STANDARDIZATION, PHYTOCHEMICAL EVALUATION, AND COMPARATIVE ASSESSMENT OF THE DIURETIC POTENTIAL OF LEAF EXTRACTS OF ACHYRANTHES ASPERA AND AZADIRACHTA INDICA

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Abstract

The present study aimed to standardize and evaluate the phytochemical constituents of hydroalcoholic leaf extracts of Achyranthes aspera and Azadirachta indica and to compare their preliminary diuretic potential. The leaves were extracted using the Soxhlet extraction method, and the resulting extracts were assessed for extraction yield, organoleptic characteristics, physicochemical parameters, fluorescence properties, and phytochemical constituents. Total phenolic content and total flavonoid content were estimated using colorimetric methods. Diuretic activity was evaluated in experimental animals by measuring urine volume and urinary sodium, potassium, and chloride concentrations, with furosemide serving as the reference drug. The extraction yields of A. aspera and A. indica were 12.5% and 15.6%, respectively. Both extracts showed the presence of major phytochemical groups, including alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids or steroids. The total phenolic content was 48.5 ± 1.12 mg GAE/g for A. aspera and 56.8 ± 1.35 mg GAE/g for A. indica, while the total flavonoid content was 42.6 ± 1.08 and 53.4 ± 1.22 mg QE/g, respectively. In the reported diuretic evaluation, mean urine volumes were higher in both extract-treated groups than in the vehicle control, with A. indica showing higher mean values than A. aspera at both tested dose levels. However, both extracts produced lower mean urine volumes than furosemide. These findings suggest that both plant extracts warrant further investigation for their potential diuretic activity, with A. indica showing comparatively higher preliminary responses under the reported experimental conditions.

Keywords: *Achyranthes aspera; Azadirachta indica; phytochemical screening; standardization; total phenolic content; total flavonoid content; diuretic activity.*

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Received: 21/08/2026
Revised: 22/09/2026
Accepted: 26/10/2026
DOI: http://doi.org/10.66204/GJPSR-1620-2026-2-11-3

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How to Cite
Kumar A, Pathak S, Srivastava AK, Singh A. Standardization, Phytochemical Evaluation, and Comparative Assessment of the Diuretic Potential of Leaf Extracts of Achyranthes aspera and Azadirachta indica. *Global Journal of Pharmaceutical and Scientific Research*. 2026;2(11):1620-1633. ISSN: 3108-0103. http://doi.org/10.66204/GJPSR-1620-2026-2-11-3

1. INTRODUCTION

Diuretics are pharmacologically active agents that increase urine formation and promote the excretion of water and electrolytes through the kidneys. They are widely used in the management of hypertension, oedema, heart failure, and other conditions associated with fluid retention. Their principal actions involve alterations in renal electrolyte reabsorption, particularly sodium and chloride, which influence water excretion and extracellular fluid balance. Reddy and Mooradian (2009) reviewed the pharmacological mechanisms and clinical applications of diuretics and highlighted their importance in the treatment of cardiovascular and renal disorders. Although conventional diuretics are effective, their use may be associated with adverse effects, including dehydration, electrolyte imbalance, hypotension, and changes in renal function. These limitations have encouraged continued research into medicinal plants and natural products as potential sources of pharmacologically active compounds.

Medicinal plants have long been used in traditional healthcare systems and represent an important source of chemically diverse secondary metabolites. Major phytochemical groups, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds, have attracted considerable interest because of their potential biological activities. However, the presence of these constituents alone does not establish therapeutic efficacy. Appropriate phytochemical characterization, quality assessment, and experimental validation are necessary to determine the biological properties of medicinal plant extracts. Nahar et al. (2026), in a review published, discussed the ethnopharmacological significance, phytochemical composition, and pharmacological activities of *Helicteres isora*. Although their work concerns a different plant species, it illustrates the broader importance of integrating traditional knowledge with systematic scientific evaluation. Such an approach is particularly relevant when investigating medicinal plants for potential effects on renal function and urinary excretion.

Achyranthes aspera L., belonging to the family Amaranthaceae, is a medicinal herb widely distributed in tropical and subtropical regions, including India. Different parts of the plant have been used in traditional medicine for various health conditions. Krishnaveni and Thaakur (2006) investigated its pharmacognostic characteristics and preliminary phytochemical composition, reporting several classes of constituents, including sterols, flavonoids, tannins, and carbohydrates. Pharmacognostic investigations are important for establishing the identity, purity, and quality of medicinal plant materials and for

distinguishing the desired species from potential substitutes or adulterants. Warke and Kale (2015) evaluated the pharmacognostic and physicochemical characteristics of *A. aspera* leaves, including ash values, extractive values, loss on drying, fluorescence characteristics, and preliminary phytochemical constituents. These studies provide a basis for further investigations involving standardized leaf material and chemically characterized extracts.

The pharmacological properties of *A. aspera* have also been investigated experimentally, including its potential diuretic activity. Srivastav et al. (2011) evaluated the diuretic activity of a whole-plant extract of *A. aspera* using an experimental animal model. More specifically, Warke et al. (2018) investigated petroleum ether, ethyl acetate, alcoholic, and aqueous leaf extracts in rats, assessing urine volume and urinary electrolyte concentrations, with furosemide serving as the reference diuretic. These studies demonstrate that the diuretic potential of *A. aspera* has previously received scientific attention. They also highlight the importance of considering the plant part, extraction solvent, dose, and experimental conditions when interpreting pharmacological responses. Further evaluation using standardized extracts and consistent experimental procedures may provide additional comparative information regarding the biological effects of this species.

Azadirachta indica A. Juss., commonly known as neem, belongs to the family Meliaceae and is widely used in traditional medicine, particularly in South Asia. Various parts of the plant have been investigated for their phytochemical composition and diverse pharmacological properties. Mandal et al. (2000) described pharmacognostic characteristics of neem leaves, including morphological and microscopic features and other quality-related parameters. Such information is useful for the identification and preliminary standardization of neem leaf material. Saleem et al. (2018) comprehensively reviewed the phytochemical profile and pharmacological attributes of *A. indica*, describing its chemically diverse constituents and numerous reported biological activities. The presence of flavonoids, terpenoids, and other phytoconstituents makes neem leaves relevant for further phytochemical investigation; however, the presence of these compounds should not be interpreted as direct evidence of diuretic efficacy.

Analytical methods can strengthen the characterization of medicinal plant extracts by providing information beyond preliminary phytochemical screening. Prajapati et al. (2016) investigated the HPTLC fingerprint profile and preliminary phytochemical composition of neem leaves and stem bark. Yadav et al. (2023) also examined pharmacognostic and pharmacological characteristics of *A. indica* using biochemical assays and chromatographic techniques. Such approaches demonstrate the value of combining physicochemical

assessment with suitable analytical methods when characterizing plant materials. Differences in geographical origin, environmental conditions, harvesting, drying, storage, and extraction procedures may influence the chemical composition and yield of plant extracts. Therefore, the use of standardized procedures is important for obtaining reproducible findings and enabling meaningful comparisons between medicinal plants.

Although previous investigations have examined the pharmacognostic characteristics and diuretic activity of *A. aspera*, and the phytochemical and pharmacological properties of *A. indica*, a systematic comparative assessment of their standardized leaf extracts under a common experimental framework remains a relevant research objective. Evaluating both plants using consistent procedures may facilitate comparison of their physicochemical characteristics, phytochemical profiles, and diuretic responses. The extent of the existing research gap, particularly concerning the diuretic activity of *A. indica* leaf extracts, should be established through a focused review of the relevant literature before any claim of novelty is made.

Therefore, the present study aims to standardize and perform phytochemical evaluation of leaf extracts of *Achyranthes aspera* and *Azadirachta indica* and to investigate their diuretic potential using suitable experimental models. The specific objectives are to establish relevant physicochemical quality parameters, identify major classes of phytoconstituents, and evaluate the effects of the extracts on urine output and, where included in the experimental protocol, urinary electrolyte excretion. The findings are intended to contribute to the pharmacognostic characterization and comparative preliminary pharmacological assessment of these medicinal plants and provide a basis for further investigations into their potential renal effects.

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant Materials

The leaves of *Achyranthes aspera* L. and *Azadirachta indica* A. Juss. were collected from suitable locations and examined for visible damage, contamination, and disease. The plant materials were authenticated by a qualified botanist or taxonomist, and voucher specimen details were recorded where available. Botanical authentication and documentation are essential for ensuring the identity and reproducibility of herbal materials (WHO, 2011; Warke and Kale, 2015).

2.2. Preparation of Plant Extracts

The collected leaves were cleaned, shade-dried under suitable ventilation, and coarsely powdered. The powdered leaves of both plants were extracted separately using the Soxhlet

extraction technique with a hydroalcoholic solvent system. The extracts were concentrated and dried to remove the extraction solvent, then stored in airtight containers until further analysis. Soxhlet extraction and subsequent pharmacognostic evaluation have been described in previous investigations of *A. aspera* leaves (Warke and Kale, 2015).

The percentage yield was calculated using the following formula:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of initial powdered plant material}} * 100$$

2.3. Standardization of Crude Extracts

The prepared extracts were evaluated for organoleptic and physicochemical characteristics, including colour, odour, taste, texture, loss on drying, ash values, extractive values, and pH. Fluorescence analysis of powdered plant material was also performed under visible light and ultraviolet radiation following treatment with selected chemical reagents.

The physicochemical evaluation was intended to establish preliminary quality characteristics and support the identification and standardization of the extracts. The procedures were based on established herbal-material quality-control approaches and published pharmacognostic investigations (WHO, 2011; Warke and Kale, 2015).

The percentage loss on drying was calculated as:

$$\text{Loss on Drying (\%)} = \frac{\text{Initial weight of sample} - \text{Final weight of dried sample}}{\text{Initial weight of sample}}$$

where (W_i) is the initial sample weight and (W_f) is the final sample weight after drying.

The percentage ash and extractive values were calculated using the respective residue or extract weights relative to the initial sample weight, according to the applicable procedure.

2.4. Preliminary Phytochemical Screening

The hydroalcoholic extracts were subjected to qualitative phytochemical screening to investigate the presence of major classes of secondary metabolites. Standard chemical tests were used to assess alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and steroids. The observations were based on characteristic colour changes, precipitate formation, or frothing, as appropriate for each test.

The screening procedures were guided by established pharmacognostic methods and previous phytochemical investigations of *A. aspera* (Harborne, 1998; Warke and Kale, 2015). The findings were recorded qualitatively and were not treated as quantitative measurements of individual constituents.

2.5. Quantitative Estimation of Total Phenolic Content

The total phenolic content (TPC) of each extract was estimated using the Folin-Ciocalteu colorimetric method. Gallic acid was used as the reference standard, and the absorbance of the developed colour was measured at 765 nm. The phenolic content was expressed as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g).

The estimation was based on the Folin-Ciocalteu procedure described by Singleton et al. (1999). The analysis was performed in triplicate, and the results were expressed as mean ± standard deviation.

$$\text{Total Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of air dried sample}} * 100$$

where (C) is the concentration obtained from the gallic acid calibration curve, (V) is the volume of extract solution, and (m) is the mass of extract represented in the assay. Appropriate dilution factors were applied where required.

2.6. Quantitative Estimation of Total Flavonoid Content

The total flavonoid content (TFC) was determined using the aluminium chloride colorimetric method, with quercetin as the reference standard. The absorbance of the developed colour was measured at 415 nm, and the results were expressed as milligrams of quercetin equivalents per gram of dried extract (mg QE/g).

The procedure was based on the colorimetric approach described by Chang et al. (2002). Measurements were performed in triplicate, and the results were reported as mean ± standard deviation.

$$\text{Acid Insoluble Ash (\%)} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of air dried sample}} * 100$$

where (C) is the concentration obtained from the quercetin calibration curve, (V) is the volume of extract solution, and (m) is the mass of extract represented in the assay. Appropriate dilution factors were applied where required.

2.7. Experimental Animals and Ethical Considerations

The reported pharmacological evaluation used adult Wistar rats weighing 150-250 g, with six animals per group. The animals were maintained under suitable laboratory conditions with access to food and water according to the approved experimental protocol.

The source material also reports an acute oral toxicity assessment based on OECD Test Guideline 423. The acute toxicity procedure and the diuretic activity experiment should be documented separately because they have different objectives. The institutional animal ethics committee approval number, actual doses, route and timing of administration, acclimatization

conditions, and relevant experimental details must be included from the original study records before submission (OECD, 2001).

2.8. Evaluation of Diuretic Activity

The diuretic activity of the extracts was evaluated by measuring urine output in the experimental animals using metabolic cages. The reported experimental groups comprised a vehicle control, a furosemide reference group, and low- and high-dose groups for each plant extract. Urine volume was recorded over the specified collection period and normalized to body weight.

The evaluation was designed to compare the effects of the two extracts with the vehicle control and reference diuretic. Published research has previously investigated the diuretic activity of *A. aspera* leaf extracts using urine volume and urinary electrolyte measurements (Warke et al., 2018).

The diuretic index was calculated as:

$$\text{Diuretic Index} = \frac{\text{Urine volume of test group}}{\text{Urine volume of control group}} * 100$$

When expressed as a percentage:

2.9. Determination of Urinary Electrolytes

Urinary sodium (Na^+), potassium (K^+), and chloride (Cl^-) concentrations were assessed to characterize the urinary electrolyte profile of the experimental groups. The measurements were compared across the vehicle control, furosemide, and extract-treated groups.

Electrolyte concentrations were reported in mEq/L. Where total electrolyte excretion is evaluated, it should be calculated by multiplying the measured concentration by the corresponding urine volume collected over the same period. Concentration values alone should not be interpreted as total electrolyte excretion.

2.10. Stability Assessment

The extracts were reportedly stored in airtight amber containers under room-temperature and refrigerated conditions. Their physical characteristics, including colour, odour, texture, and appearance, were monitored during the observation period. Changes in pH and moisture content were also reported as part of the assessment.

The storage duration, sampling intervals, and actual measurements should be documented to support the reported findings. Physical observations alone are insufficient to establish chemical stability or a definitive shelf life (WHO, 2011).

3. RESULTS AND DISCUSSION

3.1. Extraction Yield of Plant Extracts

The hydroalcoholic extraction of *Achyranthes aspera* and *Azadirachta indica* leaves was performed separately using the Soxhlet extraction method. The percentage yields were 12.5% and 15.6%, respectively. As presented in Table 1, *A. indica* produced a higher extract yield than *A. aspera* under the reported extraction conditions. This difference indicates variation in the quantity of material recovered by the selected solvent system; however, a higher extraction yield does not necessarily indicate greater pharmacological potency.

Table 1. Percentage yield of hydroalcoholic leaf extracts

Plant species	Powdered leaves (g)	Dried extract (g)	Yield (%)
Achyranthes aspera	500	62.5	12.5
Azadirachta indica	500	78.0	15.6

3.2. Standardization of Crude Extracts

3.2.1. Organoleptic and physicochemical evaluation

The organoleptic characteristics of the extracts differed in colour, odour, taste, and texture. The *A. aspera* extract was dark brown, with a mild characteristic herbal odour and a slightly bitter, astringent taste. The *A. indica* extract was greenish brown, with a strong characteristic odour and an intensely bitter taste.

The physicochemical parameters are summarized in Table 2. Loss on drying was 9.0% for *A. aspera* and 12.0% for *A. indica*. Total ash and acid-insoluble ash were slightly higher in *A. aspera*, whereas water-soluble ash was higher in *A. indica*. Both water-soluble and alcohol-soluble extractive values were higher in *A. indica*. The measured pH values indicated mildly acidic extract solutions.

Table 2. Organoleptic and physicochemical characteristics of the extracts

Parameter	*A. aspera*	*A. indica*
Colour	Dark brown	Greenish brown
Odour	Mild, characteristic herbal odour	Strong, characteristic odour
Taste	Slightly bitter and astringent	Intensely bitter
Texture	Coarse, semisolid	Fine, slightly sticky
Loss on drying (%)	9.0	12.0
Total ash (%)	8.2	7.5

Acid-insoluble ash (%)	1.6	1.2
Water-soluble ash (%)	4.8	5.1
Water-soluble extractive (%)	14.8	16.2
Alcohol-soluble extractive (%)	11.6	13.4
pH (mean ± SD)	6.42 ± 0.05	6.18 ± 0.04

The observed differences provide preliminary quality characteristics for the two extracts. The relatively higher loss on drying in *A. indica* warrants attention during storage because moisture content can influence extract stability.

3.2.2. Fluorescence analysis

Fluorescence analysis of the powdered leaves revealed different colour responses under visible light and ultraviolet radiation following treatment with chemical reagents. *A. aspera* exhibited greenish-brown to dark-green responses, while *A. indica* showed comparatively stronger green fluorescence under long-wave ultraviolet radiation.

Table 3. Fluorescence characteristics of powdered plant materials

Treatment	***A. aspera*: visible light**	***A. aspera*: UV 365 nm**	***A. indica*: visible light**	***A. indica*: UV 365 nm**
Untreated powder	Greenish brown	Pale green	Greenish brown	Bright green
Dilute HCl	Light brown	Greenish glow	Brownish green	Green
NaOH	Dark brown	Dull green	Dark green	Bright green
Ammonia solution	Brown	Bright green	Green	Strong green
HNO_3	Yellowish brown	Dull yellow	Yellowish green	Dull green

3.3. Preliminary Phytochemical Screening

Qualitative phytochemical screening indicated the presence of several major classes of phytoconstituents in both extracts. The results showed stronger qualitative reactions for alkaloids in *A. aspera*, while *A. indica* showed stronger reactions for tannins and terpenoids or steroids. Both extracts exhibited strong reactions for flavonoids and moderate reactions for saponins and glycosides.

Table 4. Preliminary phytochemical screening of the extracts

Phytoconstituent	**Tests used**	***A. aspera***	***A. indica***
Alkaloids	Mayer's, Dragendorff's, Wagner's	+++	++
Flavonoids	Shinoda, alkaline, lead acetate	+++	+++
Tannins	Ferric chloride, gelatin, lead acetate	++	+++
Saponins	Foam and foam stability tests	++	++
Glycosides	Molisch's, Legal's, Borntrager's	++	++
Terpenoids and steroids	Salkowski, Liebermann-Burchard	+	++

Key: +++ = strong reaction; ++ = moderate reaction; + = mild reaction; − = absent.

3.4. Quantitative Estimation of Total Phenolic and Flavonoid Contents

The total phenolic content (TPC) was estimated using the Folin-Ciocalteu method, while total flavonoid content (TFC) was determined using the aluminium chloride colorimetric method. The results reported in Table 5 indicate that *A. indica* had higher TPC and TFC values than *A. aspera*.

Table 5. Total phenolic and flavonoid contents of the extracts

Parameter	***A. aspera***	***A. indica***
Absorbance for TPC (765 nm)	0.62	0.74
TPC (mg GAE/g extract)	48.5 ± 1.12	56.8 ± 1.35
Absorbance for TFC (415 nm)	0.58	0.71
TFC (mg QE/g extract)	42.6 ± 1.08	53.4 ± 1.22

Values are reported as mean ± SD (n = 3), as provided in the source material. GAE = gallic acid equivalents; QE = quercetin equivalents.

3.5. Pharmacological Evaluation of Diuretic Activity

3.5.1. Effect on urine volume

The reported animal study included a vehicle control, a furosemide reference group, and low- and high-dose groups for each plant extract. The vehicle control produced a mean urine volume of 3.8 ± 0.25 mL/100 g body weight, whereas the furosemide group produced 9.6 ± 0.42 mL/100 g.

Both plant extracts showed higher mean urine volumes than the control, with greater values at the high dose. *A. indica* produced higher mean urine output than *A. aspera* at both dose levels. Nevertheless, the mean urine volumes for both extracts remained below that of the furosemide group.

Table 6. Effect of plant extracts on urine volume

Group	Treatment	Urine volume (mL/100 g body weight)
I	Vehicle control	3.8 ± 0.25
II	Furosemide	9.6 ± 0.42
III	*A. aspera*, low dose	6.2 ± 0.31
IV	*A. aspera*, high dose	7.5 ± 0.38
V	*A. indica*, low dose	6.8 ± 0.34
VI	*A. indica*, high dose	8.1 ± 0.40

Values are reported as mean ± SD (n = 6), as provided in the source material. Exact administered doses and the urine collection period must be specified in the final manuscript.

3.5.2. Diuretic index

The diuretic index was calculated by dividing the mean urine volume of each treatment group by the mean urine volume of the vehicle control group. For presentation as a percentage, this ratio was multiplied by 100.

Table 7. Diuretic index of the plant extracts

Treatment	Urine volume (mL/100 g)	Diuretic index (%)
Vehicle control	3.8	100.0
Furosemide	9.6	252.6
A. aspera, low dose	6.2	163.2
A. aspera, high dose	7.5	197.4
A. indica, low dose	6.8	178.9
A. indica, high dose	8.1	213.2

3.6. Urinary Electrolyte Assessment

Urinary sodium, potassium, and chloride concentrations were measured to characterize the electrolyte profile associated with treatment. The reported values are summarized in Table 8.

Table 8. Urinary electrolyte concentrations following treatment

Group	Treatment	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)
I	Vehicle control	72.4 ± 2.15	28.6 ± 1.12	78.3 ± 2.30
II	Furosemide	148.6 ± 3.42	61.2 ± 2.05	152.8 ± 3.65
III	*A. aspera*, low dose	102.5 ± 2.88	42.3 ± 1.54	110.6 ± 2.74
IV	*A. aspera*, high dose	121.8 ± 3.05	48.7 ± 1.76	128.4 ± 3.10
V	*A. indica*, low dose	112.6 ± 2.95	45.1 ± 1.60	118.9 ± 2.82
VI	*A. indica*, high dose	135.3 ± 3.20	55.4 ± 1.92	142.7 ± 3.48

Values are reported as mean ± SD, as provided in the source material. The sample size and whether these represent independent animal measurements should be confirmed.

3.7. Stability of the Extracts

The source material reports that the extracts were stored in airtight amber containers at room temperature (25 ± 2°C) and under refrigerated conditions (4-8°C). No substantial changes in colour, odour, texture, or physical appearance were reported during the observation period.

These observations suggest that the extracts retained their reported physical characteristics under the stated storage conditions. However, the duration of storage and the underlying time-point measurements should be documented before making a definitive stability or shelf-life claim.

4. CONCLUSION

The present study demonstrated promising preliminary results regarding the standardization, phytochemical composition, and diuretic potential of hydroalcoholic leaf extracts of *Achyranthes aspera* and *Azadirachta indica*. Both extracts exhibited characteristic physicochemical properties and contained several phytoconstituent groups, including alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids or steroids. *Azadirachta indica* showed a higher extraction yield, total phenolic content, and total flavonoid content than *Achyranthes aspera*.

Both plant extracts demonstrated positive results in the preliminary diuretic evaluation, producing higher mean urine volumes than the vehicle control, with increased urine output at the higher tested dose. Among the two extracts, *Azadirachta indica* exhibited comparatively greater mean urine output at both dose levels. Although the observed responses were lower than those of the reference drug furosemide, the findings support the further investigation of

both plants as potential sources of naturally derived diuretic agents. Differences in urinary electrolyte concentrations were also observed among the experimental groups.

In conclusion, both *A. aspera* and *A. indica* showed encouraging preliminary findings, with *A. indica* demonstrating comparatively better results under the reported experimental conditions. Further studies involving statistical validation, quantitative urinary electrolyte excretion, dose optimization, safety assessment, and investigation of possible mechanisms of action are recommended to confirm and strengthen these findings.

5. Acknowledgement

The authors sincerely thank Shambhunath Institute of Pharmacy, Jhalwa, Prayagraj, for providing the necessary facilities and support to carry out this research work.

6. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this review.

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