for a yellow, then brick red precipitate.

b) **Benedict's test**: Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

Tests for Monosaccharide:

***Barfoed's test*:** Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Observed for red precipitate.

Test for Non-Reducing Polysaccharides (Starch):

a) **Iodine test:** mix 3 ml. test solution and few drops of dilute Iodine solution. Blue colour appears; it disappears on boiling and reappears on cooling.

b) **Tannic acid test for starch:** With 20% tannic acid, test solution was observed for precipitate.

2. Tests for Proteins:

1) **Biuret test (General test):** To 3 ml test solution (T.S.) add 4% NaOH and few drops of 1% $CuSO_4$ solution observed for violet or pink colour.

2) **Million's test (for proteins):** Mixed 3 ml T.S. with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour.
3) **Xanthoprotein test (For protein containing tyrosine or tryptophan):** Mixed 3ml T.S. with 1 ml concentrated H_2SO_4 observed for white precipitate.

3. Tests for Amino Acids:

1) **Ninhydrin test (General test):** 3 ml T.S. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. observed for purple or bluish colour.
2) **Test for Tyrosine:** Heated 3 ml T.S. and 3 drops Million's reagent. Solution observed for dark red colour.
3) **Test for Tryptophan:** To 3 ml T.S. added few drops glycoxalic acid and concentrated H_2SO_4 observed for reddish violet ring at junction of the two layers.
4) **Test for cysteine:** To 5 ml. T.S. add few drops of 40% sodium hydroxide and 10% lead acetate solution. Boil. Black ppt. of lead sulphate is formed.

4. Tests for Steroid and triterpenoid:

Preparation of test extracts solution:

The extracts were refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (unsaponifiable matter) was subjected to the following test by dissolving the residue in chloroform.

1) **Salkowaski Reaction:** Mixed 2 ml of extract, 2 ml chloroform and 2 ml concentrated H_2SO_4, Shake well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.
2) **Liebermann-Burchard Reaction:** Mixed 2ml extract with chloroform add 1-2 ml acetic anhydride and 2 drops concentration H_2SO_4 from the side of test tube observed for first red, then blue and finally green colour was observed.
3) **Liebermann's reaction:** Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

5. Tests for Flavonoids:

The flavonoids are all structurally derived from the parent substance called flavone. The flavonoids occur in the free form as well as bound to sugars as glycosides. For this reason, when analyzing flavonoids it is usually better to examine the flavonoids in hydrolyzed plant extracts.

Preparation of test solution:

i. To a small amount of extract added equal volume of 2M HCl and heated in a test tube for 30 to 40 min. at 100°C.
ii. The cooled extract was filtered, and extracted with ethyl acetate.
iii. The ethyl acetate extract was concentrated to dryness, and used to test for flavonoids.

1) **Shinoda test:** To extract, add 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour was observed. To small quantity of residue, acetate solution was added, observed for yellow coloured precipitate. Addition of sodium hydroxide to the residue showed yellow colouration, which was decolourised after addition of dilute hydrochloric acid.
2) **Ferric Chloride test:** Test solution with few drops of ferric chloride solution shows intense green colour.
3) **Alkaline reagent test:** Test solution was treated with sodium hydroxide solution shows intense yellow colour which becomes colourless on addition of few drops of dilute hydrochloric acid.
4) **Lead Acetate solution test:** Test solution with few drops of lead acetate solution (10%) gives yellow precipitates.

6. Tests for Glycosides:

Preparation of test solution: The test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

A) Tests for Cardiac Glycosides:

1) **Baljet's test:** A test solution observed for yellow to orange colour with sodium picrate.
2) **Legal's test (For cardenoloids):** To aqueous or alcoholic test solution, added 1 ml pyridine and 1 ml sodium nitroprusside observed for pink to red colour.
3) **Test for deoxysugars (Keller Killiani test):** To 2 ml extract, add glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated H_2SO_4 observed for reddish brown colour at junction of the two liquid and upper layers bluish green.

4) **Liebermann's test (For bufadenolids):** Mixed 3 ml extract with 3 ml acetic anhydride. Heat and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

B) Tests for Saponin Glycosides:

1) **Foam test:** The extract was shaken vigorously with water. Persistent foam was observed.
2) **Haemolytic test:** Added test solution to one drop of blood placed on glass slide. Haemolytic zone appears.

C) Tests for Anthraquinone Glycosides:

1. **Borntrager's test**: To 3 ml. extract, add dil.H_2SO_4. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.
2. **Modified Borntrager's test:** To 5 ml. extract, add 5 ml. 5% $FeCl_3$ and 5 ml. dil. HCl. Heat for 5 min. in boiling water bath. Cool and add benzene, shake well and separate organic layer. Add equal volume dil. ammonia in organic layer. Ammoniacal layer shows pinkish red colour.

7. Tests for Alkaloids:

1. **Dragendorff's test:** To 2-3 ml filtrate added few drops Dragendorff's reagent observed for orange brown precipitate.
2. **Mayer's test:** 2-3 ml filtrate with few drops Mayer's reagent observed for precipitate.
3. **Hager's test:** 2-3 ml filtrate with Hager's reagent observed for yellow precipitate.
4. **Wagner's test:** 2-3 ml filtrate with few drops of Wagner's reagent observed reddish brown precipitate.

8. Tests for Tannins and Phenolic Compounds:

To 2-3 ml of extract, add few drops of following reagents:

1) **5% $FeCl_3$ solution:** deep blue-black color.

2) **Lead acetate solution:** white precipitate.

3) **Gelatin solution:** white precipitate

4) **Bromine water*:*** decoloration of bromine water.

5) **Acetic acid solution:** red color solution

6) **Dilute iodine solution:** transient red color.

7) **Dilute HNO_3:** reddish to yellow color.

RESULTS OF QUALITATIVE CHEMICAL INVESTIGATION

Chemical Test	Successive solvent extraction					Alcoholic extract	Aq. extract
	Pet. Ether Extract	Ben. Extract	$CHCl_3$ Extract	Acetone Extract	Et-OH Extract		
Tests for Carbohydrates							
Molish's test (General test)	-	-	-	-	-	-	+
Tests for reducing sugars a) Fehling's test b) Benedicts test	-	-	-	-	-	-	+
Test for Monosaccharides a) Barfoeds test	-	-	-	-	-	-	+
Test for Pentose sugars	-	-	-	-	-	-	+
Tests for Hexose sugars a) Selwinoff's test	-	-	-	-	-	-	+
Tests for Non-reducing polysaccharides(starch) a) Iodine test b) Tannic acid test for starch	-	-	-	-	-	-	-
Tests for Proteins:							
a) Biuret test	-	-	-	-	-	+	+
b) Millions test	-	-	-	-	-	-	-
c) Xanthoprotein test	-	-	-	-	-	-	-
Tests for Amino acids:							
a) Ninhydrin test	-	-	-	-	-	-	+
b) Test for tyrosine	-	-	-	-	-	-	+
c) Test of tryptophan	-	-	-	-	-	-	-
d) Test for cysteine	-	-	-	-	-	-	-
Tests for Steroids:							
a) Salkowski reaction	+	+	+	+	+	+	-
b) Liebermann–Burchard reaction	+	+	-	-	-	+	-
c) Liebermann reaction	+	+	+	+	+	+	-
Tests for Triterpenoids:							
a) Salkowski reaction	+	+	+	+	+	+	-
b) Liebermann– Burchard test	+	+	+	-	-	+	-

Chemical Test	Successive solvent extraction					Alcoholic extract	Aq. extract
	Pet. Ether Extract	Ben. Extract	$CHCl_3$ Extract	Acetone Extract	Et-OH Extract		
Tests for Cardiac Glycosides:							
a) Baljet test	-	-	-	-	-	-	-
b) Legal test	-	-	-	-	-	-	-
c) Keller-Killani test	-	-	-	-	-	-	-
d) Liebermann's test	-	-	-	-	-	-	-
Tests for Anthraquinone glycosides							
a) Borntraggers test	-	-	-	-	-	-	-
b) Modified Borntragger's test	-	-	-	-	-	-	-
Tests for Saponin glycosides							
a) Foam test	-	-	-	-	-	+	+
b) Haemolysis test	-	-	-	-	-	+	+
c) Bromine water test	-	-	-	-	-	-	+
Tests for Flavanoids:							
a) Ferric Chloride test	-	-	-	-	+	+	+
b) Shinoda test	-	-	-	-	+	+	+
c) Alkaline reagent test	-	-	-	-	+	+	+
d) Lead acetate test	-	-	-	-	+	+	+
Tests for Alkaloids:							
a) Dragendroff's test	-	-	-	-	-	-	-
b) Mayers test	-	-	-	-	-	-	+
c) Hagers test	-	-	-	-	-	-	-
d) Wagners test	-	-	-	-	-	-	-
e) Murexide test for purine alkaloids	-	-	-	-	-	-	-
Tannin & Phenolic Compounds							
a) 5% $FeCl_3$ solution	-	-	-		+	+	+
b) Lead acetate solution	-	-	-	+	+	+	+
c) Gelatin solution	-	-	-	+	-	+	+
d) Bromine water	-	-	-	+	+	+	+
e) Acetic acid	-	-	-	+	+	+	-
f) Dil. Iodine	-	-	-	-	+	-	+
g) Dil.HNO_3	-	-	-	+	+	+	-
h) Dil. $KMnO_4$	-	-	-	-	+	+	-

The table indicated that the extracts contain mainly sterols, flavanoids, terpenoids, phenolic compounds and tannins etc.

Determination of total phenol content

Total phenolic content was determined by Folin ciocalteu reagent. The dilute extracts (0.5ml of 1mg/ml) were mixed with Folin ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4 ml, 1 M). The mixtures were allowed to stand for 60 min and total phenols were determined using double beam UV-Vis spectrophotometer at 765nm. Total phenolic values were expressed as gallic acid equivalent in g/100g of extract which is a common reference compound. The concentration of polyphenols in samples were derived from a standard curve of gallic acid ranging from 10 to 50 µg/ml.

The results of total phenolic contents was obtained from regression equation of calibration curve ($y= 0.0041x + 0.0062$, $r^2= 0.9888$). The Values are expressed in gallic acid equivalents (GAE) for phenols (table.1).

Table 1: Phenol

Extracts	**Phenols* (GAE g/100gms)**
Pet.ether extract	1.12 ± 0.28
Aqueous	6.38 ± 0.72
Alcoholic	5.59 ± 0.51

* Each value is average ± SD (n = 3)

Total flavonoids determination

Aluminium chloride colorimetric method was used for flavonoids determination. Alcoholic and aqueous extracts of the bark (2ml) were mixed with 0.1ml of 10% w/v aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It was kept at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 415nm using double beam UV-Vis spectrophotometer. The calibration curve was plotted using quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

The flavonoids contents expressed in quercetin equivalents (QE)/100g of extract, were determined from regression equation of calibration curve ($y = 0.0165x -0.0235$, $r^2=0.9973$). Values were expressed in quercetin equivalents (QE) (table.2)

Table 2 Flavonoid content

Extracts	Flavonoids* (QE g/100gms)
Pet. ether extract	0.19 ± 0.004
Aqueous	2.13 ± 0.10
Alcoholic	3.34 ± 0.33

Each value is average ± SD (n = 3)

Free radical scavenging activity determinations

The free radical scavenging capacity of the extracts was determined using DPPH method. Plant extracts and ascorbic acid were weighed and dissolved in methanol to obtain six different concentration (1, 5, 10, 50, 100 and 500 µg/ml). Aliquots were prepared suitably by diluting with methanol.

DPPH was weighed and dissolved in methanol to make 0.004% w/v solution. 3ml of 0.004% DPPH solution was added to each test tube with the help of calibrated pipette to obtain the desired concentrations. The prepared mixtures were incubated at 37°C for 30 min. The absorbance value of each test tube was determined using UV-Visible spectrophotometer at 517nm. The percentage inhibition values were calculated using equation.

$$\text{DPPH scavenged}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of Control}} x100$$

IC_{50} was determined from % inhibition vs concentration graph. IC_{50} expressed the antioxidant activity defined as the concentration in ml that inhibits the formation of DPPH radicals by 50 %.

DPPH is the best, easiest and widely used method for testing preliminary free radical scavenging activity of a compound or a plant extract. In the present study $IC_{50.}$ values of all three extracts were calculated and compared with IC_{50} value ascorbic acid as a standard (IC_{50}= 12.42 µg/ml).

This investigation was based on the measurement of the relative inhibitory effect of extract tested at different concentrations. Table.3 represents the % inhibition of all three extracts. Figure 1 is graphical representation of % inhibitory activity Vs Concentration.

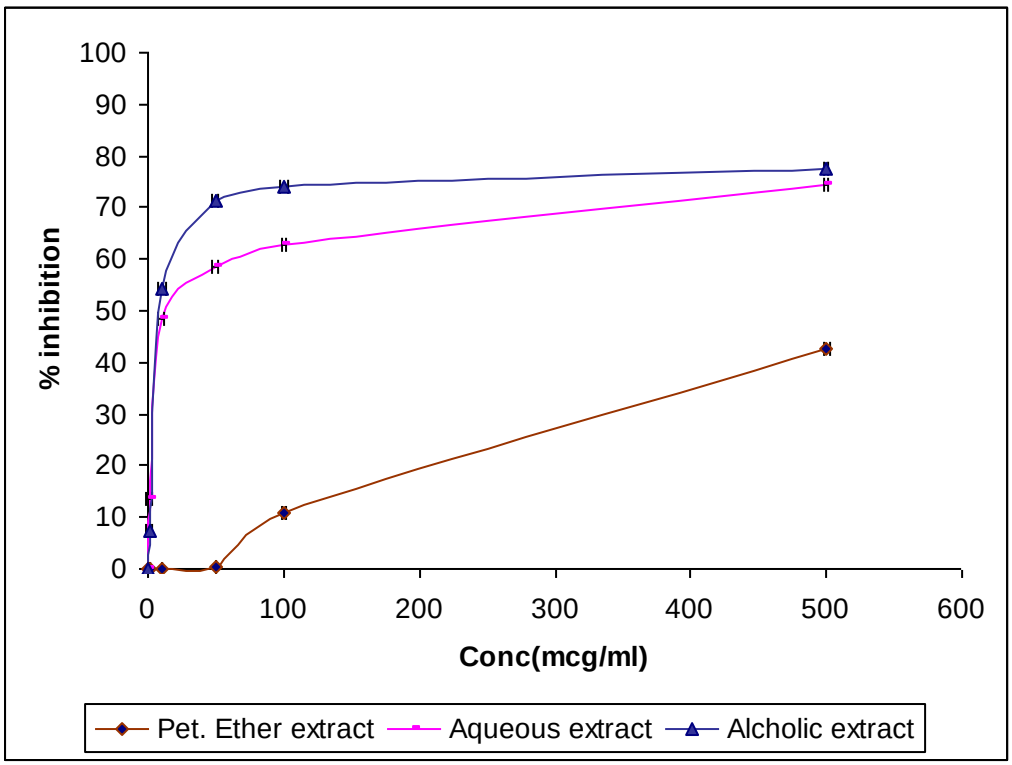
Table. 4 shows capacity of alcoholic and aqueous extract to scavenge the DPPH radical. Both the extracts showed antioxidant activity.

Table 3: % inhibition of pet ether, aqueous and alcoholic extracts

conc. (µg/ml)	% Inhibition		
	Pet. Ether	Aqueous	Alcoholic
0	0	0	0
1	0	13.63±2.36	7.55±2.11
10	0	48.51±2.22	54.13±2.63
50	0.303±0.12	58.37±2.16	71.43±2.19
100	10.891±.4	62.96±1.44	74.08±3.27
500	42.49±2.31	74.27±1.90	77.50±1.68

Table 4: Free radical scavenging activity

Extract	Pet. ether extract	Aqueous extract	Alcoholic extract
IC_{50} (µg/ml) for DPPH scavenging activity	--	17 ug/ml	9 ug/ml



Pharmacological evaluation of *Careya arborea* Roxb.

TOXICITY STUDIES

Acute Oral toxicity – Acute Toxic Class method:

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD), received draft guidelines, received from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Principle of test:

It is the principle, which is based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, with the initial dose being selected as the lower fixed dose, depending on the presence of mortality, until the study objective is achieved i.e. the classification of the test substance based on the identification of doses causing mortality, when there are no effects at the highest fixed dose.

DESCRIPTION OF THE METHOD

1. **Selection of animal species:**

   Healthy young albino mice of either sex weighing between 25 to 40g were used for acute toxicity study to determine LD_{50} of various extracts.

2. **Housing and feeding condition:**

   The temperature in the experimental room was around $25^0C \pm 1$. Lightning was artificial, the sequence being 12 hours dark, 12 hours light. The conventional laboratory diet was fed, with drinking water *ad libitium.*

3. **Preparation of animals:**

   The animals were randomly selected, marked to permit individual identification, and kept in their cages for five days prior to dosing to allow for acclimatization to the laboratory condition.

4. **Preparation of doses:**

   The pet.ether, benzene, chloroform, acetone extracts were suspended in 5% tween- 80 in water. The other extract viz. alcoholic and aqueous extracts dissolved in water.

5. Administration of doses:

The test substances were administered orally by oral feeding tube. Animals were fasted prior to dosing, following the period of fasting the animals were weighed and test substance was administered. After the dose was administered, food was withheld for a further 3-4 hrs in rats

6. Number of animals and dose levels:

In each steps six animals were used in each group. Starting dose was 300mg/kg body weight up to 5000 mg / kg body weight. The procedure of dose selection and finalizing LD_{50} cut off values is shown in the Table No. 5.

Table No. 5: Dose Selection and Finalizing LD_{50} Cut Off value of Extracts

S. No.	Name of Extract	LD_{50} Cut-Off mg/ kg, b.w
1.	Petroleum ether extract	3000 mg/kg, b.w.
2.	Benzene extract	3000 mg/kg, b.w.
3.	Chloroform extract	3000 mg/kg, b.w.
4.	Acetone extract	2000 mg/kg, b.w.
5.	Ethanolic extract	3000mg/kg b.w.
6.	Aqueous extract	5000 mg/kg, b.w.
7.	Alcoholic extract	3000mg/kg b.w.

$1/10^{th}$ of this lethal dose was taken as effective dose (therapeutic dose) for subsequent antidiabetic activity.

7. Observations:

Animals were observed after dosing at least once during the first 30 minutes then periodically during the first 24 hours. In all cases death was observed within first 24 hours. Additional observations like changes in skin, fur, eyes and mucous membranes, respiratory, circulatory, autonomic central nervous systems, somatomotor activity and behaviour pattern. Attention was also given to observation of tremors and convulsions.

EVALUATION OF ANTI-DIABETIC ACTIVITY (ACUTE STUDY)

The acclimatized animals were kept fasting for 24 hrs with water *ad libitum*, Animals were separated according to their body weight. Freshly prepared alloxan monohydrate in normal saline solution was injected intraperitoneally (i.p.) at a dose of 120 mg kg^{-1} b.w. After one hour of alloxan administration, animals were given feed ad libitum and 1 ml of (100 mg/ml) glucose orally to combat ensuring severe hypoglycemia. After 72 hrs of the alloxan injection, the animals were tested for the evidence of diabetes by estimating their blood glucose level by using Glucometer (Accu-chek active, Roche Diagnostics GmbH, Germany). The blood glucose level more than 200 mg/dl of blood was the criteria.

The animals were segregated into ten groups of six rats each, one group was normal control and others were diabetic control, petroleum ether, benzene, chloroform, acetone, ethanolic, aqueous, alcoholic extracts and standard glibenclamide group. To the animals, the test extracts and standard drug glibenclamide were administered by suspended in 5% Tween-80/ water. The blood samples were obtained through the tail vein puncturing with Lancet. A 0.2 ml of blood was withdrawn at interval of initial (0 hr), 2, 4, 6^{th} hrs of administration of single dose (for acute study).

Experimental Procedure:

The animals were divided into Ten groups (n = 6).

Table No. 9: Grouping Scheme for Antidiabetic Study

Group I	Control, rats given normal saline.
Group II	Rats induced with diabetes by Alloxan at a dose of 120 mg/kg b.w. initially
Group III	Alloxanized Diabetic rats given with Petroleum ether extract orally 300 mg/kg b.w.
Group IV	Alloxanized Diabetic rats given with Benzene extract orally 300 mg/kg b.w.
Group V	Alloxanized Diabetic rats given with Chloroform extract orally 300mg/kg b.w.
Group VI	Alloxanized Diabetic rats given with Acetone extract orally 200mg/kg b.w.
Group VII	Alloxanized Diabetic rats given with Ethanolic extract orally 300mg/kg b.w.
Group VIII	Alloxanized Diabetic rats given with Aqueous extract orally 500mg/kg b.w.
Group IX	Alloxanized Diabetic rats given with Alcoholic extract orally 300mg/kg b.w.
Group X	Alloxanized Diabetic rats given with Glibenclamide orally 5 mg/kg b.w

Estimation of Glucose:

The blood samples were obtained through tail vein by puncturing with Lancet. A drop of blood so obtained was placed on glucostrip, which was kept in the glucometer. The glucometer was kept on, then after 5 sec glucomonitor reading was recorded.

The measurement of blood glucose level at 0, 2, 4, 6 (acute study) was done after administration of dose orally and chronic study (21days) is under process. Other biochemical parameter readings will be taken in biochemical laboratory. The statistical analysis will be done by one way ANOVA followed by Dunnett's test.

Control

Animal Number	**Animal Weight (in grams)**	**Blood Glucose Levels in mg/dl at the end of**			
		0 hrs.	***2 hrs.***	***4 hrs.***	***6 hrs.***
1	212	93	94	93	94
2	187	79	79	78	79
3	179	81	80	81	82
4	195	87	88	86	87
5	176	88	90	88	89
6	185	92	91	92	91
Mean		86.66	87	86.33	87

Diabetic control

Animal Number	**Animal Weight (in grams)**	**Blood Glucose Levels in mg/dl at the end of**			
		0 hrs.	***2 hrs.***	***4 hrs.***	***6 hrs.***
1	180	301	295	294	296
2	190	285	285	285	287
3	200	285	287	286	2851
4	181	308	307	293	293
5	186	283	283	285	285
6	186	291	293	293	295
Mean.		292.16	291.6	289.33	290.16

Pet. Ether extract

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	200	276	263	245	244
2	185	300	287	260	242
3	195	315	303	271	2691
4	185	308	283	265	245
5	180	279	271	259	239
6	180	303	291	271	250
Mean.		296	283	261	248

Benzene extract

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	180	284	280	281	278
2	185	292	293	285	281
3	195	307	298	281	280
4	200	284	279	277	271
5	175	300	298	279	278
6	175	307	303	298	296
Mean.		295.66	291.83	283.5	280.5

Chloroform

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	195	300	288	285	286
2	185	276	275	271	270
3	200	284	273	276	269
4	190	284	275	271	269
5	190	261	255	251	250
6	185	284	279	273	272
Mean.		281.5	274.1	271.16	269.3

Acetone

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	180	307	297	298	293
2	190	296	293	289	285
3	201	287	287	286	283
4	183	263	243	245	239
5	196	293	289	286	287
6	190	272	266	270	261
Mean.		286.33	279.16	279.16	274.6

Ethanolic extract

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	180	301	298	283	285
2	187	263	260	261	258
3	181	287	276	273	267
4	202	279	279	275	262
5	191	291	283	276	263
6	189	273	261	253	248
Mean		282.33	276.16	270.16	263.83

Aqueous extract

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	185	273	263	250	235
2	187	298	282	261	248
3	191	288	279	259	241
4	182	300	293	281	252
5	197	265	261	256	239
6	182	271	267	251	237
Mean		282.5	274.1	259.6	242

Alcoholic extract

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	***2 hrs.***	***4 hrs.***	***6 hrs.***
1	200	300	283	261	243
2	190	290	269	251	231
3	179	301	281	259	238
4	195	305	288	263	242
5	204	304	281	259	238
6	187	297	278	253	235
Mean		299.5	280	257.6	237.83

Std. Glibenclamide

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	***2 hrs.***	***4 hrs.***	***6 hrs.***
1	191	268	243	217	209
2	203	300	271	237	215
3	187	288	248	230	211
4	190	281	253	233	215
5	183	272	241	218	209
6	192	295	259	237	214
Mean		284	252.5	228.6	121.16

(Part-II 2nd progress report)

As per the suggestion given by PEC

EVALUATION OF ANTI-DIABETIC ACTIVITY (ACUTE STUDY)

The acclimatized animals were kept fasting for 24 hrs with water *ad libitum*, Animals were separated according to their body weight. Diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (60 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycaemia. After one week of the streptozotocin injection, the animals were tested for the evidence of diabetes by estimating their blood glucose level by using Glucometer (Accu-chek active, Roche Diagnostics GmbH, Germany). The blood glucose level more than 200 mg/dl of blood was the criteria.

The animals were segregated into four groups of six rats each, one group was normal control and others were diabetic control, alcoholic extract and standard glibenclamide group. To the animals, the test extract and standard drug glibenclamide were administered by suspended in 5% Tween-80/ water. The blood samples were obtained through the tail vein puncturing with Lancet. A 0.2 ml of blood was withdrawn at interval of initial (0 hr), 2, 4, 6th hrs of administration of single dose (for acute study).

Experimental Procedure:

The animals were divided into Ten groups (n = 6).

Grouping Scheme for Antidiabetic Study

Group I	Control, rats given normal saline.
Group II	Rats induced with diabetes by Streptozotocin at a dose of 60 mg/kg b.w. initially
Group III	Diabetic rats given with Alcoholic extract orally 300 mg/kg b.w.
Group IV	Diabetic rats given with std. Glibenclamide 10 mg/kg b.w.

Estimation of Glucose:

The blood samples were obtained through tail vein by puncturing with Lancet. A drop of blood so obtained was placed on glucostrip, which was kept in the glucometer. The glucometer was kept on, then after 5 sec glucomonitor reading was recorded.

The measurement of blood glucose level at 0, 2, 4, 6 (acute study) was done after administration of dose orally and chronic study (21days) was also carried out. Other

biochemical parameter readings were taken in biochemical laboratory. The statistical analysis was done by one way ANOVA followed by Dunnett's test.

Control

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	212	93	94	93	94
2	187	79	79	78	79
3	179	81	80	81	82
4	195	87	88	86	87
5	176	88	90	88	89
6	185	92	91	92	91
Average ± SEM		86.66±2.31	87±2.50	86.33±2.43	87±2.29

Diabetic control

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	201	285	286	284	285
2	187	292	290	290	288
3	192	287	287	285	285
4	197	291	289	287	285
5	200	297	294	291	291
6	185	300	300	298	299
Average ± SEM		292.0 ± 2.33	291.0± 2.12	289.3 ± 2.08	288.8 ± 2.25

Alcoholic extract

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	187	303	292	261	260
2	193	289	286	269	259
3	201	295	279	253	243
4	189	306	287	259	251
5	200	281	269	258	249
6	185	285	271	258	254
Average ± SEM		293.1 ± 4.06	280.6± 3.77	259.5± 2.15	252.6 ± 2.61

Std. Glibenclamide

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	200	304	284	251	230
2	193	287	263	242	215
3	187	294	279	245	216
4	180	291	284	247	209
5	202	295	281	241	217
6	193	299	279	239	209
Average ± SEM		295 ± 2.43	278 ± 3.2	244.1 ± 1.79	216 ± 3.13

The results indicated that the alcoholic extract showed significant antidiabetic activity ($P<0.01$) (252.6±2.61) at the end of six hours in acute study compared to diabetic control.

Normal control

Animal Number	Animal Weight (in grams)	Blood sugar levels in mg/dl on			
		1st day.	*7th day.*	*14th day.*	*21st day*
1	212	93	91	89	87
2	187	79	77	73	75
3	179	81	80	74	76
4	195	87	84	78	79
5	176	88	87	89	85
6	185	92	90	85	86
Mean ± SEM		86.66±2.31	84.83±2.26	81.33±2.97	81.33±2.16

Diabetic control

Animal Number	Animal Weight (in grams)	Blood sugar levels in mg/dl on			
		1st day.	*7th day.*	*14th day*	*21st day*
	201	285	286	284	285
2	187	292	290	290	288
3	192	287	287	285	285
4	197	291	289	287	285
5	200	297	294	291	291
6	185	300	300	298	299
Mean ± SEM		292.0±2.33	291. ±2.12	289.16±2.08	288.83±2.25

Alcoholic extract

Animal Number	Animal Weight (in grams)	Blood sugar levels in mg/dl on			
		1st day.	*7th day.*	*15st day*	*21st day*
1	187	303	227	209	169
2	193	289	221	197	172
3	201	295	219	201	181
4	189	306	237	190	160
5	200	281	217	193	175
6	185	285	230	207	169
Mean± SEM		293.16±4.06	225.16±3.10	199.5±3.09	171±2.86

Glibenclamide

Animal Number	Animal Weight (in grams)	Blood sugar levels in mg/dl on			
		1st day.	*7th day.*	*15st day.*	*21st day.*
1	200	304	207	149	123
2	193	287	197	152	107
3	187	294	210	162	97
4	180	291	209	171	123
5	202	295	191	151	119
6	193	299	213	153	117
Mean± SEM		295±2.43	204.33±3.49	156.33±3.45	114.33±4.21

In the present study there was marked increase in blood glucose level in the diabetic control group as analysis was done on 1,7,14,21st day of study (292.00±2.33, 290.0±2.82, 290.0±3.21, 288.33±2.56) as compared with normal group. (86.66±2.31, 84.83±2.26, 81.33±2.97, 81.33±2.16).

Glibenclamide 10mg/kg significantly decreases blood glucose level (295.0±2.43, 204.33±3.49, 156.33±3.45, 114.33±4.21) as compared with diabetic control.

From these findings clearly established that the administration of alcoholic extract, exhibited better ($P<0.01$) antidiabetic activity (171±2.86, 207±2.42). at the end of 21st day.

Other biochemical parameters.

At the end of the treatment blood was collected by direct cardiac puncture and serum was separated by centrifugation at 2500 rpm. The rats were sacrificed by cervical dislocation and pancreas were excised immediately and thoroughly washed with ice cold physiological saline. The serum collected was used for biochemical estimations.

Estimation of total cholesterol and HDL cholesterol (Wybenga and Pileggi Method):

Total cholesterol and HDL cholesterol were estimated by using standard kit obtained from Biolab Diagnostics (I) Pvt. Ltd. Tarapur, Maharashtra.

Principle:

In hot acidic medium, cholesterol oxidises ferric ions to a brown coloured complex which absorbs at 530 nm and is directly proportional to cholesterol concentration.

Procedure:

The kit contents were brought to room temperature.

Procedure for estimation of total cholesterol.

Reagent	Blank (B) (ml)	Standard (S) (ml)	Test (T) (ml)
Cholesterol reagent No. 1	5.0	5.0	5.0
Distilled water	0.05	-	-
Standard reagent No. 2	-	0.05	-
Sample	-	-	0.05

The test tubes were labelled as B (Blank), S (Standard) and T (Test). Serum of test sample was added to test tube labelled T.

These were mixed well by gently shaking the test tube and kept in boiling water bath for 90 seconds. Cooled for 5 minutes under running tap water. Absorbance of the standard (S) and test sample (T) were measured against the reagent blank (B) with the help of colorimeter at 530 nm.

The total cholesterol was calculated using the formula

$$\text{Total Cholesterol(mg/dl)} = \frac{O.D.(Test)}{O.D.(S\,\text{tan}\,dard)} x200$$

Estimation of HDL cholesterol:

This procedure consists of two steps:

Step 1:

In a glass tube 0.2 ml serum was added with HDL reagent No. 3. After mixing, the tubes were incubated for 10 minutes at room temperature and then centrifuged. The clear supernatant obtained was taken for the HDL cholesterol estimation.

Step 2:

Five ml of cholesterol reagent No. 1 was put in test tubes labelled B, S and T. 0.2 ml of HDL reagent No. 3 was added to test tube labelled B and S. Then 0.2 ml of clear supernatant obtained by step 1 was added to test tube labelled T, while cholesterol standard was added to standard tube (S).

Procedure for estimation of HDL cholesterol.

Reagent	Blank HDL (B) (ml)	Standard HDL (S) (ml)	Test HDL (T) (ml)
Cholesterol reagent No. 1	5.0	5.0	5.0
HDL reagent No. 3	0.2	0.2	-
Supernatant from step 1	-	-	0.2
Cholesterol standard (200mg)	-	0.02	-

These were mixed well by gently shaking the test tube and kept in boiling water bath for 90 seconds, cooled for 5 minutes under running tap water. Absorbance of the standard (S) and test sample (T) were measured against the reagent blank (B) with the help of colorimeter at 530 nm.

The HDL cholesterol was calculated as follows:

$$\text{HDL Cholesterol(mg/dl)} = \frac{O.D.(\text{Test HDL})}{O.D.(\text{Standard HDL})} x40$$

Estimation of triglycerides (GPO-PAP Method):

Triglycerides estimation kit consists of enzyme reagents, triglyceride standard and diluent buffer, was obtained from Biolab Diagnostics (I) Pvt.Ltd. Tarapur, Maharashtra.

Principle:

Triglycerides are split into glycerol and fatty acids in the presence of lipoprotein lipase. In the presence of ATP and glycerol-kinase, glycerol is converted into glycerol-3-phosphate and ADP. Glycerol-3-phosphate oxidase dissociates glycerol-3-phosphate into dihydro-acetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine and ESPAS (N-Ethyl-N-Sulfopyropyl-n-methoxyaniline) to form a red coloured quinoneimine as indicator.

Preparation of working reagent:

The lyophilized material is dissolved with 1.5 ml buffer. A uniform solution takes place after 5 minutes which is ready to use.

Procedure:

One ml of working reagent was added to test tubes labelled B, S and T. Blank test tube was added with 0.05 ml distilled water, while 0.05 ml of standard was added to test tube labelled S and 0.05 ml of sample (serum) was added to test tube labelled T.

These were mixed well by gently shaking the test tube and incubated for 10 minutes at 37^0C.

Absorbance of the standard (S) and test sample (T) were measured against the reagent blank (B) with the help of colorimeter at 500 nm within 30 minutes.

Procedure for estimation of triglycerides.

Reagent	Blank HDL (B) (ml)	Standard HDL (S) (ml)	Test HDL (T) (ml)
Distilled water	0.05	-	-
Triglycerides standard reagent No. 3	-	0.05	-
Sample	-	-	0.05
Working reagent	1.0	1.0	1.0

The Triglycerides concentration was calculated as follows:

$$\text{Triglycerides(mg/dl)} = \frac{O.D.(\text{Test})}{O.D.(\text{Standard})} \text{ x } 200$$

Each test performed 3 times and the mean value used for the inhibitory activity of plant extracts.

Effect of *Careya arborea* Roxb. bark extracts on other biochemical parameters.

Group	Total Cholesterol	HDL	Triglycerides
Normal control	90.66±2.90	43.33±1.64	123.33±2.02
Diabetes control	173.66±7.45	33.00±1.22	194.66±3.93
Std. Glibenclamide	102.66±2.60	42.66±1.52	135.33±3.28
Alcoholic extract	107.33±4.70	41.33±1.69	132.00±3.60

Total cholesterol and triglyceride level were found to be significantly increased in diabetic control group as compared with normal control. Treatment with standard drug, alcoholic extract significantly attenuated ($P<0.01$) the elevated total cholesterol and triglyceride levels as compared with diabetic control.

HDL level decreases in diabetic control group, treatment with alcoholic extract and standard significantly increases ($P<0.05$) the HDL level as compared with diabetic control.

Study of mechanism of actions

α- glucosidase inhibitory activity

Normal healthy rats fasting for 20 hrs were sacrificed by cervical dislocation. The small intestine obtained was flushed several times with ice-cold NaCl, and 50 mM (pH 7.0) sodium phosphate buffer. The mucosa was scraped with glass slide on ice-cold glass surface. The obtained material was centrifuged and pellet homogenized in phosphate buffer containing 1% Triton X 100, further cold butanol was added to remove Triton and sample subjected to overnight dialysis. The enzyme thus obtained was used after proper dilution (Lee, 1980).

5µmol P- Nitrophenyl-α-D- Glucopyranoside(PNPG), enzyme solution (0.1 µl), in 900µl of sodium phosphate buffer (50 mM), pH 6.8 in the final volume of 1ml. Each extract 100 µg was dissolved in 20µL of distilled water and added to the test mixture before adding the substrate. Blank sample contained whole test mixture and the extract without enzyme solution. Distilled water added to the control sample (20 µl) and in the positive control 20 µL acarbose (100 µl) was enhanced. The mixture incubated at 37^0C for 30 mins, the reaction terminated by adding 3 volumes of NH_4OH solution (0.05 M). The absorbance at 405 nm was determined by Spectrophotometer. The inhibitory activity calculated using following formula

$$\text{Inhibitory activity}(\%) = \frac{O.D.(Control) - O.D.(\text{Test})}{O.D.(\text{Control})} \text{ x } 100$$

α-glucosidase inhibitory activity

Extract	% α -glucosidase inhibitory activity
Alcoholic extract	43.48±2.85

Alcoholic extract showed maximum (43.48±2.85%) α -glucosidase inhibitory activity

As per the histopathological study and discussion with histopathologist it was concluded that no effective regeneration of β- cells were observed by alcoholic extracts of bark of *Careya arborea* Roxb..

13. Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject.

- Initially pharmacognostical evaluation of bark was carried out.
- Phytochemical evaluation of bark shows presence of steroids, flavonoids, terpenoids and phenolic compounds.
- Alcoholic extract showed antidiabetic activity in streptozotocin induced diabetic rats in acute study.
- Alcoholic extract showed antidiabetic activity in streptozotocin induced diabetic rats in chronic study.
- Alcoholic extract showed improvement in parameters like lipid profiles.
- Total phenol content in

  Alcoholic extract - 5.59 ± 0.51 GAE g/100gms.
- Total flavonoids content in

  Alcoholic extracts - 3.34 ± 0.33 QE g/100gms
- Alcoholic extract showed antioxidant activity.
- Alcoholic extracts of *Careya arborea* Roxb. showed α-glucosidase inhibitory activity

14. Conclusions summarizing the achievements and indication of scope for future work.

The associated disadvantages with insulin and oral hypoglycaemic agents have led to stimulation in the research for locating natural resources showing antidiabetic activity. The traditional healers are using *Careya arborea* Roxb. in the treatment of hyperglycemia. A survey of the literature reveals that not much scientific evaluation has been conducted to check the antidiabetic potential of *Careya arborea* Roxb.

In the present study, the barks of *Careya arborea Roxb*. family Lecythidaceae were selected for pharmacognostical, phytochemical and pharmacological evaluation for possible antidiabetic activity.

The Plants were authenticated by the botanist Dr. Rajmane and Dr. S. S. Sathe, botanist, Padmabhushan Dr. Vasantdada Patil Mahavidyalaya, Tasgaon. The voucher specimen has been preserved in our laboratory for future reference. The authenticated barks were subjected to organoleptic evaluation. Microscopic evaluation was carried out by studying the transverse section of the barks as well as observing the microscopical powdered characteristics of the bark. Different staining reagents were used to study transverse section and microscopic characteristics of powdered bark.

The barks were subjected to size reduction to get coarse powder (40#). Powdered material of the barks were used for physical evaluation. Physical evaluation comprised of different ash values like total ash, sulphated ash, water soluble ash, acid insoluble ash and extractive values viz. water soluble and alcohol soluble extractives. The organoleptic, microscopical and physico-chemical parameters presented can be proposed as parameters to establish the authenticity of the said plants.

Powdered barks were extracted with alcohol using soxhlet apparatus. Aquous as well as successive solvent extraction were also be carried out. After effective extraction, solvent was distilled off by using rotary vacuum evaporator. The concentrated extracts were used for carrying phytochemical investigation, anti- diabetic activity.

All the extracts were subjected to detailed preliminary phytochemical investigation. The phytochemical investigation revealed the presence of sterols, triterpenoids, flavonoids and phenolic compounds as a major active chemical constituents.

All the extracts were studied for acute oral toxicity study using OECD guidelines as well as Miller and Trainter method. Preliminary oral LD_{50} values for alcoholic extracts of *Careya arborea* Roxb. was found to be 3000 mg/kg. $1/10^{th}$ of this LD_{50} was taken as effective dose

(therapeutic dose) for subsequent studies.

The antidiabetic activity was performed using wistar albino rats. The animals were divided into four groups, each group with six animals, two groups were selected as control, one as normal control and the other as diabetic control. One group was selected as a standard and the remaining was selected for alcoholic extract.

By observing the results of acute antidiabetic study of *Careya arborea* Roxb., it can be concluded that the alcoholic extract had shown prominent antidiabetic activity at the end of 6^{th} hour. The chronic antidiabetic study for alcoholic extract had shown prominent antidiabetic activity at the end of 21^{st} day.

At the end of the treatment, blood was collected by direct cardiac puncture and serum was separated by centrifugation at 2500 rpm. Total cholesterol, HDL cholesterol and triglycerides were estimated by using standard kit obtained from Biolab diagnostics (I) Pvt. Ltd. Tarapur, Maharashtra. The total cholesterol and triglycerides were found to be significantly increased in diabetic control group as compared with normal control. Treatment with alcoholic extract of *Careya arborea* Roxb. significantly attenuated the elevated total cholesterol and triglyceride levels as compared with diabetic controls. HDL cholesterol level decreases in diabetic control group while alcoholic extract significantly increases the HDL cholesterol level as compared with diabetic control.

The active extract (Alcoholic extract) was selected for detailed phytochemical investigation. Initially total phenol and total flavonoid content of active fractions were determined by folin-ciocalteu reagent method and aluminium chloride method respectively. The free radical scavenging capacity of the extracts were determined using DPPH method. The study also revealed that the anti-oxidant activity increases with increase in content of phenol and flavonoids in the said extract.

One of the therapeutic approaches for reducing postprandial hyperglycemia in patients with diabetis mellitus is to prevent absorption of carbohydrates after food intake. Alcoholic extracts of *Careya arborea* Roxb. showed more α-glucosidase inhibitory activity.

Histopathological study of alcoholic extract treated group revealed that no selective regeneration of β-cells were found in damaged pancreas in streptozotocin-treated diabetic rats.

14. **Procurement/ usage of equipment.**

Sr No	**Name of Equipment**	**Make/ Model**	**Cost FE/Rs.**	**Date of Installation**	**Utilization rate %**	**Remarks regarding maintenance/breaked own.**
1.	Rotary vacuum evaporator	Medica/ Evator	101650.00	30th Jan.2009	50 %	Working
2.	Six stage Joint Soxhlet Apparatus Capacity 500ml and 1000ml.	Agarval Glassware instruments	25794.00	30th Jan.2009	50%	Working
3.	TLC Kit.	Superfit laboratory instruments	9511.87	30th Jan.2009	40%	Working
4.	Glucometer	Accu-check	9150.00	20th Jan 2009	70%	Working

15. Manuscript for Publication

In the present study, the barks of *Careya arborea Roxb*. family Lecythidaceae were selected for pharmacognostical, phytochemical and pharmacological evaluation for possible antidiabetic activity.

Powdered barks were extracted with alcohol using soxhlet apparatus. Aqueous and successive solvent extraction were also be carried out. After effective extraction, solvent was distilled off by using rotary vacuum evaporator. The concentrated extracts were used for carrying phytochemical investigation, anti- diabetic activity.

All the extracts were subjected to detailed preliminary phytochemical investigation. The phytochemical investigation revealed the presence of sterols, triterpenoids, flavonoids and phenolic compounds as a major active chemical constituents.

All the extracts were studied for acute oral toxicity study using OECD guidelines as well as Miller and Trainter method. Preliminary oral LD_{50} values for alcoholic extracts of *Careya arborea* Roxb. was found to be 3000 mg/kg. $1/10^{th}$ of this LD_{50} was taken as effective dose (therapeutic dose) for subsequent studies.

By observing the results of acute antidiabetic study of *Careya arborea* Roxb., it can be concluded that the alcoholic extract had shown prominent antidiabetic activity at the end of 6^{th} hour in acute study. The chronic antidiabetic study for alcoholic extract had shown prominent antidiabetic activity at the end of 21^{st} day.

The total cholesterol and triglycerides were found to be significantly increased in diabetic control group as compared with normal control. Treatment with alcoholic extract of *Careya arborea* Roxb. significantly attenuated the elevated total cholesterol and triglyceride levels as compared with diabetic controls. HDL cholesterol level decreases in diabetic control group while alcoholic extract significantly increases the HDL cholesterol level as compared with diabetic control.

The active extracts were selected for detailed phytochemical investigation. Initially total phenol and total flavonoid content of active fractions were determined by folin-ciocalteu reagent method and aluminium chloride method respectively. The free radical scavenging capacity (Anti-oxidant activity) of the extracts were determined using DPPH method. The study also revealed that the anti-oxidant activity increases with increase in content of phenol and flavonoids in the said extracts.

The present study revealed that alcoholic plant extract can be successfully utilized for the management of diabetes due to their antidiabetic action. One of the therapeutic approaches for reducing postprandial hyperglycemia in patients with diabetis mellitus is to prevent absorption of carbohydrates after food intake. Alcoholic extracts of *Careya arborea* Roxb. showed α-glucosidase inhibitory activity.

Histopathological study of alcoholic extract treated group revealed that no selective regeneration of β-cells were observed in damaged pancreas of diabetic rats.

Signature of PI: Signature of Head of Institute

Date: Date: