CHAPTER VII

ANTIMICROBIAL STUDIES OF SYNTHESISED COMPOUNDS
Medicinal importance of plants has been known from times immemorial. Man has been exploiting nature from a very long time solving his vital problems of hunger and disease. A continued research for medicinal plants during the last several centuries has given an innumerable number of plants which are of great use in the treatment of diseases and promotion of health. Recently Moose has described a number of vegetable drugs that can be used as single drug remedies. The science of medicine has developed in the early stages around those plants which had curative properties. It is said that every disease has a drug in a plant growing in nature.

The International agencies like World Health Organisation (WHO), the International Union for Conservation of Nature and Natural Resources (IUCN), and the World Wild Fund for Nature (WWF) have jointly convened in Chiang Mai, Thailand (21-26 March 1988) an International conference on the conservation of medicinal plants in which experts of health professionals and plant conservation specialists took part to assess the use of medicinal plants in developing and industrialised countries. They recommended the urgent need for conservation of medicinal plants as the indigenous plants grown around the world hold the key for finding new drugs².
Crude plant extracts were used as such or as a decoction in drugs as antidotes to insect and snake bites, as contraceptives, as stimulants, for blood pressures, for uterine contractions etc. In India Col. R.N. Chopra started in 1920 the biological evaluation of indigenous drugs from plants. It is well known that medicinal plants represent a primary source of products for the pharmaceutical industry. The active constituents of plants are being used directly as medicaments and also serve as starting materials for the synthesis of other products. Plants have served as valuable starting materials for drug development even in advanced countries.

Chemotherapy is the treatment of infectious disease by the administration of drugs which are lethal or inhibitory to the causative organisms. The essential requirement of such a drug is that under the condition of use it must be more poisonous to the microbes than it is to the host.

An extensive effort has been made to uncover the new clinically useful antimicrobial agents. Microbiology generally refer to a study of the microscopic life forms that are closely or directly related to human welfare and activity. Activities of the microorganisms are familiar to
man from pre-historic times as decomposition of organic matter, spoiling of food, acetic acid and lactic acid fermentation and degradation of protein with the production of new and desirable flavour in certain foods. The existence of bacteria and other microorganisms was discovered almost 200 years before anything approaching a science of microbiology was evaluated. Microorganisms reproduce very rapidly, they were assigned a name "the seeds of disease" by Lucretieues in his book entitled "Drerum Nature". Francastorices of Vacona in 1546 suggested the Contagenum virum as a cause of disease and Vanplenciz accounted for the specificity of disease on the basis of microbial etiology in 1762. After 1885, in less than 20 years, more progress was made towards a true understanding of microorganisms. It has been suggested by various scientists that since 1940, we have again been in a golden period of advancement in microbiology.

In 1838, Ehrenberg made the first important attempt to classify bacteria. In 1857 the botanist Maegdi suggested the name schizomycetes, for the group of microbes, which means fission fungi. Agostino Bassi in early 1800's proved that the fungus that cause disease in silkworms is known as inuscardin.
Later on various workers successfully isolated various organisms that cause diseases in plant and animal kingdom. The first pure culture technique was developed by Joseph Hirstee in 1878. Later Koch proved that the organism that caused the disease can also be developed as a pure culture. They first used sterile humour of the age of animal as a growth medium. But later they found the other opaque medium such as potato, beef, starch, bread, egg-white, meat etc. are advantage ous. Afterwards the medium was developed by mixing gelatin with peptone solution which liquifies on warming could be sterilized by heating, easily poured into plates and readily solidify on cooling. But it liquified at relatively low temperature (20°C). Then Koch suggested the agar, seaweed extract medium which solidify below 43°C.

In 1943, Osborn screened out 2300 species from 166 families against Staphylococcus aureus and Escherechia coli. Sanders et al. screened out 15 plant extracts which were active against Bacillus subtilus and Escherechia coli. Burkholder et al. have reviewed antimicrobial agents from aquatic sources including some higher plants. Sreelaxmi and Rao studied the antimicrobial activities of the extracts of Dillenia indica against a variety of organisms. Atkinson studied several plant
extracts which were active against *S. aureus* and *S. typhi*. Lucas *et al* \(^{12}\) have contributed more work for antimicrobial screening and reported that the leaves of *Ptelea trifolia* contained substances active against *Mycobacterium tuberculosis* (in vivo). Large scale screening for antimicrobial agents from higher plants was carried out by Mitchel *et al* \(^{13,14}\). They were able to show alcoholic extracts of the stems and leaves had reproducible activity and also systematically fractioned those extracts and concluded that bioactivity was due to the alkaloids. Fong *et al* \(^{15}\) screened out biological activities from 600 plant extracts and concluded that the activity is due to phenolic compounds.

On the basis of Koch's experiments of organism transfer from one media to another, enabled us to say that various drugs can be tested, *in vitro*, for their inhibitory effects against bacteria and fungi. The natural products are known to play an important role against the micro-organisms. Nayak and Rao \(^{16}\) reviewed the antimicrobial studies of plant products. Researches have shown that plant products could be of better use against diseases because they can be easily metabolised into animal system along with curative effects. The antimicrobial drugs from the
plants have largely been investigated by many workers. Many constituents from plants like essential oils \(17-20\), alkaloids \(21-22\), glycosides/saponins \(23-25\), fatty acids \(26-29\) and pentose sugars have been studied thoroughly against number of micro organisms, following Koch's postulates which are mentioned below:

(i) The organism must always be found in the diseased animal but not in the healthy ones.

(ii) The organisms must be isolated from diseased animal and grown in pure culture away from the animal.

(iii) The organisms isolated in pure culture must initiate and reproduce the disease when reinoculated into susceptible animals.

(iv) The organism should be reisolated from the experimentally injected animals.

With the decrease of forests due to increasing human activities like cutting of the forests for providing land for agriculture, industry, shelter etc. to the day-to-day increasing population of the world, there is also depletion of many medicinal plants. Such necessities prompted researchers to prepare biologically active compounds in the laboratories. In 1907 Ehrlich synthesised "arsphenamine" an organic arsenic
compound which is active against the sporochaete of syphilis. Later on several compounds having new types of chemical structures and new derivatives of the active compounds have been prepared to produce better active drugs or the drugs having least or no side effects.

Factors affecting the antimicrobial activity:

The following factors are significant in affecting the antimicrobial activity, in vitro:

(a) Components of the medium.
(b) pH of the medium and environment.
(c) Size of the inoculum.
(d) Stability of the compounds.
(e) Incubation temperature.
(f) Length of incubation.

Considering the importance of the studies leading to the antimicrobial activity determination of natural and synthesised organic compounds, it was felt worthwhile to study the antimicrobial efficiency of the glycosides of the plant seeds and derivatives of rhodanines. Thus the glycosides extracted from the seeds of Anthocephalus cadamba, Erythrina indica, Bischofia javanica and Ailanthus excelsa and the 11 derivatives of rhodanine obtained by condensing various aldehydes and
ketones with rhodanine have been screened for their antimicrobial property against 7 bacteria and 7 fungi. The details of the investigations are given in experimental portion.

The organisms selected for the present study are:

(A) **Bacteria**:

(1) **Bacillus anthracis**: It is a gram positive bacteria. It causes anthrax, an acute specific disease of cattle, sheep and swine. Sometimes workers handling wool and hides of animals are also affected with this disease. It usually occurs as a febrile disease of animals that runs a rapid course and terminates in septicaemia. In man, it causes furuncle which ulcerates and discharges a seropurulent exudate which may heal and disappear or gangrene may set in followed by septicaemia. This usually terminates fatally in about five days.

(2) **Bacillus subtilis**: It is a gram positive bacteria. It is non-pathogenic in nature but sometimes it becomes pathogenic.

(3) **Escherichia coli**: It is a gram negative bacteria. Its shape is plump rod like (1-2 µ in length and 0.6 µ in width). Appendixitic and
peritonite epidermic diarrhoea is caused by certain specific saprophytic species of *Escherichia coli*. It causes urinary tract infections, peritonities, septicaemia and meningitis.

(4) **Klebsiella pneumoniae**: It is a gram negative bacteria, causing diseases associated with respiratory, intestinal and genito-urinary tracts of man. It may also produce otitis media, empyema, pericarditis, meningitis and septicaemia.

(5) **Pasteurella sp**: It is a gram negative bacteria causing plague in man, rats, mice, guinea pig, squirrels and rabbits.

(6) **Salmonella typhimurium**: It is a gram negative bacteria. It causes typhoid, paratyphoid in human beings. It also causes hyperplasia and narcosis of lymphoid tissues, focal nacrosis in liver and inflammation in the gall bladder.

(7) **Staphylococcus aureus**: It is a gram positive non-motile bacteria. A type of food poisoning attributable to indigestion of food is caused due to this bacteria. The disease is characterised by inflammation, narcosis and abscess formation. Every tissue and organ is susceptible to the invasion of this bacteria.
(B) **Fungi:**

(1) *Alternaria sp.* : It is a facultative parasite, mostly causing diseases of plants.

(2) *Aspergillus flavus* : It is saprophytic in nature. It affects the wheat grains. It is a soil borne fungi. Strains of this genus are of industrial importance.

(3) *Aspergillus fumigatus* : Strains of this genus are of industrial importance. It is a pathogenic fungus and it causes aspergillosis in birds and lung infection in animals.

(4) *Aspergillus niger* : It mostly occurs as saprophyte and causes fruit rots of *peragranates*, figs, dates, mangoes and also the decay of stored tobacco cigarettes etc.

(5) *Penicillium notatum* : It causes mostly diseases of the plants.

(6) *Penicillium digitatum* : It is a facultative parasite causing well known diseases of citrus fruits.

(7) *Rhizopus stolonifera* : It is a common black bread mold. It is saprophytic fungi growing over the dead organic material. It causes the spot rot of sweet potato.
**Identification of Organism:**

Bacteria and fungi were identified by using following stains. 31, 32

(1) Periodic acid - Schiff technique.
(2) Gram's stain - Nucker's modification.
(3) Zeihl - Nelson acid fast stain.
(4) Lactophenol, cotton blue stain for fungi.

**Experimental:**

**Antimicrobial Activity (in vitro)** 33, 34

There are many methods to evaluate the antimicrobial property. They are as follows:

(1) Serial dilution tube technique.
(2) Turbidimetric method.
(3) Diffusion method.
   (a) Filter paper disc diffusion method.
   (b) Agar cup method.
   (c) Agar cup cylinder method.
   (d) Agar strip diffusion method.
   (e) Replica method.

For the present study the filter paper disc diffusion plate method 35, 36 has been adopted and its details are given below:
Nutrient culture media used for antibacterial activity

Oxoid culture broth was used for preparing inoculum. It contains the following components:

- Beef extract - 3gm.
- Peptone - 10gm.
- Glucose - 25gm.
- Distilled water - 1000ml.

In the present investigation oxoid nutrient agar medium having the following composition was used for preparing the slants and plates:

- Glucose - 25gm
- Agar Agar - 20gm
- Peptone - 10gm
- Beef extract - 3gm
- Distilled water - 1000ml

Weighed quantities of the dehydrated medium were dissolved in 1000ml hot and freshly distilled water and sterilised by autoclaving at 15 lbs pressure for 30 minutes.

Nutrient culture media used for antifungal activity:

Potato dextrose medium was used for making the inoculum and the medium was prepared by taking the following composition of substances:
Potato slices - 200gm
Dextrose - 25gm
Distilled water - 1000ml.

For the present investigations potato dextrose agar medium having the following composition was used for preparing slants and plates.

Potato slices - 200gm
Dextrose - 25gm
Agar Agar - 20gm
Distilled water - 1000ml.

All the weighed quantities of the dehydrated media and the extract of potato slices were dissolved in 1000ml of hot distilled water and sterilised in an autoclave for 30 minutes at 15 lbs pressure.

Sterilisation:

Sterilisation of petridishes and other material was done by autoclaving at 15 lbs pressure for 30 minutes.

Preparation of Test Samples:

The test samples of rhodanine derivatives, saponins and the standard drugs, griseofulvin for
antifungal activity and streptomycin in antibacterial activity were prepared in dimethyl formamide (4 mg/ml) so as to make 0.4% solution.

**Stock culture and Inoculum:**

The organisms tested were subcultured on the nutrient agar slants. The inoculum of organisms were prepared by transferring a loopful of the corresponding organism from the stock culture into the sterile broth and incubated at 28±1°C for 24 hours in case of bacteria and 37±1°C for 72 hours in the case of fungi. The inoculum was shaken very well to break the colony of the fungi and bacteria and inoculate in the petridish containing nutrient media.

**Test for purity of the Media:**

20ml of potato dextrose agar medium was poured into sterilised petridishes in a bacteria free atmosphere (under laminar floor) and after gelation of the media dishes were incubated at 37°C for 72 hours. The same procedure was also followed with oxoid nutrient medium but the inoculation temperature is 28°C for 24 hours. If medium was found contaminated then it was sterilised again.
Preparation of Paper discs:

The discs of 6mm in diameter were prepared from Whatman no.1 filter paper and were sterilised by dry heat at 140°C for an hour in screw capped bottles. The required discs were taken out with sterile forceps and then moistened with the solution under investigation.

Preparation of Nutrient Plates:

The agar plates for the determination of activity were prepared with the medium which supports the heavy and rapid growth of the microorganisms tested. For the present study the plates were prepared from potato dextrose agar media for fungi and oxoid nutrient agar media for bacteria. 10ml of broth culture of the organisms were added to the cooled (at about 50°C) sterilised agar media and shaken it thoroughly until it becomes homogeneous. 20ml of this homogeneous seeded media was poured into petridishes before getting solidification. The seeded medium was allowed to solidify in petridishes.

Determination of Activity 39, 40

Paper discs moistened with the solution of the rhodanine derivatives and saponin solution were
placed at the centre of the seeded medium. It was pressed so that all parts of the paper disc come in contact with the surface of the medium. The seeded plates were incubated at \(28\pm1^\circ\text{C}\) for 24 hours in case of bacteria and at \(37\pm1^\circ\text{C}\) for 72 hours in case of fungi.

The activity was recorded at one concentration (0.4\%) and the solutions were prepared with D.M.F. Paper disc moistened with D.M.F. was also placed in the plate to see its activity and was found nil against all the organisms tested.

**Recording the Results:**

After incubation of petridishes, the relative susceptibility of the organism to the tested samples was determined by a clear zone of inhibition around the paper disc. The zones of inhibition were measured with the help of a divider to the nearest millimeter. All the tests were conducted in duplicate and the average zone of inhibition in mm are given in Tables 1 to 6. The zones recorded include the size of the filter paper disc i.e 6mm.
Table-1 Antibacterial activity of aldehyde-5-substituted Derivatives of Rhodanine.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the organism tested</th>
<th>C.</th>
<th>Name of the compound</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bacillus subtilis (+)</td>
<td>14 mm</td>
<td>17</td>
<td>15</td>
<td>14</td>
<td>25</td>
<td>16</td>
<td>11</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Bacillus anthracis (+)</td>
<td>18 mm</td>
<td>25</td>
<td>12</td>
<td>9</td>
<td>7</td>
<td>13</td>
<td>17</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Escherichia coli (-)</td>
<td>13 mm</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Klebsiella pneumoniae (-)</td>
<td>19 mm</td>
<td>11</td>
<td>12</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>5.</td>
<td>Pasteurella sp. (-)</td>
<td>20 mm</td>
<td>11</td>
<td>9</td>
<td>18</td>
<td>11</td>
<td>12</td>
<td>16</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Salmonella typhimurium (-)</td>
<td>17 mm</td>
<td>12</td>
<td>16</td>
<td>22</td>
<td>15</td>
<td>11</td>
<td>28</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Staphylococcus aureus (+)</td>
<td>15 mm</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.5</td>
<td></td>
</tr>
</tbody>
</table>

(-) = indicates that the activity is nil.

(*) = Results include the size of paper disc (6mm).

Code - 1, 2, 3, 4, 5, 6, 7 stand for
1. Compound 1 - Formaldine rhodanine.
2. Compound 2 -> Benzaldine rhodanine.
3. Compound 3 -> 4-Chlorobenzaldine rhodanine.
4. Compound 4 -> 3, 4-dimethoxy benzaldine rhodanine.
5. Compound 5 -> Acetaldine rhodanine.
6. Compound 6 -> Salicylaldine rhodanine.
7. Compound 7 -> Anisaldine rhodanine.
8. C -> Standard used - streptomycin.
### Table-2 Antibacterial activity of Ketone-5-substituted Derivatives of Rhodanine.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the organism tested</th>
<th>C</th>
<th>Name of the compound</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bacillus subtilis (+)</td>
<td>14</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>2.</td>
<td>Bacillus anthracis (+)</td>
<td>18</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>3.</td>
<td>Escherichia coli (-)</td>
<td>13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Klebsiella pneumoniae (-)</td>
<td>19</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>5.</td>
<td>Pasteurella sp. (-)</td>
<td>20</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>6.</td>
<td>Salmonella typhimurium (-)</td>
<td>17</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>7.</td>
<td>Staphylococcus aureus (+)</td>
<td>15</td>
<td>-</td>
<td>12</td>
</tr>
</tbody>
</table>

(-) = indicates that the activity is nil.

(*) = Results include the size of paper disc (6mm).

**Code** - 1, 2, 3, 4 stand for

1. Compound 1 -> Benzilidine rhodanine.
2. Compound 2 -> Vanillidine rhodanine.
3. Compound 3 -> Dibenilidine rhodanine.
4. Compound 4 -> Acetophenolone rhodanine.
5. C -> Standard used - streptomycin.
Table 3: Antibacterial activity of saponins and alcoholic extract.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the organism tested</th>
<th>C</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bacillus subtilis (+)</td>
<td>28</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>2.</td>
<td>Klebsiella pneumonia (-)</td>
<td>12</td>
<td>12</td>
<td>18</td>
<td>11</td>
<td>15</td>
</tr>
</tbody>
</table>

(*) = Results include the size of paper disc (6mm).

Code - 1, 2, 3, 4 & C stand for
1. Compound 1 -> Ailanthus excelsa saponin.
2. Compound 2 -> Anthocephalus cadamba saponin.
3. Compound 3 -> Bischofia javanica saponin.
4. Compound 4 -> Erythrina indica alcoholic extract.
5. C -> Standard used - streptomycin.
Table-4 Antifungal activity of aldehyde-5-substituted Derivatives of Rhodanine.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the organism tested</th>
<th>C</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Aspergillus fumigatus</em></td>
<td>11</td>
<td>14</td>
<td>12</td>
<td>10</td>
<td>16</td>
<td>8</td>
<td>9</td>
<td>6.5</td>
</tr>
<tr>
<td>2.</td>
<td><em>Aspergillus niger</em></td>
<td>14</td>
<td>8</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td><em>Aspergillus flavus</em></td>
<td>16</td>
<td>8</td>
<td>7.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>6.5</td>
</tr>
<tr>
<td>4.</td>
<td><em>Alternaria sp.</em></td>
<td>18</td>
<td>9</td>
<td>11</td>
<td>6.5</td>
<td>-</td>
<td>8</td>
<td>11</td>
<td>7.0</td>
</tr>
<tr>
<td>5.</td>
<td><em>Penicillium notatum</em></td>
<td>20</td>
<td>9.5</td>
<td>7</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td><em>Penicillium digitatum</em></td>
<td>24</td>
<td>7</td>
<td>8.5</td>
<td>7</td>
<td>10</td>
<td>9.5</td>
<td>9</td>
<td>6.5</td>
</tr>
<tr>
<td>7.</td>
<td><em>Rhizopus stolonifera</em></td>
<td>10</td>
<td>10</td>
<td>9.5</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>13</td>
<td>9</td>
</tr>
</tbody>
</table>

( - ) = indicates that activity is nil

( * ) = Results include the size of paper disc (6mm.)

Aldehyde 5 - Substituted Rhodanine Derivatives

Code - 1, 2, 3, 4, 5, 6, 7, C, stand for

1. Compound 1 -> Formaldine rhodanine.
2. Compound 2 -> Benzaldine rhodanine.
3. Compound 3 -> 4-Chlorobenzaldine rhodanine.
4. Compound 4 -> 3, 4-dimethoxy benzaldine rhodanine.
5. Compound 5 -> Acetaldine rhodanine.
6. Compound 6 -> Salicylaldine rhodanine.
7. Compound 7 -> Anisaldine rhodanine.
8. C -> Standard used - griseofulvin.
Table-5  Antifungal activity of ketone-5-substituted Derivatives of Rhodanine.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the organism tested</th>
<th>C</th>
<th>Name of the compound</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Aspergillus fumigatus</em></td>
<td>11</td>
<td></td>
<td>10</td>
<td>8</td>
<td>8.5</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td><em>Aspergillus niger</em></td>
<td>14</td>
<td></td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>Aspergillus flavus</em></td>
<td>16</td>
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<td>9</td>
<td>7</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td><em>Alternaria sp.</em></td>
<td>18</td>
<td></td>
<td>9</td>
<td>18.5</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><em>Penicillium notatum</em></td>
<td>20</td>
<td></td>
<td>7</td>
<td>13</td>
<td>10.5</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><em>Penicillium digitatum</em></td>
<td>24</td>
<td></td>
<td>11</td>
<td>6</td>
<td>7.5</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td><em>Rhizopus stolonifera</em></td>
<td>10</td>
<td></td>
<td>12</td>
<td>19</td>
<td>6.5</td>
<td>8</td>
</tr>
</tbody>
</table>

(-) = indicates that the activity is nil.

(*) = Results include the size of the paper disc (6mm.).

**Code** - 1, 2, 3, 4 stand for

1. Compound 1 -> Benzildine rhodanine.
2. Compound 2 -> Vanillidine rhodanine.
3. Compound 3 -> Dibenzilidine rhodanine.
4. Compound 4 -> Acetophenolone rhodanine.
5. C -> Standard used - griseofulvin.
Table-6 Antifungal activity of saponins and alcoholic extract.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the organism tested</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aspergillus niger</td>
<td>31</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Aspergillus flavus</td>
<td>2.5</td>
<td>-</td>
<td>6.5</td>
<td>-</td>
</tr>
</tbody>
</table>

( - ) = indicates that activity is nil.

( * ) = Results include the size of the paper disc (6 mm)

Code - 1, 2, 3, 4 stand for

1. Compound 1 → Ailanthus excelsa saponin.
2. Compound 2 → Anthocephalus cadamba saponin.
3. Compound 3 → Bischofia javanica saponin.
5. C → Standard used - griseofulvin.
Summary and Discussion

The antibacterial studies of rhodanine derivatives have shown good activity against all the strains of bacteria tested. Formaldehyde rhodanine has shown very good activity against *Bacillus subtilis* and *Bacillus anthracis.* Its activity is higher than the standard control streptomycin. It has not shown any activity against *Staphylococcus aureus.* Benzaldehide rhodanine has also shown good activity against *Bacillus subtilis* and *Salmonella typhimurium.* It has not shown any activity against *Escherichia coli* and *Staphylococcus aureus.* 4-chloro benzaldehyde rhodanine has high activity against *Salmonella typhimurium* and its activity is equal to the standard against *Bacillus subtilis* and *Pasteurella sp.* and no activity against *Escherichia coli.* 3,4-dimethoxy benzaldehyde rhodanine has shown very high activity against *Bacillus subtilis* and has not shown any activity against *Escherichia coli,* *Klebsiella pneumoniae* and *Staphylococcus aureus.* Salicylaldine rhodanine has shown good activity against *Salmonella typhimurium* and *Bacillus anthracis* and zero activity against *Escherichia coli,* and *Staphylococcus aureus.* Anisaldine
rhodanine has also shown good activity against *Bacillus subtilis* and *Salmonella typhimurium*. Benzilidene rhodanine has only shown high activity against *Salmonella typhimurium* and nil activity against *Escherichia coli* and *Staphylococcus aureus*. Vanillidene rhodanine has shown very good activity against *Bacillus subtilis*, *Klebsiella pneumonia*, *Salmonella typhimurium* and *Bacillus anthracis* and has shown no activity against *Escherichia coli*. Dibenilidine rhodanine has not shown any activity against *Escherichia coli* and *Klebsiella pneumoniae*. Acetophenolene rhodanine has not shown any activity against all the strains of tested bacteria.

The growth of *Bacillus subtilis* is inhibited by most of the rhodanine derivatives and against *Escherichia coli* and *Staphylococcus aureus*, most of the rhodanine derivatives have not shown significant activity and in many cases the compounds activity is nil.

Antibacterial studies of saponins extracted from seeds of the plants have shown average results i.e., they are active against all strains of bacteria but none of them have high activity than the control used.

The antifungal studies (Table 4 and 5) of 5-substituted rhodanine derivatives have shown
fairly good activity against all the tested organisms. Compound Formaldine rhodanine has shown very good activity as compared to the standard control used. It can prove to be a useful fungicidal agent.

Benzalidine rhodanine has shown average results. Against *Aspergillus fumigatus* fungi it has shown high activity than the control used and with other fungi its activity is below normal. 4-chlorobenzaldine rhodanine and 3, 4-dimethoxy benzaldine rhodanine have shown low activity than the control. Acetaldine and salicylaldine rhodanines have also shown less activity than the standard control used i.e. griseofulvin. Anisaldine rhodanine has shown less activity, and with *Aspergillus niger* and *Penicillium notatum* it has shown no activity. Salicylaldine rhodanine has not shown any activity against only one fungi strain i.e. *Penicillium notatum*. Benzaldine rhodanine has shown good activity against *Aspergillus flavus*, *Rhizopus stolonifera* when compared with the control, but against *Aspergillus niger* it has shown nil activity. Vanillidine rhodanine also has shown higher activity than the standard used against *Alternaria* sp., *Rhizopus stolonifera*, but against *Aspergillus niger* fungi it
has shown zero activity. Dibenzildine rhodanine is active against all strains of fungi but its activity is less compared to the control. Acetophenolene rhodanine activity is not very good with *Aspergillus niger*, *Alternaria* sp. and *Penicillium notatum*.

The antifungal studies of the saponins extracted from the plant seeds have not shown good activity.

The tested rhodanine derivatives inhibited the growth of bacteria more than the growths of fungi. Hence they can prove to be good bactericidal agents.

(2) *Fitoterapia*, LIx, p-365 (1988)


(4) G.N. Mukhopadhyaya, "History of Indian Medicine", Calcutta University (1923)


(6) Free Man, "Text Book of Microbiology", BOBA, W.B. Saunders Co., Toronto, p-297 (1964)


