CHAPTER-5 (PART-B)

Study of anthelmintic activity of the isolated compound MS-I, MS-II, MS-III and MS-IV
Anthelmintics are therapeutic agents that are used to destroy parasitic worm or remove them from the infected host. The majority of helminth infections are acquired by contact with:-

(a) Infected animals
(b) Ground contaminated by human or animals excrement.
(c) Water infected with cercariae
(d) Ingestion of infected meat.

Parasitic worms are dependent upon the host for permanent existence. They must therefore have some method of gaining access to the body of the host and their off springs (either eggs or larvae) must have a means of escaping from the host's body to perpetuate the species; helminth eggs or larvae are generally not able to produce an immediate infection in a new host.

A period of time, varying from a few hours in the case of oxyurids to months in the case of other parasites is necessary before the infective stage to again reached, before preventive measures against the spread of parasitic infections can be taken, an exact knowledge of this critical period in the life cycle of the helminth is essential.

Surveys have shown that one-third of the human race suffers from helminth diseases, of which a large number are multiple infection. Although helminth infections are usually associated with tropical regions more than 40 million Americans are also victims.
So many scientists and pharmacologist are working in this field to make the drugs cheaper and more effective against diseases without any side effect. Working on the same line two plants, which are reported to have anthelmintic activity were chosen (I) Ficus glomerata Roxb (N.O. Moraceae) and (II) Embelia ribes Burm (N.O. Moraceae).

**EXPERIMENTAL**

**ISOLATION OF THE COMPOUNDS**

Air dried and powdered stem bark of Ficus glomerata Roxb. was extracted with 95% ethanol in round bottom flask fitted with a reflux condensor on electric water bath and ethanolic extract was concentrated under reduced pressure. The residue obtained after concentration was partitioned into ethylacetate, chloroform, benzene, acetone, n-hexane and methanol. The methanol soluble fraction when worked up yielded compound MS-I, and MS-II.

Air dried, powdered and defatted seeds of Embelia ribes Burm were extracted with 95% ethanol in round bottom flask fitted with a reflux condensor on electric water bath. The concentrated ethanolic extract was successively extracted with various solvents. The ethyl acetate soluble fraction when worked by column chromatography yielded compounds MS-III whereas methanol soluble fraction yielded compound MS-IV.

A brief account of the isolated compound is tabulated in table.
## Table 5.10

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant</th>
<th>Part of the plant</th>
<th>Isolated compound</th>
<th>Molecular formula</th>
<th>Melting point (°C)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Ficus glo-merata</em> Roxb.</td>
<td>Stem bark</td>
<td>Lupeol-3-O-α-L-xylopyranosyl [1→4]-O-β-D glucopyranoside</td>
<td>C_{41}H_{68}O_{10}</td>
<td>190-191</td>
<td>MS - I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Root</td>
<td>β-Amyrin-3-O-β-glucopyranosyl (1→4)-O-α-Lrhamnopyranoside</td>
<td>C_{42}H_{70}O_{10}</td>
<td>200-202</td>
<td>MS - II</td>
</tr>
<tr>
<td>2.</td>
<td><em>Embelia ribes</em> Burm</td>
<td>Seed</td>
<td>Quercetol-3-O-β-D-arabinopyranoside (5,7,4' Trihydroxy flavone or Quercetol)</td>
<td>C_{20}H_{18}O_{10}</td>
<td>240-241</td>
<td>MS-III</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seed</td>
<td>Stigmasterol-3-O-β-D-arabinopyranosyl [1→4]-O-β-D-glucopyranoside</td>
<td>C_{46}H_{66}O_{10}</td>
<td>190-191</td>
<td>MS-IV</td>
</tr>
</tbody>
</table>

### ANTHelmintIC ACTIVITY OF ISOLATED COMPOUND

The anthelmintic activity\(^{54-56}\) of the compounds was tested on the various species of helminth. A detailed methods described below:
TEST WORMS

Testing of anthelmintic activity the following two species of helminth were taken for this study:

Pheritima posthuma - Earthworm

Taenia solium - Tapeworm

CHEMICAL

Piperazine phosphate and aqueous Tween 80 was included in the assay as standard reference drug as Anthelmintic agents.

EXPERIMENTAL

In the present study, the earthworms (Pheritima posthuma) and tapeworms (Taenia solium) were used and the activity of compound MS-I, MS-II, MS-III and MS-IV was compared with piperazine phosphate.

ACTIVITY AGAINST EARTHWORM AND TAPEWORMS

PREPARATION OF STANDARD AND SAMPLE SOLUTION

Emulsions of compound MS-I, MS-II, MS-III and MS-IV were prepared in Tween 80 in the concentration of 0.5% and 1.0% w/v. Piperazine phosphate was dissolved in normal saline solution to get 0.5% and 1.0% w/v solution.

PROCEDURE

10 ml volume of emulsions were transferred to petri-dishes of 4-inches diameters. Five earthworms washed with
the normal saline solution and placed in each petri-dish containing the emulsions of MS-I, MS-II, MS-III and MS-IV.

Same procedure was followed for controls (Tween 80 solution and piperazine phosphate solution) also.

Another control was maintained with normal saline solution. The movements of earthworms and tapeworm were stimulated and became more marked. The worms tried to get out of the petri-dish. Thereafter, they became progressively sluggish until death supervened. A duplicate was run for all these pentri-dishes.

The time taken for complete paralysis and death was observed. The mean paralysis time and mean death time taken by each drug was recorded. The time taken by the earthworms and tapeworm to become motionless was noted as paralysis time. To know the time taken for that of earthworm and tapeworm the motionless worms, one or more were transferred to hot water (50°C) which stimulated and induced movement of worms they alive. A number of observation were made to confirm the reading and average results were recorded.

RESULTS

The isolated compound MS-I, MS-II, MS-III, MS-IV were investigated for their anthelmintic activity against Earthworm (Phritima posthumia) and Tapeworm (Taenea solium). The results of the present investigation are summarized in the table.
Table 5.11 : Anthelmintic activity of compound MS-I of *Ficus glomerata* Roxb.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test substances</th>
<th>Concentration % (w/v)</th>
<th>Meantime in minute for paralysis and death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Earth worm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paralysis</td>
</tr>
<tr>
<td>1.</td>
<td>Compound Saponin MS-I in Aqueous Tween 80 (3%)</td>
<td>0.5</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>50</td>
</tr>
<tr>
<td>2.</td>
<td>Piperazine phosphate in Normal saline (NaCl)</td>
<td>0.5</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 5.12 : Anthelmintic activity of compound MS-II of *Ficus glomerata* Roxb.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test substances</th>
<th>Concentration % (w/v)</th>
<th>Meantime in minute for paralysis and death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Earth worm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paralysis</td>
</tr>
<tr>
<td>1.</td>
<td>Compound Saponin MS-II in Aqueous Tween 80 (3%)</td>
<td>0.5</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>35</td>
</tr>
<tr>
<td>2.</td>
<td>Piperazine phosphate in Normal saline solution</td>
<td>0.5</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>57</td>
</tr>
</tbody>
</table>
Table 5.13: Anthelmintic activity of MS-III of *Embelia ribes* Burm.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test substances</th>
<th>Concentration % (w/v)</th>
<th>Meantime in minute for paralysis and death</th>
<th>Earth worm</th>
<th>Tape worm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paralysis</td>
<td>Death</td>
</tr>
<tr>
<td>1.</td>
<td>Compound Flavonoidal glycoside MS-III in Aqueous Tween 80 (3%)</td>
<td>0.5</td>
<td></td>
<td>70</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
<td>52</td>
<td>112</td>
</tr>
<tr>
<td>2.</td>
<td>Piperazine phosphate in Normal saline</td>
<td>0.5</td>
<td></td>
<td>54</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
<td>40</td>
<td>116</td>
</tr>
</tbody>
</table>

Table 5.14: Anthelmintic activity of MS-IV of *Embelia ribes* Burm.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test substances</th>
<th>Concentration % (w/v)</th>
<th>Meantime in minute for paralysis and death</th>
<th>Earth worm</th>
<th>Tape worm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paralysis</td>
<td>Death</td>
</tr>
<tr>
<td>1.</td>
<td>Compound Saponin MS-IV in Aqueous Tween 80 (3%)</td>
<td>0.5</td>
<td></td>
<td>70</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
<td>55</td>
<td>128</td>
</tr>
<tr>
<td>2.</td>
<td>Piperazine phosphate in Saline solution</td>
<td>0.5</td>
<td></td>
<td>63</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
<td>52</td>
<td>111</td>
</tr>
</tbody>
</table>
DISCUSSION

The piperazine are present in the market in more palatable from of salts (diphenyl acetate, adipate, citrate, tartrate, dilaurate etc.) which are being used in the treatment of parasitic invasion by helminthes.

Isolated compound MS-I and MS-II of Ficus glomerata Roxb. and MS-III, MS-IV of Embelia ribes Burm exhibited marked anthelmintic activity.

The results revealed that the compounds MS-II and MS-III show significant anthelmintic activity in comparison to compound MS-I and MS-IV and so may potentially be explored as anthelmintic agents.
REFERENCES


