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Pharmacognostic Preparation, Hydroethanolic Extraction and Preliminary Phytochemical Screening of *Apium graveolens* L. Leaves

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Abstract

Apium graveolens L. (celery) is an aromatic medicinal plant whose leaves contain chemically diverse constituents with potential antioxidant, anti-inflammatory and neuropharmacological relevance. The reliability of any pharmacological investigation depends first on correct plant authentication, controlled preparation of the raw material, reproducible extraction and preliminary characterization of the resulting extract. This study therefore focused specifically on the collection, authentication, shade drying, powdering, hydroethanolic extraction and qualitative phytochemical screening of *A. graveolens* leaves. Healthy mature leaves were collected from a local herbal garden, cleaned and authenticated by their characteristic morphological and organoleptic features. The leaves were shade-dried at room temperature, coarsely powdered and stored in airtight containers. One hundred grams of dried powder was extracted with 70% ethanol in a Soxhlet apparatus at approximately 60 degrees C for 24 h. The filtrate was concentrated under controlled conditions to yield a dark greenish-brown, aromatic, semisolid extract. The dried extract weighed 12.40 g, corresponding to a percentage yield of 12.40%. Standard qualitative tests were performed for alkaloids, flavonoids, glycosides, steroids, tannins, phenolic compounds, saponins and terpenoids. All eight targeted phytochemical classes were detected; flavonoids and phenolic compounds showed the strongest reactions, whereas alkaloids, glycosides, steroids, tannins, saponins and terpenoids were present at lower qualitative intensity. The findings establish a reproducible preparation procedure and demonstrate that the hydroethanolic leaf extract is rich in secondary metabolites that justify subsequent pharmacological and chromatographic investigation.

1. Introduction

Medicinal-plant research begins long before an extract is administered to an experimental system. The identity and quality of the raw botanical material, the conditions used for drying and storage, the particle size of the powder, the polarity of the extraction solvent and the temperature and duration of extraction all influence the chemical composition of the final preparation. If these steps

are insufficiently controlled, differences in biological activity may reflect variation in plant processing rather than genuine pharmacological properties. For that reason, pharmacognostic preparation and preliminary phytochemical characterization are essential components of reproducible natural-product research (Farnsworth, 1966; Evans, 2009; Kokate et al., 2016).

Apium graveolens L., commonly known as celery, is an aromatic member of the family Apiaceae. Although it is widely consumed as a vegetable and culinary herb, different parts of the plant have also been used in traditional medicine. Reviews of celery describe a broad phytochemical spectrum that includes flavonoids, phenolic acids, phthalides, coumarins, volatile constituents and other secondary metabolites (Al-Asmari et al., 2017; Makarova et al., 2022). The leaves are particularly relevant because they are renewable plant material and can contain appreciable quantities of polar and moderately polar constituents. Their aromatic character also indicates the presence of volatile and semivolatile compounds that may contribute to the biological profile of the plant.

Flavonoids and phenolic compounds are of particular interest in the pharmacological evaluation of *A. graveolens*. These constituents are commonly associated with free-radical scavenging, modulation of inflammatory pathways and protection of cellular structures from oxidative injury. Certain flavonoids, including apigenin-related compounds reported in celery, have also attracted attention for possible effects on central nervous system signalling. However, the presence of a reported compound in the literature cannot be assumed for every plant sample. Species identity, plant part, geographical origin, harvesting season, drying conditions and extraction method may all modify the qualitative and quantitative chemical profile. The extract used for biological testing must therefore be characterized directly rather than described only by reference to previous publications.

Hydroethanolic extraction is frequently selected for preliminary medicinal-plant studies because mixtures of ethanol and water provide a broader extraction range than either solvent alone. Water improves recovery of highly polar constituents, while ethanol facilitates the extraction of moderately polar compounds and reduces some of the microbiological and handling problems associated with purely aqueous preparations. A 70% ethanol mixture is therefore suitable for extracting several classes relevant to celery, including flavonoids, phenolics, glycosides, tannins, saponins and selected alkaloid fractions (Tiwari et al., 2011). When used in a Soxhlet apparatus,

the solvent repeatedly washes the powdered plant material, improving contact between solvent and matrix and supporting efficient recovery of soluble constituents (Redfern et al., 2014).

Nevertheless, extraction efficiency should not be judged only by the mass of crude material obtained. Percentage yield is an important reproducibility parameter, but a high yield may include inactive carbohydrates, pigments and other matrix components, whereas a lower-yield extract may contain potent compounds. Yield must therefore be interpreted together with physical properties and phytochemical screening. The colour, odour and consistency of the extract provide basic descriptive information, while qualitative tests indicate which broad chemical classes are present. Such tests do not identify individual molecules and cannot replace chromatographic standardization, but they provide an important first chemical map of the preparation (Harborne, 1998).

The present paper deliberately addresses only the preparatory and phytochemical components of the larger investigation. It does not attempt to evaluate toxicity, behavioural activity or clinical usefulness. Its objective was to establish a clear workflow for collecting and authenticating *A. graveolens* leaves, preparing a hydroethanolic extract under controlled conditions, calculating extractive yield and detecting major phytoconstituent classes. By narrowing the scope to these foundational procedures, the paper provides a focused account that can support future pharmacological work and improve the traceability of the extract used in subsequent experiments.

2. Materials and Methods

2.1 Study design

A laboratory-based pharmacognostic study was conducted to prepare and preliminarily characterize a hydroethanolic leaf extract of *A. graveolens*. The workflow comprised collection of healthy leaves, botanical authentication, washing and removal of foreign matter, shade drying, coarse powder preparation, Soxhlet extraction with 70% ethanol, controlled concentration, calculation of percentage yield and qualitative phytochemical testing. The same batch of authenticated leaf powder was used throughout the extraction and screening process to maintain internal consistency.

2.2 Collection and authentication of plant material

Fresh, mature leaves of *A. graveolens* were obtained from a local herbal garden. Leaves showing visible fungal infection, insect injury, disease, physical deterioration or excessive contamination were excluded. The selected material was transferred to the laboratory in clean packaging to minimize contamination and moisture accumulation. Adhering soil and dust were removed by gentle washing with clean water, after which surface moisture was removed using clean blotting or filter paper.

Authentication was based on comparison of the plant material with standard taxonomic and pharmacognostic descriptions. Diagnostic characteristics considered included the divided green leaf blade, serrated or lobed margins, soft texture, reticulate venation and characteristic aromatic odour. A voucher specimen should be retained in an institutional herbarium or departmental repository, with the botanical name, family, plant part, collection source, date and identification details recorded. Voucher retention is important because it permits later verification of the exact material used and protects the study from error caused by substitution or misidentification (Evans, 2009; Kokate et al., 2016).

2.3 Drying, powdering and storage

The authenticated leaves were spread in a thin layer and shade-dried at room temperature under hygienic, well-ventilated conditions. Direct sunlight was avoided to limit photodegradation and thermal loss of sensitive constituents. Drying was continued until the leaves were sufficiently brittle and free from excess moisture. The material was inspected during drying, and any portion showing fungal growth, discoloration or spoilage was discarded. Shade drying was selected because gradual moisture removal helps preserve phenolic, flavonoid and volatile components while reducing the risk of microbial contamination.

The dried leaves were coarsely powdered using a mechanical grinder. The powder was passed through a suitable sieve to obtain a relatively uniform particle size. Coarse powder was preferred because it provides adequate surface area for solvent penetration while reducing the risk of compact packing in the Soxhlet thimble. The prepared powder was stored in a clean, dry, labelled and airtight container in a cool location until extraction. Protection from moisture, heat, direct light

and prolonged exposure to air was maintained to reduce oxidation and loss of extractable constituents.

2.4 Hydroethanolic extraction

A 100 g quantity of the dried coarse leaf powder was subjected to Soxhlet extraction using 70% ethanol. The powder was placed in a cellulose thimble, and the extraction solvent was allowed to reflux repeatedly through the plant material. Extraction was maintained at approximately 60 degrees C for a total duration of 24 h. The selected temperature supported solvent cycling while avoiding unnecessary exposure to excessive heat. Continuous contact with renewed solvent during Soxhlet extraction promotes efficient removal of soluble phytochemicals from the plant matrix.

After completion, the hydroethanolic extract was filtered to remove insoluble residues. The solvent was evaporated under reduced pressure or controlled low-temperature conditions until a concentrated semisolid crude extract was obtained. The extract was transferred into a labelled airtight container and kept under cool storage pending analysis. Care was taken to avoid overheating during concentration because thermolabile phenolics, flavonoids and volatile constituents may degrade under harsh conditions.

The percentage yield was calculated from the mass of dried crude extract relative to the mass of dried leaf powder using the equation: percentage yield = (weight of dried extract / weight of dried plant powder) x 100. Physical characteristics, including colour, odour, appearance and consistency, were recorded immediately after concentration.

2.5 Preliminary phytochemical screening

A portion of the dried hydroethanolic extract was dissolved in a suitable solvent and filtered when necessary. Fresh extract solutions were prepared for each qualitative test. Results were graded according to the observed intensity of colour development, precipitate, ring or persistent foam as absent (-), weakly present (+/-), present (+) or strongly present (++). Standard pharmacognostic procedures were followed (Farnsworth, 1966; Harborne, 1998; Tiwari et al., 2011).

- **Alkaloids:** Mayer's, Wagner's and/or Dragendorff's tests were applied to an acidified extract solution. Cream, brown or orange precipitate was interpreted as a positive reaction.

- **Flavonoids:** The Shinoda and alkaline reagent tests were used. Pink, red or orange colour in the Shinoda reaction, or an intense yellow colour that disappeared after acidification in the alkaline reagent test, indicated flavonoids.
- **Glycosides:** Keller-Killiani and Borntrager-type procedures were used as appropriate. A brown ring at the interface or a pink to red ammoniacal layer was taken as evidence of glycosidic constituents.
- **Steroids:** Salkowski and Liebermann-Burchard reactions were used. Reddish-brown ring formation or development of green to bluish-green colour indicated steroidal constituents.
- **Tannins and phenolic compounds:** Ferric chloride, lead acetate and/or gelatin tests were performed. Blue-black, greenish-black or dark green colour and relevant precipitate formation indicated tannins or phenolic compounds.
- **Saponins:** The extract was vigorously shaken with water. Formation of a stable persistent froth was considered a positive foam test.
- **Terpenoids:** The Salkowski test was performed by adding concentrated sulphuric acid carefully to an extract-chloroform mixture. A reddish-brown interface indicated terpenoids.

3. Results

3.1 Authentication and processed leaf material

The collected leaves displayed the expected macroscopic characteristics of *A. graveolens*: green colour, a characteristic aromatic odour, divided leaf form and serrated or lobed margins. The material was fresh and free from visible fungal growth, insect infestation and physical deterioration. Shade drying produced a coarse greenish powder that remained free from visible contamination and was suitable for Soxhlet extraction.

3.2 Physical characteristics and extraction yield

Hydroethanolic extraction produced a concentrated dark greenish-brown crude extract with a characteristic aromatic odour and semisolid consistency. From 100 g of dried leaf powder, 12.40 g of dried crude extract was recovered. The calculated percentage yield was therefore 12.40%.

Table 1: Physical characteristics of *Apium graveolens*

Parameter	**Observation**
Plant part	Leaves
Drying method	Shade drying at room temperature
Powder characteristics	Coarse greenish powder
Extraction solvent	70% ethanol
Extraction method	Soxhlet extraction
Temperature and duration	Approximately 60 degrees C for 24 h
Extract colour	Dark greenish-brown
Consistency	Semisolid crude extract
Odour	Characteristic aromatic
Dried powder used	100 g
Dried extract recovered	12.40 g
Percentage yield	12.40%

Figure 1: A) Macroscopic appearance of leaves of *Apium graveolens* and B) *Apium graveolens extract*

3.3 Qualitative phytochemical profile

The hydroethanolic leaf extract tested positive for all eight phytochemical classes specified in the study objective. Flavonoids and phenolic compounds produced the strongest qualitative reactions. Alkaloids, glycosides, steroids, tannins, saponins and terpenoids were present. The observed profile indicates that the hydroethanolic solvent system recovered chemically diverse secondary metabolites from the leaves.

Table 2: Qualitative phytochemical profile

Phytochemical group	**Test(s) used**	**Result**	**Interpretation**
Alkaloids	Mayer's/Wagner's/Dragendorff's	+	Present
Flavonoids	Shinoda/alkaline reagent	++	Strongly present
Glycosides	Keller-Killiani/Borntrager's	+	Present
Steroids	Salkowski/Liebermann-Burchard	+	Present
Tannins	Ferric chloride/gelatin	+	Present
Phenolic compounds	Ferric chloride/lead acetate	++	Strongly present
Saponins	Foam test	+	Present
Terpenoids	Salkowski	+	Present

Key: ++ = strongly present; + = present; - = absent.

4. Discussion

The quality of a medicinal-plant extract depends on the integrity of every step from collection to chemical testing. The present study used healthy mature leaves that were free from visible infection and foreign matter, and the macroscopic features were consistent with the expected morphology of *A. graveolens*. This is more than a descriptive requirement. Misidentification can introduce a

completely different chemical profile and may create both efficacy and safety problems. Recording diagnostic characters and retaining a voucher specimen are therefore central to scientific traceability (Evans, 2009; Kokate et al., 2016).

Shade drying was appropriate for the leaf material because celery contains compounds that may be sensitive to light and heat. Rapid drying at excessive temperature can accelerate loss of volatile constituents and degradation of phenolic compounds, whereas insufficient drying increases the risk of enzymatic change and microbial growth. The absence of visible contamination in the dried material and the production of a uniform coarse powder indicated that the raw material was suitable for extraction. Uniform particle size also improves solvent access and reduces variation between extraction batches.

The use of 70% ethanol reflects a balance between extraction breadth and practical handling. Pure water favours highly polar compounds but may not efficiently recover moderately polar constituents and is more susceptible to microbial contamination during prolonged processing. Absolute ethanol can exclude some hydrophilic constituents. A hydroethanolic mixture broadens the solvent polarity window and is well suited for preliminary screening of flavonoids, phenolic compounds, glycosides, tannins, saponins and selected alkaloids (Tiwari et al., 2011). The successful detection of all targeted classes supports the suitability of the chosen solvent system for *A. graveolens* leaves.

Soxhlet extraction provides repeated contact between the solvent and the powdered matrix and can produce a reproducible crude extract when time, temperature, solvent concentration and plant-to-solvent conditions are controlled. The extraction at approximately 60 degrees C for 24 h was sufficient to produce a semisolid dark greenish-brown extract without reported evidence of scorching or decomposition. The aromatic odour of the extract was consistent with the known volatile character of celery, although qualitative observation alone cannot identify or quantify volatile compounds. Future standardization should therefore include chromatographic or spectrometric analysis rather than relying on appearance and odour.

The 12.40% extraction yield provides a useful baseline for repeat experiments and dose preparation. Yield can vary with moisture content, cultivar, season, geographical source, leaf maturity, particle size and solvent ratio. It should not be interpreted as a direct measure of biological potency. Pigments, sugars and other non-active substances contribute to crude extract

mass, while pharmacologically active constituents may be present in relatively small amounts. The relevance of the yield lies mainly in process reproducibility and material balance. A future study should report extraction in replicate batches and calculate the variation in yield to assess manufacturing consistency.

The phytochemical results demonstrated a broad profile dominated qualitatively by flavonoids and phenolic compounds. This agrees with the wider literature describing celery as a source of flavonoids and phenolic acids (Al-Asmari et al., 2017; Makarova et al., 2022). Strong qualitative reactions do not provide concentration values, but they indicate that these classes are abundant enough to produce clear test responses. Flavonoids and phenolics may contribute antioxidant, anti-inflammatory and neuroprotective effects. In later pharmacological studies, their presence offers a plausible chemical basis for activity, but causality cannot be assigned without fractionation, compound identification and dose-response testing of standardized fractions.

Alkaloids, glycosides, steroids, tannins, saponins and terpenoids were also detected. Each class contains structurally diverse compounds, and a positive group test does not imply that all members of that class are present or pharmacologically relevant. Alkaloids may interact with neuronal or autonomic targets; terpenoids can contribute volatile, sedative or anti-inflammatory actions; tannins and phenolics may provide antioxidant activity; and saponins can influence membrane and inflammatory processes. The crude extract may therefore act through combined or synergistic effects. Conversely, some constituents may oppose each other or reduce bioavailability. The preliminary screen should be viewed as hypothesis-generating rather than mechanistic proof.

The study was intentionally limited to qualitative screening. Classical colour and precipitation tests are inexpensive and useful for early characterization, but they may be affected by extract colour, reagent quality, observer judgement and cross-reactivity. The dark greenish-brown colour of the celery extract may interfere with subtle colour changes, and therefore blank controls and replicate testing are important. Future work should quantify total phenolic and flavonoid contents and establish a chemical fingerprint using thin-layer chromatography, high-performance thin-layer chromatography, high-performance liquid chromatography, liquid chromatography-mass spectrometry or gas chromatography-mass spectrometry. Marker compounds such as apigenin-related flavonoids, phenolic acids and phthalide constituents should be considered for standardization.

Despite these limitations, the study provides an important foundation. It defines the plant part, preparation conditions, extraction solvent, extraction time, physical properties, yield and qualitative chemical profile. Such reporting allows the same extract to be reproduced more accurately in toxicity and pharmacological experiments. It also prevents inappropriate generalization from celery roots, seeds, essential oils or aqueous preparations to the hydroethanolic leaf extract examined here. The results apply specifically to authenticated *A. graveolens* leaves processed by the described method.

Quality assurance should be strengthened in future extraction work by documenting the plant-to-solvent ratio, number of Soxhlet cycles, final moisture content of the leaf powder and recovery conditions during solvent evaporation. These variables influence both yield and constituent stability. Repeating extraction with at least three independent plant batches would allow reporting of mean yield and batch variability rather than a single value. Reagent blanks, positive controls and duplicate or triplicate phytochemical reactions should also be included to reduce subjective interpretation. Such controls are especially important for coloured extracts because endogenous pigments may mimic or mask a weak colour reaction.

The distinction between qualitative presence and quantitative abundance is central to interpretation. A strong reaction for flavonoids or phenolics indicates greater apparent test intensity, but the response may not be linear and cannot be converted directly into concentration. Total phenolic content using a calibrated colorimetric assay and total flavonoid content using an appropriate reference standard would provide numerical estimates. These measurements could then be related to extract yield and later biological activity. A standardized extract should ideally be defined by one or more marker compounds and an acceptable concentration range rather than by colour and yield alone.

The use of leaves also has practical and sustainability advantages. Harvesting leaves is generally less destructive than collecting roots or entire plants, and cultivated celery provides an accessible raw material. However, agricultural factors such as cultivar, fertilizer use, irrigation, pesticide exposure and maturity at harvest can alter phytochemical composition. Future manuscripts should therefore report cultivation and collection conditions in sufficient detail. Authentication by morphology may also be supplemented by microscopy, chromatographic fingerprinting or DNA-

based identification when material is powdered or when closely related species could be substituted.

From a drug-discovery perspective, the present phytochemical profile supports prioritization rather than therapeutic conclusion. The strong flavonoid and phenolic reactions suggest that polar and moderately polar fractions deserve further investigation, while the detection of terpenoids indicates that less polar fractions should not be ignored. Sequential fractionation of the hydroethanolic extract into solvents of increasing polarity, followed by bioassay-guided testing, could reveal whether activity is concentrated in one fraction or depends on interactions within the crude mixture. This stepwise approach would connect pharmacognostic quality control with mechanism-based pharmacology.

Reproducibility also requires transparent reporting of negative findings. In the present focused analysis, the target phytochemical groups were detected, but the intensity of each reaction differed. Future studies should report photographic evidence of reaction tubes, reagent preparation dates, extract concentration used in each assay and the criteria used to distinguish weak from strong responses. Including these details would allow independent laboratories to compare results and would reduce the tendency to describe a plant as broadly phytochemical-rich without sufficient analytical support. Such disciplined reporting is especially important when a preliminary screening paper is later used to justify animal experimentation or product development.

5. Conclusion

Authenticated leaves of *A. graveolens* were successfully shade-dried, coarsely powdered and extracted with 70% ethanol using a Soxhlet apparatus at approximately 60 degrees C for 24 h. The process produced a dark greenish-brown aromatic semisolid extract with a yield of 12.40%. Preliminary screening detected alkaloids, flavonoids, glycosides, steroids, tannins, phenolic compounds, saponins and terpenoids, with the strongest reactions observed for flavonoids and phenolic compounds. These findings demonstrate that the hydroethanolic leaf extract contains a chemically diverse mixture of secondary metabolites and provide a reproducible basis for subsequent safety, pharmacological and chromatographic studies. Further research should quantify major constituent classes, identify chemical markers and assess batch-to-batch consistency before advanced biological or translational claims are made.

Declarations

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Authors contribution: Purnima Tiwari contributed to conceptualization, methodology, investigation, data collection, analysis, and preparation of the original manuscript. Sushila Kaura supervised the study, validated the methodology and findings, and critically reviewed and edited the manuscript. Both authors approved the final version.

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