ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 1 Issue 5

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

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

Nimisha Sharma[1*], Arvind Negi[1], Praveen Kumar Ashok[1]

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, abortifaecient, 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 control[1]. 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 area[2].

Prangos *pabularia* Lindley is a hardy perennial herb widely distributed in the alpine and sub-alpine regions. It is commonly called as Djashire – Ulufei in Iran, Komal; Krungus in India, Silphium parsley, Hay plant[3]. 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 antioxidant[4], diuretic[5], antibacterial[6], antifungal[7], cytotoxic[8] etc. The roots are used against all kinds of kidney and urinary disorders, soothing and controls urine discharge, inflammation and bleeding in the kidney[9]. The fruit is said to promote expulsion of the foetus[10]. It also possesses diuretic properties. The young leaves and flowers are used as insect repellent in paddy godowns[11]. This plant produces a large number of coumarins[6,12], 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 officinal methods prescribed in WHO[13] guidelines on quality control methods for medicinal plants materials and Ayurvedic pharmacopoeia[14]. 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 Merck 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*

S.No.	Parameters	Stem	Leaves	Flowers
1.	Colour	Green	Green	Yellow
2.	Odour	Aromatic	Aromatic	Aromatic
3.	Taste	Sharp & Aromatic	Sharp & Aromatic	Sweet & Aromatic
4.	Size	Upto 1m in length	30-45cm in length	15-45cm in length
5.	Shape	Non woody stem	Pinnate	Compound Umbels

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 2. Physical Parameters

S.No.	Parameters	Values		
1.	Foreign matter analysis	0.92 %w/w		
2.	Loss on drying	5.12 %w/w		
3.	Ash Values			
	A. Total ash	4.9 %w/w		
	B. Acid insoluble ash	1.68 %w/w		
	C. Water soluble ash	0.94 %w/w		
4.	Extractive Values	Cold Maceration (%w/w)	Hot Extraction (%w/w)	Successive Extraction (%w/w)
	A. Petroleum ether	1.27	3.93	4.01
	B. Ethyl acetate	5.00	9.76	11.24
	C. Methanol	11.43	14.78	18.56
	D. Aqueous	31.09	31.47	32.00
5.	Crude fibre content	2.38 %w/w		
6.	pH Determination			
	A. 1% solution	6.72		
	B. 10% solution	7.14		
7.	Swelling Index	Absent		
8.	Foaming Index	Less than 100		

Table 3. Observations of Phytochemical screening of *P. pabularia*

Extract constituents			PE	EtOAc	MeOH	Aq
Alkaloids	Dragendoff's Test		-	+	+	+
	Wagner Test		-	+	+	-
	Mayer test		-	-	-	-
	Hagers test		-	-	-	-
Carbohydrates	Molisch Test		-	+	+	+
	Fehlings Test		-	+	+	+
	Benedicts Test		-	+	+	+
Glycosides	Cardiac glycoside	Killer Killiani Test	-	-	+	-
	Anthraquinone glycoside	Borntragers Test	-	-	+	+
	Saponin glycoside	Haemolysis Test	-	-	-	-
Tannins & Phenols	5% $FeCl_3$ Test		-	-	-	-
	Gelatin Test		-	-	-	-
	Precipitation Test	Lead acetate	-	+	+	+
		Acetic acid	-	+	+	+
		Dil. HNO_3	-	+	+	+
		Pot. dichromate	-	+	+	+
	Zinc chloride test		-	+	+	+
Flavonoids	Lead acetate Test		+	+	+	+
	Ammonia Test		+	+	+	+
Proteins	Biuret Test		-	-	-	-
	Xanthoproteic Test		-	-	-	-
	Millon's Test		-	-	-	-
Steroids	Libermann Burchard Test		-	-	-	-
	Salkowski Test		+	+	-	-
	Liberman Test		+	+	-	-

(+) Present; (-) Absent

Table 4. Observations of Fluorescence Analysis

S.No.	Treatment	Day light	UV light (366nm)
1.	Powder as such	Pale green	Fluorescent pale green
2.	Powder in distilled water	Bluish green	Fluorescent bluish green
3.	Powder in absolute alcohol	Olive green	Fluorescent orange
4.	Powder in 10% NaOH	Light brown	Fluorescent dark brown
5.	Powder in 50% HNO_3	Yellow	Fluorescent black
6.	Powder in 50% H_2SO_4	Dark green	Fluorescent yellowish green

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 ($r^{\wedge}2$): 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.

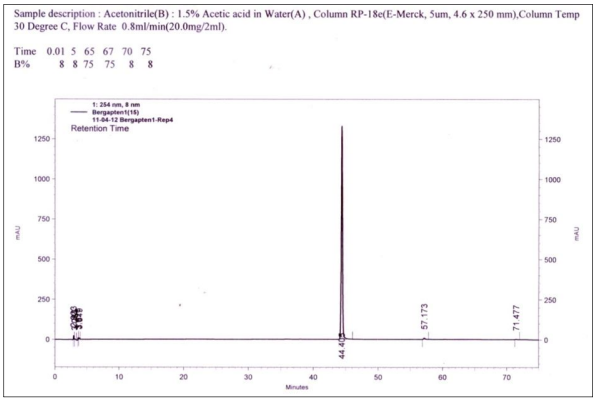

Fig 1. HPLC chromatogram of standard bergapten. Bergapten peak at Rt 44.405min detected at a wavelength of 254 nm.

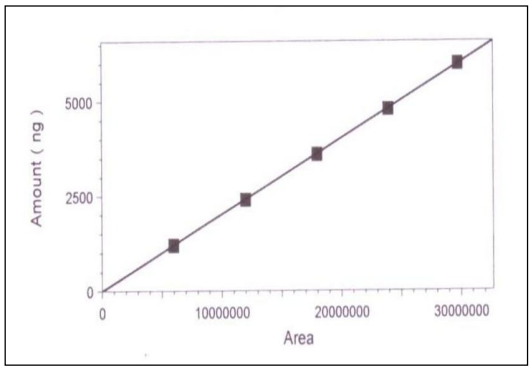

Fig 2. Calibration curve of standard bergapten.

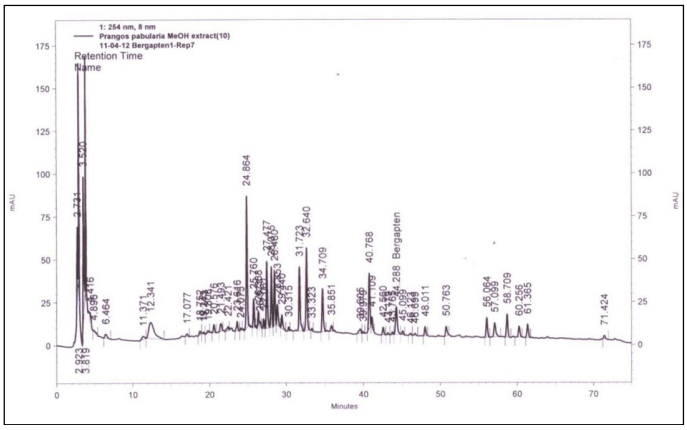

Fig 3. HPLC chromatogram of methanol extract of *P.pabularia*.

Peak at Rt 44.405min 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:

1. Anonymous. WHO policy perspective on medicines-traditional medicines growing needs and potential. WHO Geneva 2002; 2: 1-6.
2. Akerale O. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram 1993; 28: 13-19.
3. Khory RN & Katrak NN. Materia Medica of India & their Therapeutics. Edn 3, Neeraj Publishing House, Delhi, 1985, 296-297.
4. Kogure K, Yamauchi I, Tokumura A, et al. Novel antioxidants isolated from plants of the genera *Ferula*, *Inula*, *Prangos* and *Rheum* collected in Uzbekistan. Phytomedicine 2004; 11(7-8): 645-651.
5. Ballabh B, Chaurasia, OP. Medicinal Plants of cold desert Ladakh used in the treatment of stomach disorders. Indian Journal of Traditional Knowledge 2009; 8(2): 185-190.
6. Tada Y, Shikishima Y, Takaishi Y, Shibata H, Higuti T, Honda G, et al. Coumarins and γ-pyrone derivatives from *Prangos pabularia*-antibacterial activity and inhibition of cytokine release. Phytochemistry 2002; 59: 649–54.
7. Razavi SM and Zarrini G. Bioactivity of Aviprin and Aviprin-3"-O-Glucoside, Two Linear Furanocoumarins from Apiaceae. Russian Journal of Bioorganic Chemistry 2010; 36(3): 359-362.
8. Jamwal KS, Anand KK, Chopra IC. Pharmacological properties of a crystalline substance (Osthol) isolated from *Prangos pabularia* Lindl. Arch. Int. Pharmacodyn. Ther. 1962; 138: 400–411.
9. Ballabh B, Chaurasia OP, Ahmed Z, Singh S. Traditional medicinal plants of cold desert ladakh – Used against kidney and stomach disorders. Journal of Ethnopharmacology 2008; 118: 331-339.
10. Kumar Dinesh, Kumar Ajay, Prakash Om. Potential antifertility agents from plants : A comprehensive review. Journal of Ethnopharmacology 2012; 140(1): 1-32.
11. Razavi SM. Chemical and allelopathic analyses of essential oils of *Prangos pabularia* Lindl. from Iran. Natural Product Research 2011; 1-4.
12. Kuznetsova GA. Coumarins and Furocoumarins in roots, stalks, and leaves of *Prangos pabularia*. Tr. Botan. Inst. Akad. Nauk. SSSR. Ser. 5 Rast. Syr'e No. 12 1965; 49-53.
13. Anonymous. Quality control methods for medicinal plant materials. WHO: Geneva; 1998.
14. Ayurvedic pharmacopoeia of India. Edn 1, Vol. II, part I, 1999.
15. Chase CR & Pratt RS. Flourescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am. Pharmacol. Association 1949; 38: 32.
16. Kokoshi CJ, Kokoshi RJ, Sharma FT. Florescence of powdered vegetable drugs under ultraviolet radiation. J of Pharmaceutical Association 1958; 47: 715-717.
17. Khandelwal KR. Practical Pharmacognosy. Edn 14, Nirali Prakashan, Pune, 2005, 149-156.
18. Harborne JB. Methods of Extraction and Isolation. Edn 3, Chapman and Hall, London, 1998, 125-129.
19. Indian Pharmacopoeia, Chemical tests and Assays. The Controller of publications, Govt. of India, New Delhi, 1996, A34.