Standardization and Phytochemical Evaluation of the Aerial Parts of *Prangos pabularia*

Nimisha Sharma¹*, Arvind Negi¹, Praveen Kumar Ashok¹

1. Guru Ram Das Post Graduate Institute of Management and Technology, Department of Pharmacy, Rajpur Road, Dehradun, Uttarakhand, India
   [E-mail: nimihygiene@yahoo.co.in, Mob: +91-9557599209]

*Prangos pabularia* (Apiaceae) commonly known as Komal, is widely used in Ayurveda as tonic to the liver. The whole plant appears to offer a far reaching range of pharmacological effects such as antibacterial, anti-inflammatory, analgesic, diuretic, abortifacient, allelopathic, anticancer etc. since time immemorial in traditional systems of medicine. The study approaches to build up a range of specifications for standardization parameters as per WHO guidelines for this crude drug. The aerial parts of the plant were evaluated for various quantitative parameters including the study of morphological characters. Ash values, extractive value in different solvents, pH determination, fluorescence analysis, phytochemical screening, HPLC standardization and characterization of bergapten have been carried out in the study. The results generated can be utilized for its identification, authentication and prevention of adulteration.

**Keyword:** Komal, P. Pabularia, Physical Parameters, Quality, Standardization.

1. Introduction

Plants are an indispensible source of therapeutic preparations, both preventive and curative. With the resurgence in the consumption and demand for medicinal plants, WHO recognized the need for their standardization and quality control¹. In this view WHO published guidelines defining the basic criteria for evaluating the quality, safety and efficacy of herbal medicines aimed at assisting national regulatory authorities, scientific organizations and manufacturers in this particular area².

*Prangos pabularia* Lindley is a hardy perennial herb widely distributed in the alpine and sub-alpine regions. It is commonly called as Djashe – Ulufei in Iran, Komal; Krungus in India, Silphium parsley, Hay plant³. It is held in considerable repute in indigenous medicine for its roots and fruits. The roots and fruits have medicinal properties and are used in traditional medicine systems as antioxidant⁴, diuretic⁵, antibacterial⁶, antifungal⁷, cytotoxic⁸ etc. The roots are used against all kinds of kidney and urinary disorders, soothing and controls urine discharge, inflammation and bleeding in the kidney⁹. The fruit is said to promote expulsion of the foetus¹⁰. It also possesses diuretic properties. The young leaves and flowers are used as insect repellent in paddy godowns¹¹. This plant produces a large number of coumarins⁶,¹² and has been found to be relatively rich in secondary metabolic products. The constituents liable to account for such dynamic behavior comprise of osthol, isoimperatorin, coumarins and furanocoumarins viz. xanthotoxin, aviprin etc.⁴-¹¹. The present study is an attempt to
establish the phytochemical standards for this crude drug for its commercial exploitation.

2. Materials and Methods
2.1 Collection and Identification of Plant material
In the present study, the aerial parts of Prangos pabularia were collected in the month of September from Drass, Kargil. The plant was identified by Botany Division of IIIM, Jammu. A voucher specimen (Accession No. 17044) was deposited at Janaki Ammal Herbarium, IIIM, Jammu.

2.2 Morphological Characters
The dried aerial parts of Prangos pabularia were taken and observed for various parameters including shape, size, colour, taste and surface texture.

2.3 Foreign Matter Analysis
A sample of plant material was weighed. It was spread in a thin layer and foreign matter was sorted into groups either by visual inspection, using a magnifying lens or with the help of a suitable sieve according to the requirements. The remainder of the sample was sifted through a sieve no. 250; dust was regarded as mineral admixture. The portion of this sorted foreign matter was weighed. The content of each group was calculated in grams per 100g of air dried sample\[^{13}\].

2.4 Physical Evaluation
The ash values, extractive values, loss on drying and crude fibre content were determined according to the official methods prescribed in WHO\[^{13}\] guidelines on quality control methods for medicinal plants materials and Ayurvedic pharmacopoeia\[^{16}\]. Fluorescence analysis was carried out according to the method of Chase & Pratt\[^{15}\] and Kokoski\[^{16}\].

2.5 pH Determination
pH of 1% and 10% solution of powdered aerial parts of P. pabularia was determined using a standardized glass electrode.

2.6 Phytochemical Screening
The aerial parts of P. pabularia were coarsely powdered and extracted with different solvents, viz., petroleum ether, ethyl acetate, methanol and water. The extracts were then subjected to phytochemical screening as per standard methods prescribed in literature\[^{17-19}\].

2.7 Swelling Index and Foaming Index
Swelling Index and Foaming index were determined as per the methods prescribed in WHO guidelines\[^{13}\].

2.8 Development of Protocol of HPLC method for standardization of P. pabularia Extract
The dried powdered aerial parts of P.pabularia were allowed to pass through SS sieve (20 mesh). It was defatted by treating with petroleum ether (60-80°C) and then extracted to exhaustion (soxhlet) with methanol. Excess solvent was removed under vacuum to get the solid mass. 20mg of the extract was dissolved in 2ml methanol (HPLC grade) and pure compound Bergapten (2mg/5ml) was also dissolved in methanol (HPLC grade). Both solutions were centrifuged and filtered through Millipore micro filter (0.45µm) and were used for analysis.

2.9 HPLC conditions
Mobile Phase: ACN (B), Water (A); gradient, Flow rate: 0.8 ml/min, Flow type: Gradient, Column: RP-18e (Merck, 5um, 4 x 250mm.), Column temp. : 300°C, Stop time: 75 min., Wavelength: 254 nm, Retention time (Rt) for bergapten – 44.405min.

3. Analysis
An Agilant HPLC system consisting of two pumps with pump control module, an automatic sampling unit, a column oven coupled with a photodiode array detector was used for data analysis and data processing. Agilant Empower software was used for data analysis and data processing. The working solutions of Bergapten were injected in different concentrations (5 μL, 10 μL, 15 μL, 20 μL, 25 μL). The samples were analyzed at 30°C on a Merek RP-18e column (5μm, 4 x 250mm,) by UV detection at 340nm.
The calibration curve of marker compound was plotted using five levels of concentrations and goodness of fit was observed.

4. Result
The study of various physicochemical parameters of the aerial parts of *P. pabularia* like total ash, acid insoluble ash, extractive values was carried out and the result are as shown in Table 2. The loss on drying at 105°C was found to be 5.12%/w/w which gives an idea about the likely deterioration time for the crude drug. The amount of earthy materials and minerals accompanying the crude drug as indicated by the total ash was 4.9%/w/w while the siliceous matter i.e. acid insoluble ash content was 1.68%/w/w. The preliminary phytochemical tests have confirmed the presence of a wide range of chemical constituents including secondary metabolites as alkaloids, glycosides, and phenolic compounds likely to be responsible for the therapeutic effects thus justifying its traditional use.

### Table 1. Observation of Morphological Characters of Stems, Leaves and Flowers of *P. pabularia*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Stem</th>
<th>Leaves</th>
<th>Flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>Green</td>
<td>Green</td>
<td>Yellow</td>
</tr>
<tr>
<td>2.</td>
<td>Odour</td>
<td>Aromatic</td>
<td>Aromatic</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Taste</td>
<td>Sharp &amp; Aromatic</td>
<td>Sharp &amp; Sweet &amp; Aromatic</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Size</td>
<td>Upto 1m in length</td>
<td>30-45cm in length</td>
<td>15-45cm in length</td>
</tr>
<tr>
<td>5.</td>
<td>Shape</td>
<td>Non woody stem</td>
<td>Pinnate</td>
<td>Umbels</td>
</tr>
</tbody>
</table>

The HPLC method was conducted to identify and quantify bergapten from the extract of the aerial parts of *P. pabularia*. Bergapten peak from the methanolic extract was identified by comparing its Rt values with those obtained by chromatography of the standard under the same conditions. Bergapten showed a peak at Rt 44.405min.
Table 4. Observations of Fluorescence Analysis

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>Day light</th>
<th>UV light (366nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Powder as such</td>
<td>Pale green</td>
<td>Fluorescent pale green</td>
</tr>
<tr>
<td>2.</td>
<td>Powder in distilled water</td>
<td>Bluish green</td>
<td>Fluorescent bluish green</td>
</tr>
<tr>
<td>3.</td>
<td>Powder in absolute alcohol</td>
<td>Olive green</td>
<td>Fluorescent orange</td>
</tr>
<tr>
<td>4.</td>
<td>Powder in 10% NaOH</td>
<td>Light brown</td>
<td>Fluorescent dark brown</td>
</tr>
<tr>
<td>5.</td>
<td>Powder in 50% HNO₃</td>
<td>Yellow</td>
<td>Fluorescent black</td>
</tr>
<tr>
<td>6.</td>
<td>Powder in 50% H₂SO₄</td>
<td>Dark green</td>
<td>Fluorescent yellowish green</td>
</tr>
</tbody>
</table>

The compound exhibited linear response in the calibration curve, which was prepared by using the multipoint calibration curve method. Working solutions were injected and the calibration curve was plotted on the basis of five amounts (5 to 30 µl) of standard. The goodness of fit was observed ($r^2$): 0.999943. The marker compound in the methanolic extract was quantified using the calibration curve. Bergapten was found to be 0.05 %w/w in the extract.

Fig 1. HPLC chromatogram of standard bergapten. Bergapten peak at Rt 44.405 min detected at a wavelength of 254 nm.

Fig 2. Calibration curve of standard bergapten.

Fig 3. HPLC chromatogram of methanol extract of P. pabularia. Peak at Rt 44.405 min correspond to bergapten detected at a wavelength of 254 nm.

5. Conclusion
The quality control parameters as mentioned in the WHO guidelines aims at standardizing the crude drugs as well as single and compound formulations from herbal drugs. Furthermore, the stability of crude drugs and formulations thereof is also an essential parameter covered. The study of these parameters conjugated with phytochemical screening for the detection of bioactive principles present in P. pabularia will aid the researchers to exploit this plant on commercial scale. In addition, the HPLC estimation of bergapten can be utilized as an
assurance parameter for the quality of *P. pabularia* in herbal formulations.

6. References:

19. Indian Pharmacopoeia, Chemical tests and Assays. The Controller of publications, Govt. of India, New Delhi, 1996, A34.