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# Formulation And Evaluation of An Herbal Anti – Inflammatory Ointment Containing Rauvolfia Serpentina Extract

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**ABSTRACT**
Rauvolfia serpentina is an important medicinal plant in the pharmaceutical world due to the presence of its immense therapeutic properties. The plant is known for curing various disorders because of the presence of alkaloids, carbohydrates, flavonoids, glycosides, phlobatannins, phenols, resins, saponins sterols, tannins and terpenes. The plant parts, root and rhizome have been used since centuries in Ayurvedic medicines for curing a large number of diseases such as high blood pressure, mental agitation, epilepsy, traumas, anxiety, excitement, schizophrenia, sedative insomnia and insanity. The plant contains more than 50 different alkaloids which belong to the monoterpenoid indole alkaloid family. The major alkaloids are ajmaline, ajmalicine, ajmalimine, deserpidine, indobine, indobinine, reserpine, reserpiline, rescinnamine, rescinnamidine, serpentine, serpentinine and yohimbine. R. serpentina is also known for its antimicrobial, antifungal, anti-inflammatory, anti proliferative, antidiuretic and anticholinergic activities. Herbal medicines are the mainstay of primary health care for 75-80% of the world's population due to their cultural advantages, better compatibility with the human body and fewer side effects. . Therefore, we need to find alternative and natural remedies to treat millions of people around the world. Considering all these properties, this study aims to evaluate the medicinal, phytochemical and medicinal properties of R. spiral. This section on Anti – Inflammatory activity summaries research results and describes the process by which these plant reduce inflammation.

**Keywords**: Anti Inflammatory, Herbal Remedy,Serpentina,Medicinal Plant.

## Introduction:

### About Rauvolfia Serpentina

This plant belongs to the *Apocynacea* family and is usually found in tropical and tropical areas. There are about 50 species in the family that are distributed throughout the world, found in the Himalayan regions, the country of India, Burma, Indonesia and Sri Lanka, and live in India, Bangladesh and other parts of Asia . This plant was named in honor of the German physician and traveler Leonard Raulf, who published an account of his great travels in 1582. The roots of Rauvolfia serpentina (Benth) have been known since ancient times in India. The story of this plant goes back to 1000 years before Christ. Also in the works of Charaka($2^{nd}$ century AD) with the Sanskrit name "Sarpagandha".

The plant is also known by other regional names in India, such as Chandrika(Sanskrit), Chota-chand (Hindi), Chand (Bengali), Dhan-mura or Dhan-barua or Pagla-kadawa (Bihar). ) Chandra, Chota-chand, Karawi or Harkai (Bombay), Harkaya (Marathi), Patala-garud or Atalagandhi (Telugu), Covanamiloori (Tamil), Chuvanaavilppuri (Malay) and as Ahanneria (Uriah) .The roots of Rauvolfia serpentina contain reserpine, one of the 50 alkaloids found in this plant. Reserpine has been found in the roots of six Rauvolfia species (Rauvolfia hookeri, Rauvolfia micrantha, Rauvolfia serpentina, Rauvolfia tetraphylla, Rauvolfia verticillata and Rauvolfia vomitoria). Respine is a drug commonly used in the treatment of high blood pressure, usually in combination with a vasodilator or thiazide diuretic. In India, the roots of this plant are used to treat high blood pressure, insomnia, insomnia, epilepsy, pain, anxiety, anxiety, snakebite, snakebite and mental disorders. In Sida medicine, the roots of Rauvolfia serpentina are used to treat high blood pressure, headache, lethargy, amenorrhea,.Oligomenorrhea and dysmenorrhea are different.According to Rajendran and Agarwal, (2007) the fruits and seeds of this plant have also been used for their botanical and botanical flowers by the people of Tamil Nadu since time immemorial. Of the past. Dymock, India identified the presence of potassium and yellow gum in the roots of Rauvolfia snake. There are two powerful yellow crystal bases called snake and snake. The scientific classification of Rauvolfia serpentine.

**Kingdom: Plantae**
**Branch: Angiosperms**
**Order: Eudicots**
**Class: Asteroids**
**Order: Gentianales**
**Family: Apocynacae**
**Order: Rauvolfia**
**Genus : Serpentine**

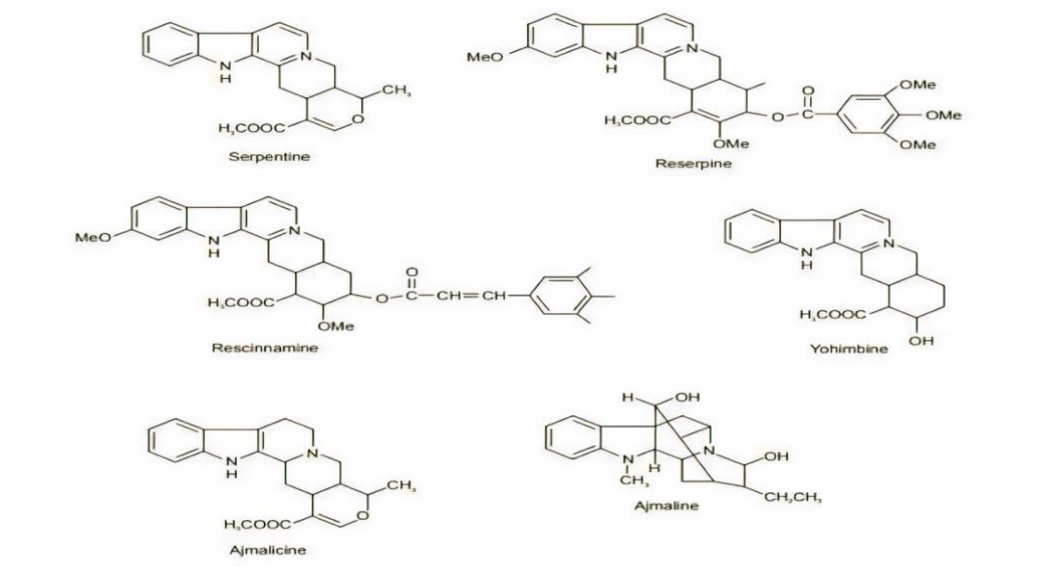

**Chemical Constituents:**

The main compound found in the roots, stems and leaves of the plant is reserpine, from 1.7 to 3.0%. The bark of the root contains more than 90% of the total alkaloids in the root.

There are three species of Rauwolfia serpentina.

1. 1.The weak basic indole alkaloids: The main alkaloids reserpine, resinamine, despyridine are tertiary indole alkaloids.
2. Indoline alkaloids of intermediate base: reserpine, ajmaline, iso-ajmaline, ravolfinine are tertiary indole alkaloids.

3. Strong anhydronium bases: Serpentine, serpentine and elotonine are strong anhydronium alkaloids. Although ajmaline, ajmalysine, chandrine, renoxidine, reserpine, sarpagin, tetraphyllicine, yohimbine, 3-epi-ayohimbine are other alkaloids found in Rauwolfia serpentina. Reserpine is the main alkaloid found in the root, stem and leaves of the plant. It contains at least 0.15% of alkaloids from the reserpine-resin amine group, known as reserpine. The percentage of alkali



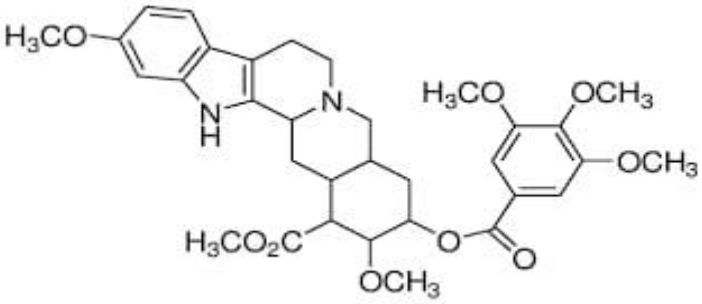

depends on the geographical location of the plant collection and the collection time. Assam samples have more percentage of potassium (2.57%) and Decemberis the best month for collection to increase percentage of potassium.

Alkaloids are organic molecules that contain a heterocyclic nitrogen ring. It is produced by plants andhas many functions, including resistance to weeds and pathogens.

4. R. serpentina alkaloids such as reserpine, ajmaline, ajmalysine, deserpidine, serpentine and yohimbine have medicinal properties. It is used as a pain reliever, sedative, antibacterial agent and a treatment for high blood pressure and breast cancer.
5. Reserpine, the most well-known alkaloid found in R. serpentina, is used as a natural preservative and cancer killer. It affects the central nervous system by reducing the storage of catecholamines and serotonin, thereby relaxing and lowering blood pressure.
6. Ajmaline, derived from R. serpentina, is a sodium channel blocker that has been used to diagnose Brugada disease, an inherited heart disease. It stimulates respiration and gastrointestinal tract and affects systemic and pulmonary blood pressure Chemical structures of some compounds in Rauwolfia serpentina. Ajmalicin is used to treat circulatory disorders and helps lower blood pressure by affecting smooth muscle function and preventing stroke. Serpentine, a topoisomerase inhibitor, has antipsychotic properties. Derived from ajmalysin by oxidation Resin amine, like reserpine, is used to treat high blood pressure. It inhibits angiotensin.
7. Yohimbine, a well-characterized alkaloid, is used as an alpha-adrenergic antagonist in the treatment of erectile dysfunction. It dilates blood vessels and increases blood flow .
8. Phenols, tannins and flavonoids are secondary plant metabolites found in R. serpentina. They have many medicinal properties, including diabetes, blood pressure and antimicrobial effects. Phenols are toxic to pests and pathogens, tannins are astringent and help heal wounds, and flavonoids act as antioxidants and have anticancer activity.

9. The saponins found in R. serpentina are foamy in nature, known for their hemolytic and bitter properties. It has been used to stop bleeding and heal wounds. R. Serpentina also contains minerals such as calcium, zinc and ascorbic acid (vitamin C). Magnesium helps with blood clotting, zinc is good for diabetes management, and ascorbic acid is important for wound healing and body function Overall, R. serpentina is a rich source of substances, minerals, and vitamins, therefore it is a vitamin. May be a source of effective drugs for the treatment of various diseases .

**Serpentine:**




The existence of enormous therapeutic properties makes Rauvolfia serpentina an essential medicinal plant in the pharmaceutical world (Khurshid et al., 2022). Because of theinclusion of alkaloids, carbohydrates, flavonoids, glycosides, phlobatannins, phenols, resins, saponins, sterols, tannins, and terpenes, the plant is used to treat a variety of ailments (Malviya and Sason, 2016). High blood pressure (Lobay, 2015), emotional agitation, epilepsy, traumas, anxiety, excitement, hysteria, sedative insomnia, and insanity (Ali et al., 2022) have all been treated with plant bits, seeds, and rhizomes for centuries in Ayurvedic medicine(Khurshid et al., 2022).Rauwolfia Serpentina is noted to have reserpine, a substance that is used to cure hypertension (Lobay, 2015). In a study by the Department of Chemistry at HNB Garhwal University, they used the roots of Rauwolfia Serpentina against Salmonella Typhimurium, Escherichia Coli (E-Coli), Citrobacter freundii, Proteus vulgaris, Enterococcus faecalis, and Staphylococcus Aureus to quantify the reserpine content of the plant and test the antimicrobial effectiveness of its menthol extract. The menthol extract of Serpentina inhibited the growth of many types of bacteria, and it was concluded that this study supports the traditional information of various herbs (Nagyi et al., 2014). Rauwolfia serpentina has been used medicinally since pre-colonial times due to the high concentration of different alkaloids in the plant. Ajmaline, ajmalysine, ajmalimine, deserpidine, indobine, indobinine, reserpine, reserpine, resin amine, serpentine and yohimbine are the most important alkaloids.

**Pharmacological Effect**:

When bacteria, viruses and fungi enter the body, attach to certain tissues or circulate in the bloodstream, inflammation occurs. Conditions such as stroke, ischemia, cancer, tissue damage and cell death can be caused by inflammation. The innate immune system, which includes mast cells, dendritic cells and macrophages, is the most important defense system against microorganisms and cancer cells. The adaptive immune system consists of more specialized cells such as B and T cells that use antibodies and receptors to fight against infectious diseases and cancer cells . Inflammatory tissue accumulates cells and secretions as a response pattern to insults and to protect against further damage. Scientists have studied inflammation for thousands of years to reduce its effects on inflammation. Celsius wrote the first description of the four symptoms of inflammation in AD 30: swelling, redness, heat and pain, or redness, heat, pain and swelling. Acute inflammation is usually accompanied by fever, erythema, edema, and significant loss of function.

White blood cells, leukocytes and serum enter the tissue to initiate early symptoms. Chronic inflammation leads to a continuous change in the type of cells found at the site of inflammation. The destruction and repair of damaged tissue is seen simultaneously as a result of inflammation. A wide variety of compounds found in plants and combined to modify specific parts of complex biological pathways are the source of herbal medicines. Since ancient times biologically active compounds have been found in various plants. Nowadays, due to toxicity and side effects of allopathic medicines, herbal medicines are used. The mechanism of the anti-inflammatory effect shows that this plant has been used in traditional medicine in South Asia and Greece. The rhizome is also cultivated for medicinal purposes in Brazil, Australia, Africa, China, India, Bangladesh, Taiwan, Mexico, Japan, Jamaica, the Middle East and some parts of the United States. The protein seems to play a minor role in the process of the inflammatory reaction, as molecular studies show that COX-1 mRNA and protein expression do not change in inflammatory sites. TNFα, lipopolysaccharide (LPS), interleukin-1 and other stimuli can activate the inducible enzyme COX-2 when tissue is damaged. Research shows that both types can be produced and influenced by physiological conditions. [1,4]Our primary focus is on carefully selected herbal ingredients that are readily available and have anti-inflammatory properties. The ability to provide new therapeutic compounds or lead molecules for drug development will be of great benefit to the pharmaceutical industry and the scientific community as a whole. The purpose of this review is to provide a comprehensive analysis of traditional medicinal plants. By incorporating experimental studies and current datasets, we aim to clarify the diversity, availability and medicinal uses of these plant materials. In addition to being important for conservation efforts, knowing which plants in the region have anti-inflammatory properties holds promise for drug development and other medical improvements.

**Anti Inflammatory Activity:**

Anti-inflammatory activities Carrageenan-induced paw edema.The animals were divided into ten groups (A – J), six mice each. Group A (control) received 0.5 ml of normal saline (vehicle), group B received indomethacin (10 mg/kg), groups C to J received the same amount of withdrawal. 1 and 10 mg/kg body weight of the extracts. The extracts were dissolved in normal saline while the indomethacin was suspended in 3% Tween 80 in normal saline. Carrageenan solution (0.1 ml of 1%) was injected into the right plantar area of the right leg of rats, 1 hour after intraperitoneal administration of normal saline, indomethacin and extract (Moody et al., 2006). ). Paw size was measured at 1, 3 and 5 hours after drug administration and withdrawal using a vibrating micrometer (SMC-20326, Sterling Manufacturing Company, Ambala Cantt, India).

The efficiency of extraction was calculated using the

**formula:Efficiency (%) = (1 – D/C) × 100.**

Where,

D represents the size of the paw average after removal until the mice were injected, and C was the size of the paws of the negative control animals.

The percentage of inflammation inhibition was calculated from the expression:

% inhibition = D0 – Dt/D0 × 100.

Where D0 is the average inflammation (hindpaw edema) of the control group at a given time. Period; and Dt inflammation in mice treated with the drug (ie, extract or reference indomethacin) at the same time.

**Methods And Preparation :**

**Method**:

**Compatibility study**:

Differential scanning calorimetric thermogram (DSC) of Extract And API optimal formulation recorded in DSC laboratory: Mettler Instrument. For analysis, all samples (2-4 mg) were carefully weighed into a standard aluminum pan and sealed with an aluminum lid. The analysis was performed in the temperature range 0-240 °C with a heating rate of 10 °C / min in a nitrogen atmosphere (30 ml / min)

**Preparation of ointment Formulation:**

The Regulation of Oil Formulation There are two bases of oil with different degrees of water or anhydrous, that is: simple USP oil (T1) and simple IP oil (T2) and the formulation contains paraffin wax combined method . In this method, the base components are assembled in a fryer and allowed to bond together at 70°C. After melting, the material was gently stirred while maintaining the temperature at 70 °C for approx. 5 minutes and cooled to 40 °C with constant stirring. The oils were stirred until smooth and stored at room temperature (25°C) and used for further analysis. An ointment formulation containing 2% salicylic acid was made by adding 2 grams of the drug to the optimized F3 sample by trituration of the oil plate with a spatula to 100 grams. Combination of all oil compositions. All finished oils are evaluated for parameters such as appearance, aroma, color, consistency, pH, dispersion, hardness, water number and viscosity measurement.

**Organoleptic Properties:**

All blank formulations (ie, formulations without active ingredients) and containing pharmaceutical formulations were tested for physical appearance, color, texture, phase separation and consistency. These activities were assessed visually. The consistency and texture were tested by squeezing a small amount of the cream and the formed skin between the thumb and forefinger. The compositional uniformity and presence of fine particles were used to evaluate texture and compositional uniformity. Skin texture (including firmness, roughness and oiliness) was also assessed

**PH:**

About 2.5 grams of all samples were taken in a dry beaker and 50 ml of water was added. The glass in the oil was heated in a water bath with a temperature of 60 to 70 degrees Celsius. The pH value of the oils was determined using a pH meter (pH Trainer, Eutech Instruments). Determination was performed in duplicate and the average of three readings was reported

**Spreadibility:**

The ability to reproduce the pattern by the proposed instrument was determined by Multimer with some modifications. It consists of a wooden blockwith a kick at one end and a glass slide attached to the block. A large amount of oil (3 grams) is also placed on the floor. Oil was applied between this plate and another glass plate of the size of a fixed plate and secured with a hook. A 1 kg weight is placed on top of the two plates for 5 minutes to expel air and create a layer of oil between the plates. A lot of oil was removed from the sides. Then the upper plate is subjected to a stress of 240 grams. With, the screw clamp is attached to the hook, and the time is set to cover the top plate 10 cm away. A shorter time means better playability. Play was calculated using the following

**formula: S = M×L/T**
where, S = play
M = weight in pan (attached to film )
L = length of moving glass plate and
T = time (in seconds) to separate all slides..

**Water value:**
The water value is the maximum amount of water that can be added to 100 grams of base at room temperature. The base was adjusted by adding distilled water with constant stirring. When no more water has entered the base indicated by the water drops, theremaining in the container is called the end point.

**Hardness/Strength**:
Texture hardness was determined using Texture Pro CT V1.3texture analysis (Formula 15 Brookfield Engineering Labs, Inc.). It is based on the speed at which the probe moves to the sample (salve) over a set distance. The probe was moved down at 2 mm/s until a surface trigger of 7 g was reached. At this point, the probe is attached to the sample screen. The probe was then continuously inserted to a depth of 4 mm at a speed of 2 mm/s. At this point, the probe returns to its original position. A needle depth of 4 mm (P/2N) and a constant load capacity of 10 kg were measured to indicate the hardness of the construct. The maximum load (up tostrength) recorded is taken as a measure of the product's strength - the higher the strength, the stronger/stronger the specimen. All tests were performed in duplicate at room temperatMedicinal cure (25±2°C) and peak strength values are expressed in grams. ontent:

**In vitro diffusion :**
study Franz diffusion cell was used for drug diffusion study. The oil was evenly coated on the surface of the cellulose membrane. A cellulose membrane was closed between the donor and the receptor of the diffusion cell. The extraction chamber was filled with phosphate buffer pH 7.4 and the assembly was maintained at 37 ± 0.5 °C under magnetic stirring. According to the guidelines for scale changes and after the approval given by the FDA, 300 mg of oil was applied to the membrane at the point of delivery, then covered with aluminum foil to prevent it from drying out. A small amount was removed from the designated areas for 1 h and the amount of salicylic acid released was analyzed at 275 nm using a UV spectrophotometer.

**In vivo Permeation Study:**
Rat skin was used to clean the skin with a mild cleanser, to remove hair and subcutaneous fat and fascia. Prepared mouse skin was mounted on a Franz diffusion cell (with an effective diffusion surface of 3.14 cm 2 and a cell volume of 7 ml) with the stratum corneum facing up. The receiver chamber was filled with phosphate buffer pH 7.4 and the assembly was maintained at 37 ± 0.5 °C under magnetic stirring. The amount of absorbed drug was transferred through the skin of the rat.

**Stability Study:**
Stability tests for oitment formulations during stability testing have been developed according to guidelines from the International Stability Conference (ICH). The manufactured oil filled in tubes can be stored and maintained under different conditions and humidity, namely 25±2°C / 60±5 RH, 30±2°C /

65±2° C RH. 5% RH and 40 °C ± 2 °C / 75% ± 5% RH for 3 months and study the shape, pH, viscosity and expansion .

**Preparation:**

**Soxhlet Extraction:**

**Procedure:**

1. A small amount of dry sample is placed in a thimble.
2. The sample is continuously washed with a solvent[ Methanol] through a cycle of boiling and condensation.
3. The desired compounds are efficiently extracted into the solvent.

**Column Chromatography**:

Mobile phase – This phase is made up of solvents and it performs the following functions:

1. It acts as a solvent-sample mixture that can be introduced in the column.
2. It acts as a developing agent – helps in the separation of components in the sample to form bands.
3. It acts as an eluting agent – the components that are separated during the experiment are removed from the column
4. Some examples of solvents used as mobile phases based on their polarity are – ethanol, acetone, water, acetic acid, pyridine, etc.

Stationary phase – It is a solid material which should have good adsorption properties and meet the conditions given below:

1. Shape and size of particle: Particles should have a uniform shape and size in the range of 60 – 200μ in diameter.
2. Stability and inertness of particles: high mechanical stability and chemically inert. Also, no reaction with acids or bases or any other solvents was used during the experiment.
3. It should be colourless, inexpensive and readily available.
4. Should allow free flow of mobile phase
5. It should be suitable for the separation of mixtures of various compounds.

**Formula.**

| SR.NO | Name Of Ingredients | Category |
|---|---|---|
| 1. | Herbal Extract { Serpentine} | Plant Extract |
| 2. | Paraffin Wax | Preservatives |
| 3. | Oleic Acid | Permeation Enhancers |
| 4. | Triethanolamine salicylate | Pain Reliever |
| 5. | Water | - |

**Conclusion:**

Rauvolfia serpentine, commonly known as indian snakeroot , has significant medicinal uses. It contains alkaloids like serpentine, which are effective in treating Anti inflammation . Overall Rauvolfia serpentina is a valuable medicinal plant,but its application require careful consideration of benefits and risks.

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