

An Investigation into the Variation in Lipid Composition during Growth and Ageing of Rauwolfia Serpentina Lea

RITESH BHAWSAR

Biotechnology

Sri Satya Sai University Bhopal M.P.

DR. SYED SOHAB AHMAD

Guide Name

Abstract

Since Rauwolfia serpentine has so many beneficial effects, this study used pale-skinned rodents to estimate the pulse, biochemical parameters, and histological engineering of the liver and kidney tissues in order to investigate the antihypertensive and antihyperlipidemic remedial reactions of R serpentine portions.

The lipid composition of Rauwolfia serpentine was examined from the time of leaf emergence through leaf fall. With the exception of the nonpartisan lipids, cell development of all significant lipid classes was associated with the progression to the early development stage; the ratio of leaf monogalactosyl diglyceride to digalactosyl fatty substance decreased from 4.6 (complete development) to 2.5 (early development) (abscised stage). Early development was the first time realistically measuring considerable amounts of free sterols and unsaturated fats was possible. When compared to the fully expanded leaf, the senescent leaf's unsaturated/soaked unsaturated fat fraction was much lower. The specific alterations in lipid composition may resemble synchronous changes in layer ultra form and function, which may be what causes irritation in the tissues of chemically large animal kinds that have alkaloid-seizing ability.

Keywords: *Variation, Lipid Composition, Rauwolfia Serpentina Lea, biochemical parameters*

1. Introduction

An evergreen, enduring, glabrous, and upright underbush known as Chandra, Sargandha, or Rauwolfia serpentina (Apocynaceae) is a restorative herb. It has been estimated to contain 50 different alkaloids, most of which are restricted to the root bark. These alkaloids, which include reserpine, yohimbine, serpentine, deserpidine, ajmalicine, and ajmaline, are used to cure hypertension and breast illness as well as to prevent hazardous reptiles from biting humans and animals and to treat dysentery. When compared to the rough plant extricates, the common sedative reserpine was thought to have a few times more hypertensive action.

Since ancient times, Rauwolfia serpentina has been used as medicine. Apocynaceae is the family in which Rauwolfia serpentina belongs. There were still about 121 chemical compounds that were separated from different healing plants that are used all over the world. 1 The name Rauwolfia serpentina is also known as Chandra in Bengali, Amalpori in Malayalam, Rauwolfia in Hindi, and Hindustani Snake Root in English. It is a sage of beneficial worth distinguished in western and ayurveda systems of healing. Its roots, seeds, leaves, and other organic materials are used to treat a variety of problems. Numerous phytochemicals, such as flavonoids, alkaloids, tannins, and phenols, are found in Rauwolfia serpentina. Since more than a billion years ago, flavonoids have been utilised to treat hypertensive activity and have a wide range of restorative applications. The alkaloid reserpine found in Rauwolfia serpentina is beneficial for treating circulatory strain and other neurological conditions. Reserpine restricts to the catecholamine in the nerve cells to produce its antihypertensive effect. When ideas are combined, the periphery and primary sensory systems are pushed downward. Reserpine also activates the parasympathetic system by depleting the synaptic material in the adrenergic neurons. Numerous neurological conditions, such as vertigo, a sleeping disorder, schizophrenia, and sexual antagonism are all treated with the drug R serpentina. The drug has similar enticing, calming, antihypertensive, and antihypercholesteremic effects. Its leaves and root concentration can be used to treat intestinal worms as well as stomach pain, liver discomfort, and loose stools. Hypertension, arrhythmia, human promyelocytic leukaemia, pneumonia, asthma, Helps, spleen issues, and skin conditions are all treated with it.

An enormous amount of effort has been put forth over the past ten years with the goal of examining crucial to optional metabolite networks for inducing alkaloid production in C. rouses. In any event, the subjective and quantitative synthesis and sequestration of therapeutically and biotechnologically important phytochemicals, such as lipids, in the leaves of Rauwolfia serpentina is still a rapidly developing and interesting area of research.

Lipids are essentially the structural and functional building blocks of plant cells and tissues. They are known to have an impact on particular film characteristics like transport, smoothness, and penetrability. Lipids in particular are sensitive to any change in physiological state as well as biotic and abiotic circumstances. The idea of sterols and the ratio of sterol to phospholipids, as well as the duration and intensity of unsaturated fat instaurations, all have an impact on how easily the film bilayer forms. As intracellular membrane compartmentation and the accessibility of intracellular (non-plasmic and periplasmic) regions improve, some metabolic changes in lipid quantity and composition may be observed. Vacuolar films may play a crucial role in the segregated storage, sequestration, and preservation of some optional metabolites, including alkaloids, since vacuoles are intracellular compartments that have the capacity to serve as gathering locations for some alkaloids.

2. Material and Methods

2.1. Plant material:-

Leaf samples were taken from *Rauwolfia serpentina* filling plants at the CIMAP trial farmhouse in Lucknow, India (26.5° N, 80.5° E, 120 m a.s.l., subtropical zone). When the leaf buds first appeared, they were given unique labels, and 7 days later, the examination process began. Additional samples were obtained every 7 days up until the time of leaf abscission, which occurred 77 days after labelling. The studies were conducted from July through October, a period defined by a day length of 12 to 15 hours and a constant, typical temperature of approximately 30 and 20°C, respectively.

2.2. Lipid extraction and analysis:

Absolute lipids were eliminated using CHCl₃-MeOH 2:1 (v/v) and decontaminated using 0.9% (w/v) NaCl in 0.2 litres of fluid (Matile, et al., 1987). The lipid component of the purge was evaporated to dryness and the accumulation was measured. Not fully settled, as lately portrayed, but completely unsaturated.

By using S-gel CC eluting in grouping with CHCl₃, CH₃)₂CO, and MeOH, lipids were separated into nonpartisan, glyco-, and phospholipids. Nonpartisan lipids, specifically pigments, sterols, and nonconjugated unsaturated fats, were present in the chloroform eluate. Glycolipids were present in the CH₃)₂CO eluate, but phospholipids predominated in the methanol eluate with hints of glycolipids. Every lipid class was quantified according to their dry weight assurance. Separate analyses of the nonpartisan lipid division's free sterols and nonconjugated unsaturated fats were conducted by Stadtman (1957) and Lowry and Tinsley (1976). The glycolipid component was cleaned of subsequent contaminations using the dissolvable framework CHCl₃-CH₃)₂CO MeOH-AcOH-H₂O 5:2:1:1:0.5 (v/v) and attention on SI-gel G plates. Genuine reference guidelines were run simultaneously to distinguish unique glycolipids, and staining with the -naphthol reagent and water shower (used to work with perceptions of clear regions on the tender loving care plates) were both used for identification and introspection. The amount of sugar released following hydrolysis was used to evaluate the separated glycolipids.

2.3. Fatty acid composition:

Methyl esters were prepared to guarantee the content of unsaturated fat. Using an AIMIL-Nucan gas chromatograph equipped with a hardened steel segment (1.8 m x 2 mm, i.d.) of 20% di-ethylene glycol short on Chromos sphere W, unsaturated fat methyl esters were separated and identified (100-120 cross section). Authentic reference norms were used to perform the identification of unsaturated fat methyl esters. By

measuring the level duplicated by the breadth of the top at half pinnacle level, the pinnacle region was identified. Each unsaturated fat's characteristics are listed as a percentage of the total weight of all unsaturated fats.

3. Results and Discussion

Beginning with explicit leaf progress modifications The percentage of leaf tissue that was damp ranged from 65 to 90%. (Table 1). The disintegration of films is one of the distinctive alterations that take place inside the cells of senescent leaves. Modifications in film porousness are reflected in changes to the composition and reliability of the cell layer. Therefore, understanding changes in lipid digestion and the lipid composition of senescent tissue is essential to understanding senescence. The production, transportation, and collection of indole alkaloids in the growing *Rauwolfia serpentine* leaf may be impacted by these intricate metabolic and administrative cell interactions. The current investigation has looked at variations in the lipid content and composition of the *Rauwolfia* leaf from the start of leaf development through leaf drop. Unquestionably, the changes in wetness rate are less obvious than those in new and dry loads (Table 1). The process of senescence is accompanied by a rapid decline in chlorophyll content. In actuality, the degradation of chlorophyll and the concomitant increase in carotenoids are fundamental aspects of senescence.

Table: 1. Leaf changes vs *R. serpentine* development

Leaf Age (Days)	Fresh Weight (mg/leaf)	Dry Weight (mg/leaf)	Leaf Moisture Content (%)
8	22.3	4.8	98
15	32.3	20.5	72
22	87.5	43.5	97
27	337.4	63.6	83
34	673.5	256.6	56
31	324.6	224.5	83
38	213.5	87.7	66
65	386.6	76.4	97
36	348.8	44.7	93
80	332.6	34.4	76
88	273.4	26.7	80

The total lipid content, expressed as g per leaf in Table 2, increased during extension to reach a maximum level of 376 g per leaf at full growth. But despite the progression of senescence and a notable drop in lipid content,

maturing did not involve any noticeable alterations. With the exception of the fact that while maturing and the beginning of senescence, the glycol- and phospholipids fell to a much more noteworthy degree than the impartial lipids, the amount of particular lipid classes per leaf varied horrifically just like all out lipids. Unbiased lipids were discovered to be the most abundant lipid class on the seventh day following multiplication. During the stages of growth, maturation, and senescence, glyco- and phospholipid levels decreased in a slow but predictable rhythm. It was immediately apparent that the amount of unbiased lipids had improved at this point.

Table: 2. R. serpentina had varied lipid distributions overall and by class at various developmental stages. Values are reported as g/leaf and represent the mean SD of three sets of trials, each set having three triplicates.

Leaf Age (Days)	Total Lipids (ug/leaf)	Neutral Lipids (ug/leaf)	Glycolipids (ug/leaf)	Phospholipids (ug/leaf)
8	4.1 ± 0.3	3.8 ± 0.3	2.6 ± 0.3	0.8±0.04
15	14.8 ± 0.6	8.9 ± 0.6	4.5 ± 0.2	2.2 ± 0.08
22	48.7 ± 2.7	24.7 ± 2.8	14.6 ± 0.7	8.4±0.5
27	157.4 ± 2.4	67.3 ± 3.4	27.2 ±2.1	41.4 ± 0.6
34	463.7 ± 4.5	353.3 ± 2.3	56.2 ± 3.5	32.7 ± 2.4
31	264.7 ± 2.2	358.3 ± 2.6	82.2 ±3.7	32.3 ± 0.6
38	263.5 ± 2.2	365.3 ± 2.5	76.2 ± 3.5	23.8± 2.4
65	263.5 ± 3.8	358.6 ± 2.3	72.1 ±3.2	23.5±0.8
36	467.4 ± 3.8	355.5 ± 2.1	72.3 ± 3.2	22.1 ± 0.4
80	464.4 ± 3.2	352.2 ± 3.8	67.3 ±2.1	20.8 ± 0.5
88	344.8 ± 2.6	288.3 ± 3.7	37.3 ± 0.8	7.7 ± 0.5

In general, when compared to the amount in the fully developed leaf, the leaf drop stage exhibited a significant increase in unbiased lipids and a notable decrease in glyco and phospholipids. Particularly, during development and up until the start of senescence, both glycolipids and phospholipids demonstrably decreased. Furthermore, it was significant that the monogalactosyl diglyceride (MGDG)/digalactosyl diglyceride (DGDG) ratio of the *Rouwolfia* leaf (Table 3) decreased from 5.2 (at full development) to 2.5 (at the stage of abscission), which may be indicative of significant changes in the ultra structure, ease, and porousness of the film. An earlier emphasis revealed a more significant reduction in the MGDG/DGDG ratio in salt-focused jojoba leaves. A significant decline in the MGDG/DGDG fraction is also mentioned in a paper on *Duboisia*'s ageing and senescence. It was hypothesized that a change in their extent would likely coincide with a change in the actual characteristics of organellar films. Furthermore, numerous investigations have shown that layers in mature tissues are in a fluid

translucent state, whereas in senescent tissue, the films—or a portion of them—are in a glasslike gel stage at physiological temperatures. In this manner, the adjustment of the layer stage may be due to subjective changes in the film lipids, such as the glycolipids, phospholipids, and MGDG/DGDG ratio.

Table: 3. Changes in glycolipid content in relation to *R. serpentine's* developmental differentiation. Values are reported as g/leaf and represent the mean SD of three sets of trials, each set having three triplicates. Digalactosyl diglycerides, Monogalactosyl diglycerides, and Sulfoquinovosyl diglycerides are all abbreviations for the same thing.

Leaf Age (Days)	MGDG(ug/leaf)	DGDG(ug/leaf)	SQDG(ug/leaf)	MGDG/DGDG Ratio
8	0.6 ± 0.02	0.5 ± 0.02	0.4 ± 0.02	2.3
15	2.3 ± 0.30	2.3 ± 0.03	0.5 ± 0.03	3.2
22	22.2 ± 2.30	2.5 ± 0.32	0.7 ± 0.02	2.2
27	37.3 ± 2.20	6.3 ± 0.32	3.2 ± 0.04	2.8
34	40.2 ± 2.50	22.6 ± 2.20	3.8 ± 0.05	3.4
31	55.3 ± 2.60	15.5 ± 2.34	8.6 ± 0.4	3.5
38	52.2 ± 2.50	23.8 ± 0.82	7.3 ± 0.3	3.8
65	52.7 ± 2.20	22.8 ± 2.22	7.2 ± 0.3	4.3
36	52.2 ± 2.20	24.5 ± 2.25	6.3 ± 0.6	5.4
80	68.2 ± 2.30	23.6 ± 2.08	5.8 ± 0.2	2.8
88	38.2 ± 3.20	22.6 ± 0.84	4.4 ± 0.3	3.4

To improve film penetrability, such spatial variations in the lipid profile have been taken into account. Given that both a change in penetrability and the presence of the gel stage occur at the same time, it is possible that the increase in porousness is partially due to the occurrence of the glass-like gel ease in the film. These variations in layers' capabilities brought on by these layer progressions can also be noticed in the maintenance and collection of auxiliary phytochemicals.

These findings may, undoubtedly to some extent, be connected to variations in alkaloid concentrations during senescence in *R. serpentina* leaf tissue. The spatial mechanism responsible for the sequestration and maintenance of indole alkaloids in leaf tissue is the subject of ongoing research. Additionally, when considered as a whole, the depiction of the lipid profile's ebbs and flows and its emphasis on the quality of metabolic systems for indole alkaloid biosynthesis may be significant for biological research aimed at considering conformational changes in the vacuolar film as a result of development and senescence and their impact on the capacity of indole alkaloids. In this way, work is also in progress.

4. Conclusion

Although there are many commercially available medication products used to treat a wide range of clinical conditions, why have healing plants generally remained the best option due to their high efficacy and security? One of the typical natural medicines with a wide range of beneficial effects is Rauwolfia serpentina. The effects of the current concentrate also revealed the methanolic concentrate of R. serpentina's hypertensive and hypolipidemic effects in rodents with light skin, with almost no injury to the liver and kidney. However, further extensive research is anticipated in order to identify the phytochemical components responsible for its healing and, if any, nearby secondary effects. In fructose-actuated T2D mice, R. serpentina reduces hyperinsulinemia, hyperglycemia, hypertriglyceridemia, and hypercholesterolemia either by preventing fructose absorption in the digestive system or by reducing insulin resistance.

5. References

1. Ben-Rais L, Alpha MJ, Bhal J (1993) Lipid and protein contents of jojoba leave in relation to salt adaptation. *Plant Physiol.* 31:547-557.
2. Bhatara VS, Sharma JN, Gupta S, Gupta YK. Images in psychiatry. *Rauwolfia serpentina: the first herbal antipsychotic.* *Am J Psychiatry* 1997; 154(7): 894.
3. Brown JH, Lynch DV, Thompson JE (1987) Molecular species specificity of phospholipids breakdown in microsomal membrane of senescing carnation flowers. *Plant Physiol.* 85:679-683.
4. Bunkar AR. Therapeutic uses of *Rauwolfia serpentina*. *Int J Adv Sci Res.* 2017;2(2):23–26.
5. Bunney WE, Davis JM. Norepinephrine in depressive reactions: a review. *Arch Gen Psychiatry.* 1965;13(6):483–494.
6. Canel C, Lopes-Cardoso MI, Whitmer S, van der Fits L, Pasquali G, van der Heijden R, Hoge JH, Verpoorte R (1998) Effects of over-production of strictosidine synthase and tryptophan decarboxylase on alkaloid production by cell cultures of *Catharanthus roseus*. *Planta* 205:4-419.
7. Chia LS, Thompson JE, Dumbroff EB (1981) Simulation of the effect of leaf senescence on membrane by treatment with paraquat. *Plant Physiol.* 67:415-420.
8. Droillard MJ, Bureau D, Paulin A (1989) Changes in activities of superoxide dismutase during aging of petals of cut carnations (*Dianthus Caryophyllus*). *Physiol. Plant.* 76:149-155.
9. Duxbury CL, Legge RL, Paliyath G, Barber RF, Thompson JE (1991) Alterations in membrane protein conformation in response to senescence-related changes. *Phytochemistry* 30:63-68.

10. Estevez JM, Cantero A, Reindl A, Reichler S, Leon P (2001) 1-deoxy-D-xylulose-5 phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. *J. Biol. Chem.* 276:22901-22909
11. Gupta J, Gupta A. Isolation and extraction of flavonoid from the leaves of *Rauwolfia serpentina* and evaluation of DPPH-scavenging antioxidant potential. *Orient J Chem.* 2015;31(special issue 1):231–235.
12. Heinz E, Siebertz HP, Linschied M, Joyard J, Douce R (1979). Investigations on the origin of diglyceride diversity in leaf lipids. In Appelqvist LA, Lilgenberg C, eds, *Advances in the biochemistry and Physiology of Plant Lipids*, Elsevier/North- Holland Biomedical Press, Amsterdam pp. 99-120.
13. Klushnichenko VE, Yakimov SY, Tuzova TP, Syagailo YV, Kuzovkina IN, Vul'fon AN, Miroshnikov AI. (1995). Determination of indole alkaloids from *R. serpentina* and *R. vomitoria* by HPLC and TLC methods. *J Chromat* 704: 357362.
14. Koiwai A, Kisaki T (1979). Changes in glycolipids and phospholipids of Tobacco leaves during flue-curing. *Argic. Biol. Chem* 43: 597-602.
15. Koiwai A, Matsuzaki T, Suzuki F, Kawashima N (1981). Changes in total and polar lipids and their fatty acid composition in tobacco leaves during growth and senescence. *Plant Cell Physiol.* 22 : 1059-1065.
16. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. Pune: NiraliPrakashan; 1998; pp 369373.
17. Kumari R, Rathi B, Rani A, Bhatnagar S. *Rauwolfia serpentina* L. Benth. ex Kurz.: phytochemical, pharmacological and therapeutic aspects. *Int J Pharm Sci Rev Res.* 2013;23(2):348–355.
18. Mishra S, Sangwan RS (1996). Dynamics of tropane alkaloid content and composition during developmental and physiological span of *Duboisia myoporoides* leaf. *J. Herbs Spices Med. Plants* 4: 61-70.
19. Pandey V, Cherian E, Patani G. Effect of growth regulators and culture conditions on direct root induction of *Rauwolfia serpentina* L. (Apocynaceae) Benth by leaf explants. *Trop J Pharm Res.* 2010;9(1).
20. Singh R, Singh A, Rath S, Ramamurthy A. A review on sarpagandha-Whole herb v/s reserpine—Its alkaloid in the management of hypertension. *Int Ayur Med J.* 2015;3:565–569.
