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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 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, 0.98% in root and 0.49% in leaf, respectively. On the other hand it was found that the field grown plant materials were 0.71% in leaf and 1.43% 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 (1987), alkaloids could play a part as nitrogen storage compounds or nitrogen transport substances. Alkaloids could also have a defense role against phytophagous animals and /or other plants. In the recent tends 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 (Harkes *et al.*, 1985; Verpoorte *et al.*, 1985; Wijnsma *et al.*, 1987; 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 bioactive compound like, ajmaline, which present the biomass in 0.9-1.8%. It has been shown that quaternary ajmalinium salts with aromatic sulfoacids gave good response like neo-gilurytmal for antiarrhythmic and antifibrillatory actions (Sergeeva *et al.*, 1996). Again Nikolaeva *et al.* (1996) reported that *R. serpentina* tissues kept *in vitro* have the ability for synthesis of such alkaloids as vomilenin perkin, 17-0-acetylajmaline, 17-0-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 (Ahmad *et al.*, 2002).
In present paper, we present the phytochemical screening including moisture content of crude plant extract and the secondary metabolites (mainly alkaloid) 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* internode 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 rectified 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 residues. 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 NH_4OH 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 eluded 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)

eluded 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 posses 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 loose thier 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 3.57 %, 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.

However, Table 4 shows the comparison of the alkaloid contents between different parts (callus, root, leaf) of the *in vitro* plant and naturally grown plant parts (leaf and root). It was found that total alkaloid was 1.17 % in callus, 0.98 % in root and 0.49 % in leaf, respectively. On the other hand field-grown leaf and root contain 0.71 and 1.43 % total alkaloid, respectively.

Previously, it was reported that *Rauvolfia serpentina* contained not less than 1% total alkaloids (WHO., 1996). Our findings are similar with the WHO's report. It is also notable that the total amount of alkaloid present in callus is highest among *in vitro* plant parts. This is probably due to better cell contact, ageing and limited differentiation of the cells in callus culture. So it is possible to produce a large quantity of callus within a short span of time (1-2 months) by using tissue culture technique and isolate the alkaloid under laboratory conditions.

Moreover, the production of the secondary metabolites can also be controlled by controlling different factors. That is why it should be quoted that manipulation of cultural conditions and composition of growth factors induced a variation in alkaloid biosynthesis both by callus cultures and regenerated plants (Illahi, 1993). Recently Ghani (1998) has also reported that in the type, number and quantity of the chemical substances present in

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

Source	Plant materials	Dry weight (gm)	Final weight (gm)	Loss of weight (gm)	% of moisture content	% of average moisture content
In vitro	Callus	100.0	6.4	93.60	93.60	92.22
	Root	6.5	0.3	6.2	95.38	
	Leaf	6.5	0.8	5.7	87.69	
Field grown	Leaf	60.0	16.3	43.7	72.83	71.24
	Root	120.0	36.4	83.6	69.66	

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

Source	Plant materials	Dry weight (gm)	Final weight (gm)	Loss of weight (gm)	% of moisture content	% of average moisture content
In vitro	Callus	0.5	0.496	0.004	0.8	0.73
	Root	0.05	0.0495	0.0005	1	
	Leaf	0.1	0.0996	0.0004	0.4	
Field grown	Leaf	5	4.45	0.55	11	11.57
	Root	14	12.3	1.7	12.14	

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

Source	Plant materials	Weight of dried plant at (45°C) (gm)	Weight of dry extract (gm)	% of yield
In vitro	Callus	6.4	0.6	9.37
	Root	0.3	0.05	16.66
	Leaf	0.8	0.1	12.5
Field grown	Leaf	16.3	0.7	4.29
	Root	36.4	1.3	3.57

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

Source	Plant materials	Dried plant materials at (45°C) (mg)	Combined $CHCl_3$ extract (mg)	Isolated total alkaloid (mg)	% yield of total alkaloid
In vitro	Callus	6400	600	74.88	1.17
	Root	300	50	2.94	0.98
	Leaf	800	100	3.92	0.49
Field grown	Leaf	16300	700	115.73	0.71
	Root	36400	1300	520.52	1.43

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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