



Stomatal Analysis of *Allium wallichii* Kunth Leaves: An Experimental Finding through Quantitative Microscopy

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Abstract

The use of microscopic techniques for the identification of medicinal plants is one of the best practices and an important quality control tool for standardization. Stomata are important organs for the study of phylogenetic relationships including plant origin, evolution, and classification. *Allium wallichii* (*A. wallichii*) Kunth, is one of the threatened medicinal herbs having important nutraceutical, medicinal and therapeutic value. The plant is one of the neglected species of *Allium* and has not been thoroughly investigated pharmacognostically. Therefore, current research work was designed to investigate the stomata types, stomata number and stomatal index through quantitative microscopy. The result presented in the current research will provide a routine identification method for *A. wallichii* with the allied species of *Allium*.

Keywords: *Allium wallichii*, Microscopy, Stomata, Stomatal Index, Quality Control

1. Introduction

Stomata are a pair of specialized cells (guard cells) mostly present in the epidermis of the plant leaves for regulation of the process of transpiration and exchange of gases¹. Both taxonomic and pharmacognostic science reveal the significance of stomata study as an identification tool of different plant taxa including several medicinal plants^{2,3}. Several studies have reported the role of stomata as a distinguishing feature for the proper identification of plants through quantitative estimation⁴⁻⁶. *Allium wallichii* Kunth (Family: *Amaryllidaceae*; locally known as Jimbu or Himalayan onion) is one of the monocot species of the genus *Allium* having high medicinal and culinary value⁷. Certainly, *A. wallichii* (Figure 1) is a higher altitude

perennial herb majorly habitat in India, Bhutan, Nepal, Myanmar and South Western China, respectively⁸⁻¹¹. Additionally, this traditional and ethnobotanical plant is majorly utilized for culinary, nutraceutical, and medicinal purposes (cholesterol level, cholera, cough, cold, infections, snake bite, body pain, cut, hypertension, headache, gastritis, bile problem, carminative, indigestion, sinusitis, skin diseases, mumps, leech removal, intestinal pain, liver diseases, wound and altitude sickness dysentery) by several local communities¹²⁻²⁷. Among secondary metabolites flavonoids, glycosides, steroids, reducing sugars and terpenoids are major class²⁸. More specifically diosgenin, 1, 2 bis (methylthio) ethene, tigogenin, 2, 4 dimethyl thiophene, dimethyl disulfide, and trisulfide are some of the reported phytochemicals during

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past studies^{29–30}. The plant was also revealed to contain carbohydrates, proteins fibre, fat, Ca, Cu, Fe, K, Mn, Rb, S, Si, Zn and vitamin C^{31–33}. Pharmacologically plants have been studied for antimicrobial, anticancer, antioxidant and anti-inflammatory activities^{11,27,28,32,34–38}. For a long, the plant is very useful and highly neglected during current research. Only few studies including pharmacognostic and phylogenetic have been carried out in past^{7,10,39}. However, no any studies so far conducted for revealing the stomata type, stomata number and stomatal index. So herein, we studied and presented the study on stomata type, stomata number and stomatal index of *A. wallichii* leaves through quantitative microscopical method.



Figure 1. Photograph of *Allium wallichii*

2. Material and Methods

2.1 Plant Material

Plant sample of *A. wallichii* was collected from the outskirts of Pitthoragarh (Uttarakhand, India) from August to September, 2021 and identified by Dr. M. C. Bharti (Department of Botany, Hemwati Nandan Bahuguna Central Garhwal University, Srinagar, Uttarakhand, India). Voucher specimens of plant (No: Herbarium/

bot./1070) have been deposited at Department of Botany, Hemwati Nandan Bahuguna Central Garhwal University, Srinagar, Uttarakhand, India.

2.2 Reagents, Chemicals and Instrument

In this current study various analytical grades chemicals and solvents are procured from Merck (Darmstadt, Germany). Camera lucida mirror type (Amtech, India), medical microscope (Aim Scientific, India), Scales: ocular micrometer and stage micrometer (Erma Inc. Japan), black chart paper A4 sheet and silver glitter ball pen or white pencil.

2.3 Microscopical Preparation

Section of leaves to study anatomy was developed including epidermal surface section of the fresh leaves for the microscopical investigations as per the standard methods reported earlier^{3–6}.

2.4 Quantitative Microscopy using Camera Lucida Method

In this method epidermal section were investigated quantitatively for determination of stomata type, stomata number and stomatal index as described in WHO guideline for medicinal plant material.

2.4.1 Determination of Stomatal Number and Stomatal Index

Stomatal number is defined as the average stomata numbers present in per square millimeter of epidermis. Same in proportion percentage with ultimate divisions of the leaf epidermis layer is termed as stomatal index. Following equation can be used for calculation of stomatal index: be

$$I = S / E + S \times 100$$

where, I = stomatal index, S = number of stomata per mm² and E = number of ordinary epidermal cells per mm².

Leaves were cleaned to peeled out both lower and upper epidermis via forceps. Both the epidermis layer were mounted separately on two different slides with glycerine water for microscopic determination. Then, a camera lucida was placed on the microscope and a drawing board with a black sheet was kept for drawing the cells. Using a stage micrometer, a square of 1 mm was drawn on it. Then the stomata type, stomata number and stomatal index of both surfaces were calculated using the above mentioned formula as per methods reported in the past^{40–42}.

3. Result and Discussion

3.1 Quantitative Microscopy

Anomocytic or weakly paracytic class of stomata were found to be present on both the surfaces of leaves (Figure 2). The result reveal in current studies are also supported in an earlier studies using several variety of *Allium* species. As per the study stomatal cell length was found to be 5-7

μm , stomatal cavity length was 3-6 μm , length of long cells was 160-300 μm and with rectangular cell shape⁴³.

3.1.1 Stomatal Number and Stomatal Index

The stomatal number of both the surfaces was calculated and was found to be 4 for the upper surface and 6 for the lower surface, respectively. Subsequently, stomatal indexes of both surfaces were calculated and were found to be 25

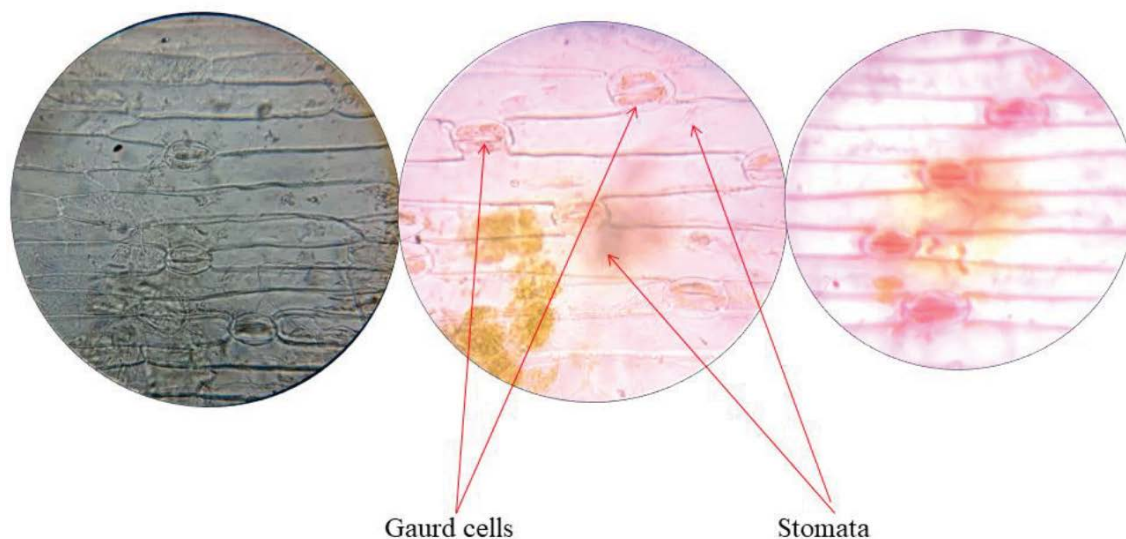


Figure 2. Type of Stomata of *Allium wallichi*

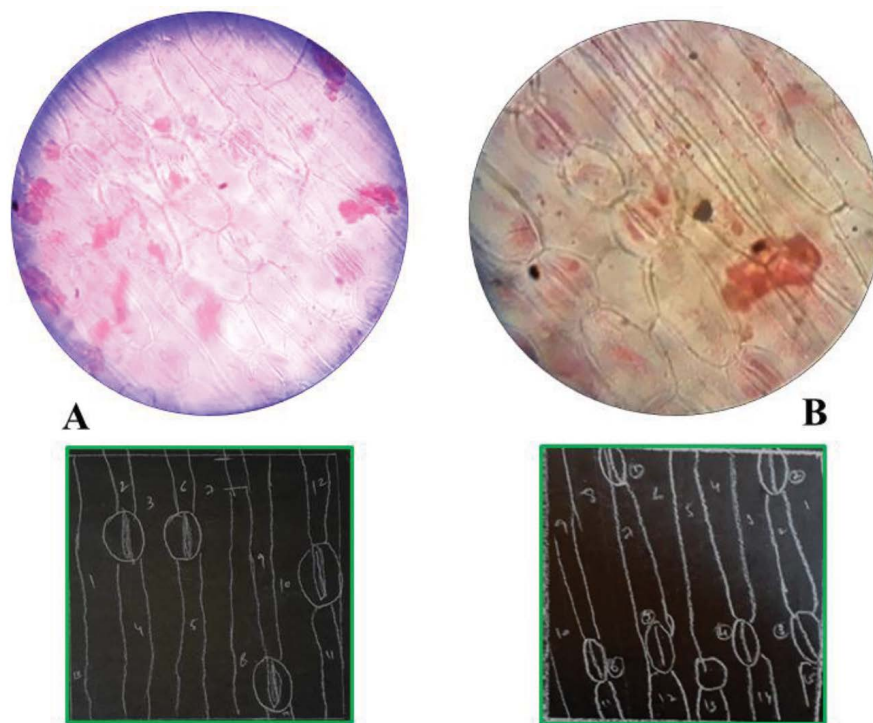


Figure 3. Stomata index of *Allium wallichii* (A. Upper epidermis and B. Lower epidermis)

for the upper surface and 28.57 for the lower surface, respectively (Figure 3). Our results are also aligned, as per earlier report published on stomatal number and stomatal index of any monocot species⁴⁴.

As per many published earlier reports measurement of some unique pharmacognostic features, including stomata number and stomatal index, quantitatively is always useful for setting standards for any crude drug. These values help in the evaluation of the purity of drugs and may act as a reliable standard for selection and identification of raw materials of optimum quality for industry production⁴⁰.

4. Conclusion

The parameters set in the current study can be considered as a distinct feature for identification and setting authenticity of *A. wallichii* in the near future for trade and industry standards.

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