Pharmacogn. Res., 2026; 18(4):1461-1466.
https://www.phcogres.com

Original Article

Phyto-Physiochemical Evaluation and Preliminary Phytochemical Screening of *Peganum harmala* L. (*Harmala*): Establishing Standardization Parameters for Quality and Safety

Aditi Padoley*, Jaimala Jadhav, Pallavi Dewangan, Dhanashri Khuspure

Department of Dravyaguna, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha, Datta Meghe Institute of Higher Education and Research (DMIHER), Nagpur, Maharashtra, INDIA.

ABSTRACT

Background: The scientific standardization of herbal medicines is necessary to ensure their quality, safety, and reproducibility in therapeutic use. Peganum harmala L., commonly referred to as Harmal or Syrian rue, is traditionally valued for its neurological, analgesic, antimicrobial, and anti-inflammatory properties, largely attributed to β-carboline alkaloids such as harmine and harmaline. Objectives: This study aimed to establish reliable pharmacognostic, physicochemical, and phytochemical standards for powdered Peganum harmala seeds to support authentication and quality control. Materials and Methods: The seed powder was evaluated through organoleptic assessment, macroscopic and microscopic examination, and physicochemical analysis, including moisture content, ash values, extractive values, and pH determination. Qualitative phytochemical screening of aqueous and ethanolic extracts was performed using standard chemical tests. High-Performance Thin-Layer Chromatography (HPTLC) was employed to generate a characteristic chemical fingerprint. Results: The physicochemical parameters were within acceptable limits of the Ayurvedic Pharmacopoeia and the WHO (loss on drying 3.8%, total ash 3.21%, water-soluble ash 1.56%, acid-insoluble ash 0.5%, water extractive 21.67%, alcohol extractive 9.56%, pH 7.8), indicating good purity and stability. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, steroids, glycosides, carbohydrates, tannins, and saponins, while proteins and amino acids were not detected. HPTLC analysis revealed consistent fluorescent bands at low Rf values (~0.05) under UV 366 nm, suggesting the presence of characteristic polar alkaloids. Conclusion: These findings provide baseline quality parameters for standardizing Peganum harmala seed powder, supporting its authentication and potential use in standardized herbal formulations. Further quantitative and pharmacological studies are recommended to strengthen clinical and regulatory applications.

Keywords: Alkaloids, Harmal, Medicinal plant, Quality evaluation, Syrian rue.

Correspondence:
Dr. Aditi Padoley
Department of Dravyaguna, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha, Datta Meghe Institute of Higher Education and Research (DMIHER), Nagpur, Maharashtra, INDIA.
Email: aditipadoley@gmail.com

Received: 05-11-2025;
Revised: 16-03-2026;
Accepted: 23-06-2026.

INTRODUCTION

Medicinal plants have long served as an important source of therapeutic agents and continue to contribute significantly to both traditional healthcare systems and modern drug discovery. With the increasing global interest in herbal medicine, scientific validation and standardization have become essential to ensure the quality safety, and reproducibility of formulations derived from plants. Herbal drugs contain complex mixtures of secondary metabolites whose composition may vary depending on geographical origin, environmental factors, harvesting practices, and processing methods. Therefore, systematic pharmacognostic, physicochemical, and phytochemical evaluation is necessary to establish reliable identification parameters and standardized quality control measures.

Many Middle Eastern therapeutic traditions, including Ayurvedic, Unani, and other medical systems, have long used *Peganum harmala* L., also known as Syrian rue or Harmal. Analgesic, antibacterial, anti-inflammatory, antioxidant, and neuroactive qualities are only a few of its many pharmacological effects that have garnered significant scientific interest. These physiological effects are mostly attributed to the presence of β-carboline alkaloids, including harmine, harmaline, and harmalol, which are known for their neuromodulatory and monoamine oxidase-inhibitory actions. In addition to alkaloids, the plant contains several other bioactive constituents, including flavonoids, phenolic compounds, tannins, and glycosides, which collectively enhance its therapeutic potential (Mahmoudian *et al.*, 2002).

OPEN ACCESS

Manuscript TECHNOMEDIA

DOI: 10.5530/pres.20260034

Despite its extensive traditional use and increasing pharmacological relevance, comprehensive standardization studies of powdered *Peganum harmala* seed material remain limited. Establishing validated baseline parameters is essential for accurate authentication, preventing adulteration, and developing consistent herbal formulations. An integrated approach combining pharmacognostic characterization, physicochemical profiling, and phytochemical screening provides a scientific framework for achieving reliable quality assurance.

MATERIALS AND METHODS

Plant Material Gathering and Verification

The seeds of *Peganum harmala* L. were procured from a local herbal market. The plant material was authenticated by a qualified botanist based on morphological characteristics, and voucher specimens were preserved for future reference.

Preparation of Plant Powder

After removing any unnecessary material, the gathered seeds were cleaned and given the proper conditions to dry. To create a coarse powder, the dried seeds were ground in a mechanical grinder. Before being kept in sealed containers for additional analysis, the powdered material was run through a 40-mesh filter to guarantee uniform particle size.

Pharmacognostic Research

Plant Material Macroscopy

The morphological features of *Peganum harmala* seeds, including size, shape, structure, and surface texture, were assessed using a basic microscope and unassisted visual inspection. Additionally, sensory evaluation was used to assess organoleptic qualities, including color, odor, and taste (Table 1).

Transverse Section Microscopy

The seed sample was initially soaked in water to soften the tissue prior to sectioning. Thin transverse sections were then carefully prepared using a sharp diamond-edged blade. Several temporary slides were mounted for microscopic observation and examined using a digital trinocular compound microscope (Evans, 1996).

Plant Material Microscopy

A 2 g sample of finely dried seed powder was prepared for microscopic examination by passing it through a mesh sieve using both wet and dry mounting methods. 50% glycerine was used to create wet mounts, and a smearing technique was used to spread the sample equally for dry mounts. Using a microscope set to 10X and 40X magnification, the prepared slides were inspected, and representative photomicrographs were taken to record the distinctive diagnostic characteristics (World Health Organization, 2011).

Physicochemical Analysis

Standard pharmacognostic procedures were employed to evaluate physicochemical parameters of the powdered drug. These parameters help assess purity, quality, and the presence of inorganic or adulterant materials (Jani and Sawant, 2013; Daniel and Mammen, 2022).

Determination of Ash Values

To assess the inorganic content and potential contamination with earthy elements, ash values were calculated.

Total Ash

A precisely weighed silica crucible was filled with approximately 3 g of air-dried powder, which was then heated to no more than 450°C until carbon-free ash was produced. After cooling in a desiccator, the crucible was weighed. The percentage of total ash content was determined for the air-dried medication.

Acid-Insoluble ash

The resulting total ash was heated for 5 min in 25 mL of diluted hydrochloric acid. Using ashless filter paper, the insoluble residue was collected, carefully washed with hot water, burned to a constant weight, and the percentage of acid-insoluble ash was computed.

Water-Soluble ash

25 mL of distilled water were used to boil the entire amount of ash for 5 min. Following filtering and washing, the insoluble fraction was burned to a consistent weight. Water-soluble ash was represented by the sum of the insoluble residue and total ash.

Determination of Moisture Content

A tared evaporating dish containing approximately 10 g of precisely weighed material was dried in an oven at 105°C for 5 hr. After cooling, the sample was weighed again. The drying loss relative to the air-dried sample was used to compute the moisture content.

Determination of pH Range

A precisely weighed 3 g of powdered *Peganum harmala* seed was suspended in 30 mL of distilled water. To ensure the extraction went well, the mixture was transferred to a closed flask and shaken periodically for 5 hr. Overnight, the suspension was left to stand undisturbed after shaking. When the solution was filtered into a

Table 1: Organoleptic Parameters.

Sl. No.	Parameters	Result
1.	Color	Brownish
2.	Odour	Characteristic
3.	Taste	Characteristic

sterile beaker the next day, the pH of the filtrate was measured by observing the color shift on pH indicator paper.

Preparation of Extracts

Approx 250 g of dried powdered *Peganum harmala* seeds were subjected to Soxhlet extraction. Initially, the powder was defatted using petroleum ether. Subsequently, exhaustive extraction was performed using ethanol as solvent for approximately 36 hr, maintaining the temperature between 40 to 50°C.

A rotary evaporator was used to evaporate the solvent under reduced pressure, producing a semi-solid extract that was vacuum-dried and stored for additional analysis and formulation experiments (Harborne, 1973).

Preliminary Phytochemical Screening

Test for Steroids

In test tubes, around 2 mL of the powdered *Peganum harmala* seed alcoholic and aqueous extracts were taken separately. 2 mL of chloroform were added to each sample, and then a corresponding amount of concentrated sulfuric acid (2 mL) was carefully added to the inside of the test tube. The presence of steroidal compounds was indicated by the formation of a pink-to-red ring at the interface (Kokate, 2007; Government of India, 1992; Mukherjee, 2001; World Health Organization, 2011; Divakar, 2002).

Test for Glycosides

About 2 mL of the alcoholic and aqueous extracts of powdered *Peganum harmala* seeds were transferred to test tubes. About 1 mL of glacial acetic acid with a few drops of a 10% ferric chloride solution was combined with each extract. After that, a clear bottom layer was created by carefully adding concentrated sulfuric acid along the sides of the test tubes. Glycosides were present because a reddish-brown ring appeared at the contact between the two layers.

Test for Alkaloids

Separately, approximately 2 mL of the alcoholic and aqueous extracts of powdered *Peganum harmala* seeds were transferred to test tubes. A few drops of diluted hydrochloric acid were added to each, and the mixtures were filtered to produce clear solutions. Each filtrate was then treated with 1 mL of Dragendorff's reagent, which is a potassium bismuth iodide solution. Alkaloids were present when a reddish-brown or orange precipitate formed.

Test for Flavonoids

A few drops of a 20% sodium hydroxide solution were added to 2 mL of the alcoholic and aqueous extracts of *Peganum harmala* seed powder in different test tubes. A strong yellow tint or precipitate was visible. The solution turned colorless upon the addition of a few drops of diluted hydrochloric acid, indicating the presence of flavonoids.

Test for Carbohydrates

A test tube was filled with roughly 2 mL of the powdered *Peganum harmala* seed alcoholic or aqueous extract. 2 to 3 drops of Molisch's reagent were then added. A distinct layer was then created by carefully introducing concentrated sulfuric acid along the inner wall of the test tube. The presence of carbohydrates was verified at the interface by the formation of a violet or purple ring.

Test for Tannins

Each test tube contained approximately 1 mL of the alcoholic and aqueous extracts of powdered *Peganum harmala* seeds, to which 5 mL of distilled water was added. Next came a few drops of a brand-new 10% ferric chloride solution. The emergence of a bluish-black or greenish-black precipitate or coloring suggested the presence of tannins.

Test for Saponins

After transferring around 2.5 mL of the alcoholic and aqueous extracts of powdered *Peganum harmala* seed into separate test tubes, 10 mL of distilled water was added. After a thorough shake, the contents were left alone for 2 min. Saponins were confirmed to be present when a stable, honeycomb-like froth formed; their absence was indicated by the lack of foam.

Fingerprint Analysis

HPTLC analysis was performed on the methanolic extract of *Peganum harmala* seeds. A 100 µL Hamilton syringe was attached to a CAMAG Linomat 5 automatic sample applicator for sample application. Precoated silica gel 60 F254 HPTLC plates (10 × 10 cm, Merck) were coated with bands 6 mm in length, containing varying quantities of the extract (2 µL, 4 µL, and 6 µL). In a presaturated twin-trough glass chamber, chromatographic separation was accomplished using toluene: Utilizing ethyl acetate (7:3, v/v) as the mobile phase, the solvent can move up to 70 mm. The plate was dried for 5 min at 60°C in an oven after development. A CAMAG TLC Scanner operating in reflectance-absorbance mode was then used to perform densitometric scanning at 254 and 366 nm. Using WinCATS software, the chromatographic data were captured and examined under ideal working circumstances.

Ethical Statement

This study involved only plant materials and did not involve human or animal subjects; therefore, ethical approval was not required.

Statistical Analysis

The data obtained from physicochemical analysis (Specific gravity, Refractive index, etc.) are expressed as absolute values observed during laboratory testing. HPTLC densitometric scanning data were analyzed using WinCATS software. No comparative

statistical tests were applied as this was a standardization study of a single plant.

RESULTS

Macroscopic Characteristics

The seeds of *Peganum harmala* are small, angular to irregularly triangular in shape, and exhibit a hard and compact structure. They are typically dark brown to black in colour, with a rough, slightly wrinkled surface texture. The seeds are flattened, with distinctctter taste and a strong, unpleasant odour. Their size is generally small, measuring approximately 2-4 mm in length.

Organoleptic Features

Transverse Section Microscopic view of *Peganum harmala* seed

The transverse section of *Peganum harmala* seed reveals well-defined anatomical layers that are important for pharmacognostic identification. The outermost layer is the testa or seed coat, which appears as a thick, dark boundary surrounding the seed and serves as a protective barrier providing mechanical strength. Just beneath this layer lies the epidermal region, or outer tegmen, composed of closely packed cells that contribute to the seed coat. The endosperm forms the largest portion of the seed and functions as storage tissue, containing reserve nutrients. Centrally located within the endosperm is the embryo that can be observed (Figure 1).

Powder Microscopy of *Peganum harmala* seed

Powder microscopy of *Peganum harmala* seeds revealed distinctive diagnostic features, including yellowish-brown lignified seed coat (testa) fragments with thick walls and irregular shapes, as well as elongated sclerenchymatous fibers. Aggregates of thin-walled endosperm parenchyma cells and reticulate surface fragments of the testa were also observed (Figure 2).

Physicochemical Parameters

Many physicochemical characteristics were assessed for the seed powder, including foreign matter, drying loss, total ash, acid-insoluble ash, pH, and extractive values for water-soluble and alcohol-soluble compounds (Table 2).

Preliminary Phytochemical Analysis

Preliminary phytochemical screening was conducted using the ethanolic and aqueous extracts made in the preceding section. To conduct the corresponding qualitative tests, around 2 mL of each extract were taken.

Fingerprinting Profile of the *P. harmala* Seed by HPTLC

The HPTLC plate observed under UV light at 366 nm revealed intense blue fluorescent bands at low R_f values (approximately 0.05), indicating the probable presence of fluorescent β-carboline alkaloids characteristic of *Peganum harmala*. The uniform band positions across all tracks demonstrate good reproducibility and consistent chromatographic behaviour. The limited migration of the bands suggests strong interaction between the relatively polar alkaloidal constituents and the silica-gel stationary phase under the selected mobile-phase conditions, resulting in decreased R_f values. Overall, the chromatographic profile provides a stable and reproducible HPTLC fingerprint, supporting the qualitative identification and characterization of *Peganum harmala* based on its characteristic alkaloid constituents (Figure 3).

Several phytochemical components were detected in the methanolic extract of *Peganum harmala* upon exposure to UV light at 254 and 366 nm, as determined by HPTLC fingerprint analysis. The presence of several UV-active chemicals was indicated by multiple peaks at 254 nm, with notable bands at R_f values of approximately 0.05, 0.72, and 0.85. The extract may contain a significant luminous alkaloid component, as evidenced

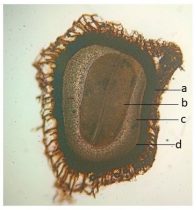

Figure 1: T. S. of *P. harmala* a. seed testa b. embryo c. endosperm d. seed rib.

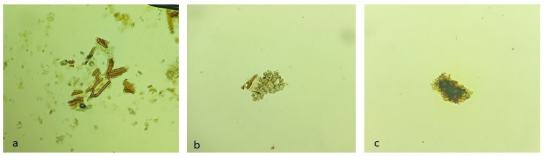

Figure2: Powder microscopy of the seed of *P. harmala*. a. fibrous sclerenchyma, b. parenchyma cells, c. testa.

Table 2: Results of Physicochemical study.

Sl. No.	Parameters	Test Result
1.	Loss on drying	3.8%
2.	Total Ash Value	3.21%
3.	Water soluble ash	1.56%
4.	Acid Insoluble ash	0.5%
5.	Water soluble Extract	21.67%
6.	Alcohol soluble extractive value	9.56%
7.	pH	7.8

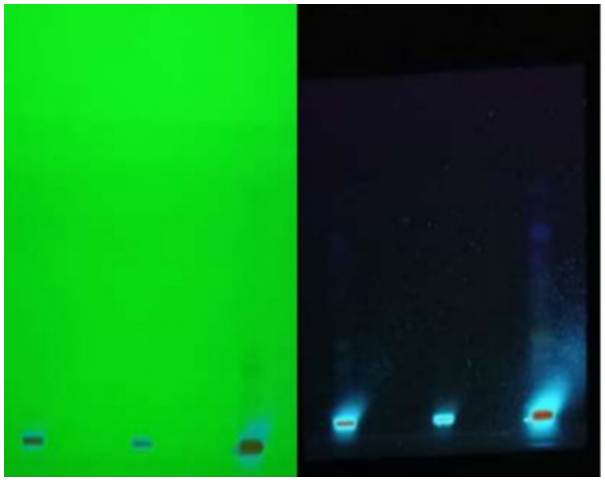

Figure 3: Visualization of the HPTLC Plate at (A) 254 nm and (B) 366 nm.

Table 3: **Preliminary phytochemical screening.**

Sl. No.	Test Parameters	Aqueous extract	Methanol extract
1.	Steroids	+	+
2.	Glycosides	+	+
3.	Alkaloids	+	+
4.	Flavonoids	+	+
5.	Carbohydrates	+	+
6.	Proteins	+	-
7.	Tannins	+	-
8.	Saponins	+	-
9.	Amino acids	-	-

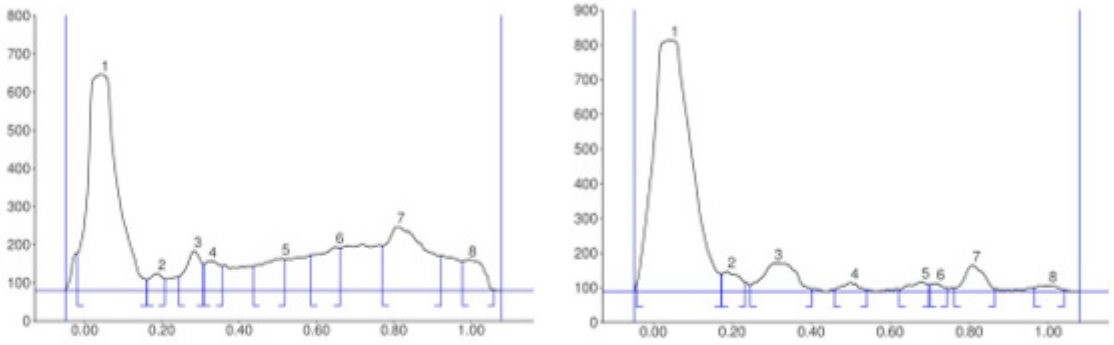

Figure 4: Profiles of Densitometric Fingerprints at (A) 254 nm and (B) 366 nm of Harmal Seed.

by the strong, constant fluorescent band seen at R_f 0.04-0.05 throughout all tracks under 366 nm light (Figure 4).

DISCUSSION

The physicochemical evaluation of *Peganum harmala* L. seed powder showed acceptable quality parameters, including low moisture content, controlled ash values, and suitable extractive values within the limits of the Ayurvedic Pharmacopoeia and the WHO. These findings suggest high purity, stability, and minimal contamination, providing useful quality-control standards and reducing the variability often seen in herbal materials.

Phytochemical screening revealed the presence of bioactive compounds, including carbohydrates, alkaloids, flavonoids, steroids, and glycosides. Since tannins and saponins were detected only in aqueous extracts, the effect of solvent polarity on extraction efficiency was highlighted. The absence of proteins and amino acids is consistent with the alkaloid-rich nature of the seeds, particularly β-carboline alkaloids like harmine and harmaline, which are known for their MAO-inhibitory, antioxidant, and neuromodulatory effects supporting traditional medicinal uses (Table 3).

HPTLC fingerprinting produced clear, reproducible bands at low R_f values under UV at 366 nm, confirming the presence of polar alkaloids and demonstrating its usefulness for authentication and quality assurance. While the findings support the therapeutic potential of *P. harmala*, further quantitative and biological studies are needed to strengthen scientific validation.

CONCLUSION

Peganum harmala L. is an important medicinal plant recognized for its broad therapeutic potential, mainly due to the presence of β-carboline alkaloids such as harmine and harmaline. This study established essential baseline quality parameters for seed powder, including low moisture content, acceptable ash and extractive values, and near-neutral pH, all of which comply with the Ayurvedic Pharmacopoeia of India and WHO standards, indicating high purity, stability, and minimal risk of adulteration.

The presence of important bioactive substances, including alkaloids, flavonoids, steroids, glycosides, carbohydrates, and tannins, was confirmed by phytochemical analysis; variations were observed depending on solvent polarity. HPTLC fingerprinting revealed consistent low-R_f fluorescent bands at 366 nm under UV, supporting the identification of polar alkaloids

and demonstrating its usefulness for authentication and quality control. Overall, the findings provide a strong foundation for standardizing *P. harmala* and for linking traditional medicinal knowledge with modern scientific validation. Future studies should focus on quantitative marker analysis, safety assessment, and *in vivo* pharmacological evaluation to support clinical use and development of standardized herbal products.

ACKNOWLEDGEMENT

The authors are grateful to Cotex Laxmi Healthcare Private Limited and Mahatma Gandhi Ayurveda College Hospital and Research Centre for providing the laboratory facilities and support necessary to conduct this study.

ABBREVIATIONS

HPTLC: High-Performance Thin-Layer Chromatography; **WHO:** World Health Organization; **UV:** Ultraviolet; **R_f:** Retardation factor; **T.S.:** Transverse Section; **MAO:** Monoamine oxidase; **cm:** Centimeter; **mL:** Milliliter; **g:** Gram; **°C:** Degree Celsius.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

AUTHOR CONTRIBUTIONS

Concept, Design, Writing, Data analysis, Interpretation, Manuscript drafting: Dr. Aditi Padoley; Data collection: Dr. Pallavi Dewangan, Dr. Dhanashri Khuspure; Final approval: Dr. Jaimala Jadhav

SUMMARY

To establish authentication and quality control parameters for *Peganum harmala* L. seed powder, this study established pharmacognostic, physicochemical, and phytochemical standardization parameters. Important diagnostic characteristics were observed by macroscopic and microscopic examinations, and physicochemical analysis revealed acceptable moisture content, ash, and extractive values, and a pH close to neutral, in compliance with Ayurvedic Pharmacopoeia and WHO standards. Important bioactive components, including alkaloids, flavonoids, steroids, glycosides, carbohydrates, tannins, and saponins, were identified through preliminary phytochemical screening. Consistent low-R_f fluorescent bands at 366 nm were produced by HPTLC fingerprinting, indicating distinctive β-carboline alkaloids. All things considered, the results offer baseline reference parameters for future development and standardization of herbal formulations based on *P. harmala*.

REFERENCES

Daniel, M., and Mammen, D. (2022). Analytical methods for medicinal plants and economic botany.

Divakar, M. C. (2002). Plant drug evaluation: A laboratory guide (2nd ed.). CD Remedies.

Evans, W. C. (1996). Trease and Evans pharmacognosy (15th ed.). Harcourt Brace and Company.

Government of India, Department of Health and Family Welfare. (1992). The Ayurvedic pharmacopoeia of India (1st ed.).

Harborne, J. B. (1973). Phytochemical methods: A guide to modern techniques of plant analysis. Toppan Company Ltd.

Jani, D. K., and Sawant, B. (2013). Advanced protocols in Dravyaguna practicals. Chaukhambha Orientalia.

Kokate, C. K. (2007). Practical pharmacognosy. Nirali Prakashan.

Mahmoudian, M., Jalilpour, H., and Salehian, P. (2002). Toxicity of *Peganum harmala*: Review and a case report. Iranian Journal of Pharmacology and Therapeutics, 1, 1-4.

Mukherjee, P. K. (2001). Quality control of herbal drugs (1st ed., pp. 183-219). Business Horizons Publications.

World Health Organization. (2011). Quality control methods for herbal materials. WHO Press.

Cite this article: Padoley A, Jadhav J, Dewangan P, Khuspure D. Phyto-Physiochemical Evaluation and Preliminary Phytochemical Screening of *Peganum harmala* L. (*Harmala*): Establishing Standardization Parameters for Quality and Safety. Pharmacog Res. 2026;18(4):1461-6.