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Comparative study of alkaloids from different parts of *Rauvolfia tetraphylla*

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Abstract

Rauvolfia tetraphylla is commonly known as Barachandrica, pinque-pinque and barachand. In India it is found in the state of Uttar Pradesh, Bihar, Orissa, Madhya Pradesh, West Bengal, Andhra Pradesh, Mysore, Madras and Kerala. The natural components which were found in *Rauvolfia tetraphylla* were ajmalicine, ajmaline, rescinnamine, reserpine, serpentine, yohimbine. It was also found that ajmaline and serpentine were present in the samples of root, stem, mature leaf, flower stem and flower. It was observed that total alkaloid from different parts of plant ranged from 0.22 to 9.0 %.

Keywords: Alkaloids, *Rauvolfia tetraphylla*, metabolites, Thin layer chromatography

Introduction

Plants are a rich source of unique natural products which are known as secondary metabolites. *Rauvolfia tetraphylla* belongs to the family Apocynaceae. *Rauvolfia tetraphylla* is a native of the West Indies but naturalized in south India (Khare, 2007; Anil Kumar, 2011) [2, 1]. It is an economically important medicinal plant because of the presence of various alkaloids. Alkaloids are low molecular weight nitrogen containing substances which, accumulate in about 20% of all plants species. These substances are important for traits such as flower color, flavor of food and resistance against microbes or herbivores. Many natural flavor compounds such as monoterpenol, C-13 norisoprenoids and skimate derived compound accumulate in fruits as flavorless precursors linked to mono- or di-glycosides and require liberation by enzymatic or acidic hydrolysis (Vasserot *et al.*, 1995) [5]. Due to the importance of numerous alkaloids in medicine and the limited occurrence of plants resources there is increasing interest in the genetic engineering of specific alkaloids (Sangwan and Sangwan, 2000) [3]. Alkaloid is an important natural component of *Rauvolfia*, it consists of a complex hexacyclic structure and almost 10 enzymes are known to participate in its biosynthetic pathway (Stockigt, 1995) [4]. One of these enzymes is β -glucosidase (Warzecha *et al.*, 2000) [6]. The present investigation was carried out to extract the alkaloids from different plant parts of *Rauvolfia tetraphylla*.

Materials and Methods

Plant Material

Different plant parts like Very young leaf, Young leaf, Mature leaf, Old leaf, Flower, Flower stem, Fruit, Stem and Root of *Rauvolfia tetraphylla* were used for the alkaloid extraction.

Chemicals

All chemical were of high analytical grade and purchased from Hi-media.

Alkaloid extraction

For alkaloid extraction, each plant part of the *Rauvolfia tetraphylla* was used. Samples were taken and dried completely. Powder was made by grinding the dried sample. There after, methanol (10 ml) was mixed to the sample. Filtration was done after 12 hrs. This process was repeated three times. Methanol was evaporated and thereafter 1 M dil. HCl was added. The contents were warmed in a water bath for 5 min at 50°C. Thereafter, basification was done by mixing 5M conc. NaOH. This solution was mixed with dichloromethane (20 ml). The separation was done by using separating funnel. This process was repeated three times. Afterwards it, evaporation was done and the dried residue is weighed and expressed as crude alkaloids.

TLC analysis

First of all TLC plate was prepared by layering silica over the plate. Thereafter, it was dried completely in oven. The alkaloids were then spotted on plate with the help of a capillary.

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After it the plate was run in the development jar with solvent for running. After completely running, plate was removed from the jar and it was dried again. Thus the alkaloids obtained were seen in UV light. There after, spraying was done using Dragendroff reagent. R_f values were calculated for different alkaloids by following formula.

$$R_f \text{ value} = \text{Distance migrated by spot} / \text{Total distance}$$

Results and Discussion

Alkaloid Extraction

Total alkaloids were extracted from each part of the plants of *Rauvolfia tetraphylla* by gravimetric estimation of indole alkaloids and also the percentage of crude alkaloids are presented in Table 1.

Table 1: Gravimetric estimation of indole alkaloids from various parts of the plant in *Rauvolfia tetraphylla*

S. No.	Parts	Wt. of tissue (mg)	Wt. of alkaloid (mg)	Yield (%)
1	Fruit	1000	2.2	0.22
2	Flower	20	1.8	9.0
3	Flower stem	685	7.4	1.08
4	Very young leaf	182	13.9	7.64
5	Young leaf	352	15.1	4.28
6	Mature leaf	1000	17.6	1.76
7	Old leaf	185	10.8	5.83
8	Stem	1000	5.8	0.58
9	Root	1000	21.6	2.16

In *Rauvolfia tetraphylla*, total alkaloid from different parts of plant ranged from 0.22 to 9.0 %. Flower and very young leaf have the highest yield of alkaloids 9.0% and 8.17 % respectively, while fruit has the lowest yield of alkaloids, 0.22 %. Among all the four leaves that are very young, young, mature as well as old showed the much variation in the yield of alkaloids as shown in Table 1 and Figure 1, flowers and

very young leaves were rich source of alkaloids having yields as high as 9.0% and 7.64% respectively. It appears that the crude alkaloids isolated from different plant parts had high percentage accumulation. However, HPLC based analysis in such kind of phytochemical studies are preferable as many other non alkaloidal component come in the extract and account for the percentage.

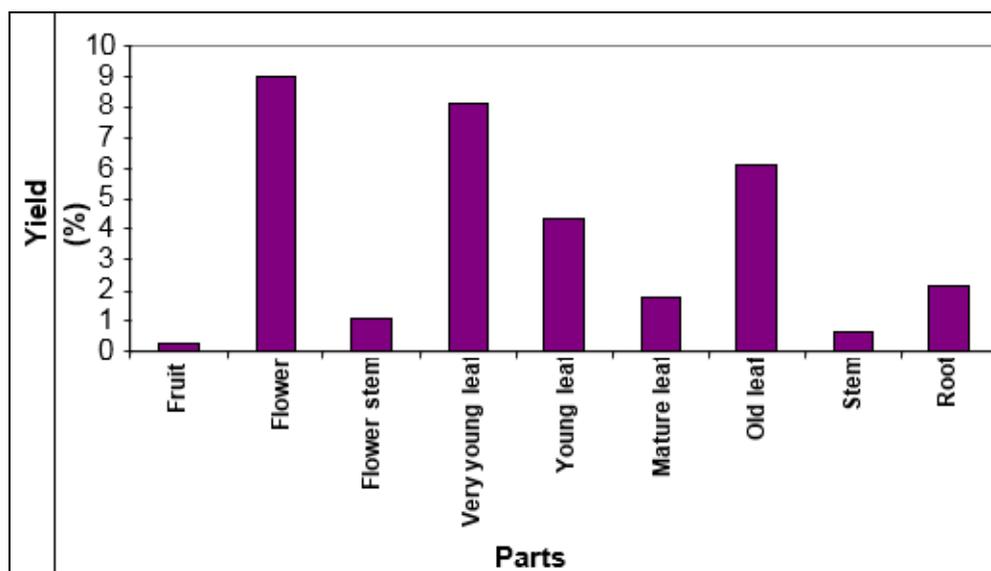


Fig 1: Yield of alkaloids in *Rauvolfia tetraphylla*

In *Rauvolfia tetraphylla*, root was found as the source of ajmalicine, reserpine, yohimbine, ajmaline and serpentine. Other important part in plant *Rauvolfia tetraphylla* was flower

stem, in which reserpine, rescinnamine, yohimbine, ajmaline and serpentine were found as shown in Table 2.

Table 2: Qualitative detection of *Rauvolfia* alkaloid constituents from various parts of the plant in *Rauvolfia tetraphylla*

Parts/ Alkaloids	Root	Stem	Mature Leaf	Flower Stem	Flower
Ajmalicine	+	-	-	-	-
Reserpine	+	-	+	+	-
Rescinnamine	-	-	-	+	+
Yohimbine	+	-	+	+	-
Ajmaline	+	+	+	+	+
Serpentine	+	+	+	+	+

The compositions of alkaloids extracted by TLC analysis are shown Figure 2. In *Rauvolfia tetraphylla*, when samples were analyzed under TLC (Figure 2), it was detected that all the

five alkaloids (except rescinnamine) were present in root samples. The maximum numbers of alkaloids were found in roots of *Rauvolfia* species.

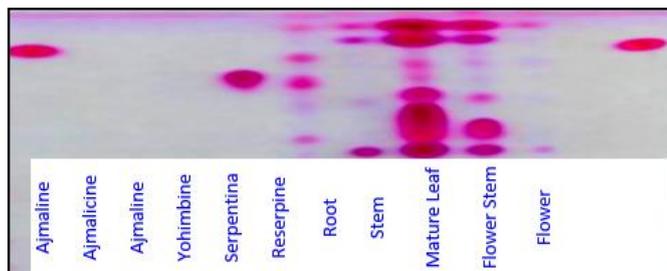


Fig 2: TLC analysis of Alkaloids for *Rauvolfia tetraphylla*

The R_f values were found for different alkaloids as

- R_f (Reserpine) = 0.776,
- R_f (Rescinnamine) = 0.704,
- R_f (Ajmalicine) = 0.856,
- R_f (Yohimbine) = 0.264,
- R_f (Ajmaline) = 0.104,
- R_f (Serpentine) = 0.024.

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