

# Comparative Analysis Of Chemical Constituent And Antimicrobial Potential Of *Acorus Calamus* Linn (Sweet Flag) In The Different Geographical Regions Of North Himalayas

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## Abstract

Sweet flag (*Acorus calamus*) is a well-known plant all over the world due to its specific bioactive components  $\beta$ - asarone in plants. The present study reports the quantitative estimation of rhizome oil of the sweet flag plant; obtain from the various district of Uttarakhand, and different geographical surroundings. GC-MS is used to estimate the quantity of rhizome oil. Although leaves are enriched by the several medicinal constituents revealed by the previous studies, rhizome, and the oil it is being investigated. The main component which was identified was Cis-ocimene, Linalool, Trans methyl isoeugenol,  $\beta$  and  $\alpha$ -asarone, and eusarone.  $\beta$ -asarone in the different samples; showed a higher percentage as compared to another component. It was observed that the Gopeshwar region sample had the 74.36 least percentage and the Pauri sample had the 92.22 highest percentage of  $\beta$ -Asarone. Meanwhile, the Gopeshwar region sample had 17.25 highest percentage, and the Pauri sample had 1.57 least percentage of  $\alpha$ -Asarone. Fungal infection is prevalent nowadays. The oil from all regions subjected to antifungal activity against the *Aspergilla fumigates*, *A. Parasiticus*, *Microsporium Canis*, *M. gypsum*, *Cladosporium*, *Aspergillus niger*. The inhibition zone diameter (IZD) against *Aspergilla fumigates*, *A. parasiticus* and was a maximum of 10cm for the Gopeshwar sample, 9cm for the Ramnagar sample as compared to the reference drug gentamicin.

**Keywords:** Sweet flag, Antifungal, Asarone, Uttarakhand.

## INTRODUCTION

The global herbal medicine market was worth USD 185.86 billion in 2020 and is expected to grow at a CAGR (Compound Annual Growth Rate) of 11.32 percent between 2021 and 2028, from USD 230.03 billion in 2021 to USD 430.05 billion in 2028 [1].

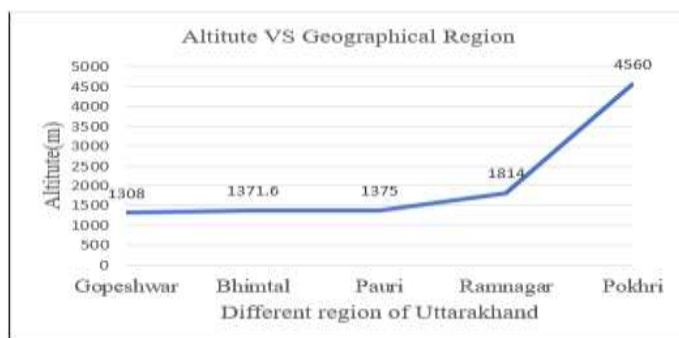
Even though there is no separate category for herbal remedies or dietary supplements, more than half of the population still appears to believe in natural drugs [2]. Sweet flag, *Acorus calamus* Linn, is a relatively uncommon but widespread semi-aquatic plant found in temperate to sub-temperate regions. The leaves are large, erect, and narrow.

Aromatic ascending from an underground rhizome, from within the rhizome is whitish pink in color and pleurably aromatic smelling of citrus, despite possessing a bitter taste. Saber-like foliage. The term "flag" refers to prostrate, iris-like leaves, and the term "sweet" refers to the aromatic odor of the plant [3-4].

## MATERIALS AND METHODS

### Materials

The Rhizome of Sweet flag was collected from different locations of Uttarakhand; selected on the altitude i.e., Gopeshwar, Bhimtal, Pauri, Ramnagar, and Pokhri in the month of December 2021, and identified in the Botanical Survey of India Dehradun, and the voucher specimen deposited at BSI Dehradun Figure 1. All the drugs and chemicals used in the study were of analytical grade. Gentamicin was obtained from Merck Limited, India. Standard cultures of *Aspergilla fumigate*, *Aspergilla Parasiticus*, *Microsporium Canis*, *M. gypsum*, *Cladosporium*, and *Aspergillus niger* were obtained from the School of Life and Applied Sciences, Uttaranchal University-Dehradun.



**Figure 1:** Geographical variation of *Acorus calamus*

### Methods

About 500g of rhizome of the different regions was subjected to sun-dried and crushed into fine powder. Finally, powdered material was subjected to hydro-steam distillation in a Clevenger apparatus for 4-5hrs. The essential oil obtained was dry over anhydrous sodium sulphate and stored in sealed glass vials in a refrigerator at 4-5°C for further use.

### Chemical analysis

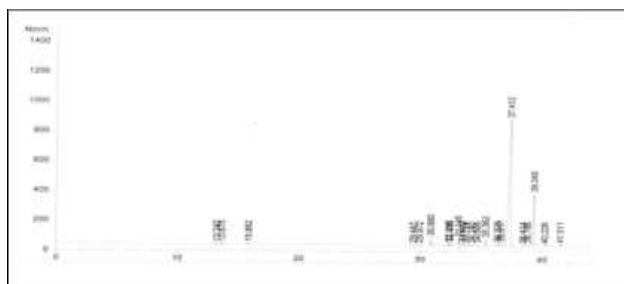
GC-MS Analysis was performed by using Perkin Elmer Clarus 500 GC, connected with PerkinElmer Clarus 500 mass spectrometer fitted with RTX-5 (60m x0.32 mm, 0.25µm film thickness) capillary column. The carrier gas was helium (1ml/ min.) injected temperature was 210°C and the oven temperature was programmed 60-210°C at the rate of 3°C/ min. The constituent of oil was identified by calculation retention indices (RI) under temperature program conditions for n alkanes.

### Anti-fungal assay:

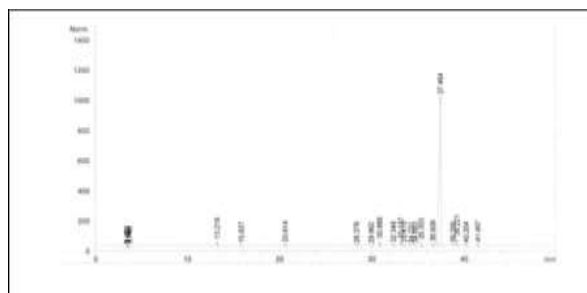
Potato Dextrose Agar (PDA) media was prepared by boiling the crushed potato: dextrose (10:1); sterilized at 121°C for 30 min. The antifungal activity of essential oil was carried out through the filter paper disc diffusion technique. The sterile filter paper disc 4mm diameter was dipped in the essential oil each essential oil disc was placed equidistance on the PDA plate for half an hour at room temperature. Gentamicin (10 µg) was used as the positive control. The inhibition zone of diameter (IZD) formed by each oil sample was recorded after 72 hrs incubation at 27°C.

## RESULT & DISCUSSION

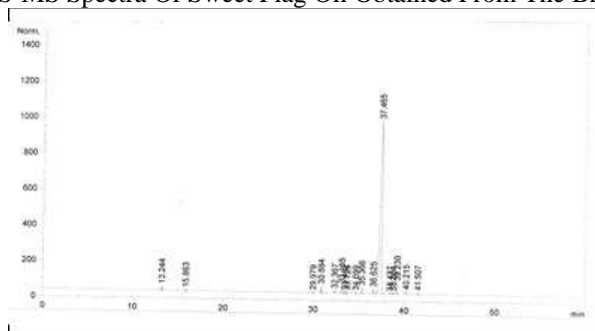
Sweet flag rhizome oil was slightly viscous, yellowish-brownish, and aromatic. The different oil samples were subjected to GC-MS for obtaining the quantitative estimation of various constituents present in different oil samples; to identify the chemotype showing the highest amount of tumor potential component which is β-asarone. The component which was identified was Cis-ocimene, Linalool, Trans methyl isoeugenol, β and α-Asarone, and Euasarone; β-Asarone showed a higher percentage, α-asarone followed the second highest constituent as compared to another component. It was observed that the species found in the Gopeshwar region had the least percentage of β-Asarone and the species of Pauri Garhwal had the highest percentage of β-Asarone. Figure2-6.



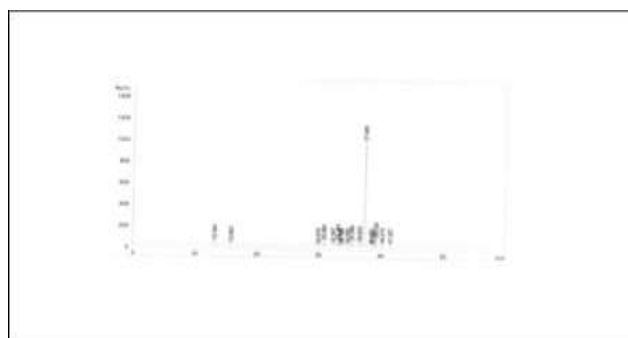
**Figure-2** GS-MS Spectra Of Sweet Flag Oil Obtained From The Gopeshwar Region



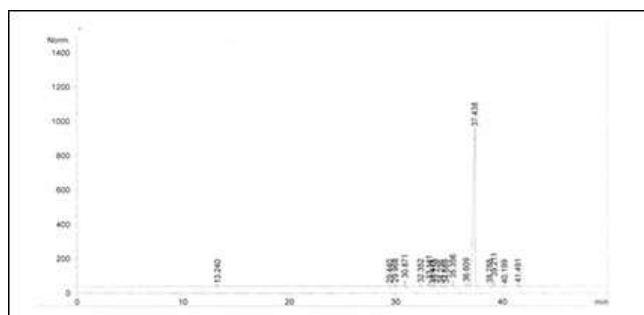
**Figure-3** GS-MS Spectra Of Sweet Flag Oil Obtained From The Bhimtal Region



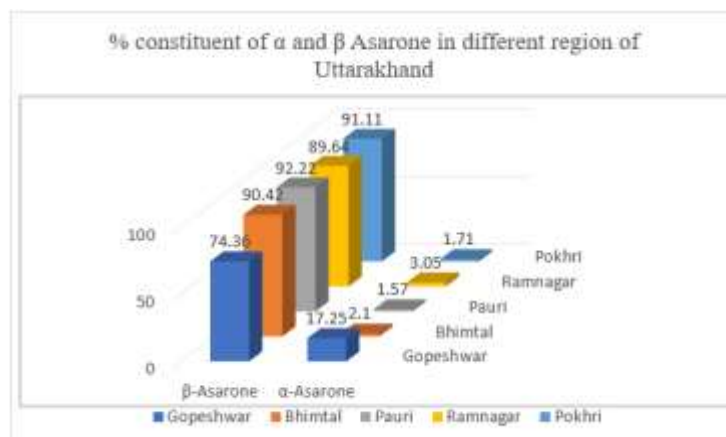
**Figure-4** GS-MS Spectra Of Sweet Flag Oil Obtained From The Pauri Region



**Figure-5** GS-MS Spectra Of Sweet Flag Oil Obtained From The Ramnagar Region



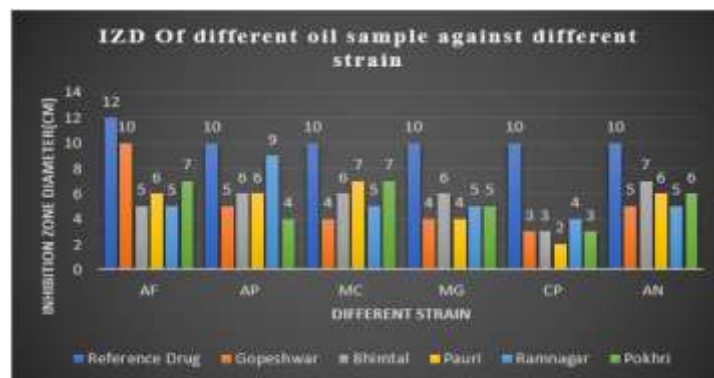
**Figure-6** GS-MS Spectra Of Sweet Flag Oil Obtained From The Pauri Region



**Figure-7** Chemical Constituent Estimation Of Sweet Flag Oil Obtained From Different Regions

Figure.7 reveals the chemical constituent percentage for all regions estimated through GS-MS. It reveals that all samples were enriched with  $\beta$ -asarone in comparison to  $\alpha$ -asarone. It was observed that the species found in the Gopeshwar region had the least percentage of  $\beta$ -Asarone and the species of Pauri Garhwal had the highest percentage of  $\beta$ -Asarone.

The screening of the antifungal activity of the essential oil of the *Acorus calamus* showed that the oil was active to towards all fungal strains. The oil from all five places showed better activity against *Aspergillus fumigates*, *A. parasitizes*, and *Microsporium canis* as compared to other fungal strains.



**Figure-8** IZD (Cm) Of Sweet Flag Oil Obtained From A Different Region

The oil from all five-region showed activity against the strain of fungus. But a sample of Gopeshwar shows tremendous IZD i.e.10 cm against *Aspergilla fumigates*, and the sample of Ramnagar shows very potent IZD i.e., 9 cm against *A. parasiticus* in comparison to the reference drug. Although the sample of pokhri exhibits 7cm IZD against the *A. parasiticus* but i.e., unsatisfactory as others Figure 8.

## CONCLUSION

According to the current investigation, the sweet flag has been used for medicinal purposes by humans since ancient times.  $\beta$ -asarone is neuroprotective, antipyretic, antitumor, and analgesic. It has been shown to have effective antifungal activity against *M. grisea* and *C. orbiculare* infections [5-7]. The sweet flag contains a high concentration of this component. Gopeshwar and Ramnagar study samples were found to have potent antifungal activity against *Aspergilla fumigates* and *Aspergillus parasiticus*, respectively. Unfortunately, there are some barriers to cultivation; therefore, we will move forward to investigate commercial production. It has positive medicinal qualities that would allow it to cross cultural barriers and gain significant use.

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## Authors Contribution Statement

For the concept, assessment and evaluation, data gathering, and data analysis and interpretation, all authors contributed their best work. No conflicts of interest were revealed by the author.

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