Studies on Moisture Content, Biomass Yield (Crude Plant Extract) and Alkaloid Estimation of *In vitro* and Field Grown Plants of *Rauvolfia serpentina*

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**Abstract:** Phytochemical screening and alkaloid estimation of *in vitro* and field grown plants of *R. serpentina* has been investigated. Under the phytochemical screening moisture content (at 45° and 105°C), biomass yield and alkaloid content in *in vitro* and field grown plant materials were determined. At 45°C, *in vitro* plant materials (leaf, root and callus) have higher moisture content compared with field grown (3 years mature plants) plant materials (leaf and root). But opposite result was observed at 105°C. Percent yield of crude plant extract (Biomass yield) of *in vitro* plant materials was higher than that of field grown plant materials. The percentage of alkaloid for *in vitro* plant materials was 1.17% in callus, 0.80% in root and 0.48% in leaf, respectively. On the other hand it was found that the field grown plant materials were 6.71% in leaf and 1.45% in root, respectively.

**Key words:** *Rauvolfia serpentina*, *in vitro*, field grown, alkaloid, moisture content

**Introduction**

*Rauvolfia serpentina* (L.) Benth. is a perennial herb belonging to the natural family Apocynaceae that is known as an alkaloid producing plant. According to Wink (1993), alkaloids could play a part as nitrogen storage compounds or nitrogen transport substances. Alkaloids could also have a defensive role against phytophagous animals and/or other plants. In the recent trends of plant research, rapid multiplication has gained considerable importance as a promising tool for propagation of medicinal plants and it has already been possible to increase certain type of alkaloids from *in vitro* grown medicinal plants (Haraks, et al., 1995; Varppore, et al., 1995; Wijesna et al., 1997; Payne et al., 1987). Among different types of alkaloids, the indole alkaloids represent a large and diverse group of plant-produced compounds and most of them can be found in the families: Apocynaceae, Loganiaceae and Rubiaceae. Recent phytochemical and pharmacological investigation have shown that the *Rauvolfia serpentina* contains potent biocative compound like, ajmaline, which present the biomass in 0.9-1.8%. It has been shown that quaternary ajmalinum salts with aromatic sulfocetids gave good response like Neo-gilurminal for anarhythmic and antitubalinatic actions (Begova et al., 1996). Again Nikolova et al. (1996) reported that *R. serpentina* tissues kept in vitro have the ability for synthesis of such alkaloids as vomilin in perkin, 17-O-acetylajmaline, 17-O-acetylnorajmaline which have not been discovered in field grown plants.

It is also noted that the process of collection of this valuable alkaloid producing genotype is mainly from wild natural habitat and that is so indiscriminate and extensive. For this reason it becomes a very rare plant in Bangladesh. For conservation and cultivation of this plant genotype we have established a protocol under laboratory conditions (Ahmed et al., 2002).

In the present paper, we present the phytochemical screening including moisture content of crude plant extract and the secondary metabolites (mainly alkaloids) of the field grown and *in vitro* plant materials in order to evaluate their percentage of presence using various biochemical tests.

**Materials and Methods**

**Plant materials:** The field grown plant parts (shoots) were collected from the medicinal plant garden, Pharmacy Department, Rajshahi University, Bangladesh during April, 1999. Subsequently we used *in vitro* grown plant parts as explants. *In vitro* internodes and leaf were used for callus derived plant and shoots were used as a rooted plant. After 8 weeks of culture of the explants callus, leaf and root were taken out from the culture media. All plant parts were washed by tap water. Then they were placed under a fan (on a blotting paper) 2-3 hours to remove water and weighed and packed in polythene bag for further tests.

**Determination fresh weight of field grown plant materials:** The leaves and roots were collected from 3 years old field grown plants in our medicinal plant garden and washed in running tap water. Then they were placed under a fan (on a blotting paper) 2-3 hours to remove water and weighed carefully with an electronic balance.

**Determination of dry weight:** After fresh weight determination, the materials were placed on petri dishes and keep in an oven for 3-4 days at 45°C for drying. Dry weight of the materials was calculated carefully with an electronic balance. Same procedure was also followed for dry weight from different parts (leaves and roots) of the field grown plants.

**Determination of moisture content:** Both *in vitro* and field grown plant materials were heated at 45°C and 105°C in an oven until constant weight was reached and the moisture content was determined using the conventional method (AOAC, 1984).

**Alkaloid extraction:** The dried plant powdered materials of different parts (leaf, root and callus) of *in vitro* and field grown plants were separately extracted, with distilled spirit for 7 days. The solvent was evaporated under reduced pressure at 40°C in a rotary evaporator (Janke and Kunkel, RV 05-ST) to obtain brownish to blackish green resids. Each of the crude extract was suspended into water and extracted with petroleum ether (50ml x 3) using a separating funnel to remove pigments and fatty substances. The organic solvents were combined and evaporated under reduced pressure to obtain greenish masses in each case of plant materials. Then the aqueous layer was separated and extracted with chloroform (50 ml x 3) and the solvent was evaporated under reduced pressure to obtain neutral chloroform extracts. The residual aqueous layer was made acidic (pH 3.0) by adding 1N HCl and extracted with chloroform (50 ml x 3) and the solvent was evaporated under reduced pressure to afford acidic chloroform extract. The remaining acidic aqueous layer was made alkaline (pH 9.0) by adding NH₄OH solution and again extracted with (50 ml x 3) and the solution was evaporated under reduced pressure to obtain a basic chloroform extract. All chloroform extracts were tested for alkaloid using Dragendorff's reagent. The chloroform extracts thus obtained were combined and subjected to a column chromatography with silica gel (60 mesh) and eluted with chloroform, chloroform-methanol with increasing polarity. The fractions that showed the presence of alkaloids in TLC were combined together and solvent was evaporated under reduced pressure to obtain crude alkaloidal mixture.

**Phytochemical screening and alkaloid isolation:** The crude alkaloidal mixture was subjected to TLC (Thin Layer Chromatography)
eluted with ethyl acetate; methanol (4:1) and spraying the plates with Dragendorff's reagent and also examined under UV light that showed the presence of two spots of alkaloids and several minor spots. The mixture was then subjected to PTLC (Preparative Thin Layer Chromatography) for the separation of alkaloid from other minor components. The corresponding bands were scraped off, washed with ethyl acetate and evaporating the solvent under reduced pressure to isolate the total alkaloid present in extract. The same procedure was also followed for isolation of alkaloids from different parts (leaf and root) of the field grown plants.

Results and Discussion

Moisture content of in vitro plant parts at 45°C was 93.60% in callus, 95.38% in root, 87.68% in leaf and on average 92.22%, respectively (Table 1). On the other hand for field-grown plant parts, moisture content was 72.83% in leaf, 69.66% in root and on average 71.24%.

It was also observed that the moisture content in root of in vitro plant materials was highest. While in case of field-grown plant, leaf possesses highest moisture. Moreover, at 45°C in vitro plant material showed higher moisture content compared to field-grown plant materials. We suggest that in vitro plant materials was not so compact as like field grown plant and grown in semi-solid medium that helps to hold more moisture than the field-grown plant parts. Besides, Table 2 showed that moisture content of in vitro plant materials at 105°C 0.8% was in callus, 1.0% in root, 0.4% in leaf and field grown plant parts was 11.0% in leaf and 12.14% in root, respectively. While the average moisture contents were 0.73% for in vitro and 11.57% for field-grown plant materials. This result was vice-versa of 45°C. It may happen due to the fact that at 105°C, the in vitro plant materials loses their moisture very rapidly because their cells are not as compact as in field-grown plant parts.

As shown in Table 3, percentage of biomass yield of in vitro plant materials was 9.37% in callus, 16.66% in root, and 12.5% in leaf, respectively. On the other hand, field-grown plant materials were in leaf 4.29% and in root 3.52%, respectively. It is revealed that the higher % of biomass yield and moisture content at 45°C of in vitro plant materials compared to field grown plant materials may be due to immature cell or cell wall to hold the water and other component within the cell.

Although it is the first report of the moisture content on in vitro and field-grown plant materials of Rauvolfia serpentina, it can give a clue to further study in this direction.

Table 1: Moisture content of various parts of in vitro and field grown plants of Rauvolfia serpentina at 45°C

<table>
<thead>
<tr>
<th>Source</th>
<th>Plant materials</th>
<th>Dry weight (gm)</th>
<th>Final weight (gm)</th>
<th>Loss of weight (gm)</th>
<th>% of moisture content</th>
<th>% of average moisture content</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>Callus</td>
<td>100.0</td>
<td>6.4</td>
<td>93.60</td>
<td>93.60</td>
<td>92.22</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>9.5</td>
<td>0.3</td>
<td>6.2</td>
<td>95.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>6.5</td>
<td>0.8</td>
<td>5.7</td>
<td>87.69</td>
<td></td>
</tr>
<tr>
<td>Field grown</td>
<td>Leaf</td>
<td>69.0</td>
<td>16.3</td>
<td>43.7</td>
<td>72.83</td>
<td>71.24</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>120.0</td>
<td>36.4</td>
<td>83.6</td>
<td>69.66</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Moisture content of various parts of in vitro and field grown plants of Rauvolfia serpentina at 105°C

<table>
<thead>
<tr>
<th>Source</th>
<th>Plant materials</th>
<th>Dry weight (gm)</th>
<th>Final weight (gm)</th>
<th>Loss of weight (gm)</th>
<th>% of moisture content</th>
<th>% of average moisture content</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>Callus</td>
<td>0.5</td>
<td>0.496</td>
<td>0.004</td>
<td>0.8</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>0.05</td>
<td>0.0496</td>
<td>0.0005</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>0.1</td>
<td>0.0596</td>
<td>0.0004</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Field grown</td>
<td>Leaf</td>
<td>5</td>
<td>4.45</td>
<td>0.55</td>
<td>11</td>
<td>11.57</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>14</td>
<td>12.3</td>
<td>1.7</td>
<td>12.14</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Percent yield of crude plant extract (biomass) of Rauvolfia serpentina

<table>
<thead>
<tr>
<th>Source</th>
<th>Plant materials</th>
<th>Weight of dried plant at (45°C) (gm)</th>
<th>Weight of dry extract (gm)</th>
<th>% of yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>Callus</td>
<td>6.4</td>
<td>0.6</td>
<td>5.37</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>0.3</td>
<td>0.05</td>
<td>16.66</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>0.8</td>
<td>0.1</td>
<td>12.5</td>
</tr>
<tr>
<td>Field grown</td>
<td>Leaf</td>
<td>16.3</td>
<td>0.7</td>
<td>4.29</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>36.4</td>
<td>1.0</td>
<td>3.57</td>
</tr>
</tbody>
</table>

Table 4: Total alkaloids isolated from in vitro grown and field grown Rauvolfia serpentina.

<table>
<thead>
<tr>
<th>Source</th>
<th>Plant materials</th>
<th>Dried plant materials at (45°C) (mg)</th>
<th>Combined CHCl3 extract (mg)</th>
<th>Isolated total alkaloid (mg)</th>
<th>% yield of total alkaloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>Callus</td>
<td>6400</td>
<td>600</td>
<td>74.88</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>300</td>
<td>50</td>
<td>2.94</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>800</td>
<td>100</td>
<td>3.92</td>
<td>0.49</td>
</tr>
<tr>
<td>Field grown</td>
<td>Leaf</td>
<td>16300</td>
<td>700</td>
<td>115.73</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>36400</td>
<td>1300</td>
<td>529.52</td>
<td>1.43</td>
</tr>
</tbody>
</table>
medicinal plants are dependent upon soil, climate, season, nature
and intensity of light, day length, stage of growth of plant, etc.
The medicinal quality of a plant or its parts therefore, varies from
sample to sample due to variation in the above (one or more)
factors. But it is more or less constant in vitro grown plant
materials as they are maintained in controlled and sophisticated
conditions.

In conclusion, we suggest that the tissue culture, in vitro
technique can be used to produce more and specific alkaloid. If this
anticipation becomes true, the possibility of the extinction of this
alkaloid producing valuable medicinal plant will come to an end.

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