CHAPTER IV

PHARMACOLOGICAL ACTIVITIES OF SWERTIA CHIRAYITA

4.1 INTRODUCTION

Pharmacology deals with the properties and effect of drugs. It can be defined as the study of interaction between chemical substance and biological system [125]. It has been realized that plants possess unique potential to provide novel structures for drug development. There is a gradual global shift away from synthetic medicines to natural products. The reason for this renewed interest in herbal products can be attributed to the ever increasing incidences of the harmful side effects of Modern Synthetic Products. The plant based products, also referred to as botanicals, phyto-pharmaceuticals and green pharmaceuticals, and it occupies a major share in the world trade and market these days. Today the scientists are visualizing a great future for plant based drugs for treatment of cancer, AIDS, chronic disease and typical viral infections. Plants also appear to be an excellent source of new bioactive compounds.

Medicinal plants are the richest bio-resource of drugs and they provide many resourceful substances that can be used in different therapeutic purpose. Extraction methods used pharmaceutically involve the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity [126]. The extracted products contain many complex phytochemicals.

Phytochemicals are natural bioactive compounds found in plants which contribute to the color, flavour, taste and aroma of plants. In addition, they form part of a plant’s natural defensive mechanism against diseases and physiological stresses. Phytochemicals are basically divided into two groups, primary and secondary constituents, according to their functions in plant metabolism. The primary constituents comprise of common sugars, amino acid, proteins and chlorophyll, while the secondary constituents consists of alkaloids, terpenoids, saponins, phenolic, flavonoids, tannins and so on. Primary metabolites make up the physical integrity of the plant cell and are involved with the primary metabolic process of building and the maintenance of living cells. Secondary metabolites do not seem to be vital
to the immediate survival of the organism that produces them and are not an essential part of
the process of building and maintaining living cells, but are responsible for their therapeutic
potentialities [5].

One of the largest group of chemicals produced by plants are the alkaloids and their
amazing effect on humans has led to the development of powerful pain killer medications
[127]. Alkaloids are basic natural products occurring primarily in plants. They occur as one
or more heterocyclic nitrogen atoms and are generally found in the form of salts with organic
acids. Alkaloids are the most efficient therapeutically significant plant substances. Pure
isolated alkaloids and their synthetic derivatives are used as basic medicinal agents because
of their analgesic, antispasmodic and anti-bacterial properties [128]. The importance of
alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic
strains has recently been reported by Kubmarawa et al. [129]. Phenolic compounds are
responsible for the antioxidant capacity in many plants. [130,131].

4.2 PHYTOCHEMICAL ANALYSIS

PREPARATION OF PLANT EXTRACT

The commonly employed technique for the separation of active principles from the
plant is called extraction. It involves the use of different solvents on the basis of their
polarity.

CHEMICALS USED

Petroleum ether, Chloroform , Acetone and Methanol (Renkem Fine Chemicals
Limited, Okhla, Delhi).

PROCEDURE

The dried powdered of whole plant of Swertia chirayita (100g) of Chakrata site was
extracted with four solvents (Petroleum ether, Chloroform, Acetone and Methanol) on the
basis of their increasing polarity, and the temperature of the mantle was regulated according
to the boiling point of the respective solvent by Soxhlet apparatus (Plate 4.1). The boiling
range of the solvent used during the extraction is given in Table 4.1.

The extracts were filtered through Whatman filter paper and reduced to small volume
in a flash rota evaporator under reduced pressure. After extraction, the percentage yields of
each fraction were determined. All these extracts were further examined for their
Phytochemical analysis and pharmacological activities (antimicrobial, antioxidant, analgesic and antipyretic).

**TABLE 4.1: Boiling Point Range Of Solvents**

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Boiling Range(Temperature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>60-80°C</td>
</tr>
<tr>
<td>Chloroform</td>
<td>75-78°C</td>
</tr>
<tr>
<td>Acetone</td>
<td>55-56°C</td>
</tr>
<tr>
<td>Methanol</td>
<td>64-65°C</td>
</tr>
</tbody>
</table>

4.3 PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACTS

All these solvent extracts, of whole plant of *Swertia chirayita*, were then subjected to phytochemical analysis for the identification of various plant constituents like alkaloids, carbohydrates, glycosides, sterols, saponins, proteins and amino acids, tannin, which are responsible for different biological activity [132].

**CHEMICALS USED FOR PHYTOCHEMICAL ANALYSIS**

Sulphuric acid, Sodium citrate, Sodium carbonate, Copper sulphate, Potassium hydroxide, Sodium Potassium-tartrate, Resorcinol, Hydrochloric acid, Copper acetate, Nitric acid, Sodium hydroxide, Mercuric chloride, Iodine, Picric acid, Ferric Chloride, Zinc dust, Glacial acetic acid, Mercury metal, Potassium iodide, Acetic anhydride, Ninhydrin, Vaniline, Sodium chloride (Renkem Fine Chemicals Limited, Okhla, Delhi.) All the chemicals used in this study were of analytical grade.

**DETECTION OF ALKALOIDS**

Different plant extracts were dissolved individually in dilute hydrochloric acid and then filtered. The filtrate was tested for the following colour tests Mayer's reagent (cream precipitate), Hager's reagent (yellow precipitate) and Wagner's reagent (reddish brown) to detect the presence of the alkaloids.
(a) **Mayer's test**

By adding Mayer’s reagent (1.36 g of Mercuric chloride in 60 ml distilled water + 5.0 g of Potassium iodide in 20 ml distilled water) in the obtained filtrate, the formation of cream precipitate indicates the presence of alkaloids.

(b) **Hager's test**

By adding Hager’s reagent (saturated aqueous solution of Picric acid i.e. 1.0% (v/v) solution of Picric acid in hot water) in a filtrate, the formation of yellow coloured precipitate indicates the presence of an alkaloids.

(c) **Wagner's test**

By adding Wagner’s reagent (1.27 g of Iodine and 2 g of Potassium Iodide in 5 ml of water and 100 ml distilled water) in a filtrate the formation of brown/reddish precipitate indicates the presence of alkaloids.

**DETECTION OF CARBOHYDRATES**

A small quantity of different extracts is dissolved separately in 4 ml of distilled water and filtered. The filtrate is subjected to the following test (Molisch's Test, Selivanoff test; Barfoed’s test, Fehling’s test and Benedict’s test).

**Molisch’s test**

Filtrate is taken in a test tube and few drops of Molisch's reagent (10 g Napthol) are added in 100 ml of 95% of alcohol and by adding 2 ml of concentrated Sulphuric acid slowly from the side of the test tubes showed a purple ring at the junction of the two liquids. This exhibits the presence of carbohydrates.

**Selivanoff's test**

Filtrate is taken in a test tube containing adding Resorcinol crystals and concentrated Hydrochloric acid. An appearance of pink color indicates the presence of carbohydrates.

**Fehling's test**

Filtrate was neutralized by adding sodium hydroxide solution and treated with Fehling A and B solutions (added in equal volumes) gives precipitate. Which indicates the
presence of carbohydrates.

**Barfoed's test**

Filtrate is taken and treated with Barfoed’s reagent (12 g of copper acetate in 200 ml distilled water and addition of 12.5 ml of 8.5 % of lactic acid solution) when boiled on water bath it gave brick red precipitate to confirm the presence of carbohydrates.

**Benedict’s test**

Filtrate when treated with Benedict's reagent and allowed to boil on a water bath, exhibits the reddish brown precipitate which confirms the presence of carbohydrates.

**DETECTION OF STEROLS**

Sterols were detected by performing following tests:

**Salkowaski's test**

By adding few drops of concentrated Sulphuric acid in the 0.5 g of extract and solution was shaken and allowed to stand, lower layer turned red indicating the presence of sterols.

**Liebermann – Burchard’s test**

When extract is treated with few drops of Acetic anhydride, and concentrated Sulphuric acid is added from the sides of the test tube, it shows a brown ring at the junction of the two layers and upper layer turn green exhibiting the presence of sterols.

**Hensen’s test**

Dried extract taken in 10 ml of chloroform and a few drops of concentrated Sulphuric acid added in it which gives an appearance of red color in acid and chloroform layers indicating the presence of sterols.

- **Detection of Saponins**

1ml of plant extract diluted with 20 ml of distilled water, on shaking in a graduated cylinder for 15 minutes. The appearance of 1 cm layer of foam indicates the presence of saponins.
TEST FOR TANNINS

Small portion of plant extract added in 100ml of distilled water, boiled and cooled, and then filtered. To this solution few drops of freshly prepared Ferric chloride (1.62g in 1L of 0.001M Hydrochloric acid) added, an appearance of greenish violet colour shows the presence of tannins.

- DETECTION OF PHENOLIC AND FLAVONOID COMPOUNDS

Small quantity of plant extract dissolved in 15ml of water and subjected for following tests to detect the presence of phenolic compounds.

Ferric chloride solution test

Extract solution gives intense green color, when it is treated with a few drops of ferric chloride solution indicates the presence of phenolic compounds.

Vanillin test

Extract solution gives red color, when it is treated with vanillin reagent (19 ml Vanillin in 10 ml Alcohol and 10 ml concentrated hydrochloric acid indicates the presence of phenolic compounds.

Zinc hydrochloric acid reduction test

Extract solution with zinc dust and few drops hydrochloric acid, gives magenta red colour which indicates the presence of phenolic and flavonoid compounds.

DETECTION OF PROTEIN AND AMINO ACID

Dissolved small quantities of extract in a few ml of water and subjected the solution to following test.

(a) Millon's Test

Extract solution when treated with Millions reagents (1 g mercury in 9 ml fuming Nitric acid volume made a solution in 100 ml with distilled water) and heated on a water bath gives red color precipitate, which indicate presence of amino acids.
(b) Ninhydrin Test

Extract solution when treated with Ninhydrin reagent (0.1 % w/v of Ninhydrin in an n Butanol) gives a blue violet color, which indicates the presence of amino acids.

(c) Biuret Test

Extract solution when treated with 40% sodium hydroxide and dilute copper sulphate solution gives, blue colour which indicates the presence of amino acids.

4.3.1 RESULTS AND DISCUSSION

PERCENTAGE YIELD OF EXTRACTION FRACTION

After extraction, the percentage yield of each fraction was determined. The results are shown in the Table 4.2.

The lowest yield 2.44% was obtained in petroleum Ether extract, while the maximum yield 13.75% was obtained in methanol extract. 5.28% yield was obtained from chloroform extract and acetone extract gave 7.8% yield.

Table 4.2: Percentage Yield of Swertia chirayita extracts in various solvents

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Extract</th>
<th>Colour</th>
<th>Yield( % w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Petroleum Ether</td>
<td>Greenish-Brown</td>
<td>2.44</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform</td>
<td>Brown</td>
<td>5.28</td>
</tr>
<tr>
<td>3.</td>
<td>Acetone</td>
<td>Dark Brown</td>
<td>7.8</td>
</tr>
<tr>
<td>4.</td>
<td>Methanol</td>
<td>Dark Greenish Brown</td>
<td>13.75</td>
</tr>
</tbody>
</table>

PHYTOCHEMICAL ANALYSIS

Phytochemical evaluation of the different solvent extracts of the Swertia chirayita were done for the presence of carbohydrates, proteins, amino acid, steroids, alkaloids, phenolic, flavonoids, tannin and saponin. The results are presented in Table 4.3 to 4.8.

Methanol and acetone extract exhibited the presence of alkaloids with Wagner’s and Hager’s tests, on the other hand chloroform extract gave positive response only with Hager’s test. However, alkaloids were absent in petroleum ether extract in all the four tests.
Table 4.3: Results of Alkaloids

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test performed</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Acetone extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wagner’s test</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>Hager’s test</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>3</td>
<td>Mayer’s test</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

(+) Presence and (-) Absence

Methanol extract shows the presence of carbohydrates in Fehling’s, Benedict’s, and Selivinoff’s tests whereas Molisch’s and Barfoed’s test gave negative results. Acetone extract exhibited the presence of carbohydrates only with Selivinoff’s test, whereas chloroform extract shows positive results for Fehling’s and Molisch’s except Barfoed’s, Benedict’s and Selivinoff’s test.

Table 4.4: Result of Carbohydrates

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test performed</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Acetone extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fehling’s test</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>Molisch’s test</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>3</td>
<td>Barfoed’s test</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>4</td>
<td>Benedict’s test</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>5</td>
<td>Selivinoff’s test</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

(+) Presence and (-) Absence

Various tests were performed with the plant extract of *Swertia chirayita* to detect the presence of sterols. Salkowski’s test shows presence of sterols with petroleum ether, chloroform, acetone, and methanol extracts. Gilberman-Buchard’s test gives positive response only with petroleum ether and chloroform extract, whereas Hensen’s test is positive only to acetone and methanol extracts.
Table 4.5: Results of Sterols

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test performed</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Acetone extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Salkowski test</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>Gilberman-Buchard’s test</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>3</td>
<td>Hensen’s test</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

(+) Presence and (-) Absence

Table 4.6: Results of Tannin and Saponin

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test performed</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Acetone extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>Saponins</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

(+) Presence and (-) Absence

Tannins and Saponins were found to be present only in methanol extract.

Table 4.7: Results of Phenolic and Flavonoid compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test performed</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Acetone extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vanillin-HCL test</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>Ferric Chloride test</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>3</td>
<td>Zinc hydrochloric acid reduction test</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

(+) Presence and (-) Absence

Phenolic and flavonoid compounds were only present in methanol extract. However acetone extract shows positive results only with Vanillin test. chloroform, petroleum ether and methanol extract gave positive results with Ferric chloride test.
Table 4.8: Results of Protein and Amino acids

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test performed</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Acetone extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Millon’s test</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>Biuret test</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>3</td>
<td>Ninhydrin test</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

(+): Presence and (-): Absence

Millon’s, Biuret, and Ninhydrin tests were performed for ascertaining the presence of proteins and amino acid in the given plant extract showed negative results for these phytochemicals with all solvents except for methanolic extract of *Swerita chirayita*.

Knowledge of the phytochemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, flavonoids, saponins, essential oils precursors for the synthesis of complex chemical substances [133].

The present phytochemical study carried out on the different solvents of plant extract of *Swertia chirayita* revealed the presence of medicinally active constituents. Analysis of plant extracts revealed the presence of carbohydrates, protein and amino acids, steroids, alkaloids, phenolic, flavonoids tannin and saponin which could be responsible for different pharmacological activities.

However, the present studies traced the presence of tannin in the samples where as the earlier findings exhibit absence of tannin. Though Nadkarni [24] in Materia medica has professed absence of tannin in *Swertia chirayita* but the result of this study which also exhibit the presence of tannin are in confirmatory with Laxmi et al. [82], which has also shown the presence of tannin in *Swertia chirayita*. Updation of information of the presence of tannin need to be done.

Different solvents have various degrees of solubility for different phytochemicals [134]. Many compounds were found in the methanolic extracts. This is because methanol is much polar than chloroform, acetone, hence extracting many of the active ingredients [132].
4.4 ANTIMICROBIAL ACTIVITY ON SWERTIA CHIRAYITA

Many naturally occurring herbs are known to possess antimicrobial functions and could serve as a source of antimicrobial agents against bacteria and fungi. Plant based antimicrobials represent a vast unexploited source of medicine which has enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is needed today [109].

An antibacterial is a substance that kills or inhibits the growth of microbes such as bacteria, viruses and fungi. Antimicrobials drugs which kills microbes are called microbicidal and those prevent the growth of microbes are called microbiostatic. In the present study an antimicrobial activity of Swertia chirayita has been carried out in four different solvent extracts under two heads that is antibacterial and antifungal by Agar disc diffusion method.

4.4.1 MATERIALS AND METHODS

PLANT COLLECTION AND EXTRACTION

The collection of the plant material and its identification was done in chapter III in 3.2. The extraction of Swertia chirayita was done by Soxhlet extraction method as described in chapter IV in 4.2.

TEST MICROORGANISMS

The cultures were obtained from the standard culture collection centre Chandigarh and were maintained on nutrient agar petri-plates, incubated at 37°C for 16-24 hours and then stored at 4°C as stock cultures for further antibacterial assay.

BACTERIAL STRAIN

The following six ESBL producing bacterial strains Staphylococcus mutans, Staphylococcus aureus, Staphylococcus epidermides, Escherichia coli, Klebsiella pneumoniae and Bacillus subtilis were used for antibacterial activity. Nutrient Agar Media (NAM) was used for the maintenance of the bacterial cultures.
FUNGAL STRAIN

Three fungal strains Aspergillus fumigates, Candida albicans and Aspergillus flavus were used for determining antifungal activity. Potato Dextrose Agar media was used for the maintenance of the fungal cultures.

PREPARATION OF EXTRACT SOLUTION FOR ANTIMICROBIAL ASSAY

The plant extracts were dissolved in DMSO (100%) solution and variable concentration of 100%, 50%, and 25% were prepared, to dissolve the different solvent extracts. 100 mg of the extract dissolved in 1 ml of DMSO solution is considered as 100%.

DIMETHYL SULPHOXIDE (DMSO)

Dimethyl sulphoxide (DMSO) is a colourless hygroscopic liquid with B.P. 189°C. It is mixed with petroleum ether, chloroform, acetone and methanol. It is used for dissolving various extracts obtained as a result of Soxhlet extraction for antimicrobial testing, as it is a good solvent for experimental purposes. With high polarity DMSO is an ideal solvent and the least toxic for assay systems.

4.4.2 ANTIMICROBIAL ACTIVITY OF SWERTIA CHIRAYITA BY AGAR WELL DIFFUSION METHOD

The antibacterial and antifungal activities of the petroleum ether, chloroform, acetone and methanol (100mg/ml) extracts of Swertia chirayita were evaluated by Agar well diffusion method as prescribed by Barry and Thornsberry [135].

The overnight culture grown was used for inoculation (The food material on which microorganism are grown is called culture medium and the growth is called culture). Nutrient Broth (liquid media) was used for inoculation of bacterial strain culture and prepared by following composition. For working stock 1ml of each bacterial strain was initially inoculated in 100ml of sterile nutrient broth and incubated for 37±1°C for 24 hr. Then 2ml of the each test organisms from the working stock was seeded into 100ml sterile nutrient agar medium in a sterile petri dish respectively. When the nutrient agar medium solidifies, four holes of uniform diameter (6mm) were made using sterilized cork borer. Then 2ml of each solvent extracts were placed in each hole separately. The plates were maintained at room temperature for 2 hr to allow the diffusion into the medium. All the bacterial plates
were incubated for 37±1°C for 18 hr and the zone of inhibition was measured. Apart from that, the comparative study also conducted with standard antibiotic Gentamycin with the test drug *Swertia chirayita*. All the diameters of inhibition zone were measured in mm.

Similarly for the antifungal activity, the stock culture were revived by inoculating in broth media and grown at 27°C for 72 hr. The agar plates of the Potato Dextrose Agar media were prepared. Each plate was inoculated with an aliquot (0.1 ml) of the fungal suspension (103 spores/ml), which was spread evenly on the plate. After 20 min, wells were made and filled with test samples of different concentrations. Gentamycin were used as positive control. All the plates were incubated at 27°C.

4.4.3 MINIMUM INHIBITORY CONCENTRATIONS (MIC) TESTING

This test is a standard method in United States and is based on the work of Bauer and Co-workers [136]. Another name for MIC is minimum lethal concentration (MLC). Minimum Inhibitory Concentration (MIC) level of any antibacterial and antifungal substances are the lowest concentration of the drug inhibiting the microbial growth. The MIC value of those microorganisms against a particular fraction is considered, which exhibit maximum activities in preliminary screening processes by disc diffusion method. MIC is determined by using the different concentrations of acetone and methanol extract.

4.4.4 RESULTS AND DISCUSSION

The results of antibacterial activities of all the four different solvent extracts in 100mg/ml of petroleum ether (PE), chloroform (CHL), acetone (ACE) and methanol (MET) for *Swertia chirayita* samples are being presented in Table 4.9. The results show that only the methanol and acetone extracts exhibit significant activity against Extended spectrum beta lacatmase (ESBL) *Staphylococcus mutans*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Staphylococcus epidermis* test isolates, compared favourably with that of standard antibiotic (Gentamcyin). On the other hand, the petroleum ether and chloroform extract did not exhibit any significant zone of inhibition.

Fig. 4.1 shows that the methanol extract of *Swertia chirayita* gives the best zone of inhibition against *Staphylococcus mutans* (14mm), *Staphylococcus aureus* (12mm) and *Staphylococcus epidermis* (15mm) whereas *Escherichia coli* and *Klebsiella pneumoniae* did not give zone of inhibition with all solvent extracts.
Table 4.9: Sensitivity of bacterial pathogens on *Swertia chirayita* in different solvent extracts (100mg/ml)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Isolates</th>
<th>Zone of inhibition in (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gentamy-cin</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus mutans</em></td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus subtilis</em></td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td><em>Staphylococcus aureus</em></td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td><em>Escherichia coli</em></td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td><em>Staphylococcus epidermis</em></td>
<td>16</td>
</tr>
</tbody>
</table>

n.z: no zone
Fig 4.1 Zone of inhibition in bacterial strain of *Swertia chirayita* in acetone and methanol Extract
4.4.5 MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF SWERTIA CHIRAYITA IN BACTERIAL STRAINS

The sensitive strains tested showed some degree of sensitivity to the methanolic and acetone extract. The MIC results of the extract on the different strains are shown in Table 4.10.

Table 4.10: MIC value for effective extract against the bacterial strains

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract used</th>
<th>MIC value (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Staphylococcus mutans</td>
</tr>
<tr>
<td>1</td>
<td>Methanol</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Acetone</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>Gentamycin</td>
<td>15</td>
</tr>
</tbody>
</table>

n.z : no zone

Minimum Inhibitory Concentrations of methanolic extracts of Swertia chirayita are very effective as 30mg/ml on Staphylococcus mutans, 10 mg/ml on Staphylococcus aureus and 20 mg/ml on Staphylococcus epidermis organisms, whereas the acetone extract showed the MIC value of 50 mg/ml on Staphylococcus mutans, 50 mg/ml on Bacillus subtilis, 20 mg/ml on Staphylococcus epidermis. The graphical representation of Minimum Inhibitory Concentrations is given in Fig. 4.2.
Fig. 4.2 Minimum Inhibitory Concentrations (MIC) of *Swertia chirayita* in acetone and methanol Extract
4.4.6 ANTIFUNGAL ACTIVITY

The sensitivity of fungal pathogens has been also performed in all the four different solvent extract in 100 mg/ml of *Swertia chirayita*. The results are depicted in Table 4.11.

Table 4.11: Sensitivity of fungal pathogens on *Swertia chirayita* in different solvent extracts (100mg/ml)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Isolates</th>
<th>Zone of inhibition in (mm)</th>
<th>Gentamycin</th>
<th>Petroleum Ether</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Meth-anol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Aspergillus fumigatus</em></td>
<td></td>
<td>17</td>
<td>n.z</td>
<td>n.z</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>2.</td>
<td><em>Candida albicans</em></td>
<td></td>
<td>16</td>
<td>n.z</td>
<td>n.z</td>
<td>n.z</td>
<td>15</td>
</tr>
<tr>
<td>3.</td>
<td><em>Aspergillus flavus</em></td>
<td></td>
<td>12</td>
<td>n.z</td>
<td>n.z</td>
<td>n.z</td>
<td>12</td>
</tr>
</tbody>
</table>

n.z: no zone

The result indicates that fungal strains *Aspergillus fumigates*, *Candida albicans* and *Aspergillus flavus*, showed best zone of inhibition on methanol extract, and on the other hand acetone extract was only effective with *Aspergillus fumigatus* strain. Petroleum ether and chloroform extract were not affected by all the three fungal strains. The graphical representation of zone of inhibition versus fungal isolates are given in Fig. 4.3.

4.4.7 MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF SWERTIA CHIRAYITA IN FUNGAL STRAINS

The results of Minimum Inhibitory Concentrations (MIC) of methanol and acetone extract are presented in Table 4.12.

The graphical representation of Minimum Inhibitory Concentrations (MIC) is given in Fig. 4.4. The result indicated that methanolic extract were more effective against *Aspergillus fumigatus* (70 mg/ml), *Candida albicans* (50 mg/ml) and *Aspergillus flavus* (30 mg/ml), whereas the acetone extract was only affected by *Aspergillus fumigatus* (80 mg/ml).
Table 4.1: MIC value for effective plant extracts against fungal strains

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract Used</th>
<th>MIC value (mg/ml)</th>
<th>Aspergillus fumigatus</th>
<th>Candida albicans</th>
<th>Aspergillus flavus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>70</td>
<td>50</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Acetone</td>
<td>80</td>
<td>n.z</td>
<td>n.z</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Gentamycin</td>
<td>50</td>
<td>45</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

n.z : no zone detected

The antimicrobial activity have been screened because of its great relevance in medical field in the recent years, infections have increased to a great extent and are resistant to the antibiotics, becomes an ever increasing therapeutic problem [137, 138]. The presence of antibacterial and antifungal substances in the plants is well established as they have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine have been used for the treatment of diseases as in done in cases of Unani and Ayurvedic system of medicines, a natural blueprint for the development of new drugs. Much of the exploration and utilization of natural product as antimicrobial arise from microbial sources.
Fig. 4.3 Antifungal activity of *Swertia chirayita* in different solvent extracts
Fig. 4.4 Minimum Inhibitory Concentrations (MIC) of *Swertia chirayita* in Fungal strains.
Natural products, extract or pure compounds provide unlimited opportunities for the development of new drugs due to the availability of chemical diversity [139]. To overcome the problem of antibiotic resistance ethnic medicinal plants have been extensively studied as an alternative treatment for diseases due to their ability to produce a variety of compounds of known therapeutic properties [132, 140] and much attention has been paid to plant extracts and their biologically active compounds [141].

Petroleum ether, chloroform, acetone and methanol extracts were used to evaluate the antibacterial and antifungal activities against the pathogenic microbes viz., *Escherichia coli*, a most common bacteria of which virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, *Klebsiella pneumoniae* which is the causative organism of pneumonia, *Staphylococcus aureus*, a wound infecting pathogen which can cause septicemia, endocarditis and toxic shock syndrome, *Staphylococcus epidermidis* which causes septicemia and endocarditis in immunocompromised patients. *Candida albicans* a causal agent of opportunistic oral and genital infections in humans and *Aspergillus niger* which causes Aspergillosis, a serious lung infection, if large amounts of spores are inhaled. The methanol extracts also showed significant activity against fungi *Candida albicans* indicating their potential to be used for treatment of severe fungal infections like Candidiasis, oral etc.

Generally the methanol extract was more active than other extracts against the selected bacterial isolates and fungus, because most of the antimicrobial agents in plants are soluble in methanol [84]. This may be attributed to the presence of soluble phenolic and polyphenolic compounds [85]. The inhibitory activity of plant extract is largely dependent on the concentration, parts of the plant used and the microbes tested [142].

The present study has been done on ESBL bacterial strain. There are few references of MIC value in research result are available and our result of MIC has proved a proper system of investigation through the established methodology.
4.5 ANTIOXIDANT CAPACITY

Several Indian medicinal plants are known for their beneficial therapeutic effects which also might have antioxidant properties [143]. Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide and hypochloric acid) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA [144]. It is well known that free radicals are the major cause of various chronic and degenerative diseases such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer [145].

Free radicals have been implicated in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging [145, 146]. That is why, antioxidants along with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases where oxidants or free radicals are implicated [147].

In the present study antioxidant capacity of *Swertia chirayita* were carried out in all the four different solvent extract (petroleum ether, chloroform, acetone and m ethanol) by Free radical scavenging capacity (DPPH assay).

4.5.1 MATERIALS AND METHODS

PLANT COLLECTION AND EXTRACTION

The collection of the plant material and its identification was done in Chapter III in 3.2. The extraction of *Swertia chirayita* was done by Soxhlet extraction method as described in Chapter IV in 4.2.

CHEMICALS

All solvents used are of analytical grade, DPPH [2, 2-diphenyl-1-picrylhydrazyl], Butylated hydroxytoluene (BHT), Gallic acid, and Folin-Ciocalteu’s phenol reagent were procured from Sigma-Aldrich Co. (USA). All other chemicals and solvents were analytical grades and obtained from Merck (Germany) companies. All solutions, including freshly prepared double distilled water.
4.5.2 FREE RADICAL SCAVENGING CAPACITY (DPPH ASSAY)

Antioxidant capacity was evaluated by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay according to the procedure described by Blios et al. [148]. A DPPH stock solution was first prepared and stored at 20°C. 2ml of a volume of 25 to 200 µl of the sample was then poured into an optical glass cuvette and the stock solution was added to a final volume of 4ml. After 30 min incubation at 28 to 30°C in dark, the absorbance at 517 nm using spectrophotometer (Thermofisher Scientif UV-2700) was measured against a blank of pure methanol and DPPH (2ml each) than compared with synthetic anti-oxidant butylated hydroxy toluene (BHT), this was used as standard. All the determinations were performed in triplicate. The antioxidant capacity was expressed as Ec50 value, which represents the concentration of the sample in cuvette necessary to decrease the initial DPPH concentration by 50%.

The Ec50 value was calculated using the following formula:

\[
\%\text{DPPH radical – scavenging} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \right) \times 100
\]

Ec50 of the extract has been determined by using SPSS software.

4.5.3 DETERMINATION OF TOTAL PHENOLIC CONTENT

Total phenols were recorded by Folin Ciocalteu reagent method as proposed by Singleton and Rossi [149] using gallic acid as a standard. Appropriate dilutions of the samples (0.25ml) were oxidized with Folin-Ciocalteu reagent for five minutes at room temperature followed by addition of 7.5% sodium carbonate solution. The absorbance of the resulting blue color was measured at 740 nm on spectrophotometer (Thermofisher Scientific UV-2700) after heating at 50°C for 5.0 min in a water bath. Quantification was done on the basis of the standard curves of gallic acid prepared from 1ml of each of the 200,150,100 and 50 ppm gallic acid solutions assaying in the manner similar to that used for the extracts. The results were expressed as % gallic acid equivalents (GAE).
4.5.4 RESULTS AND DISCUSSION

DPPH RADICAL SCAVENGING ACTIVITY

All the four extracts obtained by using extraction of the whole plant of *Swertia chirayita* with solvents namely Petroleum ether (PE), Chloroform (CHL), Acetone (ACE) and Methanol (MET) were screened for their antioxidant capacity using DPPH free radical scavenging assay. DPPH is a stable, free radical which accepts electrons and becomes a stable diamagnetic molecule. The purple colour or violet colour of the DPPH solution changes to yellow as diamagnetic molecule form and the absorbance at 517nm wavelength maximum decreases. The decrease in absorbance of DPPH caused by antioxidant is due to the reaction between the antioxidant molecule and its radical which results in the scavenging of the radical by H-donation.

Table 4.13: Antioxidant capacity of *Swertia chirayita* in Methanolic Extract

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration of extracts (µg/ml)</th>
<th>Antioxidant capacity (%) Methanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>25</td>
<td>51.508</td>
</tr>
<tr>
<td>2.</td>
<td>50</td>
<td>66.810</td>
</tr>
<tr>
<td>3.</td>
<td>75</td>
<td>66.810</td>
</tr>
<tr>
<td>4.</td>
<td>100</td>
<td>64.655</td>
</tr>
<tr>
<td>5.</td>
<td>125</td>
<td>85.162</td>
</tr>
<tr>
<td>6.</td>
<td>150</td>
<td>85.162</td>
</tr>
<tr>
<td>7.</td>
<td>175</td>
<td>90.417</td>
</tr>
<tr>
<td>8.</td>
<td>200</td>
<td>90.417</td>
</tr>
<tr>
<td>9.</td>
<td>Ec50</td>
<td>27.704</td>
</tr>
</tbody>
</table>

Data shown in the Table 4.13 reveals that the extract MET had DPPH radical scavenging activity from 51% to 90% at a concentration of 17µg/ml. MET scavenging is more than 90% of the DPPH radical compared to the BHT control. The BHT control was arrayed at 100 µg/ml, corresponding to the maximum allowable concentration for BHT.
additions to food stuffs [150] values between 25-175 µg/ml were used to determine the inhibition concentration (Ec₅₀), which is the amount of antioxidant required to deplete the initial DPPH by 50%. The Ec₅₀ value of MET was 27.70 µg/ml, compared to 17.75 µg/ml. It is also observed that the petroleum ether, chloroform and acetone extract were inactive towards antioxidant capacity.

**TOTAL PHENOLIC CONTENT**

The result of total phenolic content in methanolic extract has been shown in Table 4.14.

<table>
<thead>
<tr>
<th>Solvent Used</th>
<th>Total Phenol content (%)</th>
<th>Ec₅₀ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.234</td>
<td>27.704</td>
</tr>
</tbody>
</table>

It has been observed that the scavenging effects on the Perspectives, DPPH radical increases sharply with the increasing concentration of the samples and standards to a certain extent [151] and hence are said to be strongly dependent on the extract concentration. DPPH free radical scavenging capacities of the plant extractive are attributed to their phenolic constituents. A large number of studies have been reported [152, 153] wherein the free radical scavenging capacity of the plant positively correlated with their phenolic constituents. Thus the extracts MET conferring free radical scavenging capacity was also examined for its TPCₖ, TPC₅ in MET extract as determined by Folin Ciocalteau reagent method were found to be 0.24% GAE.

It was observed that the phenolic contents of MET extracts are associated with an increased radical scavenging capacity. This effect may be due to the electron donating ability of the phenolic compounds present in the MET. Studies conducted on free radical scavenging activity of medicinally important plants have shown that the efficiency of each plant species differs depending on the particular assay methodology, reflecting the complexity of the mechanisms involved in total antioxidant capacity [154].
4.6 ANALGESIC ACTIVITY

Pain is defined as an “unpleasant sensation that is caused by actual or perceived injury to body tissues and produces physical and emotional reactions” [155]. Most probably, pain sensation has evolved to protect our bodies from harm by causing us to perform certain actions and avoid others. Pain might be called a protector, a predictor, or simply a hassle. Pain arising from the skin and from the deep structures like muscles, bones, and joints is also termed as somatic pain. It is usually well defined and is generally caused by inflammatory reaction in the tissues, it may be accompanied by contraction of the surrounding skeletal muscles as in patients with rheumatoid arthritis. Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. Non steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain, fever and inflammation. However these drugs have no side effects especially on the gastrointestinal tract [155]. There are several ways to classify pain, but the first distinction usually made is that between acute and chronic pain. Pain is a subjective sensation which cannot be measured objectively [109]. Analgesic drugs are those which reduce the pain. The analgesic activity of Swertia chirayita was performed in methanolic extract.

4.6.1 MATERIALS AND METHODS

PLANT COLLECTION AND EXTRACTION

The collection of the plant material and the identification of plant material were done in chapter III in 3.2. The extraction of Swertia chirayita plant was done by Soxhlet extraction method which is described in Chapter IV in 4.2.

METHOD: Two methods were used for analgesic activity

1. Eddy’s hot plate (Elico India)
2. Analgesiometer (Elico India)

EXPERIMENTAL ANIMALS

Young Albino mice (18–25 g) and Wistar rats (180–200 g) of either sex were used in the present study which were acquired from Shri Guru Ram Rai Institute of Technology and Science, Dehradun. All animals were kept in the animal house under 12/12 hr light and dark
cycles with free access of food and water ad libitum. All the experiments were performed after the approval of animal ethical committee and experiments were performed as per guidelines of care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

CHEMICALS AND DRUG

Nimusulide was used as the standard drug for analgesic activity, which was gifted by Panesia Biotech. carboxy methyl cellulose was purchased from LOBA Chemmie. Pvt Ltd Mumbai.

4.6.2 EDDY'S HOT PLATE

EXPERIMENTAL DESIGN

Twenty four Albino mice (18–25 g) were employed in this activity. Animals were divided into four groups each group comprised of six animals.

Group I
Normal saline (10 ml/kg, i.p) was administered.

Group II
Test drug [100 mg/kg orally methanol extract of Swertia chirayita] was administered 30 minutes before the experiment.

Group III
Test drug [200 mg/kg orally methanol extract of Swertia chirayita] was administered 30 minutes before the experiment.

Group IV
Nimusulide (10 mg/kg i.p) was administered 30 minutes before the experiment.

PRINCIPLE

The heat method is used for the induction of pain. This method was first described by Eddy and Leimbach [156]. Hot plate device consist of a water bath in which a metallic
cylinder (diameter 20 cm and height 10 cm) is placed. The temperature of the cylinder is set at 55±1°C (Plate 4.4).

**PROCEDURE**

The animals were weighed (Plate 4.2) and appropriately marked. After 30 minutes of drug administration the animals were individually placed on a hot plate and the basal reaction time was taken by observing hind paw licking or jump response (whichever appears first) was taken as end point. A cut off period of 15 sec was maintained to avoid damage to the paw. Animals that showed short reaction time were selected for the study. The reaction time in second was recorded at the interval of 15, 30, 45, and 60 minutes of drug administration with a cut off period of 15 sec.

**4.6.3 TAIL FLICK METHOD**

**EXPERIMENTAL DESIGN**

Twenty four Wistar rats (180–200 g) were employed for the study of this method. Animals were divided into four groups each group comprised of six animals.

**Group I**

Normal saline (10 ml/Kg,i.p) was administered.

**Group II**

Test drug [100 mg/kg orally methanol extract of *Swertia chirayita*] was administered 30 minutes before the experiment (Plate 4.3).

**Group III**

Test drug [200 mg/kg orally methanol extract of *Swertia chirayita*] was administered 30 minutes before the experiment.

**Group IV**

Nimusulide (10 mg/kg i.p) was administered 30 minutes before the experiment.
PRINCIPLE

An analgesiometer (Plate 4.5) was used to record the flicking time of tail [reaction time] of the animals using the heated nichrome wire as the source of heat stimulus. The strength of the current passing through the naked nicrome wire was kept constant at 5 ampere. Cut-off reaction time was 10 sec to avoid any tissue injury during the process [157].

PROCEDURE

The animals were weighed and appropriately marked. After 30 minutes of drug administration to the animals, the basal reaction time was taken by placing the tip (last 1-2cm) of the tail of animals on the radiant heat source. The tail-withdrawal from the heat (flicking response) was taken as the end point. Normally a mouse withdraws its tail within 3-5 sec. A cut off period of 10-12 sec was observed to prevent damage to the tail. Administered drug to each group animal and reaction time was noted at the 15, 30, 45, and 60 minutes. As the reaction time reaches 10 sec it is considered maximum analgesia and the tail is removed from the source of heat to avoid tissue damage.

STATISTICAL ANALYSIS

Data is expressed as mean ± S.E.M. statistical difference between groups were determined by using one-way ANOVA P≤0.05 was considered as significant.

4.6.4 RESULTS AND DISCUSSION

Results of analgesic activity by Hot Plate are depicted in the following Table 4.15. The result showed a significant increase in the latency period of the extract treated animals as compared to the control group. The finding reveals that methanolic extract of *Swertia chirayita* possesses analgesic activity. The crude extract was used in two doses as 100 mg/kg and 200 mg/kg. The results of the extract treated animals, when compared with standard (Nimusulide) showed their analgesic activity comparable with Nimusulide. It is found that, the administration of methanolic extract of *Swertia chirayita* at the doses of 100 mg/kg and 200 mg/kg shows most significant response after 15 minutes of dose administration as compared to control group. Both doses show significant response during the test time durations. It is observed that the standard drug Nimusulide increases significantly the basal reaction time. Fig. 4.5 exhibits the graphical presentation of analgesic activity by Hot Plate method.
Table 4.15: Analgesic activity of *Swertia chirayita* (Methanolic) extract on Swiss albino mice by Hot plate method

<table>
<thead>
<tr>
<th>Time</th>
<th>15min</th>
<th>30min</th>
<th>45min</th>
<th>60min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug Treatment</strong></td>
<td><strong>PL</strong></td>
<td><strong>JR</strong></td>
<td><strong>PL</strong></td>
<td><strong>JR</strong></td>
</tr>
<tr>
<td>Control</td>
<td>5sec ± 0.09</td>
<td>7sec ± 0.11</td>
<td>5.6sec ± 0.11</td>
<td>6.5sec ± 0.12</td>
</tr>
<tr>
<td>Test-1(100 mg/kg)</td>
<td>11.3sec ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.6sec ±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.1sec ±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.1sec ±0.14</td>
</tr>
<tr>
<td>Test-2(200 mg/kg)</td>
<td>9.8sec ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11sec ±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.5sec ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.8sec ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>10.5sec ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.8sec ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11sec ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.5sec ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Fig. 4.5 Analgesic activity of *Swertia chirayita* by Hot plate method

Each group (n=6) represents mean ± SEM a=p≤0.001 Vs control group, b=p≤0.01 Vs control group
Results of analgesic activity by tail flick method are depicted in the following Table 4.16. The result showed a significant increase (on an average about 40 to 50%) in tail flick time i.e., an increase in the latency period of the extract treated animals as compared to the control group. The findings suggested that methanolic extract of Swertia chirayita possess analgesic activity. The crude methanolic extract was used in two doses of 100 mg/kg and 200 mg/kg of tested animals under test (Group II and Group III).

Table 4.16: Analgesic activity of Swertia chirayita (Methanolic) extract on Wistar’s rats by Tail flick method

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Time 15min</th>
<th>Time 30min</th>
<th>Time 45min</th>
<th>Time 60min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.6 sec ± 0.11</td>
<td>4.8 sec ± 0.10</td>
<td>5.6 sec ± 0.12</td>
<td>5.8 sec ± 0.15</td>
</tr>
<tr>
<td>Test-1(100 mg/kg)</td>
<td>7.3 sec ± 0.16 (^a)</td>
<td>6.5 sec ± 0.11 (^b)</td>
<td>7.1 sec ± 0.13 (^b)</td>
<td>6.6 sec ± 0.14</td>
</tr>
<tr>
<td>Test-2(200 mg/kg)</td>
<td>6.6 sec ± 0.10 (^b)</td>
<td>7.8 sec ± 0.09 (^c)</td>
<td>7 sec ± 0.18 (^b)</td>
<td>8.5 sec ± 0.14 (^b)</td>
</tr>
<tr>
<td>Standard</td>
<td>7.5 sec ± 0.11 (^a)</td>
<td>7.6 sec ± 0.13 (^c)</td>
<td>8 sec ± 0.14 (^b)</td>
<td>8.3 sec ± 0.13 (^b)</td>
</tr>
</tbody>
</table>

Test I (100 mg/kg) showed significant analgesic effect at 15 minutes (7.3 sec ± 0.55), 30 minutes (6.5 sec ± 0.34) and 45 minutes (7.1 sec ± 0.6). However in Test II (200 mg/kg) produced significant results at 15 minutes (6.6 sec ± 0.42), 30 minutes (7.8 sec ± 0.30), 45 minutes (7 sec ± 0.35) and 60 minutes (8.5 sec ± 0.21). The data of the extract treated animals, when compared with control group showed significant difference. Standard drug (Nimuslide) gave significantly increased tail flicking of rats at in all observed time interval as compared to the control group. The graphical representation of analgesic activity by tail flick method between the response time and activity time on the animals using methanolic extract has been presented in Fig. 4.6.
Fig. 4.6 Analgesic activity of *Swertia chirayita* by Tail flick method

Each group (n=6) represents mean ± SEM, a = P ≤ 0.01 Vs Control group, b = P ≤ 0.05 Vs Control group, c = P ≤ 0.001 Vs Control group
Animal tests of analgesic drugs commonly measure nociception and involve testing the reaction of an animal to painful stimuli [158]. The analgesic properties were also studied using sensitive models that could provide different grades of noxious stimuli (in thermal stimulus and chemically induced tissue damage). In the present study the thermal test was selected because of several advantages including the sensitivity to strong analgesics and limited tissue damage.

The hot plate and tail flick method involve spinal reflexes and is regarded as one of the most suitable methods for studying the involvement of centrally acting analgesics [159]. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase [160].

An increase in reaction time is generally considered as an important parameter of analgesic activity in Heat conduction method. In these models, increase in stress tolerance capacity of the animals indicates the possible involvement of a higher centre [161]. The finding reveals that methanolic extract of *Swertia chirayita* possesses analgesic activity. The phytochemical analysis of this extract revealed that it contains flavonoids, carbohydrate, alkaloids, phenolic and saponin. Of these, flavonoids and saponins are well known for their ability to inhibit pain perception. The above results are in agreement with the findings of Das *et al.* [95]. The Hot plate and Tail flick test gives us indication that the *Swertia chirayita* has got good analgesic property which can be used for human being and will be able to replace synthetic analgesic drug. Therefore, the result of this study proved the uses of this plant in folklore medicine for the management of pain.
4.7 ANTIPYRETIC ACTIVITY

Medicinal plants are the easy reachable health care alternative for most of the people and the traditional medicines remain a part of our basic health system. Fever is one of the most common signs of illness and it is best defined as an increase in body temperature over what is normal for a given individual at that particular time of day [162]. The normal body temperature is regulated by a center in the hypothalamus, which ensures a balance between heat loss and heat production. Fever occurs when there is a disturbance of this hypothalamic thermostat that leads to the set point of body temperature being raised [163].

Antipyretic are drugs, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between the production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever, this set point is elevated and drug like paracetamol do not influence the body temperature when it is elevated by factors like exercise or increase in ambient temperature. Antipyretic activity is commonly mentioned [164, 165] as a characteristic of drugs or compounds which have an inhibitory effects on prostaglandin-biosynthesis [166].

In the present study antipyretic activity of Swertia chirayita was evaluated in methanolic extract by Brewer’s yeast induced hyperpyrexia method in albino mice.

4.7.1 MATERIALS AND METHODS

PLANT COLLECTION AND EXTRACTION

The collection of the plant material and the identification were done in Chapter III in 3.2. The extraction of Swertia chirayita plant was done by Soxhlet extraction method as described in Chapter IV in 4.2.

DRUGS AND REAGENTS

Paracetamol was used as the standard drug, which was gifted by Panesia Biotech. carboxy methyl cellulose which was purchased from LOBA Chemmie. Pvt Ltd Mumbai, and Brewer’s yeast was purchased from the local market.

PREPARATION OF FEVER INDUCING AGENT

In this investigation 0.5 % carboxy methyl cellulose solution was prepared in normal saline. 15 % of yeast was suspended in this prepared 0.5 % w/v carboxy methyl cellulose solution.
EXPERIMENTAL ANIMALS

Young Albino mice (18–25 g) of either sex were used in the present study which was acquired from Shri Guru Ram Rai Institute of Technology and Science, Dehradun. All animals were kept in the animal house under 12/12 hr light and dark cycles with free access of food and water ad libitum. All the experiments were performed after the approval of animal ethical committee and experiments were performed as per guidelines of care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

EXPERIMENTAL DESIGN

Twenty four Albino mice (18–25 g) were employed in this activity. Four groups were designed, each group comprised of six animals. In all the groups 15% yeast (0.1 ml s.c) was administered.

Group I
Normal saline (10 ml/kg, i.p) was administered.

Group II
Test drug [100 mg/kg orally methanol extract of Swertia chirayita] was administered.

Group III
Test drug [200 mg/kg orally methanol extract of Swertia chirayita] was administered.

Group IV
Paracetamol (10 mg/kg i.p) was administered.

4.7.2 ANTIPYRETIC STUDIES BY BREWER’S YEAST INDUCED HYPERPYREXIA METHOD

The antipyretic activities of plant extract were evaluated using Brewer’s yeast induced pyrexia [167] in albino mice. Before yeast injection the basal rectal temperature of mice was recorded by inserting digital clinical thermometer to a depth of 2 cm into the rectum and after recording the temperature mice were given subcutaneous injection of 0.1 ml, subcutaneous of 15 % w/v yeast suspended in 0.5 % w/v methyl cellulose solution for
elevation of body temperature of mice. Mice were then returned to their housing cages. After the 24 hours of yeast injection, the control, standard drug and test drugs were administered in to respective groups. Paracetamol was administered orally to standard group of animals. The methanolic extract of *Swertia chirayita* plant was administered orally at a dose of 100 mg/kg and 200 mg/kg of body weight of respective groups. Rectal temperature was recorded by clinical thermometer after 0, 2, and 4 hr after drug administration.

**STATISTICAL ANALYSIS**

Data an expressed as ± S.E.M. statistical difference between mean were determined by one-way ANOVA $P \leq 0.05$ were as considered significant.

**4.7.3 RESULTS AND DISCUSSION**

The effect of methanolic extract of *Swertia chirayita* against antipyretic activity has been shown in Table 4.17.

**Table 4.17: Antipyretic activity of Swertia chirayita (Methanolic) extract on Albino mice**

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Normal body temperature</th>
<th>Body temperature after 24 hr administer of yeast</th>
<th>Body temperature after drug administer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 hr</td>
</tr>
<tr>
<td>Control</td>
<td>37.8±0.14</td>
<td>39.1±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.0±0.15</td>
</tr>
<tr>
<td>Test-1(100 mg/kg)</td>
<td>38.1±0.24</td>
<td>39.5±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.8±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-2(200 mg/kg)</td>
<td>37.0±0.19</td>
<td>39.6±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.0±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>38.0±0.24</td>
<td>39.8±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.4±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Fig. 4.7 Graphical representation of Antipyretic activity of *Swertia chirayita*.

The mean standard error value of each group (n=6) represent mean ± SEM. a=p≤0.05 Vs normal body temperature of respective group. b=p≤0.05 Vs body temperature after 24 hour administer of yeast of respective group.
The data obtained after the experiment revealed that the rectal temperature of 37.2°C at 0 hour was markedly elevated to 38°C in each groups for 24 hr after the administration of subcutaneous injection of yeast suspension.

The drug treatment with extracts at the dose of 100 mg/kg and 200 mg/kg body weight and the administration of Paracetamol decreased the yeast induced body temperature in rats after 2 and 4 hour interval of drug administration. A significant reduction in the yeast elevated rectal temperature was observed in the test drug as compared to control group. The graphical presentation of antipyretic activity of Swertia chirayita has been presented in Fig. 4.7. The impact of the Yeast Induced fever is called pathogenic fever. The antipyretic activity studies revealed that, the methanolic extract of the plant possess a significant antipyretic effect against elevated rectal temperature induced by yeast suspension in rats. The effectiveness of antipyretic activity were observed at doses of 100 mg/kg and 200 mg/kg, and the effects were comparable with the standard drug paracetamol. The antipyretic effects increased with time, upto 4 hr and it was found to be statistically significant when compared with the control group. The present results showed that the Swertia chirayita at dose 200mg/kg, at 4 hr have significant antipyretic activity and can be used as antipyretic drug in fever. Various studies [168] have reported that the presence of alkaloids and flavonoids are responsible for antipyretic activity in the plant. The phytochemical analysis of methanol extract gives the confirmatory results. Hence, the Swertia chirayita plant can be used as harmless antipyretic drug.