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Pharmacognostic investigation of *Astragalus graveolens* leaf and their traditional ethnomedicinal utilization

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ABSTRACT

Background & Aim: The current study was performed to evaluate the ethnobotanical uses, chemical constituents of *Astragalus graveolens* Buch. Leaves and their pharmacological activities through literature search. The preliminary phyto-evaluation of this plant has produced a number of bioactive chemicals, some of which have been proven to have antibacterial and cytotoxic potential as a result of its traditional therapeutic applications.

Experimental: We conducted a survey in diverse databases (such as Google scholar, Scopus, and Web of Science, etc.) and professional websites with a key word related to our study. Firstly, we evaluated the traditional utilization of *Astragalus graveolens* Buch. and then the pharmacological studies.

Results: *Astragalus graveolens* has a number of health advantages, including the ability to treat toothaches, strengthen tooth roots, nourish and treat chest infections, kidney, stomach and toothaches. Renal issues, burns, hypertension, and demulcent. Back pain, kidney pain, tooth pain, diabetes, tooth pain, bone pain, bone pain in the bone and tooth pain. They used in food, as a sweetener, a tonic, a headache and stomach pain reliever and to speed up the healing of deep wounds that are infected. The leaves of *Astragalus graveolens* have been investigated for various chemical compounds with pharmacological activities, such as antifungal, antimicrobial, antimalarial, antioxidant, anticancer, anti-inflammatory, anti-diuretic, analgesic and insecticidal activities. The leaves are not well explored for chemical constituents and pharmacological activities.

Recommended applications/industries: *Astragalus graveolens* Buch. need to be subjected to scientific verification based on their traditional utilization, which may lead to a better and effective alternative phytoconstituents for the management of various diseases.

1. Introduction

Plants have been the primary source of therapeutic agents for curing human diseases for a longtime. As medicinal plants have historically been used to treat veterinary ailments, natural resource-based medications are crucial for disease prevention (Shoaib *et al.*, 2021) and human diseases in different areas (Shah *et al.*, 2021). As a result, pharmacognosy is essential to the discovery, characterization, and manufacture of these medications (Cahlíkova *et al.*, 2020). The rising usage of herbal treatments around the world is seen as a positive development (Jones *et al.*, 2006). The pharmacognostic study will help to discover drugs and is the best approach to molecular and genetic discoveries (Alamgir, 2018; Harvey, 2000). Besides,

more than half of the modern drugs have been originated from traditionally used plants (Abbasi *et al.*, 2010). There is an urgent need to document the ethnomedicinal uses of plants worldwide and collaborate with chemists to analyze the compounds in medicinal plants. Such information needs verification through in vitro and in vivo scientific investigation and clinical trials in order to ascertain and establish their utility. Phytochemicals are responsible for the medicinal activity of plant species. They can cure various ailments and possess potential anti-inflammatory, anti-bacterial, analgesic, diuretic, anti-oxidant and anti-fungal properties. Natural products from medicinal plants, both as pure compounds or as extracted, provide opportunities for new drugs because of the unmatched availability of chemical diversity and are not as costly as their chemical counterparts (Nirumand *et al.*, 2018). Due to the rising demand for chemical diversity in screening programs, seeking therapeutic drugs from herbal products is quite interesting worldwide. Botanicals and herbal preparations for medicinal purposes contain various bioactive compounds like flavonoids, alkaloids, saponins, sterols, etc.

A medicinal plant of *Astragalus graveolens* shrub species (*A. graveolens*) (Fig.1) belongs to the family Fabaceae. It is classified as belonging to the second-largest dicotyledons family.

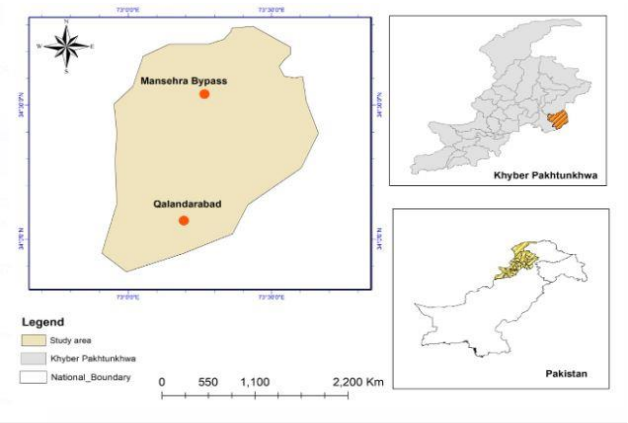

Figure 1. Map of study area

There are 1100 species and 100 genea in India that comprise the family. Based on the floral features, the family was separated into 3 sub-families. According to several botanists, the three subfamilies can be divided into the Papilionaceae, Caesalpiniaceae and Mimosaceae families (Patel *et al.*, 2014). *Astragalus graveolens* has a number of health advantages, including the ability to treat toothaches, strengthen tooth roots, nourish and treat chest infections, kidney, stomach and toothaches. Renal issues, burns, hypertension, and demulcent. Back pain, kidney pain, tooth pain, diabetes, tooth pain, bone pain, bone pain in the bone and tooth pain. They used in food, as a sweetener, a tonic, a headache and stomach pain reliever and to speed up the healing of deep wounds that are infected (Anderson and Grant, 1998).

2. Materials and Methods

The plant sample was collected from district (Mansehra) during session June 2021 and Identified by Prof. Dr Ghulam Mujtaba Shah, Chairman, Department of Botany, Hazara University Mansehra KP, Pakistan. After identification the voucher Number (15059) was assigned to the species and specimens were deposited in the Herbarium of Hazara University (HUP) for permanent record.

2.1. Literature Survey

Initially, pharmacognostic and ethnomedicinal information of the species was gathered from a variety of databases and authentic websites. Google Scholar, Web of Science, Science Direct, PubMed, CAB Abstracts, Springer, Taylor, and Francis were consulted using a variety of keywords.

2.2. Macroscopical features

The macroscopical observations of the leaves and organoleptic characters viz., odour, taste, texture along with colour size, shape, fracture, fracture surface, presence or absence of rootlets, margin, venation, apex, duration, type, phyllotaxis, presence and absence of petiole and stipule were analyzed by following the standard procedure proposed by Puruis *et al.* (1996). Marphological observations of the leaves of plants were examined using digital microscope (Portable USB Digital Microscope20x-800x).

2.3. Anatomical features

Microscopic and anatomical features of the leaves of the species were examined by free hand section cutting. Leaves were preserved in the fixative i-e Formalin Acetic Acid (FAA) for 24-48 hours. Fixative composition was: FAA = 5:5:90 5ml Formalin, 5ml Acetic acid, 35 90 ml Ethanol. Transverse free hand-

section cutting of the leaves of *Astragalus graveolens* was carried out from fresh material. Using a sharp microtome and 1% safranin for two to three minutes, thin slices were created; afterwards these sections were gradually dehydrated by treating with different alcoholic grades for 2-3 minutes in each grade i-e 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and finally 100% alcohol (ethanol). Section of plants after dehydration were placed on the petri dish and treated with a drop of light green for 2 minutes. Afterwards the sections were mounted on a permanent glass slides, with a drop of Canada balsam (Wallis *et al.*, 1985).

2.4. Powdered microscopy

Powdered drug analysis of *A. graveolens* was supported by suceeding the standard method of (Youngkin *et al.*, 1948), with certain modifications to study of the physical and microscopical characteristics of the drug samples. A drop of this solution is placed on a glass slide and analyzed by using research microscope having objective lenses of 10X, 40X and 100X for observation of different structures, and microphotographs were captured with Nikon microscope fitted with camera (Evans *et al.*, 2002).

2.5. Stomatal number (SN) and Stomatal index (SI)

The (SN) and (SI) are most specific and distinguishing character for authentication of leaf of crude drugs (Chaudhary *et al.*, 1997). Average number of stomata present per square mm of both the surfaces of leaves (abaxial and adaxial) is called the stomatal number and stomatal index in the stomatal percentage to the whole stomatal number found in the epidermis of leaf (Bozoglu *et al.*, 2006).

2.6. Technique

Fresh leaf surfaces from the abaxial and adaxial surfaces were chopped into small pieces, cooked in 30% nitric acid with 2.0 g of potassium chloride for 2 to 3 minutes, and then rinsed with distilled water. After drying, both epidermis layers were peeled off with a forcep and kept in a solution of 60% NaOH for two hours. The peeled parts were then mounted on glass slides and covered with cover slips after being bathed in lactic acid Size, shape and number of stomata per square mm was observed under 100x 37 magnifications. A standard formula proposed by was used to find out the stomatal index (Barkatullah *et al.*, 2012).

3. Results and discussion

The macroscopic and microscopic features were investigated in pharmacognostic studies of powdered drug.

3.1. Organoleptic characters

Organoleptic approach was used to macroscopically study and record the powder drug's organoleptic properties, which include its flavour, consistency, aroma and colour. *Astragalus graveolens* has been found to have the following organoleptic effects (Fig. 2 and Table 1). The powdered leaves look to be light green in color, have a fine texture, a flavor that is slightly bitter and have an aromatic aspect.

Fig 2. Image of *Astragalus graveolens* Buch, showing habit Anderson *et al.*(1988).

Table 1. Leaf macroscopical characters of *Astragalus graveolens*.

S. No	Character	Observation
1	Odour	Aromatics
2	Color	Light green
3	Textur	Fine
4	Taste	Slightly bitter

3.2. Microscopic evaluation of Astragalus graveolens leaf

A microscopic feature is considered useful for the herbal drugs and their quality study, such as qualitative microscopy of leaf transverse section (T.S) with or without staining under photomicrograph (Alamgir, 2018). Microscopic features of the transverse section of the *A. graveolens* leaf and anomocytic type of stomata are shown in (Fig. 3). The species of the family Rubiaceae has mostly paracytic stomata in leaves (Bahadur *et al.*, 1971).The many tracheid kinds,

parenchymal cells, epidermal cells, non-glandular trichomes, sclerenchymal cells, etc. were measured in the current study to assess the powder treatment of *Astragalus graveolens*. The powder was found to be tasteless and henna green in colour. Spiral trachieds, Parenchyma cells, epidermal cells, Cork cells and stone cells were discovered when the medication's powder was studied (Fig. 3).

Table 2. Microscopical characters of *Astragalus graveolens* leaf.

Cells types	Length (µm)	Width (µm)
Eipdermal cell	10µm	5.75µm
Cork cell	18.5µm	14.7µm
Spiral tracheids	12.25µm	3.75µm
Vessels	17.25µm	12.5µm
Parenchymatous cells	70µm	20µm
Stone cells	50µm	25µm

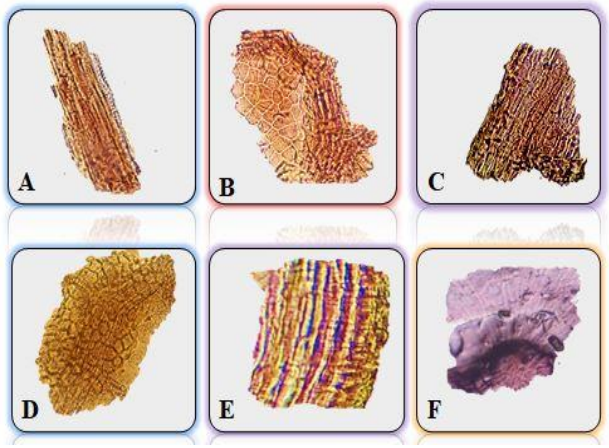

Fig. 3. Powdered drug study of leaves A- Trachieds and vessel, B- Paranchyma cells, C- Cork cell, D- Epidermal cell, E- Spiral tracheids and F- Stone cells, Maiti *et al.* (2002).

Various other scientists also conducted the powdered drug analysis of different plants belonging to different families. Maiti *et al.* (2002) performed the 92 histochemical analysis of the various plants of family Solanaceae for authentication and classification of medicinal plants. Mishra *et al.* (2008) performed the powdered drug analysis of leaf of Tridax procumbens. Patil *et al.*, (2009) analyzed epidermal cells, fibers, trichomes and calcium oxylate crystals of Ficus bengalensis in powdered drug. Murti *et al.* (2010) studied the powdered drug of *Calotropis procera*. Nirmal *et al.* (2009) revealed the presence of xylem vessels, pitted xylem fibers, xylem parenchyma, calcium oxylate crystals and starch grains and multiseriate medullary rays in powdered drug analysis of Sesbania sesban.

3.3. Quantitative characters

A quantitative investigation of the leaves of *Astragalus graveolens* revealed that the abaxial surface (lower) contained 180–184 stomata per square millimetre. In addition, the length and width of guard cells and epidermal cells were measured. The readings were taken using the 10x lens on the microscope. The results were displayed in (Table 3).

Table 3. Quantitative analysis of *Astragalus graveolens* leaf.

S.No	Characters	Observation
1.	Number of Stomata per square mm	154µm
2.	Length of stomata	40 µm
3.	Width of Stomata	20 µm
4.	Number of epidermal cell per square mm	280µm
5.	Epidermal cells length	70 µm
6.	Epidermal cell Width	40 µm
7.	Length of Guard cells	30µm
8.	Width of Guard cells	15µm

3.4. Qualitative characters of Astragalus graveolens

To analyzing the qualitative characteristics of *Astragalus graveolens* leaves, such as the stomata, guard cells, epidermis, epidermis layer and trichome is absent. The outcome was shown in (Table 4).

Table 4. Qualitative characters of *Astragalus graveolens*.

S. No.	Characters	Observation
1.	Stomata type	Aminocytic
2.	Guard cells	Bean shaped
3.	Epidermal cell layer	Double
4.	Epidermal type	Irregular polygonal
5.	Presence of trichome	Absent

3.5. Number of stomata (SN) and index of stomata (SI) analysis

The stomatal index was calculated using a quantitative analysis of the number of epidermal cells and stomata per square millimetre. A technique created by Chaudary and Imran in 1997 was used to determine the stomatal index (Evan, 2002). The final result is shown in (Table 4).

Table 4. Stomatal index.

Numerical Data	
Number of epidermal cells per square mm	280 µm
Number of Stomata per square mm	154 µm
Stomatal index	35.48µm

Stomatal index (SI) = S×100/S+E

- SI = Stomatal index
- E = Number of epidermal cell per unit area
- S = Number of stomata per unit area.

3.6. Section cutting of leaf

The transverse section of a leaf was examined under a microscope at 4X and 10X, revealing the presence of the following distinct cells (Fig. 4).

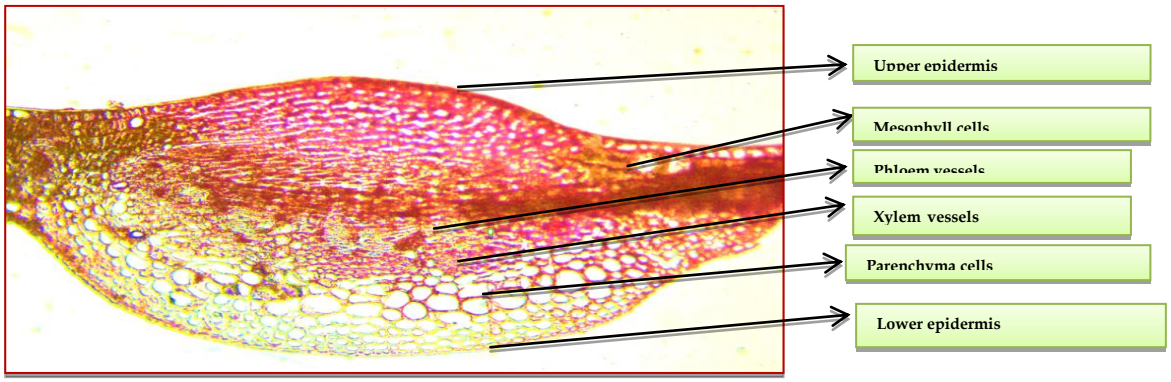

Fig 4.Transverse section of *Astragalus graveolens* Buch. Leaf.

4. Traditional utilization and chemical constituents

The leaves of *A. graveolens* were traditionally used to treat various ailments. The well-known traditional utilization of root and stem is usuall for herpes, bone pain, wound healing and diabetes (Kavita *et al.*, 2016). Leaves powder has been used against viral infections. It has been practiced by the tribal of Rajasthan (India) against snake bites, scorpion stings, and diabetes (Govil *et al.*, 1993). The wood is reported to be used to make gunpowder charcoal (Ravi Shankar and Shukla, 2007) and leaves as an insecticide for the stored grains. The leaves paste is used externally for joint pain traditionally (Pradhan and Badola, 2008). The leaves of *A. graveolens* have been investigated for phytochemicals and various pharmacological activities. The traditional utilization of its parts for specific disease may be due to the bioactive chemical constituents present inside the plant parts. Previous studies demonstrated that the leaves of *A. graveolens* are rich in alkaloids, flavonoids, tannins, phenolics, carboxylic acids, amides, carbohydrates, and saponins (Table 5) (Govindappa *et al.*, 2014; Kavita *et al.*, 2016; Silva *et al.*, 2014). However, no clear evidence is available on chemical constituents and pharmacological activity of their leaves. The traditional knowledge of S. suaveolens should be considered for further clinical trials of the chemical constituents, especially on leaves, to investigate their efficiency and better utilization.

Table 5. Pharmacological activity and chemical constituents of *Astragalus graveolens* leaves.

Plant part	Pharmacological activities	Chemical/functional groups	Chemical constituents/ extract	References
Leave	Antifungal and antimicrobial	Carbohydrates	Cyclobutanol, 4-acetoxy-3- methoxy styrene	Dahpour *et al.*, (2012)
	Antimalarial	Alkaloids	Cyclohexane,	Ertas *et al.* (2015); Gaur, (1999)
	Antioxidant and anticancer	Flavonoids and phenolics	Decyl, 1-pentadecene, palmitaldehyde (Di iso pentylacteal)	Govindappa *et al.* (2014); Igwe and Okwu, (2013)
	Anti-diuretic	Tannins	Benzene Ethan amine,	Nakamura *et al.* (1986)
	Insecticidal	Saponins	3,4- benzyloxy-2,5-difluoro-.1-octadecene	Papitha *et al.* (2017)
	Analgesic and Anti-inflammatory activities.	Terpenoids, aromatic, amines, amides, carboxylic acids and alkanes	Ethanone, 1-(3-methylene cyclo pentyl).	Sathyaprabha *et al.* (2010)

5. Pharmacological activities of chemical constituents

Chemically, this species enrich having various useful secondary metabolites. The major chemical groups found in *A. graveolens* were alkaloids, saponins, flavonoids, tannins, phenolics, etc., containing various types of bioactive compounds (Table 5). Medicinal significance of these chemicals has also been reported by worker, Coates (2006) investigated the chemical constituents of this speciesthrough GC-MS analysis using either different or single extraction methods (i.e. petroleum ether, chloroform, ethyl acetate, and methanol). Similarly, antiviral activity was attributed to compounds such as adamantine methylamine, alpha-methyl. Tetramethyl-2-hexadecen showed anti-inflammatory, analgesic and antipyretic activities (Tsunoda *et al.,* 1965). Octadecyn and phytol were reported to have anti-bacterial (Zhu *et al.,* 2011) and anti-inflammatory (Dangoggo *et al.,* 2012) activities, respectively. Cyclotrisiloxane and hex methyl was reported to possess anti-bacterial activity (Al-Wathnani *et al.,* 2012) and phenol existed anti-fungal, anti-microbial, antimalarial, UV-stabilizer and anti-oxidant properties for hydrocarbon-based products (Dahpour *et al.,* 2012). Tetramethyl-2 hexadecen-1-ol use full as anti-microbial, anticancer, anti-inflammatory, and anti-diuretic activities (Govindappa *et al.,* 2014). Pentatriacontene is a herbistat (Sadananda *et al.,* 2014), and Octadecane is known to have anti-fungal activity (Abubacker and Devi, 2015). Hexadecanoic acid, benzenedicarboxylic acid, ethylhexyl ester, and sistosterol was reported to have anti-bacterial activities (Sathyaprabha *et al.,* 2010; Yinusa *et al.,* 2015).

6. Conclusion

In summary, the traditional utilization of leaves and the chemical constituents from *A. graveolens* has shown good efficacy against the toothache especially the oral cavity, viral infections, demonstrating that the plant has the great potential against viral diseases and considering the present Gums diseases, which is a serious threat to human life, this potential could be very important. Further clinical trials are required regarding the efficacy and safety of *A. graveolens* against oral infection and other pharmacological activities need to be subjected to scientific verification, which may lead to finding a better and effective alternative phytoconstituents for the treatment of toothache and other several diseases. Pharmacognostic studies of their leaves are further recommended for future work.

7. Acknowledgements

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