

# Phytochemical Analysis of Leaves Extract of Plant *Skimmia Laureola* Obtained from the High-Altitude Region of Uttarakhand, India

\*<sup>1</sup>Piyush Panthri and <sup>2</sup>Yogita Dobhal

<sup>1</sup>M. Pharm Scholar, Sardar Bhagwan Singh University, Balawala, Dehradun, Uttarakhand, India

<sup>2</sup>Head of Pharmacology Department, Sardar Bhagwan Singh University, Balawala, Dehradun, Uttarakhand, India

\*Email ID: [piyushpanthari990@gmail.com](mailto:piyushpanthari990@gmail.com)

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**Abstract-** The medicinal shrub *Skimmia laureola* (Rutaceae) is recognized for its health benefits, yet little is known about its chemical composition and bioactivity. Ethyl acetate and methanol were utilized in this study to extract the dried leaves, which were then evaluated using both qualitative and quantitative methods. Simple chemical tests detected alkaloids, flavonoids, and phenolic compounds. According to quantitative analysis, each gram of ethyl acetate extract had a total alkaloid content of  $7.11 \pm 4.67$  mg atropine equivalents, total flavonoid content of  $4.16 \pm 3.13$  mg quercetin equivalents, and total phenolic content of  $1.45 \pm 1.21$  mg gallic acid equivalents. The abundance of alkaloids and flavonoids in *S. laureola* is associated with its reported antiinflammation and antimicrobial properties, providing a scientific basis for its traditional uses. This study established a foundation for the potential application of *S. laureola* extracts in the pharmaceutical and nutraceuticals sectors.

**Keywords:** *Skimmia laureola*, Total flavonoid, Total alkaloid, Total phenolic

## Introduction

The pharmaceutical industry has long used plants as a source of new bioactive chemicals, and *Skimmia laureola* has great potential. Flavonoids, alkaloids, and essential oils are abundant in this medicinal plant, adding to its potent therapeutic effects<sup>[1]</sup>. Traditional medicine has used this plant for its therapeutic properties for ages, establishing the foundation for contemporary pharmacological study<sup>[2]</sup>.

### *Skimmia laureola*

It is a species in the Rutaceae family that has a long and illustrious history that begins in the Himalayan region<sup>[3]</sup>. *S. laureola*'s distribution throughout Japan and the Philippine Islands demonstrates its tolerance to a variety of Asian environments<sup>[4]</sup>. In India, western and eastern Himalayan regions, which include states like Jammu & Kashmir, Himachal Pradesh and Uttarakhand are home to the majority of the *Skimmia* genus, especially *Skimmia anquetilia* and *Skimmia laureola*<sup>[5]</sup>. Although they are mostly found between 1700 and 3100 meters above sea level, certain populations can be found at lower elevations in shady ravines, indicating their capacity to adapt to different micro-climates<sup>[6]</sup>. Morphologically, a crucial identifier is simple, alternating, gland-dotted

leaves that emanate an aromatic scent when crushed<sup>[7]</sup>. Its taxonomic distinctiveness is enhanced by the thyriform inflorescences (type of flower cluster where the main stem produces side flower groups one after another, from bottom to top, like a raceme) and polyandry of its flowers<sup>[8]</sup>. They produce drupaceous berries with 1–5 wrinkled pyrenes, a fruit structure atypical for Rutaceae, aiding in taxonomic differentiation<sup>[9]</sup>. Male and female plants are often dioecious, with male specimens historically favored for ornamental cultivation<sup>[10]</sup>.

### Chemical Composition

An article published by *Darmwal et. al 2024* performed the (GC-MS), it is found on analysis of the essential oil extracted from *S. laureola* leaves, has identified 28 constituents. The composition is dominated by monoterpenes which account for over 93.5% of the total oil, whereas sesquiterpenes only make up roughly 0.3%<sup>[11]</sup>.

**Primary Components-** Linalyl Acetate (50.5%) is an important substance with potential medical uses, such as antispasmodic action and fragrant qualities<sup>[12]</sup>. Linalool (13.1%) is known for its antibacterial and sedative qualities<sup>[13]</sup>. Geranyl Acetate (8.5%) and Cis-p-menth-2-en-1-ol (6.2%)<sup>[14]</sup>. These substances contribute to the scent of the oil and may also possess other bioactive properties<sup>[15]</sup>. The plant has variability in composition<sup>[16]</sup>, like the composition of essential oils<sup>[17]</sup> variance is based on environmental factors and geographic location<sup>[18]</sup>. Linalyl acetate, for instance, has been demonstrated to exhibit antispasmodic effects, it has significant antibacterial and antifungal activity against various pathogenic strains, such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*<sup>[19]</sup>. Some Quinoline alkaloids like oxirane and

coumarins like scopoletin glucoside inhibit calcium channels and lower pro-inflammatory mediators like histamine<sup>[20]</sup>. Its effectiveness in a variety of inflammatory diseases is explained by this multi-target mechanism<sup>[21]</sup>.

**Medicinal Use-** The plant possesses many properties that can be used to treat numerous diseases, making it most widely used in research and pharmaceutical uses.

**Antinociceptive Property-** The essential oil of *S. laureola* leaves (SLO) exhibits peripheral analgesic effects by inhibiting prostaglandin synthesis and blocking pain signal transmission<sup>[22]</sup> acetic acid-induced writhing tests, SLO (200 mg/kg) reduced abdominal contractions in mice by 67.2%, comparable to aspirin (64.8%)<sup>[23]</sup>. The oil also increased pain latency in hot plate tests (55.6% at 200 mg/kg), suggesting central analgesic pathways<sup>[24]</sup>.

**Antipyretic Activity-** SLO has shown promising effects in modulating fever responses through its action on the hypothalamus, where it suppresses the levels of prostaglandin E2 (PGE2)<sup>[25]</sup>. PGE2 is a significant mediator of the febrile response, and its reduction can lead to notable decreases in body temperature during hyperthermic conditions<sup>[26]</sup>.

**Anthelmintic Activity-** The anthelmintic activity of *Skimmia laureola* has been extensively studied, especially its essential oils and extracts, which show promising results against parasitic worms<sup>[27]</sup>. In-vitro studies demonstrated significant efficacy against *Haemonchus contortus* adult worms, with essential oils at 50 µL/10 mL causing rapid immobilization and death within hours<sup>[28]</sup>. Ethyl acetate extracts induced paralysis in earthworms within few minutes and

death within few minutes afterwards at 100 mg/mL, comparable to albendazole<sup>[29]</sup>.

## **In - Vitro Anti-inflammatory**

### **Mechanisms**

Human red blood cell (HRBC) membranes are stabilized by *S. laureola* leaf extracts, verified in vitro anti-inflammatory test. At 400 mg/kg, methanol extracts show a 67.53% suppression of hemolysis, outperforming the fractions of ethyl acetate and chloroform<sup>[30]</sup>. Alkaloids like skimminan which interfere with arachidonic acid pathways, are responsible for the concentration-dependent anti-inflammatory effect (up to 90.70% inhibition at 400 mg/mL ethyl-acetate extract)<sup>[31]</sup>.

### **Material and Methods**

There are quantitative and qualitative tests performed on the leaf extract of *Skimmia laureola*, and during the test, various chemical reagents and instruments are required which are mentioned below.

### **Chemicals**

The chemicals used in this research study were methanol and ethyl acetate, which were obtained from the chemical store facility of Sardar Bhagwan Singh University, Dehradun, India.

### **Plant Sample Collection and Identification**

*S. laureola* plant was collected in August 2024 from the national forest department nursery, which was present in high altitude region of Deoban, situated in Chakrata, Uttarakhand, India. The plant was, then taxonomically identified by the Botanical Survey of India.

### **Sample Extraction**

Plant leaves of *S. laureola* were shade-dried at room temperature. The fully dried leaves were

powdered in an electric blender. About 50g of powder of *Skimmia laureola* leaves was inserted in a Soxhlet apparatus, and the solvent extract was siphoned. Afterwards, the solvent obtained was recovered using a distillation assembly to prevent wastage of solvent.

### **Extraction Yield Determination**

The formula shown in **equation-1** helps to determine how much extract material is obtained from given amount of plant material this metric is commonly used to find efficiency of extraction solvent and the method used

$$\% \text{ Yield} = \frac{\text{weight of dry extract after solvent evaporation}}{\text{weight of dry leaves}} \times$$

100

### **Preliminary Phytochemical Screening**

Phytochemical screening was conducted on methanol and ethyl acetate extracts to identify the presence of metabolites, including proteins, amino acids, alkaloids, phenols and flavonoids.

### **Total Phenolic Content**

First, an ethyl acetate extract is made from dried and finely ground plant material to find the total phenolic content of *Skimmia laureola*. The Folin–Ciocalteu reagent, is then added to a determined portion of the extract in test tubes so that it can react with the phenolics of the plant extract<sup>[32]</sup>. To ensure full color development, the mixture is left to incubate for approximately half an hour at room temperature. Then, a UV-Vis spectrophotometer is used to measure the absorbance of the blue complex at about 760 nm. Using a standard, such as gallic acid, a calibration curve is created that helps to link known concentrations to their absorbance measurements<sup>[33]</sup>.

### **Total Flavonoid Content**

One often used technique for determining

the total flavonoid content (TFC) in extracts of *Skimmia laureola* is the colorimetric assay with aluminum chloride. This process involves combining aluminum chloride with a methanolic and ethyl acetate extract of dried, finely crushed plant material. The aluminum chloride then combines with flavonoids to create a stable compound that has a distinctive yellow hue<sup>[34]</sup>. A UV-Vis spectrophotometer is used to measure the absorbance of the colored complex at about 415 nm after it has been incubated at room temperature for a while, usually around 30 minutes. The TFC can then be expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g) by creating a calibration curve with quercetin as a standard<sup>[35]</sup>.

### Total Alkaloid Content

An acid-base extraction followed by a colorimetric estimate is a commonly used technique to ascertain the total alkaloid content in *Skimmia laureola* extracts. An acidic solvent is initially used to extract the dried and finely crushed plant material to aid in solubilizing the plant's alkaloids. After filtering, the alkaloids

are precipitated by adding an appropriate reagent, such as Dragendorff's reagent or bromocresol green, which forms a colored complex with the alkaloids after the extract's pH has been adequately adjusted<sup>[36]</sup>. A UV-Vis spectrophotometer is then employed to detect the color's intensity at a wavelength of approximately 470 nm. To quantify the number of alkaloids in the extract, a calibration curve is created using a recognized alkaloid standard, like atropine<sup>[37]</sup>.

### Results

On conducting qualitative and quantitative tests, all the information is converted into tables, and the formulas and chemicals used are mentioned in the respective sections.

### Qualitative

They are analytical procedures that are designed in a way to detect the presence or absence of a particular class of compounds in your plant extract. **Table-1** shows the result by performing various phytochemical constituents present in the plant extract.

**Table-1 Phytochemical compounds of *Skimmia laureola* identified in screening tests**

|                                | Tests            | Methanol | Ethyl Acetate | Color                  |
|--------------------------------|------------------|----------|---------------|------------------------|
| <b>Alkaloid</b>                | Dragendorff's    | ++       | ++            | Reddish brown ppt      |
|                                | Wagner           | ++       | ++            | Brown/reddish ppt      |
|                                | Mayers           | ++       | ++            | Cream white/yellow ppt |
|                                | Hager            | ++       | ++            | Creamy white ppt       |
| <b>Flavonoid</b>               | Shinoda          | +        | +             | Pink to crimson colour |
|                                | Zn hydrochloride | +        | +             | Magenta                |
| <b>Phenol</b>                  | Ferric chloride  | -        | -             | Green ppt              |
|                                | Iodine sol       | -        | -             | Transient red colour   |
| <b>Protein and amino acids</b> | Million reagents | -        | -             | White ppt              |
|                                | Ninhydrin test   | -        | -             | Deep Blue              |

(+) Depicts the presence, (-) Depicts the absence, + low, ++ high

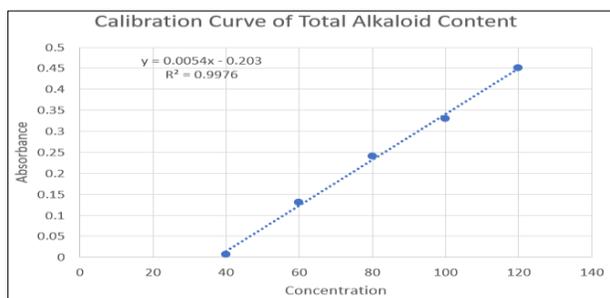
## Quantitative

These tests are conducted to determine the quantity of specific classes of compounds present in plant extracts by measuring the absorbance against a series of known standards. These tests provide an accurate and precise measurement of the compounds' concentrations.

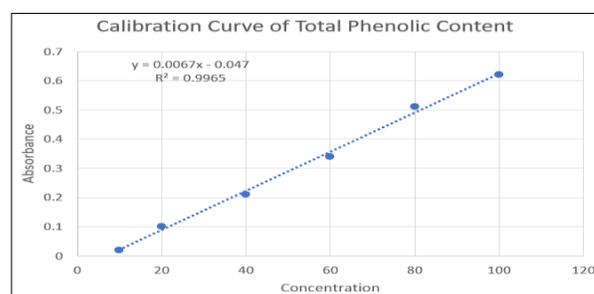
## Discussion and Conclusion

*S. laureola*'s plenty of alkaloids and flavonoids are linked to reported anti-inflammatory and antimicrobial properties, and also has some anti-anxiety effect, giving its traditional uses a scientific basis. These encouraging findings indicate that SLO is an excellent source of bioactive compounds and natural antioxidants.

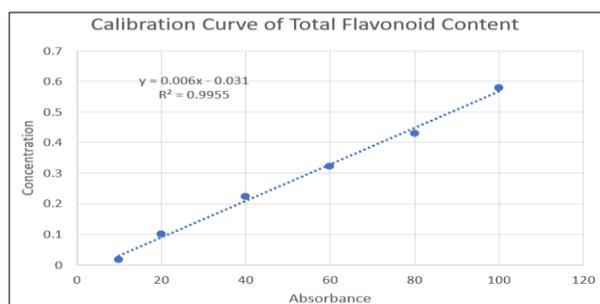
As per the study conducted, the results of qualitative test are shown in **Table-1** and for quantitative the standard curves of alkaloid, flavanoid and phenolic content are shown in **Figure-1, 2** and **3** and the result obtained of plant material are mentioned in **Table-2, 3** and **4**. To support the advancement of plant-based therapeutics, future research will concentrate on separating individual compounds, evaluating their biological activities in animal and laboratory models, and investigating sustainable cultivation techniques. This study laid out the foundation for the possible use of *S. laureola* extracts in pharmaceutical and nutraceutical applications.



**Figure-1** Calibration curve of TAC



**Figure-2** Calibration curve of TPC



**Figure-3** Calibration curve of TFC

**Table-2** Total alkaloid content of solvents

| S. No. | Extracts      | Total Alkaloid Content (% w/w) |
|--------|---------------|--------------------------------|
| 1.     | Ethyl Acetate | 2.67 %                         |
| 2.     | Methanol      | 2.34 %                         |

**Table-3 Total flavonoid content of solvents**

| S. No. | Extracts      | Total Flavanoid Content (% w/w) |
|--------|---------------|---------------------------------|
| 1.     | Ethyl Acetate | 1.49 %                          |
| 2.     | Methanol      | 1.32 %                          |

**Table-4 Total phenolic content of solvents**

| S. No. | Extracts      | Total Phenolic Content (% w/w) |
|--------|---------------|--------------------------------|
| 1.     | Ethyl Acetate | 0.86 %                         |
| 2.     | Methanol      | 0.83 %                         |

Conduct in future bioassay-guided fractionation to isolate and characterize individual phytochemicals responsible for the specialized activities, such as anticancer, antidiabetic, neuroprotective, or cardio-protective effects in targeted bioassays<sup>[38]</sup>.

Perform detailed mechanistic studies at the cellular and molecular levels to identify specific enzymatic or signaling pathways modulated by *S. laureola* constituents<sup>[39]</sup>.

Evaluate the in-vivo pharmacological efficacy of standardized extracts in relevant animal models of inflammation, infection, and oxidative stress<sup>[40]</sup>.

Investigate potential synergistic or antagonistic interactions between *S. laureola* compounds and other herbal or conventional pharmaceuticals<sup>[41]</sup>.

Apply genomics and metabolomics approaches to elucidate the biosynthetic pathways underlying key secondary metabolites and their regulation<sup>[42]</sup>.

Explore endophytic and rhizospheric microbial associations that may enhance phytochemical production or confer additional bioactivities<sup>[43]</sup>.

Formulate nanocarrier-based delivery systems (e.g., nanoparticles, liposomes) to improve the solubility, stability, and bioavailability of active constituents<sup>[44]</sup>.

Implement conservation strategies and germplasm repositories to protect wild populations and support sustainable harvesting practices<sup>[45]</sup>.

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### Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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