Phytochemical Extraction and Characterization of Solanine

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***Abstract*—This study is about extracting and analyzing α-solanine from potato tubers (Solanum tuberosum) using an eco-friendly method. Potato peels are a rich source of steroidal glycoalkaloids, which have useful medicinal properties.The researchers used a step-by-step extraction process: first with Natural Deep Eutectic Solvents (NADES), then with acidified ethanol to get better yields. They also used ultrasound to make the extraction more effective.The extracts were then tested through phytochemical screening and analyzed using modern lab techniques like HPLC, HPTLC, UV-Visible spectroscopy, and FTIR. The study also covers the physical and chemical properties of α-solanine, its potential health benefits, and its toxic effects.Overall, this work promotes green chemistry by turning potato waste into a valuable source of bioactive compounds for pharmaceutical use. It also gives an overview of modern methods for extracting and studying solanine from potatoes.This research shows how we can turn everyday kitchen waste into useful compounds for medicines in a clean and sustainable way.**

***Index Terms*—α-Solanine, Glycoalkaloids, Natural Deep Eutectic Solvents (NADES), Ultrasound-assisted extraction, HPLC**

I. INTRODUCTION

Potato plant, Solanum tuberosum L., is a fundamental part of food security and pharmaceutical potential in the world. It is part of the Solanaceae family and presently ranks as the fourth largest staple crop globally after maize, wheat and rice, producing over 368 million tonnes annually. This herbaceous perennial plant reaches a height of up to 100 cm and has a wide range of uses with all parts of the plant used both traditionally and as medicine.[1] The plant's developmental stages are areas of major change in the secondary metabolic profile due to environmental factors, such as: sone sprout development, shoot emergence, tuber initiation, bulking and maturation. On a biochemical level, the Solanum tuberosum tuber is not just a storage depot for starch (which accounts for 9% to 30% of its weight) but a complex mixture of bioactive components.[4] These are proteins (2%), lipids (0.1%) and various micronutrients including Vitamin C, Vitamin B-complex, potassium, and phosphorus. The most important secondary metabolites with respect to the pharmacy practitioner are the steroidal glycoalkaloids, namely α-solanine and α-chaconine. They are the plant's main chemical defence against herbivores, pathogenic fungi and insect predators – an evolutionary adaptation that shows a high degree of sophistication.[5]

TABLE 1: Taxonomical Classification and Morphological Characteristics [12]

The taxonomy of Solanum tuberosum can give insight in its genetic diversity and metabolic capacity. The species is part of Order Solanales, which is well-known for their high content of potent alkaloids and Family Solanaceae.The habit is rosette or semi-rosette, with the leaves growing close to the base of the stems. The flowers are bisexual and promote self-pollination, and the tubers in the ground are modified stems that are used for storage of nutrients and for asexual reproduction. [21,23]

Botanical Hierarchy	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	*Solanum*
Species	*Solanum tuberosum* L.

The phytochemical profile of the tuber is influenced by its color—ranging from yellow to blue and red—and the specific cultivar. For instance, pigmented varieties often contain higher concentrations of anthocyanins and phenolic acids, such as chlorogenic acid, which contribute to the plant's antioxidant capacity. However, the distribution of toxic glycoalkaloids is predominantly concentrated in the periderm (peel) and the sprouts, where the concentrations can be up to ten times higher than in the flesh. [13]

II. CHEMICAL ARCHITECTURE OF STEROIDAL GLYCOALKALOIDS

The primary steroidal glycoalkaloids in *Solanum tuberosum* are α-solanine and α-chaconine, which together account for approximately 95% of the total glycoalkaloid (TGA) content. These molecules are nitrogen-containing steroidal glycosides, biosynthesized through the mevalonic acid pathway, specifically via the sterol branch that produces cholesterol. Structurally, they consist of a C27 steroidal aglycone, solanidine, which is linked to different trisaccharide moieties.[25]

Structural Specificity of α-Solanine and α-Chaconine

The aglycone, solanidine (C_{27}H_{43}NO), has a solanid-5-ene backbone, with the 22β,25R configuration. The hydrophobic steroidal core is crucial for the molecule's interaction with lipid bilayers in biological membranes. The hydrophilic part of α-solanine consists of a solatriose unit which is made up of galactose, glucose, and rhamnose. [26,35] On the other hand, α-chaconine has one glucose moiety and two rhamnose moieties in a chacotriose unit.

TABLE 2: Molecular and Physical Properties of α-Solanine[28,18]

Property	Specification
Molecular Formula	$C_{45}H_{73}NO_{15}$
Molecular Weight	868.06 g/mol
Melting Point	285°C (with decomposition)
Optical Rotation	-59.45° (in pyridine)
Solubility (Organic)	Methanol, Pyridine, Hot Ethanol
Solubility (Aqueous)	Sparingly soluble in H_2O (25 mg/L at pH 6.0)
pKa	6.66 (at 15°C)

The structural difference between α-solanine and α-chaconine, though seemingly minor, significantly alters their biological activity. α-Chaconine is generally considered more toxic and exhibits stronger membrane-disrupting properties than α-solanine, which is attributed to the specific orientation of its rhamnose units.

III. MECHANISMS OF ACTION AND PHARMACOLOGICAL SIGNIFICANCE

Historically, solanine was primarily viewed through the lens of toxicology. However, contemporary research in pharmacy practice has shifted toward its potential as a therapeutic agent, particularly in oncology and immunology. The pharmacological significance of *S. tuberosum* extends beyond simple nutrition, encompassing anticancer, antimicrobial, anti-inflammatory, and neuroprotective activities. The following mechanisms are summarized from reported pharmacological studies available in scientific literature. [3,6]

Anticancer and Pro-apoptotic Effects

α-Solanine has demonstrated significant anticancer potential against multiple human cancer cell lines, including liver, breast, lung, and prostate cancers. The compound promotes programmed cell death through mitochondrial-mediated apoptotic pathways involving caspase activation. In addition, α-solanine suppresses tumor progression by reducing cellular migration and invasion through regulation of matrix metalloproteinases and inflammatory signaling pathways. These combined actions highlight its potential as a promising phytochemical for future anticancer research

Antimicrobial and Anti-inflammatory Properties

Solanum tuberosum extracts have demonstrated potent antimicrobial activity against several pathogenic bacteria. The mechanism involves the disruption of the microbial cell membrane, leading to the leakage of intracellular components. Studies have shown that potato peel extracts are effective against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Additionally, the high content of phenolic acids, such as chlorogenic acid, provides antioxidant protection against reactive oxygen species (ROS), which are

implicated in atherosclerosis, diabetes, and tissue damage.[17]

IV. MODERN EXTRACTION STRATEGIES: THE NADES REVOLUTION

Traditional extraction of glycoalkaloids from potato tubers has historically relied on volatile organic solvents (VOS) such as methanol, chloroform, and acidified aqueous solutions. While effective, these methods present environmental and health risks. The shift toward green chemistry has popularized Natural Deep Eutectic Solvents (NADES), which are eco-friendly, non-toxic, and highly efficient alternatives.

Principles and Advantages of NADES

α-Solanine most dramatically activates caspases 3 and 9, leading to mitochondrial apoptosis in various human cancer cell lines, without the involvement of the tumor suppressor protein p53. This is especially important in Oral Squamous Cell Carcinoma (OSCC) because mutations in p53 are related with chemoresistance. Moreover, α-Solanine has been found to be a suppressor of the expression of MMP-2 and MMP-9, which help in the invasion and metastasis of tumors. These enzymes break down the extracellular matrix and are necessary for the movement of cancer cells. Solanine further regulates the inflammatory microenvironment which is usually conducive to tumour progression by modulating the MAPK pathway and interfering with the phosphorylation of ERK1/2 and JNK.[31,32]

Synergistic Combination: Sequential Extraction (F1 and F2) [29]

In this study, a sequential extraction procedure with two different extraction steps is used: a main extraction step with NADES and a short extraction step with acidified ethanol.

First Extraction (F1 - NADES Step): This step uses the special glycoalkaloid and phenolic compound solvents (NADES - Choline Chloride: Citric Acid) that target the most easily extracted glycoalkaloids and phenolic compounds. The disruption of the plant cell walls by acoustic cavitation during the Ultrasound-Assisted Extraction (UAE) increases the mass transfer. The material from step 1 is then treated with acidified ethanol (95% ethanol adjusted with acetic acid to pH 2–6),

Second Extraction (F2 - Acidified Ethanol Step): This second extraction step may improve the recovery of residual alkaloids remaining within the plant matrix

Fig 1: Systematic Extraction of Solanine from Solanum tuberosum Tuber via Acidified Solvent Maceration.[28]

TABLE 3: Standardized Ingredient List for Sequential Extraction[15]

Ingredient	Role in Process
Choline Chloride	Hydrogen Bond Acceptor (NADES)
Citric Acid	Hydrogen Bond Donor (NADES)
Ethanol (96% or 95%)	Primary Organic Solvent (F2)
Glacial Acetic Acid	pH Adjustment (Acidification)
Ammonium Hydroxide	Precipitating Agent (pH 10-11)
Distilled Water	Solvent/Diluent

V. PROCEDURAL METHODOLOGY FOR PHYTOCHEMICAL ISOLATION

The extraction of solanine from *Solanum tuberosum* requires meticulous preparation of the plant material to prevent the degradation of heat-sensitive glycoalkaloids.

Preparation of Plant Material

Uniform tubers of *Solanum tuberosum* are selected, washed to remove soil, and peeled manually to a thickness of approximately 1.5–3.0 mm. To increase the glycoalkaloid concentration, tubers may be exposed to artificial light to induce greening. The peels are then dried in an oven at 40–60°C or freeze-dried at -50°C for 48 hours. The dried material is powdered using a motar and pestle to a fine powder (mesh size 35 or 0.2–0.8 mm) and stored in airtight containers in the dark at -18°C or -20°C until use.[33]

Fig 2: Size reduction and trituration of dried *Solanum tuberosum* peels using a porcelain mortar and pestle[11]

Detailed Sequential Extraction Protocol [14,24]

The laboratory process consists of a computed sequence in which two fractions are obtained F1 and F2.

- NADES Formulation: Choline chloride and citric acid are mixed in the molar ratio of 1:1 or 1:2 and heated with stirring at 80 °C till a clear liquid is obtained.
- The NADES mixture (1:10 w/v) is prepared by adding 10 g of the plant powder to 100 mL of the mixture, which is now called Fraction 1 (F1) Extraction. The mixture is treated by Ultrasound-Assisted Extraction (UAE, 100-300W for 30 minutes at 50°C). The mixture is then filtered through a Whatman No. 4 filter paper.
- After first filtration the residue (raffinate) is immediately mixed with 100 mL of 95% ethanol acidified with 5% glacial acetic acid (Fraction 2 Extraction). The mixture is stirred for 15 minutes at room temperature and filtered.
- Concentration & Purification: The F1 and F2 filtrates are concentrated to 40-45°C in the rotary vacuum evaporator. The concentrated extract is adjusted to pH 10 with 25% ammonium hydroxide and glycoalkaloids are precipitated. The mixture is prevented from becoming sterile and is heated to 70°C for 30 minutes to ensure complete precipitation and is then cooled and centrifuged to harvest the pellet.

TABLE 4: Calculated Process Flow and Mass Balance[34]

Step	Output/Parameter	Calculation/Formula
Initial Weight	Plant Powder (W_p)	10.00 g (Standard)
F1 Yield	NADES Extract (W_{F1})	Weight of dried F1 extract (g)
F2 Yield	Ethanolic Extract (W_{F2})	Weight of dried F2 extract (g)
Total Yield	Combined Extract (W_t)	W_{F1} + W_{F2}
Percentage Yield	% Extract Recovery	(W_t / W_p) \times 100

TABLE 5: Physicochemical Standardization Data[34]

Parameter	Standard Value (%)
Moisture Content	13.05%
Total Ash Value	10.60%
Water-Soluble Extractive	6.66%
Total Glycoalkaloid (TGA)	20–150 mg/kg (Fresh Weight)

VI. QUALITATIVE EVALUATION AND PHYTOCHEMICAL SCREENING

Phytochemical screening is essential to confirm the

presence of alkaloids and other bioactives in the F1 and F2 fractions. These tests rely on specific colorimetric reactions between the secondary metabolites and chemical reagents. [2,8]

Alkaloid Identification Tests [9,10]

- Dragendorff's Test: A few drops of Dragendorff's reagent (potassium bismuth iodide) are added to 1 mL of the extract. The formation of an orange-red precipitate indicates a positive result for alkaloids.
- Mayer's Test: The extract is treated with Mayer's reagent (potassium mercuric iodide). A yellowish-buff precipitate confirms the presence of alkaloids.
- Wagner's Test: The addition of Wagner's reagent (iodine in potassium iodide) produces a reddish-brown precipitate.
- Hager's Test: A yellow precipitate with picric acid solution is indicative of alkaloids.
- Foam Test (Saponins): 0.2 g of the extract is shaken vigorously with 2 mL of water. The formation of persistent foam (lasting 10 minutes) indicates the presence of saponins.
- Ferric Chloride Test (Phenols): 1 mL of the extract is treated with 5% ferric chloride solution. A dark green or bluish-black color signifies the presence of phenolic compounds.

TABLE 6: Evaluation Test Matrix for F1 and F2 Fractions[19,20]

Test Name	Reagent Used	Expected Observation	Inference
Alkaloid Test	Dragendorff's Reagent	Orange-red precipitate	Alkaloids Present
Alkaloid Test	Mayer's Reagent	Cream/Yellow precipitate	Alkaloids Present
Saponin Test	Distilled Water +	Persistent foam	Saponins Present
Flavonoid Test	10% NaOH	Yellow color change	Flavonoids Present
Tannin Test	1% FeCl_3	Blue-green/Black color	Tannins Present

VII. CHARACTERIZATION BY ADVANCED ANALYTICAL TECHNIQUES

The complexity of the glycoalkaloid matrix requires precise analytical methods for the identification and quantification of α-solanine. Modern pharmacy practice utilizes Chromatography and Spectrophotometry for these purposes.[22]

High-Performance Liquid Chromatography (HPLC)

HPLC is the primary method for quantifying α-solanine. Due to the lack of a strong chromophore, UV detection is typically performed at wavelengths between 202 nm and 218 nm.

TABLE 7: HPLC Operational Parameters for Solanine Analysis [36]

Parameter	Optimized Setting
Column	Reversed Phase C18 (250 \times 4.6 mm; 5 μm)
Mobile Phase	Acetonitrile: 1.0 mM Ammonium Acetate (pH 4.2) (65:35 v/v)
Flow Rate	0.6 – 1.0 mL/min
Injection Volume	5 – 20 μL
Detection Wavelength	218 nm
Retention Time	α-Solanine: 4.43 – 4.88 min

The method is validated for linearity, limit of detection (LOD), limit of quantification (LOQ), and accuracy. α-Solanine typically establishes a linearity range of 1–100 μg/mL with an R^2 value of 0.9992.

HPTLC and Spectrophotometry

High-Performance Thin-Layer Chromatography (HPTLC) provides a rapid means of screening multiple samples. Using a mobile phase of Chloroform: Methanol: Ammonia (7:3:0.5 v/v), solanine displays an R_f value of approximately 0.05. For visualization, the plates are dipped in modified Carr-Price reagent (antimony (III) chloride) and heated, producing distinct red zones.

UV-Vis spectrophotometry can also be used for determining total phenolic content (TPC) and total flavonoid content (TFC) using the Folin-Ciocalteu and aluminum chloride methods, respectively.[37]

VIII. STANDARDIZATION AND PROXIMATE ANALYSIS

Standardization of the *S. tuberosum* tuber extract involves determining its proximate values according to Association of Official Analytical Chemists (AOAC) guidelines. This ensures the reproducibility and safety of the pharmaceutical product.

Moisture Content and Ash Value

The moisture content of the dried potato peel powder is a critical parameter for stability. High moisture levels (above 15%) can lead to microbial spoilage and enzymatic degradation of alkaloids. The standard moisture content for S. tuberosum peel powder is determined to be approximately 13.05%. The total ash value reflects the total inorganic residue (minerals) in the sample. For potato peels, this value is approximately 10.60%, while the acid-insoluble ash is significantly lower, indicating low levels of siliceous contamination.

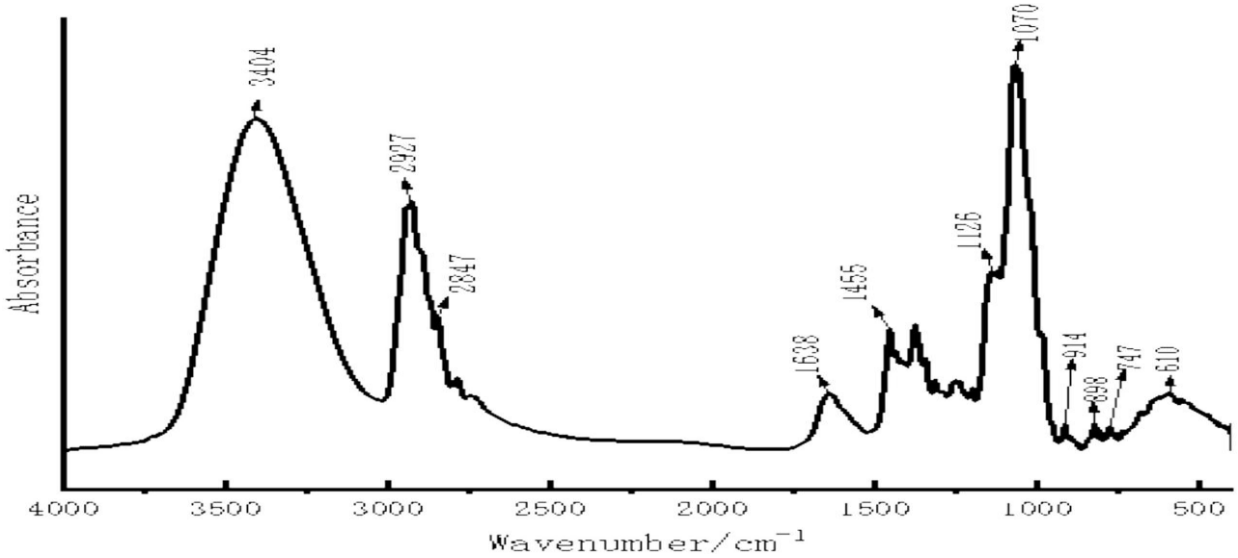

Fig 3: FTIR Spectrum of Solanine Extracted from Solanum tuberosum (Potato) Tubers [38]

TABLE 8: FTIR Peak Assignments for Solanine from Solanum tuberosum [38]

The reported FTIR peak assignments, summarized from previously published literature, indicate the characteristic functional groups associated with α-solanine and related steroidal glycoalkaloids.

S. No.	Absorbance Peak (cm^{-1})	Functional Group	Vibrational Assignment
1	3200–3400	O–H	Broad stretching vibration of hydroxyl group
2	2920–2935	C–H	Asymmetric stretching of aliphatic alkane
3	2850	C–H	Symmetric stretching of CH_2/CH_3 groups
4	1640–1660	C=C	Stretching vibration of steroidal alkene
5	1450–1465	CH_2	Bending vibration
6	1370–1385	CH_3	Symmetric bending vibration
7	1250–1270	C–N	Stretching vibration of alkaloid amine group
8	1150–1080	C–O–C	Glycosidic ether stretching
9	1020–1080	C–O	Alcohol stretching vibration
10	850–950	=C–H	Out-of-plane bending vibration

Toxicological Thresholds and Safety

While α-solanine has pharmaceutical potential, its narrow therapeutic index requires careful monitoring. The presently recognized upper limit of safety for human consumption is 200 mg TGA per kg of fresh potato. Doses exceeding this threshold can lead to severe toxicity, characterized by rapid, shallow breathing, shallow pulse, delirium, and coma.

IX. SUSTAINABILITY AND BIO-VALORIZATION [39]

The extraction of solanine from potato side-streams (peels and sprouts) aligns with the principles of the bio-economy. Potato peels are often discarded as industrial waste, but they represent a high-value source of steroidal alkaloids for the pharmaceutical industry.

Environmental Factor (E-factor) Analysis

The "greenness" of the extraction process can be assessed using the E-factor (environmental factor), which measures the ratio of the total mass of waste generated to the mass of the product obtained. Modern techniques such as UAE combined with NADES significantly lower the E-factor compared to traditional Soxhlet extraction by reducing solvent consumption and extraction time.

The integration of ultrasound and NADES technologies marks a significant step toward sustainable pharmaceutical manufacturing. By utilizing renewable, non-toxic solvents and maximizing the recovery of bioactives through sequential extraction (F1 and F2), the pharmaceutical scientist can produce high-quality extracts with minimal environmental impact.[39]

X. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research work.

XI. CONCLUSION

The phytochemical extraction and characterization of solanine from *Solanum tuberosum* tubers exemplify the evolving landscape of pharmacy practice, where traditional botanical knowledge meets advanced green chemistry. Through the implementation of a sequential extraction strategy—employing Natural Deep Eutectic Solvents for the primary Fraction (F1) and acidified ethanol for the secondary Fraction (F2)—maximum recovery of steroidal glycoalkaloids is achieved.

The detailed characterization of α-solanine via HPLC, UV-Vis spectrophotometry, and standardized phytochemical screening confirms its identity and establishes a foundation for quality control. Pharmacological studies highlight its significant potential as an anticancer and antimicrobial agent, provided that its toxicological profile is strictly managed. As the global demand for plant-based therapeutics grows, the valorization of potato industrial waste into high-purity bioactive extracts represents a sustainable and innovative pathway for the development of new pharmaceutical interventions. Future research should continue to explore

the synergistic effects of F1 and F2 fractions and the potential for their encapsulation to improve the stability and targeted delivery of solanine in clinical settings.

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