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Physicochemical evaluation for *Allium Hookeri* Thw. Enum leaves

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Abstract

Awareness and general acceptability of the natural products is increasing day by day because of its lesser side effect and cost factor. Above 80% of the world population is depending upon herbal medicines. Use of plants as medicine is the oldest practice that has been used in all countries from ancient time. Based on those old practices medicines of modern days are derived either directly or indirectly from the natural products. Manipur is one of the north eastern states of India and one of the hot spot area of biodiversity, blessed with the amazing variety of flora and fauna. *Allium hookeri* belongs to Alliaceae family and is a perennial herb growing in dampy soil in every kitchen garden in Manipur. It is locally known as Maroi Napakpi and used as spice and condiment in almost all Manipuri dishes. Its taste is preferred over onion in Manipur. Fresh leaves and fibrous roots are used as medicine in reducing temperature, in swelling as an antimicrobial and antioxidant and reducing blood cholesterol etc. It is reported that its root has antioxidant, free radical scavenging, antimicrobial and anti-inflammatory action. In Manipur people are taking fresh green leaves for many ailments like reducing temperature, reducing blood cholesterol, etc.. So the present study has been carried out to evaluate pharmacognostic and physicochemical analysis of shade dried leaves of *Allium hookeri*.. The reported information will provide data which will be useful in proper identification and authentification of this plant for future research work.

Keywords : *Allium hookeri* leaf, phyto physico-chemical evaluation., antioxidant, anti-inflammatory.

Introduction

Herbs have been used as medicine since the beginning of civilization and some derivatives have become mainstay of human pharmacotherapy. Any biological activities of herbal products depend on its phytochemical constituents. Herbal products are traditionally considered harmless and increasingly being consumed by the people without any prescription. Herbal products are not considered scientifically valid if it is not tested for authentification and characterised it in order to ensure the reproducibility in the manufacturing process. So the major setback in promoting traditional medicine is the lack of standardisation, proper identification and authentification. It is because of the fact that the herbal product can cause health problems by itself, by interacting with other drugs and it may not be effective to the body. Increasing report of adverse drug reaction and toxic effect has drawn the attention of the regulatory bodies and agents on the need of standardisation of herbal plants and its products. Standardisation is an important step for establishing biological activities, toxic effect, allergic reactions, and effect of contamination. Methods to develop authentic analytical techniques which can profile the phytochemical composition is a major challenge to the scientists. So the WHO gives guidelines that herbal products need to be standardised for making it safe and effective before its entry to the market. According to the WHO guidelines, microscopical and macroscopical, pharmacognostic evaluation is the first step towards establishing its authentification, quality and purity standard of herbal plants..

Conventional methods of standardisation of herbal products include botanical authentification microscopic and macroscopic evaluation and chemical profiling.

In the present study we try to evaluate and standardise the leaf of *Allium hookeri* Thaw. Enum. Which belongs to genus lliaceae. The Allium is the largest and most representative genus of Alliaceae that comprises 700 species, widely distributed in the northern hemisphere, North America, North Africa, Europe and Asia. *Allium hookeri* plant species is native to India, Burma, Bhutan and south western China. Common names include hooker chives, Phulun Zung (in India) kuan ye jiu in China .It is also known as winter leek.

Allium hookeri is a wild perennial herb growing in wet and damp soil in wide range without any significant bulb but with fibrous root. In Manipur one of the north eastern state of India, *Allium hookeri* is grown in every kitchen garden . It is used as spice , condiments

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and in the preparation of vegetable stew, soup, fish curry , chicken curry etc.

Allium is well known for its therapeutic uses [1]. It is also reported that *Allium hookeri* is used as antioxidant, anti -inflammatory, anticancer [2,3]. The plant also contains a good amount of nutritiuos compounds like protiens, sugar, fibre ascorbic acid. phytosterols and phenols [4]. its leaves are thick fleshy green , linear with prominent midribs and parellel veination.

In Manipur local people are using the fresh green leaves for many ailments like reducing temperature, blood cholesterol level etc.

Allium hookeri has fibrous root. .In Manipur, local people are using its roots and leaves almost every day in all the preparation as garnishing stuff in Eromba (special dish of Manipuris) vegetable stew (kangshoi), baked food like (Paknam) etc.

Materials and Methods

Collection and authentication of plant materials

Fresh leaves were collected in the month of June-July 2014 from some kitchen gardens of khongman zone 2 Imphal east district of Manipur, India and authenticated by Dr Sunita Garg chief scientist reference no NISCAIR/RHMD/CONSULT/2014 2479-58 dated 8/7/2014 and submitted the sample in CSIR-NISCAIR herbarium for future reference.

Preparation for Sample for analysis

The matured fresh leaves of *Allium hookeri* were collected in the month of July and it is thoroughly washed and cleaned to be free from foreign matters and other contaminants. These were chopped into pieces and shade dried for nearly a month and crushed in powdered form with the help of a grinder. Then successive extraction is performed by using different solvents with different polarity by Soxlet apparatus(ie hot extraction) and evaluation were performed as per IP and ayurvedic pharmacopiea (standard methods.)

Macroscopic examination

The fresh leaves of *Allium hookeri* were examined and characterised according to the shape, colour, odour, texture, taste i.e. organoleptic characteristics.

Microscopic examination

Microcopic studies of the leaves of the plant Allium hookeri was carried out. Thin cross sections from the middle portion of matured portion of matured leaf of Allium hookeri are obtain by sharp razor blade and stained with safranine [5].The sections were examined under light microscope (OlympusBX41) under different magnifications. The adaxial and abaxial epedermis of matured leaves was peeled for observation of stomatal type. The area of stomatal apparatus is calculated by using the formula π / w [5]. and the stomatal index is calculated by following formula [6].

$$\text{Stomatal index (\%)} = \frac{\text{stomatal density}}{\text{Stomatal density + Epidermal cell density}} \times 100$$

All the sections were photographed under light microcope and digital images were manually analysed using Adobe Photoshop.

Physicochemical parameters

Powdered samples were subjected to physicochemical analysis including water and alcoholic soluble extractive ,total ash insoluble ash, water soluble ash, sulfated ash, PH ,moisture content fluorescence analysis microbial and heavy metal contamination were also conducted as per WHO guideline and I.P.

Thin layer Chromatography Analysis

The chemical finger-prints were determined using thin layer chromatography (TLC) for all the extracts were applied to the activated silica gel plates and run in developing chamber and analyzed the results.

Preliminary Photochemical analysis

Test for alkaloids, glycosides tanins and phenolic compounds, flavanoids phytosteroids saponins, protiens, carbohydrates fixed oils and fats are conducted with different reagents.

Fluorescence analysis

The fluorescence characteristics of the powdered crude drug with different chemical were observed in day light and ultraviolet light. The powdered drug was treated with various solvents like picric acid, acetic acid,conc HNO_3, Conc H_2SO_4 ,Conc HCL, ferric chloride, aq. KOH, Iodine solution, ammonia solution 30%v/v and observe under day light UV 254nm and UV 366 nm .

Physicochemical parameters

We determine ash value like total ash, acid insoluble ash, water soluble ash, extractive value, moiture content, PH of the crude drug, determination of microorganisms like *E.coli*, salmonella and determination of heavy metal by atomic absorption spectroscopy. Determination of aflatoxins B1, G1,B2,G2 etc and determination of total phenolic and total flavonoid content taking gallic acid and quarcetin as standard respectively

Pharmacognostic evaluation

Macroscopical characters

Allium hookeri which is a grassy perennial plant having thick and fleshy leaves belonging to the Alliaceae family. Leaves are having parallel veination (figure 1). Flowers are white umbilical shape (2a) ,roots are white and fibrous(figure b). L.S of leaf shows abaxial

epidermal surface with dense distribution of stomata (figure 3) there is sparse distribution of stomata in adaxial epidermal surface.(figure 4).T.S of leaf (figure 5) showing vascular bundle (conjoint, co(p) and mesophyll cells and llateral and closed type) showing xylem tissue(x) phloem tissue. T.S of midrib portion of leaf (figure 6) showing vascular bundle (conjoint, collateraland closed type) showing xylem tissue (x),phloem tissue (p), mesophyll cells, palisade cells and cuticles.

TLC plates. The plates were developed using different solvent and viewed under UV florescence light at wavelenght 254 nm and 365 nm and finally sprayed with the detection reagent like Dragendroff;reagent,Kmno4 reagent, Ninhydrin reagent, and DNS reagent etc to determine the compounds present.(Figure 7a,b,c,d,e).

Plant Profile

Figure 1: Allium hookeri

Figure 2(a): Flower

Figure 2(b)

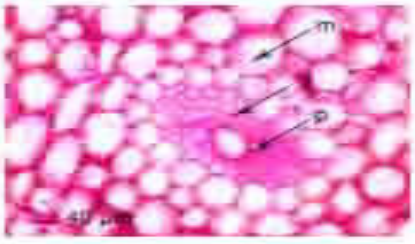

Figure 3

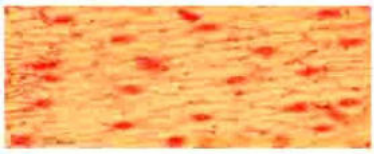

Figure 4

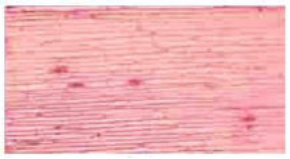

Figure 5

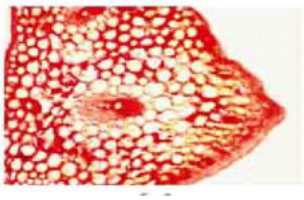

Figure 6

Figure (7a)

Figure (7 b)

Figure(7c)

Figure(7d)

Figure (7e):TLC chromatogram.

Microscopic studies :- Mesophyll cells are not differntiated. The middle vascular bundles are ovate in shape The stomata were observed on both the upper and lower surface and they were of the anomocytic type and oblong in shape. The shape of the guard cells is suborbicular to orbicular. Wax cells on both adaxial and abaxial surface were observed .

Results and discussion

Macroscopic and microscopic evaluation

The organoleptic charaacteristics of *A. hookeri* leaves are green in colour roots and flower are white it has pungent aroma tasteless parallel veination leaf thickness 805.0±5.7 (µm) mesophyll thickness74.2 ±8.9 (µm) length of middle vascular bundle 225.2±5.2 µm stomatal index is 24.05 ±0.16% and stomatal apparatus area 294.64±9.5 μm^2 chloroplast were observed within the guard cells in the cross section of the leaf. Cuticle of adaxial surface was found thicker than the abaxial surface. Stomatal distribution, size, density, morphology and behavior are closely associated with plant transpiration [7].Wax secreting cells on both sadaxial and abaxial surface were observed. They act as sunscreen and aids in shedding water so that the leaf cells do not become saturated with water and burst. [8]. The above characteristics will be essential in preliminary identification of the plant. Table (1) is showing the extractive value which is more in methanolic extract i.e 9.6 gms Table 2 showing the TLC spot and Rf value with different solvent ratio .. Table (3) indicating the importance of fluorescence which is exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in many natural products (eg alkaloids like berberine), which do not visibly fluoresce in day light. If the substances are not fluorescent themselves they may be often converted into florescent derivative by applying different reagents. The plants extract were spotted using capillary tube on TLC plates .The plates were developed using different solvents and the viewed under UV fluorescence light at wave length 254nm and 364nm and finally sprayed with the required detecting reagents like Dragendorff reagent, $KMNO_4$ reagent, Ninhydrin reagent, DNS reagent, etc. to determine the compounds present and solvent system which give the best observation. It was noted that various leaf extract showed the presence of alkaloids, nitrogen compounds, and surfactants detected by Dragendorff reagent .The leaf extract also shows the presence of amino acids, amines ,amino sugars detected by Ninhydrin reagent, the presence of the fatty acid derivative and organic compounds are detected by $KMNO_4$.

Table 1. Weights of the plant leaf extracts of *Allium hookeri.*

Plant Part	Extracts	Extracts weight
Leaf Powder (1000 g)	Hexane	6.5 g
	Chloroform	4.2 g
	Ethyl acetate	2.8 g
	Methanol	9.6 g

Table (2) TLC of different extract of *Alium hookeri* leaf performed on precoated silicagel 60 f_{254} Plates using different solvent systems

EXTRACT	SOLVENT SYSTEM	NUMBER OF SPOTS	Rf value
Ethyl acetate	Hexane : Ethyl acetate (50:50)	6	0.29; 0.42; 0.64; 0.73; 0.82; 0.96
Ethanol	Dichloromethane : methanol (95:5)	4	0.19; 0.28; 0.83; 0.92
methanol	Chloroform : Methanol (90:10)	9	0.12; 0.43; 0.52; 0.59; 0.62; 0.73; 0.79; 0.84; 0.87
Aqueous	Butanol : Acetic Acid: water (4 :1:2)	3	0.24; 0.48; 0.76

Table 3:- Fluorescence analysis of leaf powder of *Allium hookeri*

S.no.	Reagent	Day light	Short wavelength [254nm]	Long wavelength [366nm]
1.	Acetic acid	Yellowish green	Green	Blake brown
2.	Conc. HCL	Yellowish Green	Green	Black brown
3.	Conc. H_2SO_4	Yellowish green	Dark green	Black
4.	$FeCl_3$	Yellowish green	Green	Black
5.	Conc. HNO_3	Orange yellow	Yellowish green	Black
6.	Picric acid	Pale Yellow	Green	Black
7.	Aq. KOH	Pale yellow	Yellowish Green	Black
8.	Alcoholic KOH	Pale yellow	Yellowish Green	Black
9.	Iodine solution	Yellow	Green	Black
10.	Ammonia solution	Yellowish green	Green	Black

Table 4.1:- Different Physicochemical Parameter

S. No.	Parameters	Result
1.	Total Ash value (%w/w)	14.91
2.	Acid insoluble ash (%w/w)	0.56
3.	Water soluble ash (%w/w)	6.15
4.	Sulfated ash	19.98
9.	Soluble ash	5.22
10.	PH of the powdered drug	5.92

Table 4.2:- Aflatoxin and Total Bacterial Count

S. No.	Parameters	Result
1.	Test of Aflatoxins	
	Aflatoxin B1	Not detected
	Aflatoxin B2	Not detected
	Aflatoxin G1	Not detected
	Aflatoxin G2	Not detected
2	S. aureus/g	Absent
3	E. coli/g	Absent
4	Salmonella/10g	Absent
5	P.aeruginosa/g	Absent

Table 4.3:- Heavy Metals

S. No.	Parameters	Result
1.	Heavy metals	
	Arsenic, As	0.178ppm
	Lead, Pb	0.570 ppm
	Mercury, Hg	Not detected
	Cadmium, Cd	Not detected

Conclusion

The pharmacognostic standards for *Allium hookeri* leaves are being reported in this study. The standard established in this study will be helpful in minimizing the adulteration and it will help the researcher, manufacturer and individuals in selecting the right plant materials for various purposes. Its TLC chromatogram shows that it has many nutrient elements like sugar, amino acid, fatty acid and its derivative and other organic compounds. Hence it can be used as food supplement and in manufacturing of nutraceuticals in future. It has high flavanoid and phenolic content so it is a potent antioxidant. By establishing pharmacognostic standards, plants which are very prone to contamination, deterioration and variation in composition can be controlled, monitored and evaluated by sophisticated modern analytical techniques. This may lead to new formulation of good quality, therapeutically effective, safe, economic and better acceptability. We can extend our study by determining its total phenolic and total flavanoid content and check its antioxidant potential by DPPH assay and FRAP assay. So we can also further evaluate its biological activities like anti-diabetic, reducing blood-cholesterol level and other metabolic syndrome in animal model in future studies.

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Conflict of Interest

The authors declare no conflict of interest.

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