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Phytochemical and Biological investigations of the active constituents of various parts of Solanum indicum

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ABSTRACT

Solanum indicum, commonly known as Indian nightshade, has demonstrated diverse pharmacological activities across its various extracts. Studies have shown that leaf extracts possess significant antioxidant properties, potentially due to the presence of phenolic compounds and flavonoids. The fruit extracts have exhibited notable antimicrobial effects against several pathogenic bacteria and fungi, suggesting their potential use in treating infectious diseases. Additionally, root extracts have displayed anti-inflammatory and analgesic activities in animal models, indicating possible applications in pain management. Some research has also highlighted the potential antidiabetic properties of *S. indicum* extracts, with observed reductions in blood glucose levels in experimental studies. Furthermore, preliminary investigations have suggested antitumor activities in certain extracts, though more comprehensive research is needed to fully elucidate these effects and their underlying mechanisms. To guarantee quality, efficacy, and repeatability, this study focused on standardising and testing Solanum indicum extracts and isolated components. Flavonoids, phenolic acids, alkaloids, and terpenoids are examples of moderately polar compounds that can be extracted using C6H14. The extracts, particularly the C2H5OH and CH3COOC2H5 extracts, exhibited significant antibacterial, antioxidant properties and Anthelmintic properties.

Keywords: *Solanum indicum, pharmacolgocial activity, Phytochmcial screening.*

1. INTRODUCTION

The Solanum genus, comprising over 1,500 species, is known for its diverse range of plants, many of which have medicinal value. Solanum nigrum (black nightshade) and *Solanum tuberosum* (potato) have been traditionally used in various cultures for their therapeutic effects. Therefore, the pharmacological potential of these species should be explored. Owing to the side effects and drug resistance, the search for new and effective pharmacological agents is ongoing. Plant-based compounds have historically contributed to the development of many drugs, and Solanum species offer a promising reservoir of bioactive substances. Investigating these plants can reveal new avenues for drug development, especially for conditions where current treatments are ineffective. *Solanum indicum's* pharmacological potential extends beyond its antioxidant, antimicrobial, anti-inflammatory, and analgesic properties. Recent studies have explored its hepatoprotective effects, with liver enzyme levels showing improvement in animal models treated with S. indicum extracts. This suggests a possible role in managing liver disorders. The plant's extracts have also demonstrated antiulcer activities, protecting gastric mucosa against various ulcerogenic agents. These findings indicate potential applications in treating gastrointestinal disorders. Moreover, *S. indicum* has shown promise in cardiovascular health. Extracts from the plant have exhibited hypotensive effects in experimental models, suggesting potential use in managing hypertension. Some studies have also reported cholesterol-lowering properties, which could be beneficial in addressing cardiovascular risk factors. Additionally, preliminary research has indicated possible neuroprotective effects, with certain extracts showing promise in models of neurodegenerative diseases. However, as with many of its potential applications, further research is necessary to fully understand the mechanisms of action and to establish safe and effective therapeutic protocols for S. indicum-derived treatments. This article will examine the pharmacological potential of Solanum species plant extracts using in-vivo and in-vitro experimental models to determine their efficacy, mechanism of action, and therapeutic potential.[1-15]

2. MATERIAL & METHODS

Extraction of Plant Material

Powdered plant materials *Solanum indicum* (100 g) from different parts (rhizomes, shoots, leaves, pollen) were subjected to successive Soxhlet extraction using solvents in ascending polarity (C6H14, CHCL3, CH3COOC2H5, mC2H5OH). After each extraction, the plant residue was dried before the next solvent cycle. Extracts were concentrated using rotary evaporation, dried, and stored in desiccators. Yield was expressed as a percentage of dry weight.

Preparation of Aqueous Extracts

Each 100 g sample of dried herbs was boiled in 1 L of distilled water for 1 hour, filtered three times, and reduced to 10 mL under vacuum at 50°C. Dried extracts were weighed and stored in water for later use. Yield was calculated as dry mass obtained per 100 g of raw herb.

Phytochemical Screening

Qualitative chemical tests were performed on extracts of *Solanum indicum* following IP2018 protocols. Tests were conducted in triplicate and the results tabulated.

Antimicrobial Activity of Extracts and Isolated Compounds

Pure bacterial cultures obtained from the Department of Microbiology at AND College of Pharmacy (ANDCP), Babhnan, were employed in this study. These strains, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus* species, were maintained on nutrient agar and Sabouraud's dextrose agar.

Inoculum Preparation

Stock cultures were stored at 4°C on nutrient agar slopes. Active cultures for testing were prepared by inoculating nutrient broth with a loopful of stock culture and incubating at 37°C for 24 hours. These cultures were further diluted for experiments.

Media Preparation

Agar media were prepared by dissolving components in distilled water and sterilizing by autoclaving at 121°C for 15 minutes. These were then used for antimicrobial assays.

Assessment of Antibacterial Activity

The disc diffusion method was employed using nutrient agar from Himedia (Mumbai). Extracts were applied to 3 mm sterile discs, allowed to dry briefly, and placed on inoculated agar plates. After 24 hours of incubation at 37°C, inhibition zones were measured in millimeters. Each test was repeated three times, with 10 microgram/disc penicillin used as the standard.

Minimum Inhibitory Concentration via Serial Dilution

Tests were carried out in broth containing 10^6–10^7 CFU/mL. Crude extracts and standard penicillin were evaluated at concentrations of 1000, 500, 250, 125, 62.5, and 31.25 microgram/mL. DMSO was used to dissolve both the extracts and the standard. Serial dilutions were prepared across six tubes for each sample. Following incubation at 37 ± 1°C, turbidity was measured to calculate MIC. DMSO controls showed no microbial inhibition.

Extracts with MIC values below 100 microgram/mL were considered highly active; values between 100–500 microgram/mL indicated moderate activity; 500–1000 microgram/mL weak activity; and above 1000 microgram/mL no activity.

Antimicrobial Susceptibility Testing

The disc diffusion method was also used for antifungal and antibacterial testing. NA plates were prepared with 15 mL of molten media and inoculated with 0.11% microbial suspension. After drying, extract-impregnated discs were applied, and the plates were incubated for 24 hours at 37°C. Zones of inhibition were measured in millimeters. For fungal strains, Sabouraud's dextrose agar was used. Each test was conducted in triplicate with penicillin (10 microgram/disc) as the standard.

DPPH Radical Scavenging Activity and IC_{50} Determination

4.9.2.5 DPPH Radical Scavenging Assay

Aliquots (20 µL) of extracts were mixed with 80 µL of 100 mM Tris-HCl buffer (pH 7.4) and 100 µL of 250 µM DPPH in C2H5OH. Samples were incubated in the dark at room temperature for 20 minutes, then absorbance was measured at 517 nm. Percentage decolourisation was calculated as:

DPPH Scavenging Activity (%)=(A0−A1)×100

Where:

- A0 = Absorbance of the **control** (without sample)

- A1 = Absorbance of the **test sample** (with antioxidant)

Statistical Analysis

Results were expressed as mean ± SD from triplicates. A p-value < 0.05 was considered statistically significant.

Although phenolic and flavonoid contents did not always correlate directly with antioxidant capacity, synergistic effects of various phytochemicals likely contribute significantly. This suggests potential for using wetland medicinal plants as accessible sources of natural antioxidants. Further research is warranted to isolate specific antioxidant constituents.

4.13 Anthelmintic Activity of *Solanum indicum*

Extract suspensions were prepared in 1% Tween 80 to obtain final concentrations of 1%, 2.5%, and 5%. Similarly, albendazole, used as the reference standard, was dissolved in distilled water at equivalent concentrations.

Two milliliters from each concentration of the extracts and albendazole were further diluted with normal saline to a total volume of 10 mL and placed in Petri dishes. The experimental setup was divided into five groups: Group I received only normal saline (negative control), Group II was treated with the standard drug albendazole (positive control), while Groups III to V were exposed to the three concentrations (1%, 2.5%, and 5%) of the different plant extracts. Each dish contained six adult earthworms (*Pheretima posthuma*) of approximately equal size.

The time taken for each worm to become paralyzed and subsequently die was recorded in minutes. Paralysis was defined as the loss of motor function that did not reverse when transferred to normal saline, while death was confirmed by complete immobility and discoloration of the worm's body. The earthworm model was selected due to its structural and physiological resemblance to parasitic helminths in the human intestine. Extracts from all parts of the plant were evaluated for anthelmintic potential using this method.

3. RESULTS

Yields of Extracts (as % w/w of dry weight)

Plant Part	C_6H_{14}	$CH_3COOC_2H_5$	$CHCl_3$	C_2H_5OH	Water
Root	2%	4%	5%	10%	12%
Stem	1%	2%	3%	7%	10%
Leaf	2%	5%	6%	12%	15%
Aerial Parts	2%	5%	6%	12%	18%
Fruit	3%	6%	7%	15%	20%

The yield of the extracts varies depending on the polarity of the solvent, type of plant material, and chemical composition. Quantitative extraction results (in grams and % w/w yield) of 200 g of each plant part (root, stem, leaf, aerial part, and fruit) of Solanum indicum using C_6H_{14}, $CH_3COOC_2H_5$, $CHCl_3$, C_2H_5OH, and aqueous solvents. The extraction yield of Solanum indicum varied across different plant parts and solvents used. Overall, the fruit exhibited the highest extraction yield in all solvents, with water extraction yielding the most (20%), followed by C_2H_5OH (15%). The aerial parts also showed high yields, particularly in water (18%) and C_2H_5OH (12%). Among all plant parts, water and C_2H_5OH were the most effective solvents, consistently producing the highest yields across roots, stems, leaves, aerial parts, and fruits. Conversely, C_6H_{14} consistently yielded the least, typically around 1–3%, indicating minimal extraction of non-polar compounds.

Quantity of Extracts from 200 g of

Solvent	Plant Part	Extract Quantity (g)	Yield (% w/w)
C_6H_{14}	Root	4.2	2.1%
	Stem	3.8	1.9%
	Leaf	6.5	3.3%
	Aerial Part	7.0	3.5%
	Fruit	5.6	2.8%
$CH_3COOC_2H_5$	Root	7.8	3.9%
	Stem	6.2	3.1%
	Leaf	10.4	5.2%
	Aerial Part	11.0	5.5%
	Fruit	8.7	4.4%
$CHCL_3$	Root	6.5	3.3%
	Stem	5.3	2.7%
	Leaf	8.9	4.5%
	Aerial Part	9.2	4.6%
	Fruit	7.4	3.7%
C_2H_5OH	Root	12.5	6.3%
	Stem	10.8	5.4%
	Leaf	18.2	9.1%

	Aerial Part	19.4	9.7%
	Fruit	15.0	7.5%
Aqueous	Root	10.2	5.1%
	Stem	8.5	4.3%
	Leaf	14.7	7.4%
	Aerial Part	15.8	7.9%
	Fruit	13.0	6.5%

Solubility tests and other phytochemical tests for extracts of **Solanum indicum** obtained using C6H14, CH3COOC2H5, CHCL3, C2H5OH, and water were performed to determine the solubility profile and the presence of bioactive compounds in different extracts.

1. Solubility Tests

The solubility of the extracts in different solvents was determined.

4. RESULTS

Extract	C6H14	CH3COOC2H5	CHCL3	C2H5OH	Water
Root	+	+	++	++	--
Stem	--	+	++	++	+
Leaf	+	++	++	++	++
Aerial Parts	--	++	++	++	++
Fruit	++	++	++	++	++

Soluble ++ Insoluble -- Partially soluble +

Solubility Summary of *Solanum indicum* Extracts in Different Solvents:

The solubility of *Solanum indicum* extracts varies depending on the plant part and the solvent used:

Fruit and aerial parts showed the **highest overall solubility**, being **fully soluble in all solvents except C6H14 (for aerial parts)**.

Leaf extracts were **soluble in all solvents**, making them highly versatile for various extraction processes.

Root and stem extracts exhibited **limited solubility**, with roots being **insoluble in water** and **partially soluble in non-polar solvents** like C6H14 and CH3COOC2H5. Stem extracts were **insoluble in C6H14** and only **partially soluble in water and CH3COOC2H5**.

Among the solvents, **CHCL3 and C2H5OH** proved to be the most effective, yielding **good solubility across all plant parts**.

C6H14 was the **least effective solvent**, showing poor solubility especially for stems and aerial parts.

2. Phytochemical Tests

To identify the presence of bioactive compounds such as alkaloids, flavonoids, tannins, phenolics, saponins, and terpenoids.

Summary of Results Across Extracts

Phytochemicals	C6H14	CH3COOC2H5	CHCL3	C2H5OH	Aqueous
Alkaloids	-	+	++	++	+
Flavonoids	-	++	+	++	++
Tannins	-	+	-	++	++
Phenolics	-	++	+	++	++
Saponins	-	-	-	+	++
Terpenoids	+	++	++	+	-
Glycosides	-	-	+	++	++
Steroids	++	+	+	+	-
Carbohydrates	-	-	-	++	++
Proteins	-	-	-	+	++

The phytochemical screening of *Solanum indicum* using various solvents revealed the following key findings:

C2H5OH and aqueous extracts exhibited the **broadest and strongest presence** of phytochemicals, including **alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, carbohydrates, and proteins**. **CHCL3 extracts** also showed a **rich phytochemical profile**, particularly for **alkaloids, terpenoids, and glycosides**, but were less effective for saponins and tannins. **CH3COOC2H5**extracts demonstrated significant presence of **flavonoids, phenolics, and terpenoids**, suggesting moderate polarity extracts phytochemicals efficiently. **C6H14 extracts** were the **least effective**, with only terpenoids and steroids detected, indicating its limitation in extracting most bioactive constituents. **Terpenoids** were detected in all extracts except aqueous, with CHCL3 and CH3COOC2H5showing strong presence. **Steroids** were most abundant in C6H14, while **saponins, carbohydrates, and proteins** were most abundant in aqueous extracts. Overall, the results suggest that **C2H5OH and water are the most suitable solvents** for extracting a **wide range of bioactive phytochemicals** from *Solanum indicum*.

Results for Standardization Tests and Biological Activities of Solanum indicum Extracts

Standardisation methods and additional tests on **Solanum indicum** extracts (roots, stems, leaves, aerial parts, and fruits).

1. Physicochemical Parameters

Parameter	Root	Leaf	Stem	Aerial parts	Fruits
Moisture Content (%)	9	9	12	13	18
Total Ash (%)	7	6	8	8	5
Water-Soluble Ash (%)	3	4	6	6	3
Water-Soluble Extractive	12	10	15	18	22
Alcohol-Soluble Extractive	8	6	10	12	15
Loss on Drying (%)	8	6	10	12	15

Antimicrobial Activity (Zone of Inhibition in mm) Concentration: 50 microgram/m. Tested against *E. coli*, *S. aureus*, and *Candida albicans*.

Microorganism	C6H14	CH3COOC2H5	CHCL3	C2H5OH	Aqueous
E. coli	9.2	14.5	13.7	16.3	11.4
S. aureus	8.5	13.8	12.5	15.2	10.6
C. albicans	7.8	12.4	11.9	14.8	9.7

The antimicrobial activity of *Solanum indicum* extracts was tested against *E. coli*, *S. aureus*, and *Candida albicans* using a concentration of 50 microgram/mL. The results, presented as the zone of inhibition (in mm), show varying effectiveness across different extracts and microorganisms. **C2H5OH extract** demonstrated the **strongest antimicrobial activity**, producing the largest zones of inhibition against all three microorganisms:*E. coli*: 16.3 mm, *S. aureus*: 15.2 mm, *C. albicans*: 14.8 mm **CH3COOC2H5extract** showed moderate activity, with inhibition zones of:*E. coli*: 14.5 mm, *S. aureus*: 13.8 mm, *C. albicans*: 12.4 mm. **CHCL3 extract** also exhibited considerable activity:*E. coli*: 13.7 mm, *S. aureus*: 12.5 mm, *C. albicans*: 11.9 mm The **aqueous extract** produced smaller inhibition zones: *E. coli*: 11.4 mm, *S. aureus*: 10.6 mm, *C. albicans*: 9.7 mm. **C6H14 extract** had the **lowest activity**, with the smallest inhibition zones: *E. coli*: 9.2 mm, *S. aureus*: 8.5 mm, *C. albicans*: 7.8 mm. The C2H5OH extract was the most effective against all tested microorganisms, followed by the CH3COOC2H5and CHCL3 extracts.

1. Antioxidant Activity Screening

Assay Methods: DPPH radical scavenging,

Extract/Compound	DPPH IC_{50} (microgram/mL)
C6H14	160.5
CH3COOC2H5	45.7
CHCL3	87.6
C2H5OH	33.4
Aqueous	54.2
Solasodine (Isolated)	22.3

Antioxidant Activity of *Solanum indicum* Extracts (DPPH Assay)

The antioxidant potential of *Solanum indicum* extracts and the isolated compound solasodine was evaluated using the DPPH radical scavenging assay, expressed as IC_{50} values (microgram/mL). Lower IC_{50} values indicate stronger antioxidant activity. **Solasodine (isolated compound)** showed the **strongest antioxidant activity** with an IC_{50} of **22.3 microgram/mL**.Among the crude extracts, the **C2H5OH extract** was the most potent (IC_{50} = **33.4 microgram/mL**), followed by **CH3COOC2H5**(IC_{50} = **45.7 microgram/mL**), and **aqueous extract** (IC_{50} = **54.2 microgram/mL**). **CHCL3 extract** exhibited moderate antioxidant activity (IC_{50} = **87.6 microgram/mL**). The **C6H14 extract** showed the **least activity** with a high IC_{50} value of **160.5 microgram/mL**. These results suggest that the C2H5OH and CH3COOC2H5extracts, along with solasodine, are particularly rich in antioxidant compounds.

2. Antimicrobial Screening

Assay Method: Disc diffusion method, Minimum Inhibitory Concentration (MIC) determination

Extract/Compound	Zone of Inhibition (mm)	MIC (microgram/mL)	MIC (microgram/mL)	MIC (microgram/mL)
C6H14	12.5	50	55	48
CH3COOC2H5	15.8	20	25	21
CHCL3	14.2	45	50	43
C2H5OH	18.7	10	12	15
Aqueous	10.3	65	70	60
Solasodine (Isolated)	20.4	5	8	7

Antimicrobial screening of different Solanum indicum extracts was conducted using the disc diffusion method and Minimum Inhibitory Concentration (MIC) determination. Among all extracts, C2H5OH extract of the plant parts exhibited the highest antimicrobial activity with a zone of inhibition of 18.7 mm and low MIC values (10–15 microgram/mL), indicating strong efficacy. The CH3COOC2H5and CHCL3 extracts showed moderate activity with zones of inhibition of 15.8 mm and 14.2 mm, respectively, and MICs ranging from 20–50 microgram/mL. C6H14 extract had lower efficacy (zone of inhibition: 12.5 mm; MIC: 48–55 microgram/mL), while the aqueous extract displayed the weakest antimicrobial effect (zone of inhibition: 10.3 mm; MIC: 60–70 microgram/mL). The isolated compound solasodine demonstrated the highest potency with the largest inhibition zone (20.4 mm) and the lowest MIC values (5–8 microgram/mL), highlighting its potential as a strong antimicrobial agent.

Anthelmintic Activity of *Solanum indicum* Extracts at Various Concentrations

Plant Part	Solvent	Concentration (1%) Paralysis/death (in Minutes)	Concentration (2.5%) Paralysis/death (in Minutes)	Concentration (5%) Paralysis/death (in Minutes)	Albendazole Paralysis/death (in Minutes)
Root	C6H14	20 / 30	18 / 25	15 / 20	10 / 15
	CH3COOC2H5	18 / 28	15 / 22	12 / 18	10 / 15
	CHCL3	22 / 32	20 / 28	16 / 23	10 / 15
	C2H5OH	15 / 25	12 / 20	10 / 15	8 / 12
	Aqueous	30 / 40	28 / 35	25 / 30	12 / 20
Stem	C6H14	22 / 32	20 / 28	17 / 22	10 / 15
	CH3COOC2H5	18 / 28	16 / 24	13 / 18	10 / 15
	CHCL3	24 / 34	22 / 30	19 / 26	10 / 15
	C2H5OH	20 / 30	18 / 26	15 / 22	8 / 12
	Aqueous	32 / 42	30 / 38	27 / 33	12 / 20
Leaf	C6H14	18 / 28	16 / 24	14 / 20	10 / 15
	CH3COOC2H5	14 / 24	12 / 18	10 / 15	8 / 12
	CHCL3	16 / 26	14 / 20	12 / 18	8 / 12
	C2H5OH	12 / 22	10 / 16	8 / 12	6 / 10

	Aqueous	28 / 38	25 / 32	22 / 28	10 / 16
Aerial Part	C6H14	24 / 34	22 / 30	19 / 26	10 / 15
	CH3COOC2H5	20 / 30	18 / 26	15 / 22	10 / 15
	CHCL3	22 / 32	20 / 28	17 / 23	10 / 15
	C2H5OH	18 / 28	15 / 22	12 / 18	8 / 12
	Aqueous	30 / 40	28 / 35	25 / 30	12 / 20
Fruit	C6H14	20 / 30	18 / 25	15 / 20	10 / 15
	CH3COOC2H5	16 / 26	14 / 20	12 / 18	8 / 12
	CHCL3	18 / 28	16 / 22	14 / 20	8 / 12
	C2H5OH	14 / 24	12 / 18	10 / 16	6 / 10
	Aqueous	25 / 35	23 / 30	20 / 26	10 / 16

The anthelmintic activity of *Solanum indicum* extracts was evaluated using different solvents (C6H14, CH3COOC2H5, CHCL3, C2H5OH, and Aqueous) at three concentrations (1%, 2.5%, and 5%). The time required for paralysis and death of earthworms (*Pheretima posthuma*) was noted, with the results compared to the reference drug, albendazole. **C2H5OH Extract** showed the most significant anthelmintic activity across all plant parts, with the shortest times for paralysis and death of earthworms. The 5% concentration was the most effective, with paralysis occurring within 8 to 12 minutes, and death within 12 to 18 minutes, depending on the plant part. **CH3COOC2H5Extracts** also demonstrated good activity, with similar results to C2H5OH, though the times for paralysis and death were slightly longer. The 5% concentration was the most effective, with paralysis observed within 12 to 18 minutes and death within 16 to 22 minutes. **CHCL3 Extracts** exhibited moderate anthelmintic activity, with times for paralysis and death being longer compared to C2H5OH and CH3COOC2H5extracts. The 5% concentration generally showed paralysis within 14 to 20 minutes and death within 18 to 26 minutes. **C6H14 Extracts** displayed weaker anthelmintic effects, with paralysis times ranging from 15 to 22 minutes and death times from 20 to 30 minutes at the highest concentration (5%). **Aqueous Extracts** exhibited the least anthelmintic activity. The 5% concentration resulted in paralysis occurring within 25 to 35 minutes and death occurring within 30 to 40 minutes. Overall, **C2H5OH** and **CH3COOC2H5extracts** from the various plant parts of *Solanum indicum* showed the most potent anthelmintic effects, making them suitable candidates for further pharmacological studies in the development of anthelmintic agents.

5. SUMMARY AND DISCUSSION

This study evaluated the standardization parameters and biological activities of Solanum indicum extracts prepared using C6H14, CH3COOC2H5, CHCL3, C2H5OH, and aqueous solvents. Among these, extracts obtained with C2H5OH, CH3COOC2H5, CHCL3, and water demonstrated superior bioactivity and quality characteristics. Key physicochemical assessments included moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractives, and loss on drying. Chromatographic profiling identified these solvents as efficient in extracting major phytochemicals. The extracts exhibited notable antioxidant, antimicrobial, and cytotoxic activities against Escherichia coli, Staphylococcus aureus, and

Candida albicans, with the most effective extract showing low moisture content and high stability. Pharmacological evaluations included DPPH radical scavenging, ABTS assay, FRAP, disc diffusion, MIC determination, cytotoxicity, anti-inflammatory, antidiabetic, and antiviral tests. These findings underscore the potential of Solanum indicum extracts for diverse therapeutic applications.

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