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PALYNOLOGICAL INVESTIGATION FOR TAXONOMIC AUTHENTICATION OF MEDICINAL FLORA FROM THE HINDUKUSH RANGE OF NORTHERN PAKISTAN

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Abstract

The current study sought to evaluate the morphological variations in pollen grains between 38 species of the Hindukush range of Northern Pakistan. A total of 38 taxa have been analyzed using microscopic techniques to examine the qualitative and quantitative characters of pollen. Pollen size, shape, ornamentation, polar diameter, equatorial diameter, exine thickness, colpi length, colpi width and mesocolpium were studied. Pollen grains of the most taxa studied were tricolporate and prolate. Majority of the pollen recorded have small-medium size and its shape were oblate, peroblate. The study declared that people living in the rural areas were primarily dependent on the medicinal plants. In this study 150 peoples were interviewed through random sampling technique. These medicinal plants were used for the treatment of different diseases i.e digestive, respiratory and dermatological problems in Gilgit. Under a Scanning electron microscope reticulate, echinate, psilate, and perforate have been examined. In addition to being a useful tool for specific delimitation, palynological studies can be used as a key for identifications of taxonomic characters on species, genus and family level. Exine ornamentations provide useful identifying traits to differentiate closely related species. Thus, it is evident that pollen morphological characteristics can both be helpful in distinguishing between taxa at a particular level.

Key words: Pollen, Medicinal plants, Taxonomic features, Microscopy, Identifications.

Introduction

Palynology is the scientific study of pollen and spores, practically applied in genetics, forensics and systematic studies (Noor *et al.,* 2017). Palynological studies serve as essential tools across a range of disciplines including melissopalynology, aeropalynology, criminology, paleobotany, allergy research, and stratigraphic correlation of coal and gas deposits in rock formations. The micrograph imaging of pollen using light and scanning electron microscopy are helpful for morphological descriptions and taxonomic implications (Umber *et al.,* 2022; Khan *et al.,* 2023) investigated that pollen morphological study aids in finding out the phylogenetics and systematics information's of angiosperms. Pollen morphological studies offer insights into genetic diversity and adaptive traits critical for crop evolution, aiding in the selection of cultivars with desired genetic traits for improved agricultural practices. The pollen morphological studies investigated via scanning electron microscope were very useful for taxonomic study due to its high resolution of images (Jones & Bryant, 2007; Alam *et al.,* 2023). The palynological features are used for intrageneric and intraspecific classification (Gentry, 1986). This study is conducted to play an important role in the modern taxonomy for identification and delimitation of species. (Sufyan *et al.,* 2018) recorded that pollen size, shape, and ornamentation are very important distinguishing attributes. A palynological study was conducted to elucidate the morphological characteristics of pollen, serving as a crucial tool for plant taxonomists in species delimitation. This investigation aids in identifying and resolving taxonomic challenges spanning from the species to genus level, unraveling intricate relationships within plant classifications (Singh *et al.,* 2020). Exine sculpture, polar diameter, equatorial diameter, P/E ratio, number of colpi and pores are the important features for species identifications. It has made its link directly with aerobiology, forestry, horticulture, archaeology, geology, molecular biology, plant ecology and agriculture.

In Himalayan highly medicinal plants were recorded within the high altitudes (Shinwari *et al*., 2006; Kala & Ratajc, 2012; Shaheen *et al.,* 2023; Tariq *et al*., 2024) Ethnobotanical knowledge has been collected from the local peoples to know about the local uses of plants. (Sivasankari *et al.,* 2014; Shinwari *et al*., 2009) investigated the importance of herbal medicine to be used by the indigenous communities and local peoples in many countries. Ethnobotanical research documents the rich knowledge of cultural interactions between people and plants. It delves into the integration of plants into religious and cultural traditions, unveiling how communities have historically utilized them for diverse purposes, offering profound insights into human-plant relationships across time and geography. (Balick & Cox, 1996; Shinwari *et al*., 2011; Manzoor *et al.,* 2023a; Mirzaman *et al.,* 2023; Gillani *et al.,* 2024a).

In many developing nations, various rural or indigenous communities view herbal medicines as extremely important (Shinwari *et al*., 2013; Sivasankari *et al.*, 2014). Globally, people have a preference for herbal remedies over conventional ones. The World Health Organization estimates that up to 80% of people worldwide rely on traditional medicine, and 60% of Indians who live in rural areas take herbal remedies (Manzoor *et al.*, 2023b; Gillani *et al.*, 2024b; Kayani *et al.*, 2024). The percentage of people using herbal supplements rose from 2.5% to 12% in the past few years (Stickel & Schuppan, 2007). Study of taxonomy and pollen morphology is very important for the scientists working in the field of paleobotany, melissopalynology, aeropalynology, stratigraphic correlations of coal and oil-bearing rocks. In flowering plants, the application of palynology is the best evidence. An array of scientific disciplines, stand to gain valuable insights from the study of pollen morphology and taxonomy. The most outstanding instances of the application of pollen morphology in plant taxonomy are found in flowering plants, particularly in the angiosperms (Attique *et al.*, 2022). The angiosperm plants have the widest range of pollen morphology. Similar to other academic fields, pollen grains play a significant role in the current problem of plant taxonomy (Noor *et al.*, 2024; Sadia B *et al.*, 2024).

Microscopic research serves as a powerful tool for taxonomic studies of traits among closely related genera, aiding in the refinement of taxonomic terminology regarding pollen ornamentation and enabling identification to a finer taxonomical granularity. Attributes such as shape, size, sculpturing, and other morphological characteristics of pollen grains provide invaluable aids in floral taxa identification, with exine sculpturing particularly prized in the realms of taxonomy and systematics (Pospiech *et al.*, 2021). Additionally, it is noted that pollen apertures are a crucial component of pollen sculpturing. Both of them use the fundamental criterion for classifying flora based on pollen apertures and shapes. In the past, palynology was used more broadly and as a tool to identify major plant families and related taxa (Hameed *et al.*, 2020; Noor & Ahmad, 2021). All plants are natural resources with a variety of applications; however, only certain plants have active chemical components that are utilized medicinally (Hazrat *et al.*, 2007). Approximately 400–600 medicinal plants are known to exist in Pakistan and are utilized in the traditional medical system (Hamayun, 2005; Manzoor *et al.*, 2024a). Yaseen *et al*., (2015) investigated that medicinal plant as health benefits against different diseases. Khan *et al*., (2011) reported the phytochemical analysis of important medicinal plants, Shinwari & Khan (2000) investigated 50 plants for the different health benefits. The current study was carried out in order to authenticate and document the medicinal uses of plants using scanning electron microscopy.

For the preservation, production, and protection of herbal medications, medicinal evaluations of plant species are crucial (Vitalini *et al.*, 2013). The Himalayan region meets another challenge, the loss of valuable medicinal and economically significant plant species due to habitat degradation (Shaheen *et al.*, 2024). Hence, it becomes imperative to validate and harness the ethnomedicinal properties of plants within the study area, with a focus on addressing health concerns, disease management, market potential, commercial viability, and raising awareness about pertinent issues. The current study aimed to find out the morphological differentiations within the different taxa of the Himalayan and to examine the palynological features of the related species and genera with each other.

Material and Methods

Sampling and plant identification: The study region was the Hindukush range of Northern Pakistan. The plant specimens were collected during various field trips during July and August 2022. The collected specimens were identified with the help of Botanists, Flora of Pakistan and also compared with already deposited specimens in ISL Herbarium. The plant specimens were dried in newspapers and further preserved using Ethanol and mercuric chloride solution and afterwards mounted on standard sized herbarium sheets. The preserved herbarium specimens were given proper accession number and then submitted to ISL Herbarium of Quaid-i-Azam University.

Light microscopy: Light microscopy serves as an indispensable tool in pollen studies, enabling detailed examination of pollen morphology and facilitating taxonomic classification. With the help of needle, the anthers of flowers were separated and kept on the slides. One drop of acetic acid was put on the slide and crushed the anthers to extract the pollen grains. For staining purposes one drop of glycerin were put on the slide and then covered with a cover slip. The glycerin jelly was prepared for staining using the protocol of Meo & Khan (2005). Using light microscopy different quantitative and qualitative characters of pollen grains were examined (de Oliveira Souza *et al.*, 2021). The pollen photographs were taken at 40X magnification lens using Nikon FX-35 Camera fitted with photo-micrographic system (Ullah *et al.*, 2018). Five readings were taken for each parameter of pollen grain Qualitative features include; polar diameter, equatorial diameter, colpi length, colpi width, exine thickness, spine length and spine width. The qualitative features of pollen include colpi shape, exine sculpture and pollen shape.

Scanning electron microscopy (SEM) of melliferous pollen: Scanning Electron Microscopy was employed to study pollen morphology, providing high-resolution images for detailed analysis. The pollen grains were separated from the anthers, treated with 45% acetic acid, crushed and then transferred to the SEM stubs. The actolyzed pollen grains were sputtered with a gold palladium coating. Then, peculiarities of pollen grains and their surface ornamentation were observed under a scanning electron microscope in the laboratory (Butt *et al.*, 2018). The comprehensive terminology of Punt *et al.*, (2007) was employed to describe the pollen characters. Palynological investigation of melliferous plants was studied using scanning electron microscopy (Nabila *et al.*, 2022). For SEM investigations, pollen grains were detached from anthers, treated with a few drops of 45% acetic acid, and then crushed and directly mounted on prepared stubs. After that, sputtering of acetolyzed pollen samples was done with a gold palladium coating. Then, peculiarities of pollen grains and their surface ornamentation were observed under a scanning electron microscope in the laboratory (Butt *et al.*, 2018). The descriptive terminology of Punt *et al*., (2007) was employed to describe the pollen features.

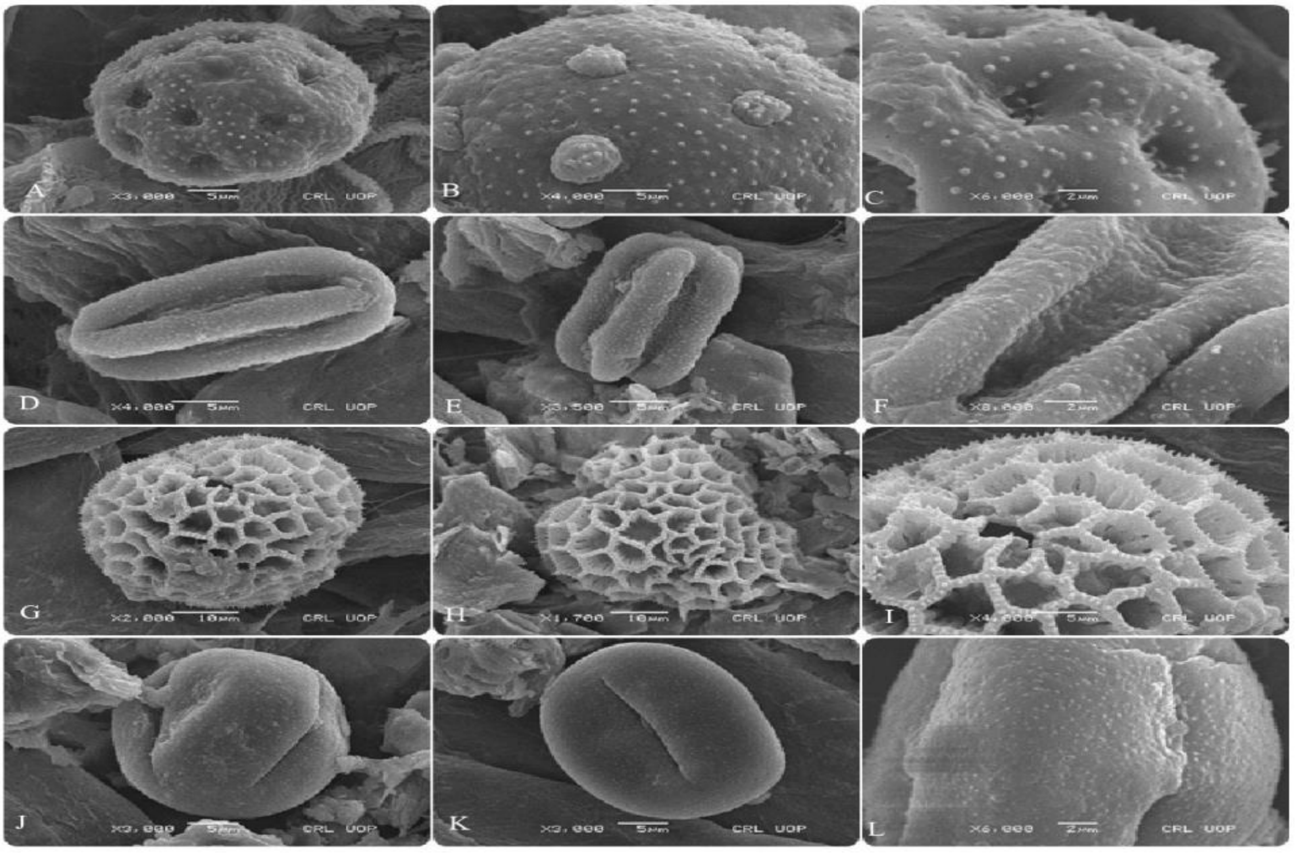

Fig. 1. Scanning electron microscopy pollen micrographs. (A-C) *Chaerophyllum reflexum* Aitch. (D-F) *Onosma hispidum* Wall. Ex G.Don (G-I) *Acontholimon libanoticum* Boiss. (J-L) *Bistorta affinis* (D.Don) Greene.

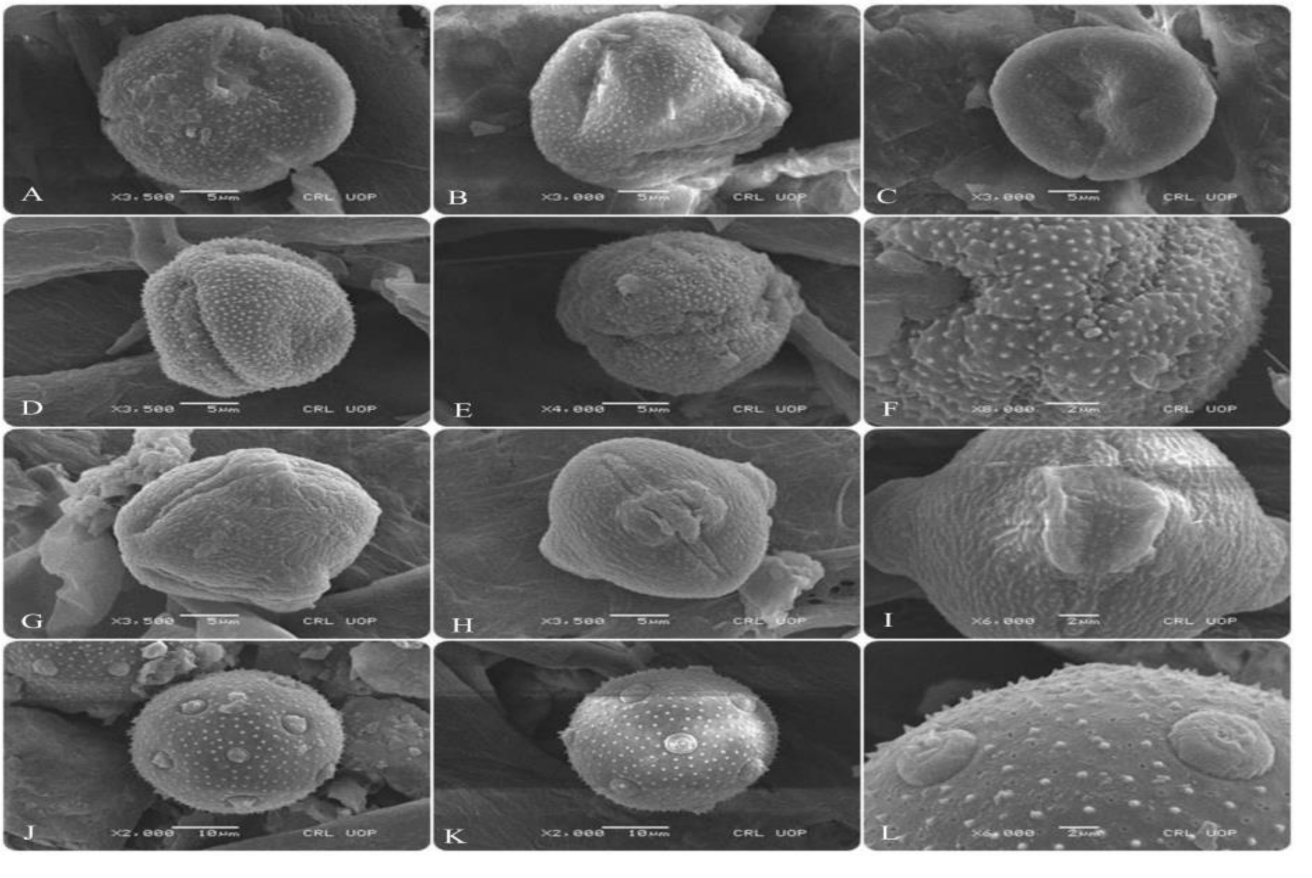

Fig. 2. Scanning electron microscopy pollen micrographs. (A-C) *Polygonum biaristatum* Aitch. & Hemsl. (D-F) *Papaver nudicaule* Linn. (G-I) *Potentilla crantzii* (Crantz) Fritsch. (J-L) *Silene kunawarensis* Benth.

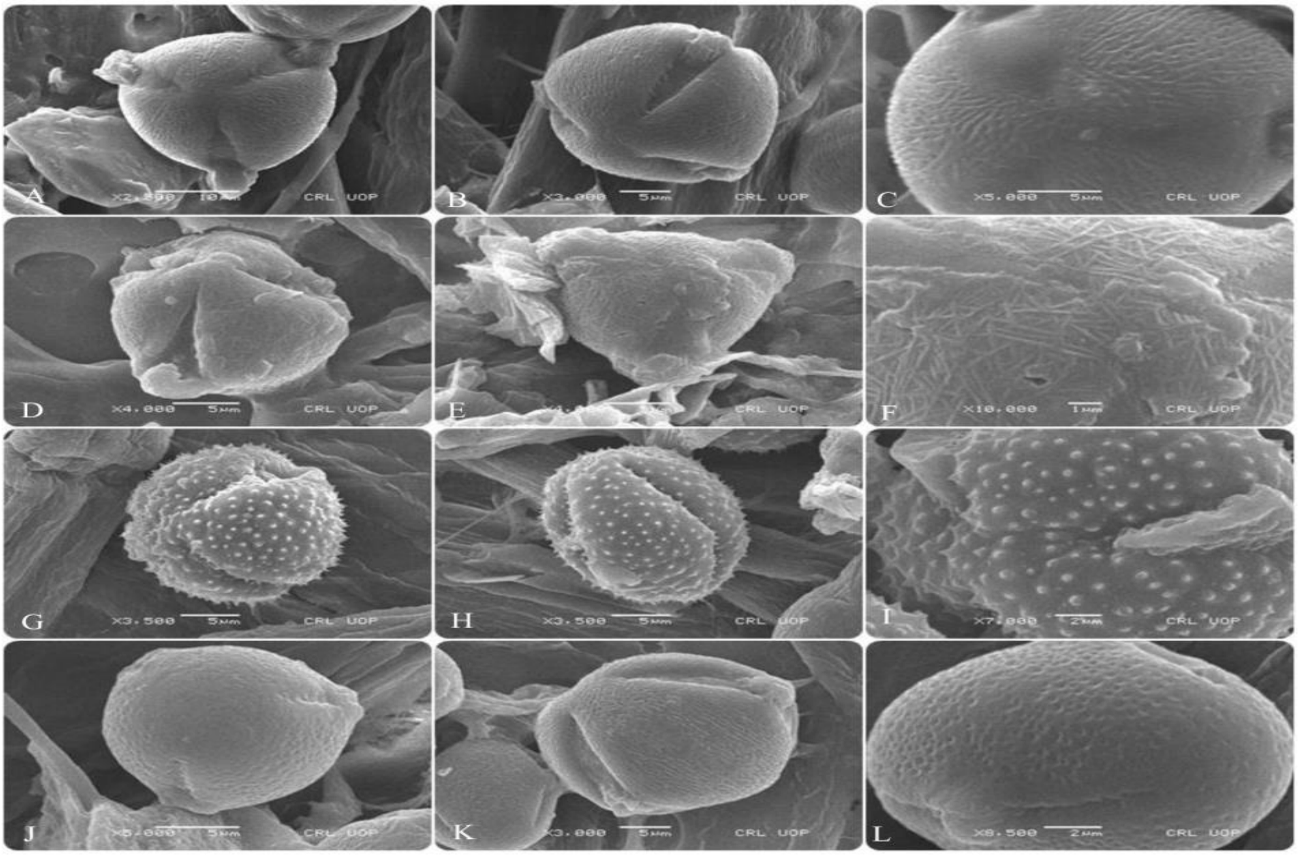

Fig. 3. Scanning electron microscopy pollen micrographs. (A-C) *Gentiana autumnalis* L. (D-F) *Sedum ewersii* Ledeb. (G-I) *Potentila reptans* L (J-L) *Astragalus rhizanthus* Benth.

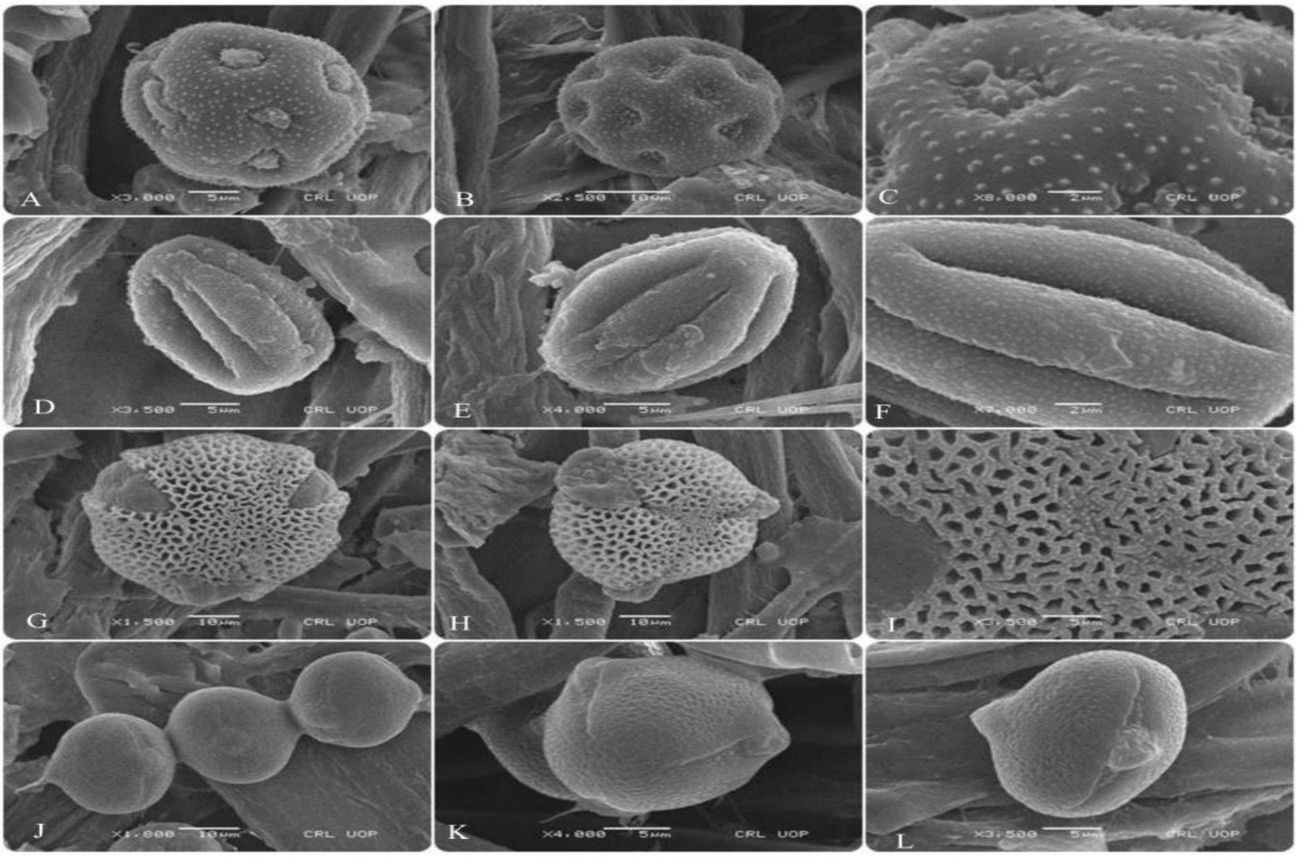

Fig. 4. Scanning electron microscopy pollen micrographs. (A-C) *Pulsatilla wallichiana* (Royle) Ulbr. (D-F) *Galium verum* L. (G-I) *Limonium gilesii* (Hems) Rech.f. and Koeie (J-L) *Astragalus graveolens* Benth.

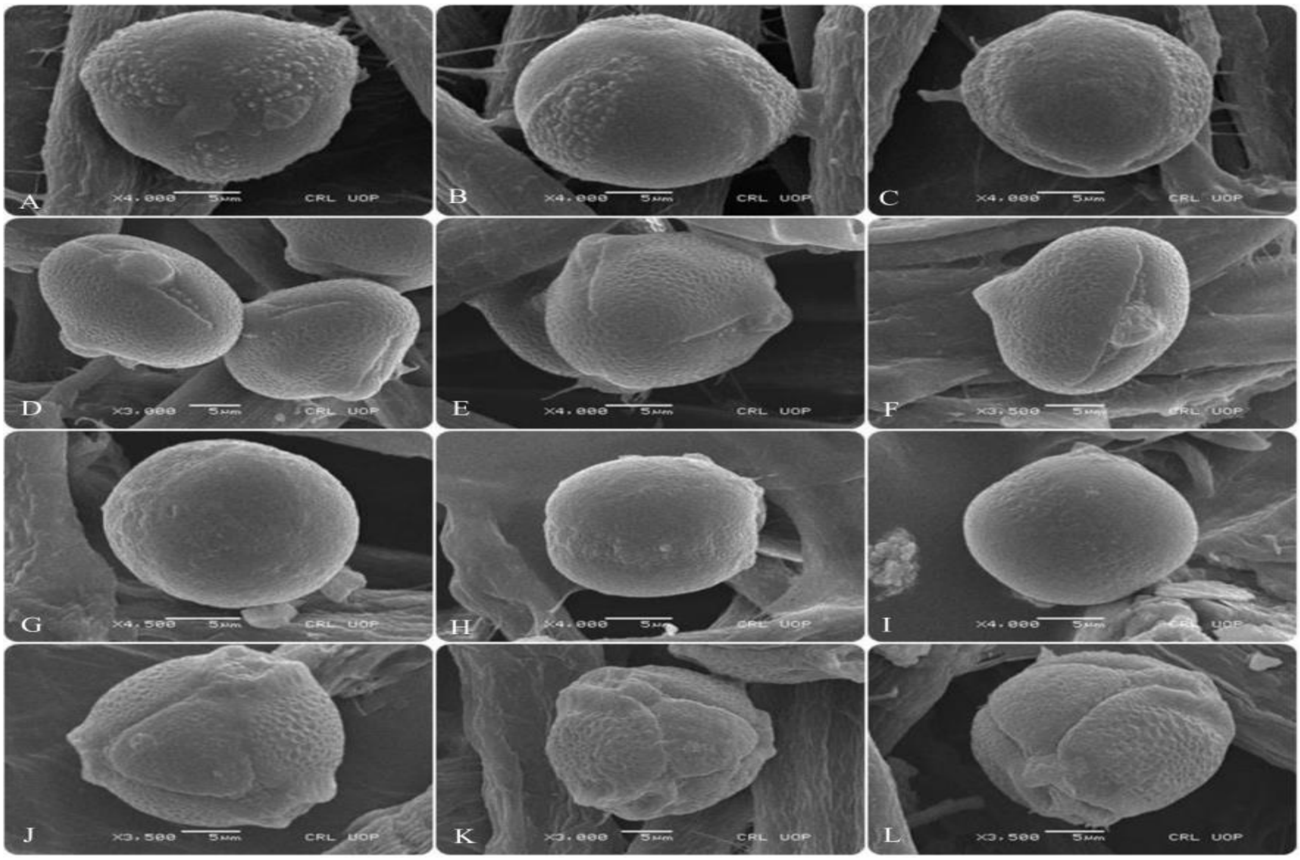

Fig. 5. Scanning electron microscopy pollen micrographs. (A-C) *Carum carvi* Linn. (D-F) *Medicago polymorpha* Linn. (G-I) *Pedicularis pyramidata* Pall. Ex steven (J-L) *Thalictrum pedunculatum* Edgew.

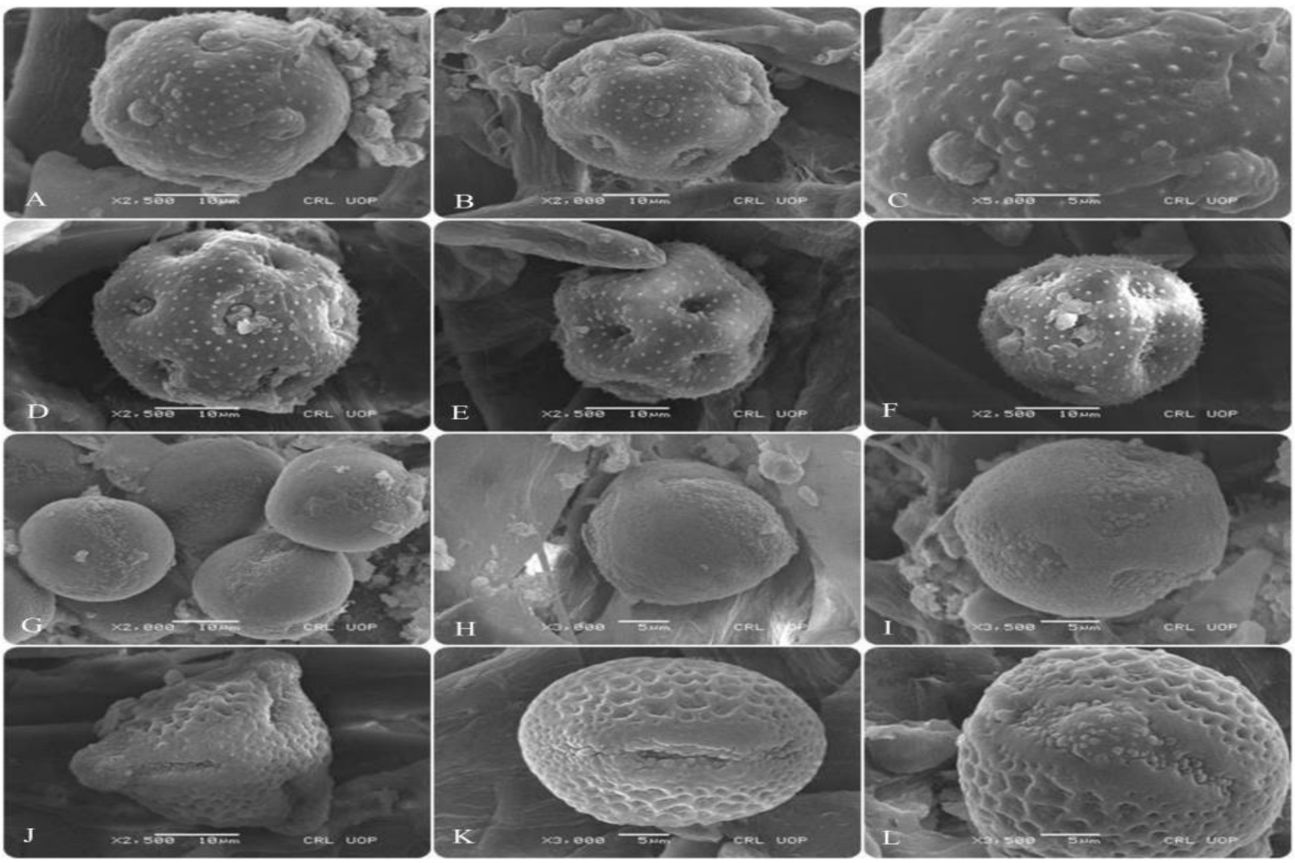

Fig. 6. Scanning electron microscopy pollen micrographs. (A-C) *Silene vulgaris* (Monech Garcke) (D-F) *Silene gonosperma* (Rupr.) *Bocquet* (G-I) *Veronica alpina* L (J-L) *Trifolium pratense* L.

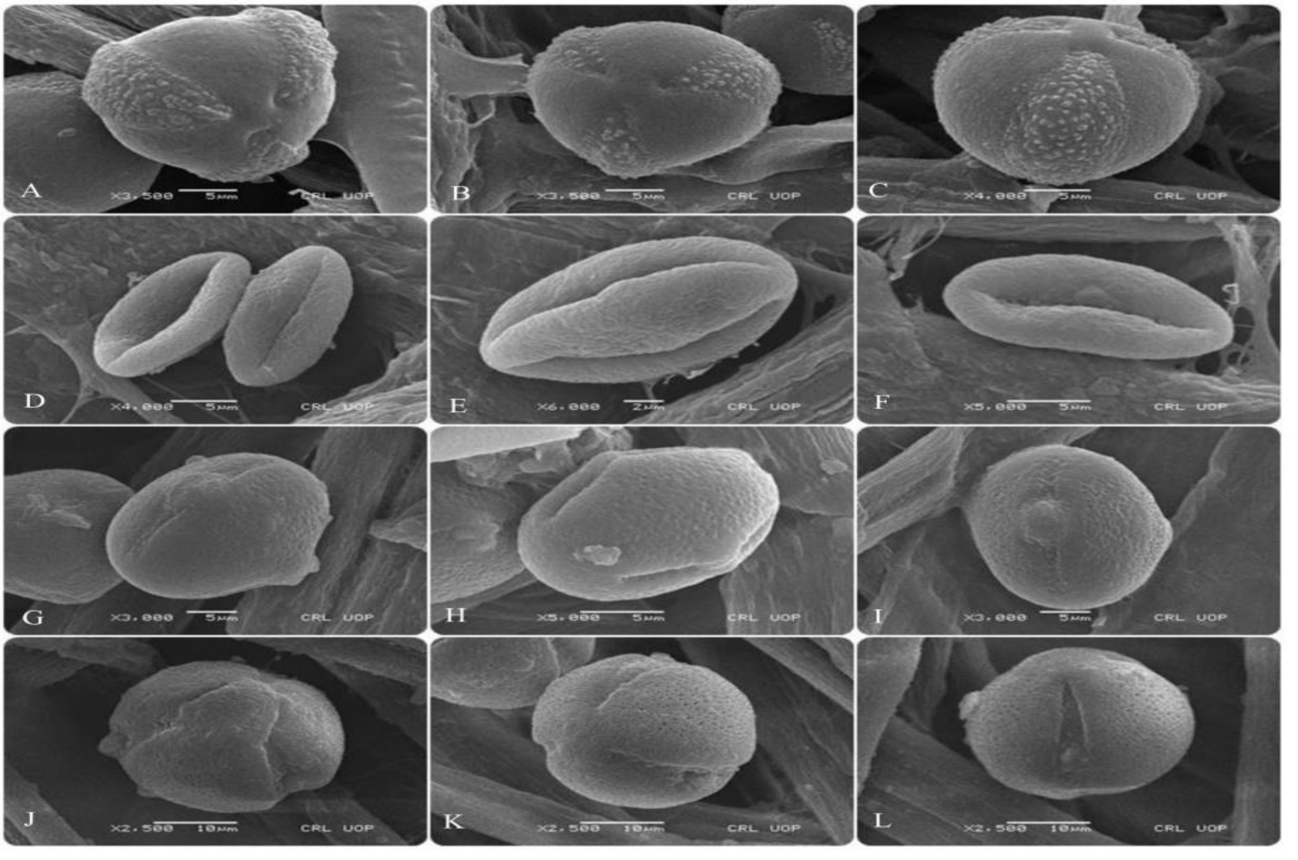

Fig. 7. Scanning electron microscopy pollen micrographs. (A-C) *Aconitum heterophyllum* Wall.ex Royle (D-F) *Clematis grata* Wall. (G-I) *Astragalus graveolens* Benth. (J-L) *Primula macrophylla* D. Don.

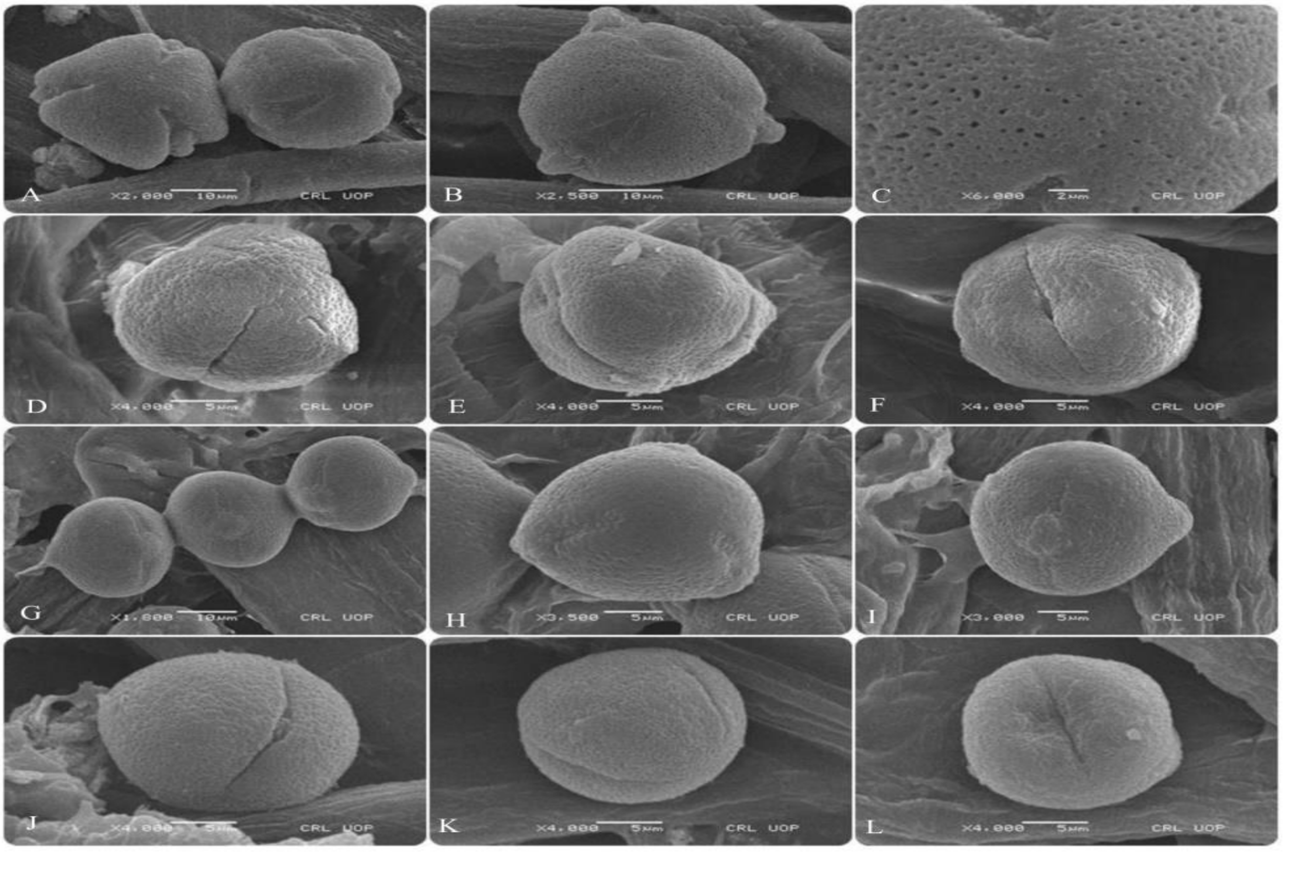

Fig. 8. Scanning electron microscopy pollen micrographs. (A-C) *Swertia petiolate* D. Don (D-F) *Rumex nepalensis* Spreng. (G-I) *Astragalus breviscapus* B.Fedtsch. (J-L) *Oxyria digyna* (L.) Hill.

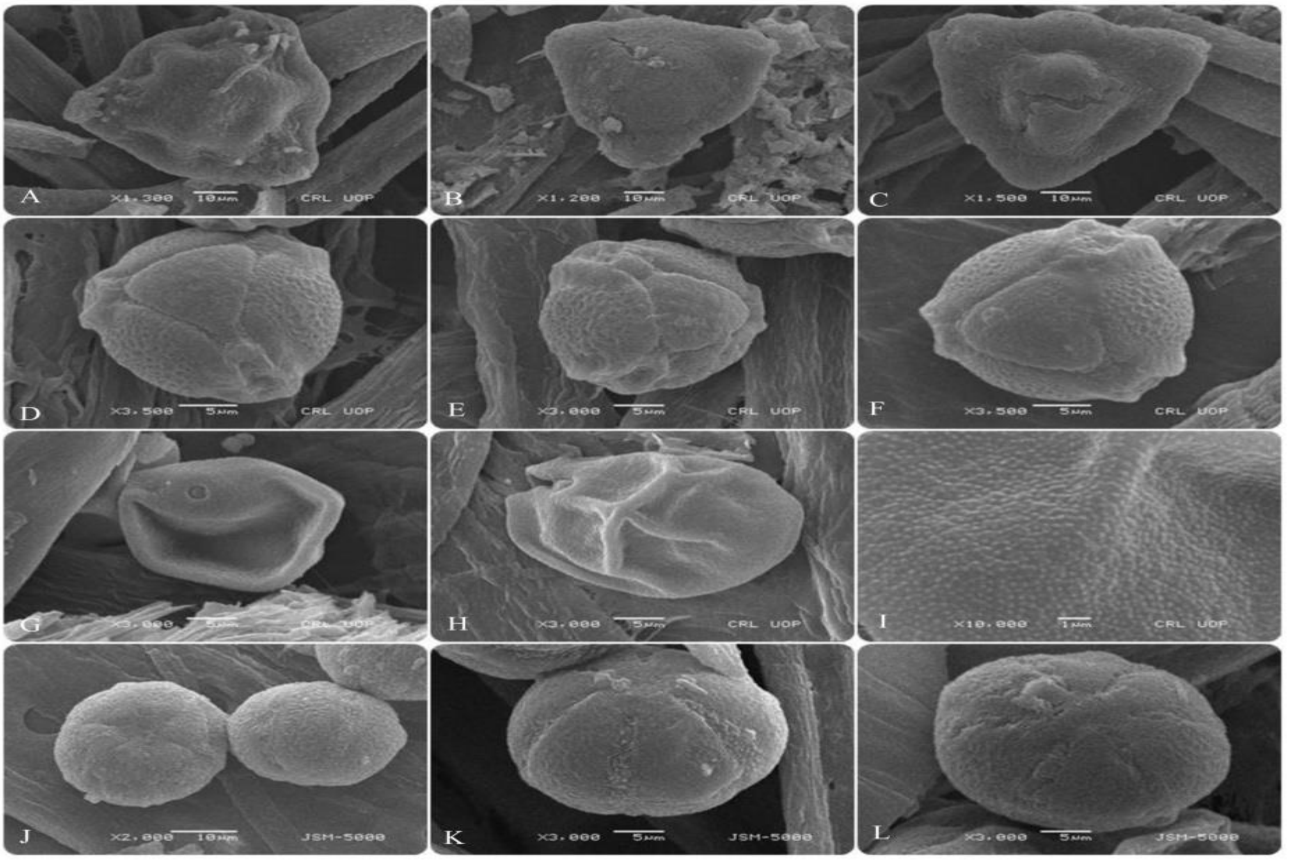

Fig. 9. Scanning electron microscopy pollen micrographs. (A-C) *Epilobum angustifolium* L. (D-F) *Thalictrum pedunculatum* Edgew. (G-I) *Festuca rubra* L. (J-L) *Actaea spicata* L.

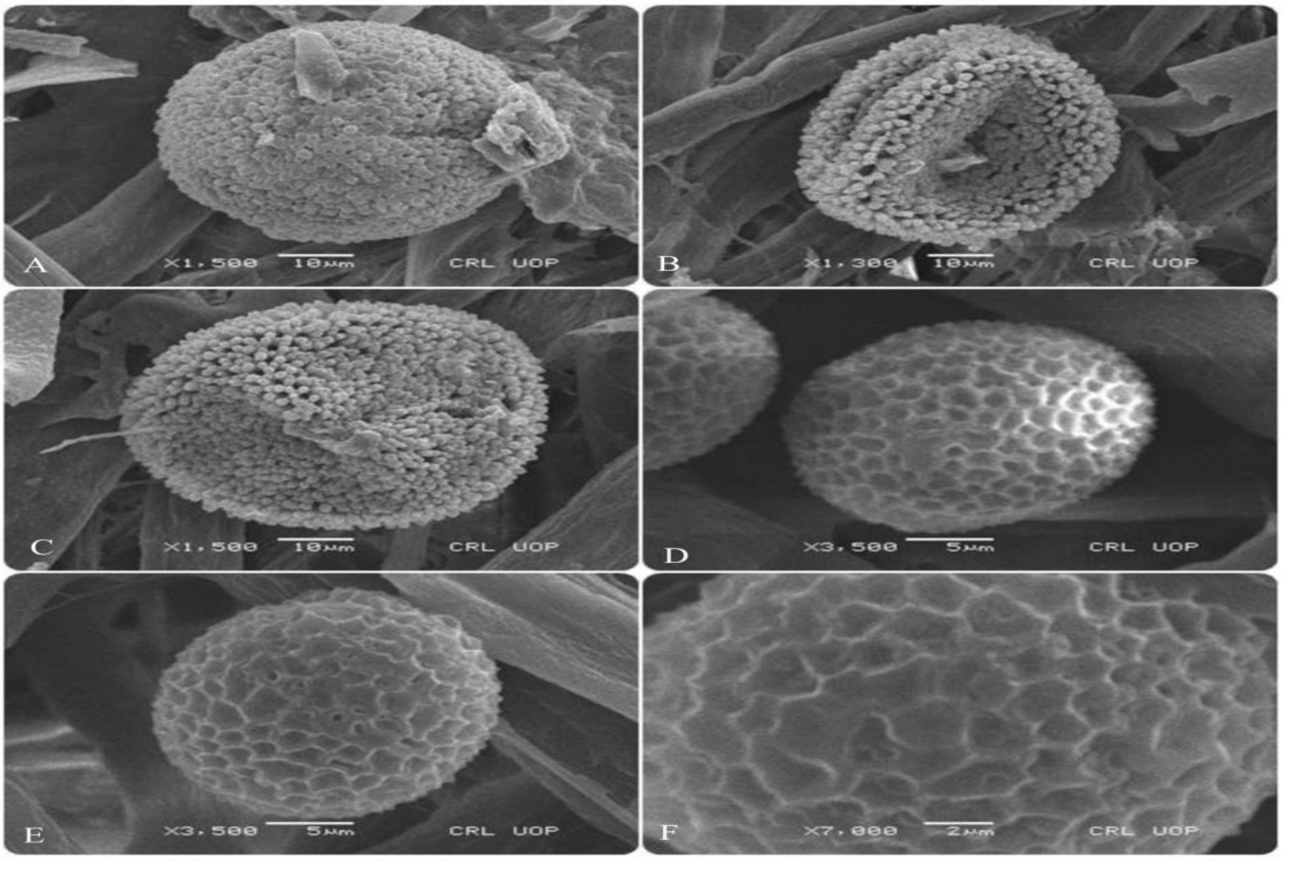

Fig. 10. Scanning electron microscopy pollen micrographs. (A-C) *Geranium pratense* L. (D-F) *Impatience edgeworthii* Hook. F.

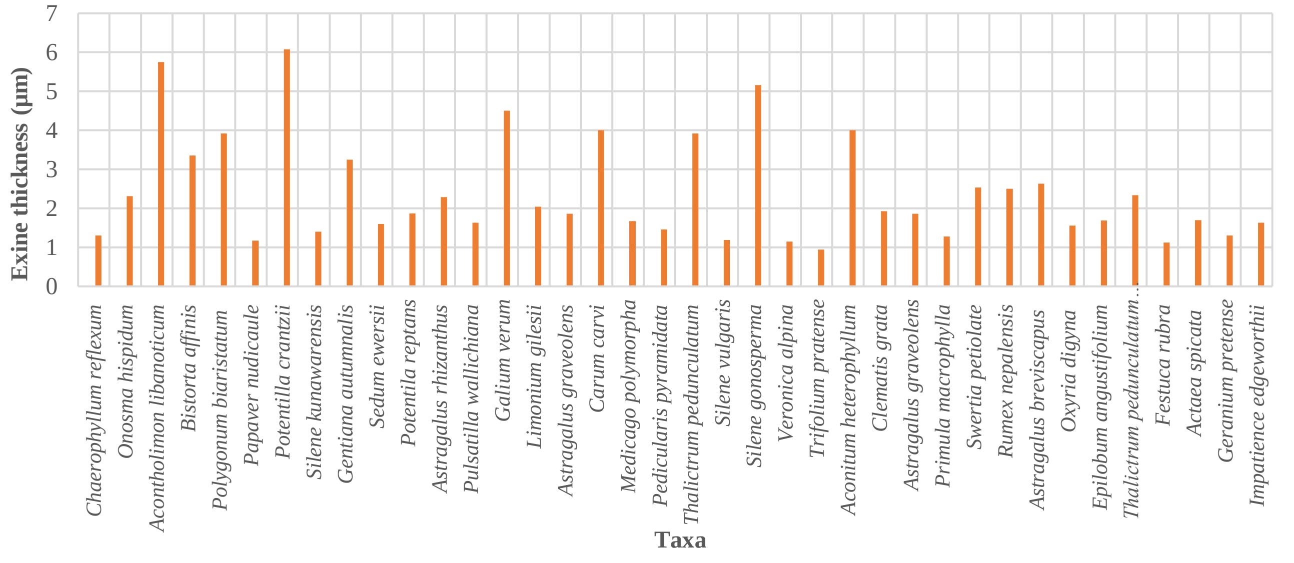

Fig. 11. Variations in the values of exine thickness among palynological studies of selected medicinal plants of Himalayan.

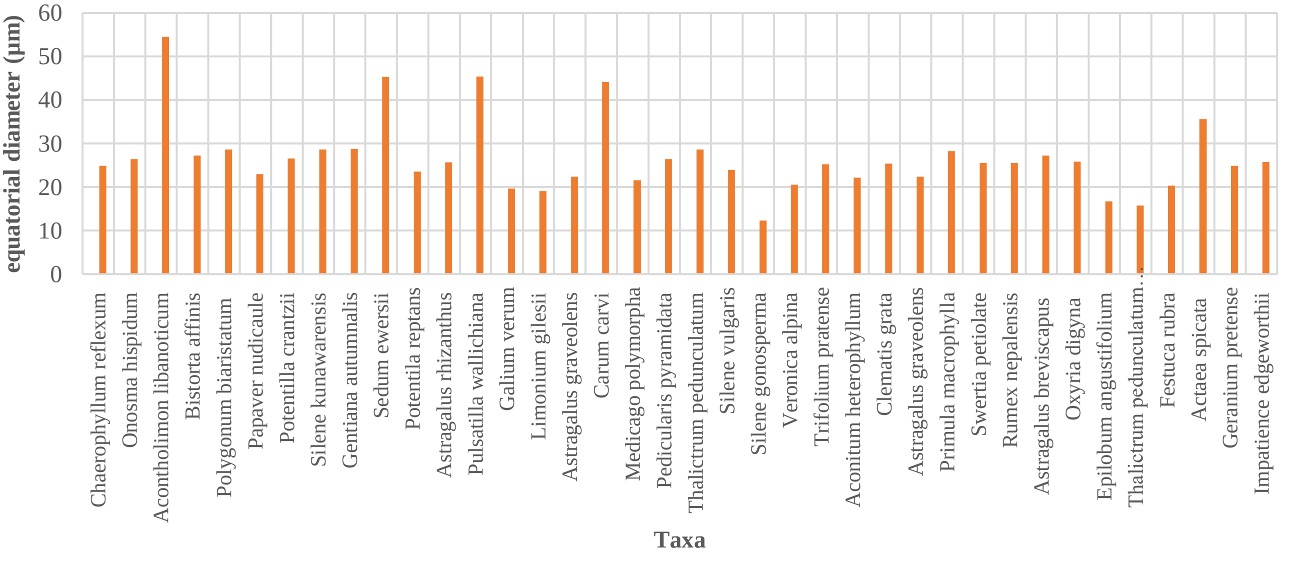

Fig. 12. Variations in the equatorial diameter among the palynological studies of selected medicinal plants of Himalayan.

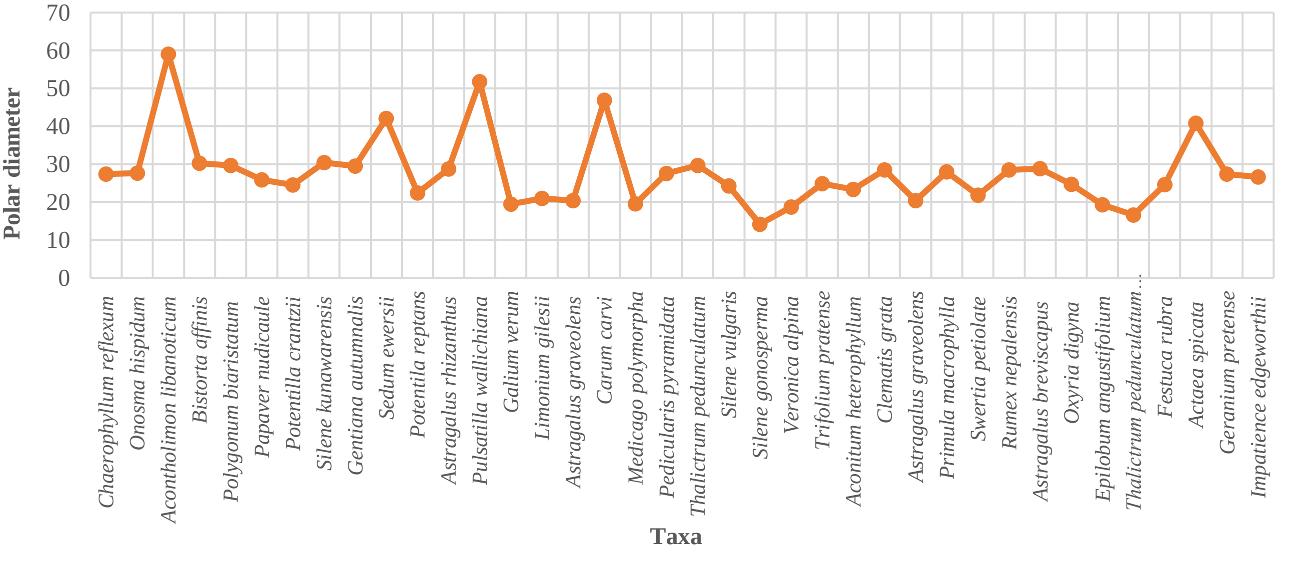

Fig. 13. Variations in the polar diameter among the palynological studies of selected medicinal plants of Himalayan.

Table 1. Medicinal uses of the plants used by the local communities in the study area.

S. No	Botanical name	Family	Part used	Medicinal uses	Diseases categories
1.	*Papaver nudicaule* Linn.	Papaveraceae		Cough, *whooping cough, Asthma*	Respiratory
2.	*Aconitum heterophyllum* Wall.ex Royle	Ranunculaceae	Leaves	Analgesic, antipyretic, anti-inflammatory	Digestive
3.	*Silene kunawarensis* Benth.	Caryophyllaceae	Root	Cough	Respiratory
4.	*Cicer microphyllum* Benth.	Fabaceae	Leaves	*Vomating, indigestion*	Digestive
5.	*Oxytropis deflexa* (Pall.) DC.	Fabaceae	Leaves	*Pain, common cold, and bleeding*	Dermatological
6.	*Gentiana autumnalis* L.	Gentianaceae	Leaves	*Fever, hypertension, muscle spasms*	Dermatological
7.	*Clematis grata* Wall.	Ranunculaceae	Leaves	Skin diseases	Dermatological
8.	*Acontholimon libanoticum* Boiss.	Pluminginaceae	Whole plant body	Cold, cough, skin diseases	Respiratory, Dermatological
9.	*Silene vulgaris* (Moench) Garcke	Caryophyllaceae	Seeds	*Rheumatic pains*	
10.	*Limonium gilesii* (Hems].) Rech.f. and Koeie	Plumbaginaceae	Leaves	Pulmonary hemorrhage, Gastrointestinal issues, and bronchorrhea	Digestive, Respiratory
11.	*Astragalus graveolens* Benth.	Fabaceae	Whole plant body	Diabetes, hay fever and kidney disease	Respiratory, Renal
12.	*Primula macrophylla* D. Don	Primulaceae	Leaves	Fevers, wounds and ulcers.	Digestive
13.	*Swertia petiolate* D. Don	Gentianaceae		*Hepatitis, cholecystitis, pneumonia*	Respiratory
14.	*Rumex nepalensis* Spreng.	Polygonaceae	Leaves	Pain, inflammation, bleeding, tinea, tumor, and constipation	Digestive, Dermatological
15.	*Carum carvi* Linn.	Apiaceae	Roots	Indigestion, pneumonia	Respiratory, digestive
16.	*Astragalus breviscapus* B.Fedtsch.	Fabaceae	Leaves	Asthma, (hay fever)	Respiratory
17.	*Pulsatilla wallichiana* (Royle) Ulbr.	Ranunculaceae	Roots	Headache, Skin diseases.	Rheumatic
18.	*Oxyria digyna* (L.) Hill	Polygonaceae	Leaves	Scurvy, dysentery	Digestive
19.	*Epilobum angustifolium* L.	Onagraceae	Whole plant body	Urogenital diseases, stomach and liver disorders, skin problems,	Digestive
20.	*Silene gonosperma (*Rupr.) Bocquet	Caryophyllaceae	Leaves	Wounds treatment	Dermatological
21.	*Convolvulus arvensis* L.	*Convolvulaceae*	Leaves	wounds and fever	Dermatological
22.	*Cynoglossum wallichii* G.Don.	Boraginaceae	Leaves	wounds, burns, and infections.	Dermatological
23.	*Medicago polymorpha* Linn.	Fabaceae	Leaves	intestinal ulcers, gastritis, liver disorders, bleeding gums, asthma, high blood pressure, eczema, anemia, constipation,	Digestive
24.	*Impatience edgeworthii* Hook. f	Balsaminaceae	Roots	inflammation of the nails, scurvy, carbuncles, dysentery, bruises, foot diseases	Digestive
25.	*Pedicularis pyramidata* Pall. Ex steven	Orobanchaceae	Leaves	leucorrhoea, fevers, sterility, rheumatism, general debility, collapse, and urinary problems	Rheumatic
26.	*Thalictrum pedunculatum* Edgew.	Ranunculaceae	Leaves	Fever and urinary problems	Renal
27.	*Bistorta affinis* (D.Don) Greene	Polygonaceae	Leaves	Dysentery, coughs, tonsillitis and fevers	Respiratory
28.	*Geranium pratense* L.	Geraniaceae	Leaves	Cholera, diarrhea dysentery	Digestive
29.	*Onosma hispidum* Wall. Ex G.Don	Boraginaceae	Leaves	Hypertension	
30.	*Heracleum maximum* W.Bartram	Apiaceae	Leaves	Tuberculosis	Respiratory
31.	*Festuca rubra* L.	Poaceae	Leaves and shoots	hay for livestock	Respiratory
32.	*Astragalus rhizanthus* Benth.	Fabaceae	Roots	Hay fever, diabetes, kidney disease	
33.	*Sedum ewersii* Ledeb.	Crassulaceae			
34.	*Actaea spicata* L.	Ranunculaceae	Roots	Inflammation, nerve diseases, lumbago, scrofula and chorea,	Digestive
35.	*Potentilla crantzii (*Crantz) Fritsch.	Rosaceae	Leaves	Dysentery	Digestive
36.	*Chaerophyllum reflexum* Aitch.	Apiaceae	Seeds	Pain, cold, and cough	Digestive
37.	*Galium verum* L.	*Rubiaceae*	leaves, stems and flowering shoots	Urinary disorders	Digestive
38.	*Papaver nudicaule* Linn.	Papaveraceae	Leaves	Anti-inflammatory	Digestive

Table 2. Qualitative micromorphological features of pollen.

S. No.	Taxa	Family	Pollen size	Pollen shape	Colpi/Pore P/A	Number of Colpi/Pore	Exine sculpturing
1.	*Chaerophyllum reflexum* Aitch.	Apiaceae	Medium	Per-oblate	Tricolpate	P	Psilate-echinate
2.	*Onosma hispidum* Wall. Ex G.Don	Boraginaceae	Medium	Per-oblate	Tricolporate	P	Psilate-echinate
3.	*Acontholimon libanoticum* Boiss.	Plumbaginaceae	Large	Oblate	Tricolpate	P	Reticulate
4.	*Bistorta affinis* (D.Don) Greene	Polygonaceae	Medium	Per-oblate	Tricolporate	P	Psilate-echinate
5.	*Polygonum biaristatum* Aitch. & Hemsl.	Polygonaceae	Small	Per-oblate	Tricolpate	P	Granulate
6.	*Papaver nudicaule* Linn.	Papaveraceae	Medium	Per-oblate	Tricolpate	P	Echinate
7.	*Potentilla crantzii (*Crantz) Fritsch.	Rosaceae	Small	Per-oblate	Tricolporate	P	Regulate
8.	*Silene kunawarensis* Benth.	Caryophyllaceae	Medium	Per-oblate	Tricolporate	P	Echinate
9.	*Gentiana autumnalis* L.	Gentianaceae	Medium	Per-oblate	Tricolporate	P	Psilate-striate
10.	*Sedum ewersii* Ledeb.	Crassulaceae	Medium	Spheroidal	Tricolporate	P	Regulate
11.	*Potentila reptans* L	Rosaceae	Small	Per-oblate	Tricolpate	P	Echinate
12.	*Astragalus rhizanthus* Benth.	Fabaceae	Medium	Per-oblate	Tricolporate	P	Perforate
13.	*Pulsatilla wallichiana* (Royle) Ulbr.	Ranunculaceae	Large	Per-oblate	Tricolporate	P	Echinate
14.	*Galium verum* L.	*Rubiaceae*	Small	Per-oblate	Tricolporate	P	Echinate
15.	*Limonium gilesii* (Hems) Rech.f. and Koeie	Plumbaginaceae	Small	Per-oblate	Tricolpate	P	Reticulate
16.	*Astragalus graveolens* Benth.	Fabaceae	Small	Spheroidal	Tricolporate	P	Perforate
17.	*Carum carvi* Linn.	Apiaceae	Medium	Per-oblate	Tricolpate	P	Psilate-granulate
18.	*Medicago polymorpha* Linn.	Fabaceae	Small	Spheroidal	Tricolporate	P	Perforate
19.	*Pedicularis pyramidata* Pall. Ex steven	Orobanchaceae	Medium	Per-oblate	Tricolpate	P	Psilate
20.	*Thalictrum pedunculatum* Edgew.	Ranunculaceae	Medium	Per-oblate	Tricolporate	P	Reticulate
21.	*Silene vulgaris* (Moench) Garcke	Caryophyllaceae	Medium	Spheroidal	Pantaporate	P	Echinate
22.	*Silene gonosperma (*Rupr.) Bocquet	Caryophyllaceae	Small	Per-oblate	Pantaporate	P	Echinate
23.	*Veronica alpina* L.	Plantaginaceae.	Small	Per-oblate	Tricolporate	P	Regulate
24.	*Trifolium pratense* L.	Fabaceae	Small	Per-oblate	Tricolporate	P	Reticulate
25.	*Aconitum heterophyllum* Wall.ex Royle	Ranunculaceae	Small	Spheroidal	Tricolporate	P	Regulate
26.	*Clematis grata* Wall.	Ranunculaceae	Medium	Per-oblate	Tricolporate	P	Regulate
27.	*Astragalus graveolens* Benth.	Fabaceae	Small	Per-oblate	Tricolporate	P	Regulate
28.	*Primula macrophylla* D. Don	Primulaceae	Medium	Per-oblate	Tricolporate	P	Regulate
29.	*Swertia petiolate* D. Don	Gentianceae	Small	Per-oblate	Pericolporate	P	Regulate
30.	*Rumex nepalensis* Spreng.	Polygonaceae	Medium	Per-oblate	Tricolpate	P	Reticulate
31.	*Astragalus breviscapus* B.Fedtsch.	Fabaceae	Medium	Per-oblate	Tricolporate	P	Reticulate
32.	*Oxyria digyna* (L.) Hill	Polygonaceae	Small	Per-oblate	Tricolporate	P	Regulate
33.	*Epilobum angustifolium* L.	Onagraceae	Small	Spheroidal	Tricolporate	P	Regulate
34.	*Thalictrum pedunculatum* Edgew.	Ranunculaceae	Small	Per-oblate	Tricolporate		Regulate
35.	*Festuca rubra* L.	Poaeceae	Small	Per-oblate	Monoporate	P	Regulate
36.	*Actaea spicata* L.	Ranunculaceae	Medium	Per-oblate	Tricolporate	P	Reticulate
37.	*Geranium pratense* L.	Geraniaceae	Small	Per-oblate	Tricolporate	P	Regulate
38.	*Impatience edgeworthii* Hook. F	Balsaminaceae	Small	Per-oblate	Tricolpate	P	Echinate

Table 3. Quantitative micromorphological features of pollen.

S. No.	Taxa	P/E ratio	Exine mean (Min-Max) SE (µm)	Polar diameter mean (Min-Max) SE (µm)	Equatorial diameter mean (Min-Max) SE (µm)	Length of colpi mean (Min-Max) SE (µm)	Width of colpi mean (Min-Max) SE (µm)
1.	*Chaerophyllum reflexum* Aitch.	1.2	1.30 (0.78-1.92) ± 0.35	27.38 (19.64-31.50) ± 1.24	24.86 (20.72-26.33) ± 0.61	3.74 (2.34-6.38) ± 0.83	4.82 (3.2-7.2) ± 0.73
2.	*Onosma hispidum* Wall. Ex G.Don	1.01	2.31 (0.91-3.41) ± 0.17	27.60 (16.80-32.82) ± 0.93	26.42 (25.74-28.01) ± 0.25	8.74 (6.37-9.67) ± 0.64	5.80 (3.68-7.96) ± 0.73
3.	*Acontholimon libanoticum* Boiss.	1.08	5.75 (5.25-6.25) ± 0.29	59.03 (58.25-60.25) ± 1.04	54.50 (53.50-55.25) ± 0.62	12.50 (10.75-14.75) ± 1.18	3.90 (3.50-4.50) ± 0.30
4.	*Bistorta affinis* (D.Don) Greene	1.11	3.35 (1.51-5.41) ± 0.23	30.25 (29.75-30.75) ± 0.28	27.25 (26.75-27.75) ± 0.29	3.33 (3.00-3.75) ± 0.29	4.0 (3.75-4.25) ± 0.14
5.	*Polygonum biaristatum* Aitch. & Hemsl.	1.03	3.92 (1.82-4.61) ± 0.32	29.63 (28.44-30.40) ± 0.62	28.65 (25.81-30.52) ± 1.57	8.74 (4.32-12.52) ± 1.23	6.21 (3.63-9.73) ± 1.3
6.	*Papaver nudicaule* Linn.	1.12	1.17 (0.70-1.80) ± 0.18	25.84 (19.04-29.14) ± 0.23	22.97 (19.13-24.90) ± 0.61	A	A
7.	*Potentilla crantzii (*Crantz) Fritsch.	1.0	6.08 (5.75-6.50) ± 0.22	24.50 (23.25-26.00) ± 1.02	26.58 (24.50-28.25) ± 0.8	4.25 (3.75-4.75) ± 0.3	2.8 (2.25-3.25) ± 0.30
8.	*Silene kunawarensis* Benth.	1.06	1.40 (1.08-1.81) ± 0.15	30.42 (25.80-34.65) ± 1.52	28.62 (21.41-33.70) ± 1.80	9.21 (5.34-16.54) ± 1.30	8.12 (1.73-8.91) ± 0.91
9.	*Gentiana autumnalis* L.	1.02	3.25 (3.75-3.50) ± 0.41	29.50 (28.75-30.25) ± 0.75	28.75 (27.75-30.25) ± 1.32	3.50 (3.25-3.75) ± 0.25	3.83 (3.50-4.25) ± 0.14
10.	*Sedum ewersii* Ledeb.	0.92	1.60 (1.31-1.98) ± 0.16	42.02 (45.12-46.30) ± 1.05	45.32 (31.75-59.230 ± 2.20	A	A
11.	*Potentila reptans* L	0.95	1.87 (1.45-3.12) ± 0.15	22.40 (19.60-24.45) ± 0.76	23.56 (19.30-25.82) ± 1.6	8.9 (7.30-9.10) ± 0.72	8.21 (6.45-11.35) ± 0.82
12.	*Astragalus rhizanthus* Benth.	1.11	2.29 (2.00-2.15) ± 0.39	28.70 (28.70-29.50) ± 0.5	25.66 (25.66-26.35) ± 0.62	4.52 (2.90-7.72) ± 0.92	3.5 (3.25-3.75) ± 0.46
13.	*Pulsatilla wallichiana* (Royle) Ulbr.	1.13	1.63 (1.20-2.50) ± 0.41	51.74 (46.50-58.25) ± 1.96	45.42 (35.75-54.82) ± 2.86	18.25 (10.76-26.46) ± 2.54	14.33 (11.50-17.14) ± 1.61
14.	*Galium verum* L.	0.98	4.5 (4.25-4.75) ± 0.14	19.41 (18.25-20.25) ± 0.60	19.66 (17.75-23.25) ± 1.79	A	A
15.	*Limonium gilesii* (Hems) Rech.f. and Koeie	1.09	2.04 (1.43-2.76) ± 0.86	20.92 (14.43-26.06) ± 0.78	19.06 (17.15-21.13) ± 0.45	5.42 (3.02-7.54) ± 0.94	3.65 (1.92-4.52) ± 0.34
16.	*Astragalus graveolens* Benth.	0.91	1.86 (1.06-2.25) ± 0.34	20.34 (17.45-23.83) ± 1.24	22.35 (16.73-27.04) ± 0.62	4.79 (3.63-7.45) ± 0.94	4.82 (4.85-6.74) ± 0.83
17.	*Carum carvi* Linn.	1.06	4.00 (3.75-4.25) ± 0.25	46.91 (46.25-47.75) ± 0.77	44.16 (42.73-46.55) ± 0.53	7.75 (7.20-8.30) ± 0.14	6.0 (5.31-6.69) ± 0.6
18.	*Medicago polymorpha* Linn.	0.90	1.67 (1.30-1.73) ± 0.63	19.50 (17.52-21.47) ± 0.92	21.54 (16.65-25.78) ± 1.8	A	A
19.	*Pedicularis pyramidata* Pall. Ex steven	1.04	1.46 (1.4-1.86) ± 0.26	27.54 (26.32-29.53) ± 0.63	26.41 (24.52-27.64) ± 0.67	A	A

Table 3. (Cont'd.).

S. No.	Taxa	P/E ratio	Exine mean (Min-Max) SE (µm)	Polar diameter mean (Min-Max) SE (µm)	Equatorial diameter mean (Min-Max) SE (µm)	Length of colpi mean (Min-Max) SE (µm)	Width of colpi mean (Min-Max) SE (µm)
20.	*Thalictrum pedunculatum* Edgew.	1.03	3.92 (1.82-4.61) ± 0.32	29.63 (28.44-30.40) ± 0.62	28.65 (25.81-30.52) ± 1.57	8.74 (4.32-12.52) ± 1.23	6.21 (3.63-9.73) ± 1.3
21.	*Silene vulgaris* (Monech Garcke)	1.03	1.19 (0.67-1.72) ± 0.32	24.23 (19.94-28.10) ± 0.10	23.90 (22.31-26.45) ± 0.61	9.92 (6.65-16.63) ± 2.10	4.12 (1.62-6.30) ± 0.72
22.	*Silene gonosperma (*Rupr.) Bocquet	1.14	5.16 (4.75-5.50) ± 0.22	14.16 (13.25-14.75) ± 0.46	12.33 (12.00-12.75) ± 0.22	3.00 (2.75-3.25) ± 0.14	2.66 (2.25-3.00) ± 0.22
23.	*Veronica alpina* L.	0.91	1.15 (0.71-1.92) ± 0.28	18.67 (14.80-22.70) ± 0.81	20.51 (12.34-27.95) ± 2.1	6.43 (2.12-11.85) ± 1.83	5.34 (2.91-8.37) ± 1.63
24.	*Trifolium pratense* L.	0.98	0.94 (0.48-1.86) \ ± 0.36	24.82 (22.27-26.85) ± 0.38	25.23 (23.60-26.60) ± 0.54	12.25 (8.64-14.30) ± 1.6	6.76 (4.73-9.26) ± 1.3
25.	*Aconitum heterophyllum* Wall.ex Royle	1.05	4.00 (3.70-4.35) ± 0.26	23.33 (22.75-24.25) ± 0.46	22.16 (21.75-22.80) ± 0.30	7.75 (7.30-8.25) ± 0.23	5.38 (2.73-9.00) ± 1.16
26.	*Clematis grata* Wall.	1.12	1.93 (0.70-1.70) ± 0.4	28.45 (26.60-29.10) ± 0.92	25.43 (22.80-29.12) ± 0.83	12.23 (10.72-14.60) ± 1.25	8.43 (4.92-12.41) ± 2.5
27.	*Astragalus graveolens* Benth.	0.91	1.86 (1.06-2.25) ± 0.34	20.34 (17.45-23.83) ± 1.24	22.35 (16.73-27.04) ± 0.62	4.79 (3.63-7.45) ± 0.94	4.82 (4.85-6.74) ± 0.83
28.	*Primula macrophylla* D. Don	0.98	1.28 (1.15-2.32) ± 0.83	27.94 (26.71-30.67) ± 0.85	28.27 (25.27-29.74) ± 0.85	6.10 (4.13-7.35) ± 0.8	8.79 (5.70-11.56) ± 0.95
29.	*Swertia petiolate* D. Don	0.85	2.53 (2.31-2.10) ± 0.15	21.77 (17.80-23.00) ± 0.62	25.55 (18.50-27.72) ± 1.37	8.76 (7.31-9.16) ± 0.60	9.23(7.70-11.14) ± 0.64
30.	*Rumex nepalensis* Spreng.	1.11	2.50 (0.98-3.20) ± 0.32	28.45 (22.21-32.30) ± 0.81	25.51 (19.72-27.81) ± 1.42	13.81 (11.45-17.30) ± 1.2	6.3 (4.23-8.47) ± 0.42
31.	*Astragalus breviscapus* B.Fedtsch.	1.05	2.63 (1.11-3.34) ± 0.46	28.78 (27.21-29.62) ± 0.73	27.22 (24.53-29.72) ± 0.94	11.90 (10.32-12.34) ± 1.54	8.43 (7.73-9.34) ± 0.96
32.	*Oxyria digyna* (L.) Hill	0.95	1.56 (0.75-1.65) ± 1.54	24.66 (17.71-28.81) ± 1.34	25.84 (21.47-29.41) ± 1.56	11.46 (8.86-13.36) ± 1.54	7.52 (4.21-10.92) ± 1.64
33.	*Epilobum angustifolium* L.	1.14	1.69 (1.36-2.12) ± 0.14	19.25 (16.56-23.00) ± 0.98	16.74 (15.56-19.75) ± 0.85	A	A
34.	*Thalictrum pedunculatum* Edgew.	1.05	2.34 (1.5-1.91) ± 0.42	16.60 (12.45-20.52) ± 0.62	15.76 (12.17-17.40) ± 0.83	8.68 (7.42-9.32) ±1.4	4.73 (1.63-7.60) ± 1.42
35.	*Festuca rubra* L.	1.21	1.12 (0.73-1.71) ± 0.18	24.59 (19.91-28.95) ± 1.34	20.32 (19.45-23.40) ± 0.94	10.98 (7.76-16.35) ± 1.96	7.73 (7.65-16.24) ± 0.96
36.	*Actaea spicata* L.	1.14	1.7 (0.92-2.61) ± 0.45	40.75 (38.20-42.40) ± 0.72	35.63 (32.00-38.32) ± 1.36	6.07 (4.43-7.51) ± 0.56	4.72 (3.66-6.32) ± 0.61
37.	*Geranium pratense* L.	1.2	1.30 (0.78-1.92) ± 0.35	27.38 (19.64-31.50) ± 1.24	24.86 (20.72-26.33) ± 0.61	3.74 (2.34-6.38) ± 0.83	4.82 (3.2-7.2) ± 0.73
38.	*Impatience edgeworthii* Hook. F	1.03	1.63 (1.57-1.83) ± 0.65	26.57 (25.84-27.64) ± 0.87	25.73 (21.64-24.73) ± 0.74	8.72 (8.62-11.23) ± 0.83	3.42 (1.93-3.65) ± 0.53

Results

The pollen morphology of 38 selected medicinal plants was investigated showing differentiations in pollen shapes and size. The variation in exine thickness, equatorial diameter and polar diameter were represented in Figs. 1-13. The quantitative and qualitative features of pollen grains along with the medicinal uses were described in tables 1-3. Pollen grains of the reported species were noted to be monad, apolar, radially symmetrical, reticulate and peroblate. The detailed micromorphological features of qualitative and quantitative characters of pollen were noted in tables 1, 2 while micrographs were shown in the plates 1 and 2. Pollen grains of the species varied in colpi, number of pores, spine length and spine width. The qualitative and quantitative features of selected taxa of Himalayans were showed in Table 1, 2 and its micrographs were presented in Figs. 1-10.

Symmetry, size, and shape: Pollen grains of the study were examined to be monad, isopolar and radially symmetrical. Pollen grains were varied in size and orientations. The majority of species exhibited oblate, prolate, and peroblate pollen shapes, with sizes ranging from small to large. Maximum exine thickness was examined for *Acontholimon libanoticum* 5.75 µm while the minimum in *Cynoglossum wallichii* 0.94 µm. Maximum exine thickness was examined for *Acontholimon libanoticum* 59.03 while the minimum in *Silene gonosperma* 16.60 µm. Maximum polar diameter was examined for *Acontholimon libanoticum* 59.03 µm while the minimum in *Silene gonosperma* 15.76 µm. Maximum length of colpi was examined for *Pulsatilla wallichiana* 18.25 µm while the minimum in *Heracleum maximum* 2.66 µm. Maximum colpi width was examined for *Oxytropis deflexa* 10.60 µm while the minimum in *Potentilla crantzii* 2.8 µm.

P/E ratio: P/E ratio for each species was determined by dividing the means values of polar diameter over equatorial diameter. The highest P/E was calculated for *Melilotus* indica 1.23 and lowest for Stellaria media 0.77. Maximum P/E ratio was examined for *Heracleum maximum* (1.14), while the minimum in *Swertia petiolate* (0.85).

Exine ornamentation: Exine ornamentation serves as a crucial pollen feature for delineating selected taxa of the Himalayas, displaying patterns such as psilate, regulate, reticulate, and echinate. The highest exine thickness was noted in *Acontholimon libanoticum* Boiss. (5.75 µm) and the lowest in *Veronica alpina* L (1.15 µm).

Shape and size: The investigated species exhibit a wide range of variations in their shape and size. The majority of the pollen grains have peroblate shape followed by spheroidal and oblate while size of the pollen grains was small, medium and large.

Discussion

Taxonomically, the study revealed that exine ornamentation held greater significance than quantitative characteristics. Notably, exine ornamentation emerges as a crucial pollen trait for distinguishing taxa within specific families, such as Fabaceae. Therefore, the information obtained from Palynological as well as Characteristics of the studied taxa may aid in the taxonomy of certain Himalayan taxa. Pollen morphological studies provide valuable insights into the genetic resources of crops, offering clues to their evolutionary history and genetic diversity. Understanding these morphological traits facilitates targeted breeding efforts, leading to the development of resilient and high-yielding crop varieties essential for agricultural evolution. Under a SEM, psilate, reticulate, and perforate have been observed. In addition to being a useful tool for specific delimitation, pollen morphology can be used as a key for taxonomic features and to identify and classify taxa at both the specific and generic levels. Exine sculpture's diversity provides useful identifying traits for distinguishing closely related species. Thus, it is evident that pollen's qualitative and quantitative characteristics can both be helpful in distinguishing between taxa at a particular level. Fabaceae (06), Rananculceae (06), Polygonaceae (03), Apiaceae (03), Caryophyllaceae (03) have been investigated as the dominant families followed by Gentinaceae (02), Papaveraceae (02), Bignoniaceae (02), Rosaceae, Plumbaginaceae, Onagraceae,

Convolvulaceae, Orobanchaceae, Geraniaceae, Poaceae, Primulaceae, Balsaminaceae, Crassulaceae and Rubiaceae. It is recommended to utilize scanning electron microscopy for comprehensive analysis of pollen morphology, particularly the intricate exine patterns, surpassing the limitations of light microscopy. Most of the pollen grains observed in this study were tricolporate, peroblate, prolate, and sub-prolate. Under a scanning electron microscope (SEM), the pores on the surface of all pollens are visible, but they are not easily visible under a light microscope. Pollen grains of *Chaerophyllum reflexum* Aitch, *Pulsatilla wallichiana* (Royle) *Ulbr* noted were medium-sized, tricolpate and psilate-echinate. Pollen grains of *Chaerophyllum reflexum* examined *by* (Anjum & Muhammad, 2007) were isopolar, tricolporate, radially symmetrical, sexine and nexine have equal thickness and colpi membrane is psilate to granulate. Pollen grains of *Onosma hispidum* Wall. Ex G. Don, *Acontholimon libanoticum* Boiss, *Papaver nudicaule* Linn, *Bistorta affinis* (D. Don) Greene noted were medium sized, tricolpate and psilate-echinate while the pollen grains of *Onosma hispidum* Wall. Ex G. Don, were single, radially symmetrical, isocolpate, isopolar or heteropolar, tricolporate and size in medium range look similar to our current result (Mazari & Liu, 2019). The pollen of *Polygonum biaristatum* Aitch. & Hemsl, *Potentilla crantzii (*Crantz) Fritsch. were small peroblate, tricolporated and echinated. The pollen of *Potentilla crantzii* (*Crantz) Fritsch* were prolate to prolate-spheroidal, sized in medium range and triangular-circular in polar view (Faghir *et al.*, 2012). *Astragalus rhizanthus* Benth, *Medicago polymorpha* Linn. noted were medium sized, tricolpate and perforated. *Galium verum* L were large, peroblate, tricolporate and echinate. The palynological study of *Limonium gilesii* (Hems) Rech. f. and Koeie, *Thalictrum pedunculatum Edgew*. showed large, peroblate, tricolporate and reticulated ornamentations. *Astragalus graveolens* Benth, *Medicago polymorpha* Linn. pollen grains were small, spheroidal, tricolpoarte and perforate. Pollen grains of *Carum carvi* Linn. were medium sized, peroblate, tricolporate and psilate-granulated. *Pedicularis pyramidata*

Pall. Ex steven were medium sized, peroblate, tricolporate and psilated. *Silene vulgaris* (Moench) Garcke, *Silene gonosperma* (Rupr.) *Bocquet* and *Silene kunawarensis* Benth were medium sized, spheotdal-peroblate, pantaporated and echinated. Pollen grains of *Silene vulgaris* (Moench) Garcke examined in the previous study was spheroidal to prolate-spheroidal, isopolar, sexine thicker or thinner than nexine and exine ornamentations reticulate or reticulate-scabrate (Anjum & Muhammad, 2007). The pollen morphology of *Veronica alpina* L., *Trifolium pratense* L., *Aconitum heterophyllum* Wall.ex Royle, *Clematis grata* Wall, *Astragalus graveolens* Benth, and *Primula macrophylla* D. Don. have shown small-medium sized, tricolporated and regulated. Pollen grains of the *Primula macrophylla* D. noted in the previous study, were prolate-spheroidal to spheroidal and trizonocolporate with reticulate exine sculpture (Sarkar *et al.,* 2021). Fabaceae pollen grains noted in this study was tricolporate and look similar to the previous results of (Luz *et al.,* 2013). Many Asteraceae, Lamiaceae, Polygonaceae and Orobanchaceae have been recorded from Himalayan and look similar to our results. The taxa were used for the treatments of Joint pains, fumigant toxicity, astringent, diuretic, antitumor, antibacterial effects bacterial infections, inflammation, coughing, fever, arthritis, pimples, bone pain, febrifuge wounds healing, antitussive, diabetes, uterine hemorrhage respiratory diseases (Hameed *et al.,* 2023).

Analyzing the morphological traits of pollen, including polar axis, equatorial diameter, shape, colpi length, pore diameter, exine thickness, and exine ornamentation, enables precise identification of plants. This, in turn, facilitates the study of genetic resources and crop evolution, unveiling variations among different taxa of the Himalayas. Scanning electron microscopy was used rather than a light microscope to examine all of the morphological features of the pollen, especially the exine pattern. It is concluded that perprolate, prolate, and sub-prolate pollen taxa were nearly all tricolporate. Most of the pollen grains have pores that are visible under a scanning electron microscope (SEM), but they are not as visible under a light microscope. Therefore, taxa at both the specific and generic levels are identified and classified using the palynological features of different selected taxa.

Pollen grain morphology has emerged as a pivotal component in the multidisciplinary collaboration of plant systematics and evolution. By unraveling taxonomic puzzles at various levels family, generic, and specific this tool not only enriches our understanding of plant diversity but also contributes to the exploration of genetic resources and the evolutionary relationship of crops (Gabr, 2018). Many researchers have utilized the differences in the polar unit, shape, symmetry, aperture, and wall sculpture of pollen grains to distinguish between different taxa. Pollen varies in size, shape (equatorial diameter, polar diameter, exine thickness, colpi length, colpi width) and exine ornamentations. These results are the main findings for species identifications based on morphological characters. Light and scanning electron microscopy are the significant characters for the identifications of the complex flora in the field of plant taxonomy, aeropalynology and melissopalynology. The current study encourages the taxonomists for the identifications of taxa.

Scanning electron microscopy revealed a broad spectrum of variations among selected Himalayan species, underscoring the crucial role of pollen morphology in systematic plant studies. This detailed analysis not only enhances our understanding of plant diversity but also brightens the genetic resources essential for crop evolution. Our results show similarity to the previous reported knowledge from Margalla hills of Pakistan (Sufyan *et al.,* 2018). The current study gives the details of palynological study of medicinally and economically important taxa in the study area. Pollen morphology plays an important role in the recent issues of plant taxonomy, systematics and their implications. The study revealed that indigenous plants have been used for the treatment of numerous respiratory, digestive, dermatological, rheumatic and renal diseases. So, it was necessary to identify local medicinal plants and conservation-based strategies in need of development for the protection of flora (Manzoor *et al.,* 2024b).

Conclusion

Pollen morphological studies have been used as a taxonomic tool in solving the problems of plant systematic studies. However, the study project's goal is to record the use of medicinal plants and identify them using SEM-observed pollen micromorphological features which can be useful for properly collecting the area's priceless medicinal flora. The findings of this study highlight the rich diversity of medicinal plants thriving in the Northern Pakistan, underscoring their integral role in the regional healthcare system. Furthermore, these findings contribute to our understanding of the evolutionary relationships among medicinal plant species in this ecologically diverse region. Therefore, to safeguard the selected flora, it is imperative to determine the true source of traditionally used medicinal plants and to develop conservation-based strategies. Herbal medicines were extracted from these medicinal plants and used by indigenous peoples for different diseases. The study helps to identify the morphological descriptions and main source of local knowledge used for medicinal plants.

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