CHAPTER - 4
MATERIALS
AND
METHODS
4.1. COLLECTION AND AUTHENTIFICATION OF PLANT MATERIALS

In the present study, the naturally developed fruitlets of *Lagenaria siceraria* were obtained from the neighborhood business sector of Bardoli, Gujarat. The crisp leaves of *Moringa oleifera* and *Ocimum gratissimum* were gathered from Vidyabharti trust campus and was authenticated from botanist Botanist, Dr. B. R. Patel, Botanist, Patidar Gin Science College, Surat.

After authentication, fruits and leaves were properly washed and cleaned with water and dehydrated beneath shadow at room environment. After completely drying, the material are subjected to evaluation using different parameters.

Then the leaves and fruit were taken for the size reduction using cutter mill in order to get coarser to a fine powder. After size reduction, the powder passed through 40# sieve to get uniformly sized powder. The final powdered material are kept in air tight wide mouth bottle for future use. The powdered material are subjected to various standardization parameters as per pharmacopoeias / literatures.

4.2. PHARMACOGNOSTIC STUDY

♦ Anatomical Studies:

Plant materials were subjected to morphological examinations using reported method and the results were then compared with reported characters. Fresh plant material was studied for color, odor and taste, determination of shape, surface characteristics and appearance, etc. (Khandelwal, 2008).

♦ Histological Studies:

The plants were subjected to microscopic evaluation in the whole as well as in powder form. The transverse section of fruits and leaf were obtained by using a rotary microtome. Permanent mount of plant materials was prepared. After preparation the plant material are seen under microscope and the photo has been taken.

Powder Microscopy and Photomicroscopy:
Dried powder of the plant was treated by chloral hydrate. Then stained with 1:1 phloroglucinol: HCl. On the slide, a droplet of glycerin was put and secured with a spread slip. The arranged slide was seen under magnifying instrument (Khandelwal, 2008).

Photomicrographs taken at diverse amplifications which rely on anatomical features of the examining cells. Photomicrography was done utilizing Olympus magnifying lens that was appended with Magnus MIPS Polaroid (Khandelwal, 2008).

4.3. PHYTOCHEMICAL PARAMETERS

4.3.1. Determination of Loss on Drying (Anonymus, 2002):

Accurately weighed 5 - 6 g of powder separately and placed in a tarred vanishing dish and afterward dehydrated for 4 h. at 110 °C. The sample was cooled. The dehydrating and weighing was proceeded at one-hour interim until steady weight was arrived at. The calculation of loss on drying was based on the moisture content present in the sample and the formula to calculate are

\[
\text{Loss on drying (\%)} = \frac{\text{weight of powder after drying in g}}{\text{Initial weight of the powder in g}} \times 100
\]

4.3.2. Ash Values (Anonymus, 2002):

♦ Total ash:

Accurately take 2 gram of the pulverized air dried powder and set in an at one time lighted crucible (typically platinum crucible or silica) and spread in an even layer. The crucible is then burnt by progressively growing temperature up to 600 °C awaiting white, showing deficiency of carbon. Material was cool in a desiccator and weigh up. Ash which are carbon free is not acquired by along these lines, the cauldron was cooled and deposit was dampened with 2 ml water or soaked result of ammonium nitrate. It was dried up on water bath and again blazed to relentless mass. The surplus permitted to calm in desiccator for 35 min, and it was reweighed on the double. The total ash value was measured in fraction with respect to the dehydrated material. The formula are

\[
\text{Total ash value (\%)} = \frac{Z - \text{Weight of empty crucible}}{\text{Weight of drug taken}} \times 100
\]
Where, \( Z = \text{Weight of crucible + ash (after complete incineration)} \)

♦ **Acid insoluble ash:**

Twenty five ml HCL was mix in container containing the total ashand closed with glass plate. The crucible was simmered mildly for 5-7 min. on the water bath. After 5-7 min, the glass was splashed with warm water (5ml) and liquid poured to the pot. The unsolvable substance was collected on an ash-less mesh. The insoluble matter then washed with warm water till the remainder was found to be neutral. After washing unsolvable stuff, shifted to the crucible from the filter paper. Crucible containing material was withered upon hot plate, burnt to persistent weight. The excess was permitted for 30 min to calmin a desiccator, and weighed instantaneously. Ash (acid insoluble) was calculated in term of percentage with respect to the dehydrated plant material.

♦ **Water soluble ash:**

25 ml water (purified) was poured in the crucible (silica) holding total ash. Simmered for 5 min. The unsolvable substance was transferred to the crucible and washed with hot water. After washing, the unsolvable stuff was moved to the crucible and burnt for 15 minutes at 450 °C. The excess was allowed to cool for 30 - 40 min. in a desiccator, furthermore it was weighed at once. The weight of the deposit was deducted from the weight of aggregate ash. Water dissolvable ash remains was ascertained in term of rate with reference to dehydrated plant material.

4.3.3. Extractive Values (Anonymus, 2002):

♦ **Alcohol extractive value:**

Around 5.0 gram of coarsely pulverized air dried solid was weighed. Transferred it in a conical flask with stopper. Macerated the powder for 6 h with 100 ml methanol with infrequent shaking. After it was permitted to remain for 18 h. After 18 h, it was filtered speedily and care was taken not to miss any amount of solvent. After this, the 25 ml of
remainder was transferred to a flat bottomed tarred dish and vaporized to dryness. The extract was dehydrated for 6 h at 105 °C, chilled in desiccators for 30 min and balanced immediately. The rate of the liquor dissolvable extractive regarding the air dried powdered medication material was ascertained.

♦ Water extractive value:

About 5.0 gram of the drug was mixed with chloroform (100 ml) and allowed to macerate for 24 h in a closed flask with occasional shaking for initial 6 h and then permitted it to stand another 18 h. The solutions sieved quickly, 25 ml of remainder was vaporized to dehydration in tare plane bottomed bowl, and dry up at 105 °C, weigh up. The fraction of the water extractive with respect to dehydrated powdered drug material was calculated.

4.3.4. Estimation of Total Tannin Content: (Kumazawa et al., 2002):

Weigh about 2 g of drug powder. Extract with 50 ml water in Iodine flask in the water bath for 1 h. After 1 h. filter the solution makes up volume with water upto 150 ml. From that pipette out 10 ml extract solution. Add 10 ml indigo carmine dye and 100 ml water heat the solution at the temp 60 – 70 °C on the water bath and titrate this solution with 0.1 N KMnO₄, until blue colour changes to parrot green to yellow colour and repeat the procedure without taking extract. Each ml 0.1 N KMnO₄ solution = 0.0004157 g of tannins.

4.3.5. Estimation of Total Phenolic Content: (Kumazawa et al., 2002):

Take 0.5 ml solution of crude extract (10 µg/ml) and blend with Folin Ciocalteu (0.5 ml) and 0.5 ml of 10 % sodium bicarbonate. Altogether shake the mixture and spot at room enviornment for 1 h.

At that point take absorbance at a wavelength of 760 nm. Get ready polyphenol calibration curve utilizing gallic acid as standard in fixation range 2 - 10 mg/ L. Figure the aggregate polyphenol in gram of gallic acid / 100 gram of rough concentrate.
4.4. PREPARATION OF EXTRACTS

The shade dried fruits of *Lagenaria siceraria*, leaves of *Ocimum gratissimum* and *Moringa oleifera* were decreased to a fine triturate (# 40 lattices) and nearby 200 grampulverized powder presented to progressive hot uninterrupted (soxhlet device) extraction using methanol. Ultimately, powder was macerated using water.

After powerful extraction prepare, solvents dense off and concentrate was focused on the water bath. Last concentrate accomplished with every dissolvable will be weighed immediately. Its proportion will be calculated with respect to dehydrated mass of plantmatter. The colour of the extracts and uniformity will be noted down. The acquired extracts will be imperiled to chemical exploration, pharmacological screening and for formulation development.

4.5. QUALITATIVE CHEMICAL INVESTIGATION OF EXTRACTS (Kokate, 2002)

Different qualitative chemical examinations were conducted for all the extracts of fruits of *Lagenaria siceraria*, leaves of *Ocimum gratissimum* and leaves of *Moringa oleifera* to identify their various phytoconstituents. The various tests and reagents used are as follows:

4.5.1. Carbohydrates Identification:

**Molish's test (General test):**

To 3-4 ml fluid concentrate included 2 - 3 α-naphthol drops result in liquor, shaken for few seconds and afterward included concentrated sulphuric acid from outskirts of the test tube. At that point watched for violet ring at the convergence of two fluids.

**Tests for Reducing Sugars:**

*a) Fehling's test:* Mixed Fehling's A solution (1 ml) with Fehling's B solution (1 ml) and boiled for 1-2 minute. Equivalent volume of trial solution was added in the
above. It was then warmed in steaming water bath for 6 - 11 min. At first, yellow then block red hasten was watched.

b) *Benedict's test:* Test solution and Benedict's reagent in equal volume taken and mixed with in the test tube. Warm this mixture in steaming water bath for 4-5 min. The mixture might show up green, yellow or may be red subjected on the amount of diminishing sugar exhibit in test result of test tube.

**Tests for Monosaccharides:**

*Barfoed's test:* Equivalent volume of the sample to be evaluated and Barfoed's reagent were blended and warmed for 2-3 min, in steaming water bath and chilled. Observed for red coloured precipitate.

**Tests for Hexose Sugars:**

*Cobalt-chloride test:* 3 ml of test solution taken and mixed 2 ml of cobalt chloride in it, boiled and cooled. Then few drops of NaOH solution was added. Result observed for purple colour (Fructose), greenish blue colour (glucose), or greenish blue upper layer and purplish lower layer (Glucose and fructose mixture).

**Tests for Non-Reducing Sugars:**
a) The samples fails the benedict test and also fails the fehling’s test.

**Test for Non-Reducing Polysaccharides:**

a) *Tannic acid test for starch:* In the test solution add 20 % tannic acid and the solution was observed for precipitation.

b) *Iodine test:* In 3-4 ml of sample solution added little droplets of diluted Iodine solution and mix it. Blue color shows up and it continues bubbling and return for chilling of the mixture.

**4.5.2. Proteins Identification:**

a) *Million’s test (for proteins):* In 3-4 ml of examination sample add 5-6 ml of Million's reagent. Precipitate of white colour appears. Heated up the precipitate, it goes block red or it gives red color when precipitate dissolves.
b) **Biuret test (General test):** Take 3-4 ml of the sample to be tested and add 4 % NaOH solution and little droplets of 1 % CuSO₄. The violet or pink colour of solution was observed.

c) **Test for sulphur containing protein:** In 5-6 ml test sample, include 2 ml of 40 % sodium hydroxide and 2 drops of lead acetic acid (10 %) solution. Result was turned dark or earthy when bubbled because of Pbs establishment was observed.

d) **Xanthoprotein test (For tyrosine or thetryptophanprotein):** Take 1 ml if sulfuric acid and mixed with 3 ml of sample and then observed for white precipitate occurrence.

4.5.3. Tests for Amino Acids:

a) **Test for Tyrosine:** Heated 3-4 ml investigation sample and 3 globules Million's reagent. Solution observed for dark red colour.

b) **Ninhydrin test (General test):** 3 milliliter test sample mixed with few drops of 5 % Ninhydrin solution. The result was warmed for 10-12 min in boiling water. The result was observed for development of bluish colour or purple.

c) **Test for Tryptophan:** To 3 ml test sample added 2 - 3 drops of glyoxalic acid and H₂SO₄ (concentrated). Then observed the sample for reddish violet ring at joint of the two layers.

d) **Test for cysteine:** To 5 ml. test sample adds few drops of 40 % NaOH and 10 % lead acetate solution. Boil it. Black precipitate of lead sulphate is formed.

4.5.4. Tests for Steroid:

a) **Salkowski reaction:** In 2 ml investigation sample, add 2 ml sulphuric acid and chloroform respectively. Shaked fine and check whether red coloured layer of chloroform layer and layer of acid displayed fluorescence of greenish yellow.
b) **L-Burchard reaction**: Mixed 2 ml testing sample with chloroform. In this add 2 drops of H₂SO₄ and 1 to 2 ml acetic anhydride through sideways of tube and observed for generation of red to blue and lastly green.

c) **Libermann’s reaction**: Mixed 3-4 ml of investigating sample with acetic anhydride (3 ml). Warmed and cooled. After cooling added few drops H₂SO₄ (concentrated) and observed for blue colour.

4.5.5. **Flavonoids Identification**:

   a) **Shinoda test**: Take dehydrated concentrate and included 5 ml of 95 % ethanol, little droplets of hydrochloric acid and 0.5 gram turnings of magnesium. Pink shade was watched.

4.5.6. **Glycosides Identification**:

Tests for **Cardiac Glycosides**:

a) **Baljet's test**: The sample is mixed with sodium picrate. A test result watched for yellow to orange.

b) **Legal’s test (For cardenoloids)**: In the investigating solution, added 1 ml of pyridine. After this add 1 ml of sodium nitroprusside. The pink coloured turned red.

c) **Test for deoxysugars (Kellar Killani test)**: In 2 ml test sample, mix one drop of sulfuric acid and few drop of glacial acetic acid. In this add 1 drop of FeCl₃(5%) and watched for the formation of rosy tan shade at the intersection of two fluid and somewhat blue green upper layers.

d) **Libermann’s test (For bufadenolids)**: In 3-4 ml of investigating sample, add 3-4 ml of acetic anhydride. Warmed and cooled. Littleglobules of H₂SO₄ was added an observed for blue colour.

Tests for **Saponin Glycosides**:

a) **Haemolytic test**: Test result was added to one drop of human blood set on a glass slide. Watched for the presence of haemolytic zone.
b) **Foam test:** The medication concentrate or dry powder was shake overwhelmingly with water. Industrious froth in the result was watched.

**Tests for Anthraquinone Glycosides:**

*a) Brontager’s test:* To 3 ml extract, add diluted H$_2$SO$_4$. Boil and filter. Add anequal volume benzene or chloroform to the cold filtrate. Jiggle well. Then isolated the organic layer. Ammonia was incorporated. The ammonia layer goes pink coloured or red color.

*b) Modified Brontager’s test:* In 5-6 ml of sample, add 5 % FeCl$_3$(5 ml) and dil. HCl (5 ml). Warmed for 5-6 min. in steaming water and then cool the solution. Then include benzene or any natural dissolvable. Jiggle well and separate natural layer. Include break even with volume weakened Ammonia. Pinkish red colored ammoniacal layer occured.

4.5.7. **Tests for Alkaloids:**

*a) Dragendroff’s test:* In the invenstigation smaple (2-3 ml) Dragendroff’s reagent is added in little amount dropwise. Orange or brownish orange precipitate obtained.

*b) Mayer’s test:*Mayers reagent is added in the test filerate and observed for the formation of precipitate.

c) **Hager’s test:** Hagers reagent is mixed with the few ml of the test filerate and observed for the formation of yellow color preprecipitate.

d) **Wagner’s test:** A small amount of wagners reagent is added in 3-4 ml of the test filerate and observed for the development of precipitate of reddish brown color.

4.5.8. **Tannins and Phenolic Compounds identification:**

To 3-4 ml test solution, added littleglobules of whether showed subsequent was observed:-

*a) Lead acetate solution:* - Development of white precipitate observed.

*b) Gelatin solution:* - Development of white precipitate observed.
c) 5% FeCl₃ solution: - Development of dark bluish black color.

d) Bromine water: - It causes discoloration of bromine water.

4.5.9. Vitamins Identification:

a) Test for Vitamin D: Break down a amount proportionate to about vitamin D 1000 units in chloroform and include antimony trichloride (10 ml), a pinkish-red shade shows up on the double.

b) Vitamin A: Dissolve a amount correspondent to 10 - 15 units in 1 ml of chloroform. In this, mix 5 ml of antimony solution. A short-lived blue colour is immediately formed.

4.6. CHROMATOGRAPHIC STUDY:

4.6.1. Thin Layer Chromatography for Saponin Compounds (Sethi and Charegaonkar, 1992):

Preparation of TLC Plate:

The thin layer chromatography studies of extracts of Lagenaria siceraria, Ocimum gratissimum, and Moringa oleifera were carried out to confirm the presence of saponin compounds.

Silica gel G is used as adsorbent in thin layer chromatography. Distilled water is used to prepared slurry of silica gel. In this technique the Silica gel GF254 (for TLC) was used as an adsorbent and plates were prepared by spreading technique, air dried over-night and activated for one hour at 110°C and used. The slurry was applied to acquire a thin layer of 0.4 mm thickness over a clean and dry glass plate of 10 x 20 cm size by an applicator.

The details of the thin layer chromatographic plate and the methods and the details of the solvent systems and the detection system for the saponins compounds are shortened in table below.
4.6.2. Thin Layer Chromatography for Phenolic Compounds (Sethi and Charegaonkar, 1992):

Preparation of TLC Plate:

The thin layer chromatography studies of extracts of *Lagenaria siceraria*, *Ocimum gratissimum*, and *Moringa oleifera* were carried out to confirm the presence of Phenolic compounds.

Silica gel G is used as an adsorbent in TLC (thin layer chromatography). Distilled or purified water in the preparation of slurry which is used to form the TLC plate. This prepared slurry was applied to get a thin layer of 0.4 mm thickness over a clean and dry glass plate of 10 x 20 cm size by an applicator. The plate was activated at 110°C for one hour. The solvent system used for the TLC is Toluene (3) : Acetone (3) : Acetic acid (2). The absorbance taken at 366 nm wavelength. The detection was carried out using vaniline-sulphuric acid and visible method.

The details of the thin layer chromatographic plate and the methods and the details of the solvent systems and the detection system for the phenolic compounds are summarized in table 7.
<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Name Parameter</th>
<th>Parameter</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Adsorbent / Stationary phase</td>
<td>Silica gel GF254</td>
</tr>
<tr>
<td>2</td>
<td>Plate size</td>
<td>10 x 20 cm</td>
</tr>
<tr>
<td>3</td>
<td>Thickness</td>
<td>0.4 mm</td>
</tr>
<tr>
<td>4</td>
<td>Activation temperature</td>
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<td>5</td>
<td>Solvent system / Mobile phase</td>
<td>Toluene : Acetone: Acetic acid (3:3:2)</td>
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<tr>
<td>6</td>
<td>Detection</td>
<td>Vaniline-sulphuric acid and Visible method</td>
</tr>
<tr>
<td>7</td>
<td>Absorbance / Wavelength</td>
<td>366 nm</td>
</tr>
</tbody>
</table>

4.7. PHARMACOLOGICAL SCREENING

Albino wistar rats of whichever sex weighing in the middle of 130–220 g were used in this investigate. They were engaged for assessing acute toxicity study, pain relieving activity study, anti-pyretic activity study and anti-inflammatory activity study. Animals were given marketable laboratory animal feedstuff and water in ample quantity. All the experimental animals were maintained at normal environmental condition of work space. They were housed at 25 ºC and 12 h day / night cycle in a group of six rats in hygienic cages. The beds of the cages was changed every day.

Before conducting the investigation, ethical or moralpermission was acquired from IAE Committee, Vidyabharti Trust College of Pharmacy, Umrakh (CPCSEA / VBT / IAEC / 14/01/60). Six extracts of plant material were utilized for the screening of anti-inflammatory, analgesic and anti-pyretic potential. These are listed below.

1. Methanolic concentrate of *Lagenaria siceraria*
2. Aqueous concentrate of *Lagenaria siceraria*
3. Methanolic extract of *Ocimum gratissimum*
4. Aqueous extract of *Ocimum gratissimum*
5. Methanolic extract of *Moringa oleifera*
6. Aqueous extract of *Moringa oleifera*

4.7.1. Acute Toxicity Study:
**Acute oral toxicity:**

The intense oral noxiousness study was done according to the principles set by the OECD (Organization for Economic Co-operation and Development) current rules 423 B, got from the CPCSEA (board of trustees with the end goal of control and supervision of investigates creatures), MSJE under Indian administration.(Walum, 1998, Diener and Schlede, 1998).

**Principle of Test method:**

The test is taking into account a stepwise system with the utilization of the most minimal amount of creatures for every step; essential data is gotten on the intense danger of the trial substance empower its characterization. The part is offered orally to gathering of investigational subjects at one clear measurements. The substance will be tried utilizing a stepwise process, every step utilizing three rats of the same sex (females typically). Nonattendance or vicinity of compound linkeddeath of creatures cured at one stage will characterize the following step.

- No additional analysis is required.
- Administer themedicine to three supplementarycreatures with the similar dose.
- Medicating of three creatures at the subsequent greater dose level or the succeeding lower dosage.

The method aids a judgment with respect to categorizing the investigation substances to one of the sequences of noxiousnesslessonsdistinct by stablecutoffLD\(_{50}\)values.

**Methods Description:**

- **Assortment of Animal Type:**

  Healthy young wistar rats of any gender, considering in the middle of 130 - 220 gram were utilized for acute toxicity investigation to determine LD\(_{50}\) of various extracts. Four groups were prepared comprising three animal in each.

- **Housing and Feeding Condition of Experimental room:**
The temperature in the experimental room was around 25°C. Lightning was non-natural, the arrangement being 12 h dark, 12 h light. The conventional work room diet was fed, with an unlimited supply of water for drinking.

- **Preparation of Animals:**
  Randomly selected animal are stamped to allow singular recognizable proof, and kept in their confines for seven days earlier to medicating to permit for acclimation to the work room condition.

- **Preparation of dosages:**
  All the extracts were prepared in a form of suspension by triturating with 2% of gum acacia.

- **Dosage Administration:**
  The investigating substances are given in a solitary dose by gavage by means of a gastrointestinal tube. Animals were fasted prior to dosing. After completion of the subsequent fasting period, the experimental animals were weighed and investigating substance was directed. After the dose was given to subjects, foodstuff was suspended for a additional 3 - 4 h in subjects.

- **Dose and Number of Animals:**
  In every step, three animals were used in every set. Meanwhile, there was no data on the material to be tried (i.e. concentrates), beginning measurements was 100 mg/ kg b. wt. up to 3000 mg/ kg b. wt. The procedure of dose selection and finalized cutoff values (LD50) are exposed in the table no. 18.

- **Observations:**
  Creatures were watched at first in the wake of dosing in any event once all through the initial 30 minutes, intermittently amid the initial 24 hours. In all cases, demise was noticed in 24 hours after administration. Further observations like changes in eyes mucous films, skin, and additionally respirational, cardiovascular,
autonomic and focal anxious frameworks and romatomotor action and conduct design. Convulsions and tremors are also take into consideration.

4.7.2. Screening for Analgesic Activity:

Animal assortment:

Rats of either one gender weighing 130 to 200 gram were picked for the experimentation. They were utilized for evaluating pain relieving potential of the sample. Albino wistar rats are alienated into eight clusters containing six rats. The comforter material of the confines was altered each day.

Investigation Material:

a. Methanolic and aqueous extracts of *Lagenaria siceraria*.
b. Methanolic and aqueous extracts of *Ocimum gratissimum*.
c. Methanolic and aqueous extracts of *Moringa oleifera*.
d. Pentazocine (Standard drug)

Dose selection:

1. *Lagenaria siceraria* methanolic extract (200 mg / kg b.wt)
2. *Lagenaria siceraria* aqueous extract (200 mg / kg b.wt)
3. *Ocimum gratissimum* methanolic extract (100 mg / kg b.wt)
4. *Ocimum gratissimum* aqueous extract (100 mg / kg b.wt)
5. *Moringa oleifera* methanolic extract (500 mg / kg b.wt)
6. *Moringa oleifera* aqueous extract (500 mg / kg b.wt)
7. Control gum acacia (p.o.) (5 ml / kg) as
8. Standard: Pentazocine 5 mg / kg body weight (i.p.)

Method:

*Tail immersion method (Chandrashekar et al., 2004)*

The method of Chandrashekar was utilized to assess pain killing action of the sample concentrate of plants. The animal were allocated into eight clusters (each cluster comprising six rats). The first cluster was worked as control group and taken 5 ml / kg b. wt. (orally) of 5% acacia solution only, then next cluster of animals was assisted as standard and Pentazocine was administered (5 mg / kg b. wt., i.p.). The rats of remaining clusters were cured with different concentrates of *Lagenaria siceraria, Ocimum gratissimum* and *Moringa oleifera*. The analgesic responses of the extracts of different plant parts were assessed by means of the
method known as tail immersion. The rats were initially weighed and stamped in this procedure. The animals are set into individual controlling pens forgetting the tail hanging uninhibitedly. The creatures are permitted to acquaint to the confines for 30 min. previously the commencement of the test. The bottommost 5 cm tailportion is spotted with marker. This portion of the rat tail is dipped in a mug of newly packed water of accurately 55 ± 5°C temperature. Inside a couple of seconds, the rodent reacts by withdrawing the tail from the container. Now the standard, investigational and control drug or extracts dosages were given to the rats and the response interval was observed at 0, 30, 60, 90, 120 and 180 minute time interval for assessing the activity of the plants extracts.

4.7.3. Screening for Anti-pyretic Activity:

Animal assortment:

Rats (albino wistar) of either one gender weighing 130 to 200 gram were taken for the test. They were engaged for estimating fever reducing repose of the plant extracts. The experimental subjects were allocated into eight clusters, each cluster consuming six rats. The comforter material of the confines was changed every day.

Investigating Material:

a. Methanolic and aqueous extracts of *Lagenaria siceraria*.

b. Methanolic and aqueous extracts of *Ocimum gratissimum*.

c. Methanolic and aqueous extracts of *Moringa oleifera*.

d. Paracetamol as standard drug

e. 15 % suspension of Brewer’s yeast in normal saline

f. Digital thermometer to measure rectal temperature of experimental rats.

Dose Used:

1. *Lagenaria siceraria* methanolic extract (200 mg / kg b.wt)
2. *Lagenaria siceraria* aqueous extract (200 mg / kg b.wt)
3. *Ocimum gratissimum* methanolic extract (100 mg / kg b.wt)
4. *Ocimum gratissimum* aqueous extract (100 mg / kg b.wt)
5. *Moringa oleifera* methanolic extract (500 mg / kg b.wt)
6. *Moringa oleifera* aqueous extract (500 mg / kg b.wt)
7. Control: 1ml / kg body weight. (p.o.)
8. Standard: 200 mg / kg body weight. (i.p)
9. Brewer’s yeast: 2 ml/kg wt. (subcutaneously)

**Method:** (Jalalpure et al., 2002)

Rats (albino wistar) of whichever gender weighing 130 to 250 gram were carefully chosen and alienated into eight clusters, each cluster having six rats. At the commencement of the test, standard rectal temperature was checked by thermometer. Fever was persuaded by administering 2 ml/kg b.wt. of brewer’s yeast suspension (15 % w/v) previously prepared in saline via subcutaneous route. The experimental rats remained abstained for the interval of the testing (24 h.). Later, 18 h. of the yeast administration, the extracts were administered by oral route to all groups except the control, which was given 1 ml/kg b.wt. normal saline solution (p.o.). A standard set of rats was received standard drug Paracetamol 200 mg/kg b.wt. (i.p.). The rectal temperatures of every last one of creatures were noted at 30 min. interim till 3 h. After actuating the test drug through oral course, the rectal temperature was taken by embeddings 2 cm of advanced thermometer, greased up with glycerine into the rectum part for 2 min. Temperatures noted were arranged and contrast in the ascent of temperature from that of ordinary rodent temperature was registered. The mean estimation of results in of gathering of experimental rats were measured and noted.

4.7.4. Screening for Anti-Inflammatory Activity:

**Animal assortment:**

Albino wistar rats of whichever gender weighing 150 to 250 gram were designated for the testing of the plants extracts. They were employed for evaluating anti-inflammatory potential of the concentrate of plants material. The testing animals were separated into eight clusters, each cluster having six rats. The eiderdown material of the crates was altered each day.

**Investigational Material:**

a. Methanolic and aqueous extracts of *Lagenaria siceraria*.
b. Methanolic and aqueous extracts of *Ocimum gratissimum*.
c. Methanolic and aqueous extracts of *Moringa oleifera*.
d. Diclofenac sodium as standard drug
e. Carrageenan : 0.1 ml of 1 % solution
f. Plethysmometer
**Dose Used:**

1. *Lagenaria siceraria* methanolic extract (200 mg / kg b.wt)
2. *Lagenaria siceraria* aqueous extract (200 mg / kg b.wt)
3. *Ocimum gratissimum* methanolic extract (100 mg / kg b.wt)
4. *Ocimum gratissimum* aqueous extract (100 mg / kg b.wt)
5. *Moringa oleifera* methanolic extract (500 mg / kg b.wt)
6. *Moringa oleifera* aqueous extract (500 mg / kg b.wt)
7. Control: 5 ml / kg b.wt. (p.o.) normal saline
8. Standard: intra peritoneal 100 mg/ kg body weight.
9. Carrageenan: 0.10 ml of 1.0 % w/v solution in normal saline.

**Method:**

*Carrageenan induced paw oedema (Shah and Seth, 2010):*

Wister albino rats of whichevergender weighing in the middle of 150 g - 200 g were contained in regular metal confines. They were delivered with food and liquid in sufficient quantity. The animals were permitted one-weekadaptation period earlier the investigational schedule. The animals were distributed into eight clusters (each clustercomprising six rats). The first cluster was assisted as a control group and given normal saline (5 ml / kg) only, thesecondcluster of rats was assisted as standard and were given Diclofenac sodium as standard (100 mg / kg i.p.). Remaining groups of rats were managed with different extracts through oral route. A spot was made with the marker on the both hind paws of rats just below the tibiotarsal joint so each one time the paw could be dunked in the section of the plethysmograph up to the spot to guarantee the steady paw volume of rats. Following 30 minutes of the above treatment, an incendiary edema was incited in the left rear paw by infusing 0.1 ml of carrageenan 1 % w/v in saline, in the grower tissue of every last one of creatures. The paw volume was checked at the outset hour and emulated by consistently up to the fourth hour after the organization of carrageenan to each one gathering. The contrast between the starting and ensuing perusing gave the genuine edema volume. Swelling was measured as % restraint by utilizing the recipe, \[ \% \text{ Inhibition} = 100 \times (1-\frac{v_t}{v_c}) \], where "vc" speaks to edema volume in control and "vt" edema volume in the gathering treated with test compound.

**4.7.5. Statistical Analysis**
Information were communicated as mean ± SEM (standard error mean). Information were examined by utilizing examination of change took after by Dunnett's t-test. Contrasts were thought to be huge at P < 0.05