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# Discovery of Potential Plant-Based Anti-HCV Agents: Anti-Replicative Activity of Solanum surattense and Its Bioactive Flavonoids Against HCV-3a

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## ABSTRACT

Hepatitis C virus (HCV) infection remains a global health burden, with genotype 3a (HCV-3a) being highly prevalent in South Asia and associated with accelerated disease progression and reduced response to certain direct-acting antivirals (DAAs). Resistance-associated substitutions (RASs) continue to limit therapeutic efficacy, emphasizing the need for novel, cost-effective antiviral agents. In this study, we evaluated the anti-HCV potential of Solanum surattense leaves and flowers against HCV-3a replication in Huh-7 hepatoma cells. Methanolic extracts were prepared, and cytotoxicity was assessed via MTT assay, revealing cell viability >90% up to 105 µg/mL for both extracts. Huh-7 cells were transfected with full-length HCV-3a and NS3-expressing plasmids and treated with extracts at 52.5 and 105 µg/mL. Antiviral activity was quantified using qRT-PCR and NS3 protein expression analysis. Leaf extract demonstrated robust, dose-dependent inhibition of HCV-3a replication, achieving 65.1% and 88.2% inhibition at 52.5 µg/mL and 105 µg/mL, respectively, while flower extract exhibited moderate inhibition (42.7% and 57.9%). NS3 expression was similarly suppressed by leaf extract (61.4–85.7%). Notably, the antiviral efficacy of leaf extract at higher concentrations was comparable to daclatasvir (73.5% inhibition). These findings suggest that S. surattense leaves harbor bioactive flavonoids and related phytochemicals capable of targeting multiple stages of HCV replication without significant cytotoxicity.

## INTRODUCTION

Hepatitis C virus (HCV) infection remains a major global health challenge, affecting approximately 71 million individuals worldwide and contributing substantially to chronic liver disease (CLD), cirrhosis, and hepatocellular carcinoma (Alberts et al., 2022; Sallam & Khalil, 2024; Veracruz et al., 2022; Pham et al., 2024). Despite the availability of direct-acting antivirals (DAAs), which have dramatically improved sustained virologic response rates, treatment failures continue to occur, primarily due to the emergence of resistance-associated substitutions (RASs) within the viral genome (Izhari, 2023; Popping et al., 2021; Dietz et al., 2023; Khalil et al., 2024). These limitations underscore the urgent need for novel antiviral agents that are both effective and affordable, particularly in resource-limited regions where HCV prevalence is highest (Said & El-Sayed, 2022; Toma et al., 2025; Tabll et al., 2023).

Natural products have historically served as a prolific source of therapeutic agents, with plant-derived bioactive compounds demonstrating broad-spectrum antiviral activities (Thomas et al., 2021; Venkataraman, 2022). Secondary metabolites, such as flavonoids, alkaloids, and saponins, are known to interfere with viral replication, inhibit critical viral enzymes, and modulate host antiviral pathways (Venkataraman, 2022; Dhamija & Mangla, 2022; Adeosun & Loots, 2024). Ethnomedicinal plants have therefore emerged as promising candidates for the development of complementary or alternative antiviral therapies (Saifulazmi et al., 2022; Alhazmi et al., 2021). Among these, Solanum surattense, a plant traditionally utilized in South Asian medicine for hepatoprotective and anti-inflammatory purposes, has attracted attention for its potential antiviral properties (Hasan et al., 2024). Nevertheless, systematic investigations evaluating its efficacy against HCV, particularly genotype 3a (HCV-3a), remain limited.

HCV-3a is highly prevalent in South Asia and is associated with accelerated disease progression and lower response rates to certain DAAs compared with genotype 1 (Chuaypen et al., 2022). Genotype-specific therapeutic challenges necessitate the identification of novel compounds capable of targeting HCV-3a replication (Mandal & Hazra, 2023). Plant-derived agents that inhibit essential viral proteins, such as the NS3 protease, which

plays a central role in polyprotein processing and replication complex formation, may effectively suppress viral propagation while minimizing cytotoxic effects on hepatocytes (Gabbianelli et al., 2023). Such interventions could offer a cost-effective strategy to address the increasing incidence of antiviral resistance and improve treatment accessibility in under-resourced regions.

Recent studies have highlighted the antiviral potential of bioactive flavonoids and related phytochemicals against HCV, demonstrating inhibition of viral entry, replication, and protein expression (Bachar et al., 2021). Given its reported hepatoprotective and antioxidant properties, S. surattense may provide dual benefits by both inhibiting HCV replication and mitigating virus-induced oxidative stress. Additionally, the differential distribution of bioactive compounds in various plant tissues suggests that specific plant parts, such as leaves or flowers, may exhibit varying degrees of antiviral potency, warranting systematic comparative analyses (Denaro et al., 2020; Behl et al., 2021).

In this context, the present study aims to investigate the anti-HCV potential of S. surattense leaves and flowers, focusing on their effects against HCV-3a replication and NS3 protein expression in Huh-7 hepatoma cells. By assessing dose-dependent antiviral activity alongside cytotoxicity, this work seeks to identify plant-derived candidates with favorable therapeutic indices for further development as novel anti-HCV agents. This approach integrates traditional ethnomedicinal knowledge with contemporary molecular virology, providing a translational framework for the discovery of effective, low-cost, plant-based therapeutics. Ultimately, elucidating the antiviral properties of S. surattense could contribute to the development of alternative or adjunctive therapies for HCV, addressing both the challenges of drug resistance and global disparities in treatment access.

## METHODOLOGY

The primary in-vitro model of an Hepatitis C virus (HCV) infection was human hepatoma (Huh-7) cells. Cells were cultured in Dulbecco Modified Eagle Media (DMEM; Gibco) with one-tenth heat-inactivated fetal bovine serum (FBS), 10 percent penicillin-streptomycin, and 10 percent L-glutamine. Cultures were incubated at 37 C with 5% CO 2, in the humid environment. All assays were done in cells at 70-80 percent confluence in order to have maximum viral replication capacity.

Authenticated botanical sources were used to collect the fresh leaves and flowers of Solanum surattense and wash the leaves and flowers to remove debris. A standard solvent-extraction protocol was used to shade-dry samples, power them, and extract them. Concisely, 100 g of dry vegetation was crushed in 70% methanol over 72 h with the constant shaking. Rotary evaporator was used to concentrate the filtrate under low pressure and dried further to produce crude extracts. Stock solutions were used in DMSO (dimethyl sulfoxide) and diluted in culture medium to get nontoxic working concentrations (<0.1% DMSO).

Cytotoxicity of all the extracts was tested using MTT assay to determine safe working doses. Seeded Huh-7 cells of 96-well plates (1 × 10 4 cells/well) were subjected to serial doses of leaf and flower extracts of 13.12 up to 210 µg/mL at the end of 48 h. The spectrophotometric measures of cell viability were taken at 570 nm. The concentrations with 90% or more cell viability were chosen to determine antiviral effects.

Two molecular vectors were used, which include a complete genome HCV genotype 3a (HCV-3a) and a NS3-expressing plasmid. Transfection transpired before purifying the plasmids under endotoxin-free kits. Lipofectamine 3000 was used to transfect Huh-7 cells according to the protocols of the producer. Incubation was stopped after 6 h, and media were substituted with new complete media with the predetermined nontoxic concentrations of S. surattense extracts in.

In order to determine the inhibitory effect of S. surattense extracts, HCV constructs-transfected cells were subjected to leaf extract, 52.5 µg/mL, and flower extract, 105 µg/mL. Daclatasvir (105 nM) is a DAA that has been clinically approved and was used as the positive control. At the end of 48 h of treatment, a total RNA was extracted with the TRIzol reagent. An HCV replication was quantified by qRT-PCR of the 5'UTR region with SYBR Green chemistry. The percentage of viral inhibition was determined as compared to untreated controls.

Each experiment was performed 3 times. Findings were given in mean (SD). One-way ANOVA was determined to measure statistical significance, after which a post-hoc test was carried out (Tukey), where p =.05 to indicate statistical significance.

## RESULTS

An evaluation of cytotoxicity ensured the well-tolerance of the leaf and flower extracts of S. surattense to up to 105 µg/mL and cell viability of over 90 percent in Huh-7 cells (Table 1). Significant cytotoxic effects were not detected at these concentrations, which were used in antiviral assays, and this showed a favorable therapeutic window.

**Table 1**

*Cytotoxicity of S. surattense Extracts in Huh-7 Cells (MTT Assay)*

| Extract Type | Concentration (µg/mL) | Cell Viability (%) ± SD |
|---|---|---|
| **Leaf** | 26.25 | 98.2 ± 1.1 |
| **Leaf** | 52.5 | 96.5 ± 1.4 |
| **Leaf** | 105 | 91.8 ± 1.7 |
| **Flower** | 26.25 | 97.5 ± 1.3 |
| **Flower** | 52.5 | 94.7 ± 2.0 |
| **Flower** | 105 | 90.5 ± 2.3 |

### Antiviral Activity Against Full-Length HCV-3a

The efficacy of the leaf extract in treatment showed potent dose-dependent inhibition of HCV-3a virus replication. Viruses replicated at 52.5 ug/mL were inhibited 65.1 per cent, viruses at 105 ug/mL were inhibited 88.2 per cent (Table 2). Comparatively, daclatasvir (105 nM) lowered HCV replication by 73.5 percent. The antiviral activity of flower extract was modest with an inhibitory activity of 42.7% and 57.9% at 52.5 µg/mL and 105 µg/mL respectively.

**Table 2**

*Inhibition of HCV-3a Replication by S. surattense Extracts in Huh-7 Cells*

| Treatment | Concentration | HCV Replication (%) ± SD | % Inhibition vs Control |
|---|---|---|---|
| Leaf Extract | 52.5 µg/mL | 34.9 ± 2.1 | 65.1 |
| Leaf Extract | 105 µg/mL | 11.8 ± 1.4 | 88.2 |
| Flower Extract | 52.5 µg/mL | 57.3 ± 2.5 | 42.7 |
| Flower Extract | 105 µg/mL | 42.1 ± 1.9 | 57.9 |
| Daclatasvir | 105 nM | 26.5 ± 1.7 | 73.5 |
| Control (untreated) | — | 100 ± 0 | — |

### Antiviral Activity Against HCV-NS3 Plasmid

HCV-NS3 protein expression was also greatly suppressed with leaf extract having 61.4 percent and 85.7 percent inhibition at 52.5 µg/mL and 105 µg/mL, respectively (Table 3). Extracts of flowers were again observed as moderate. Such findings indicate that leaf parts of S. surattense attack different HCV replication periods, which is in line with their high antiviral activity.

**Table 3**

*Inhibition of HCV-NS3 Expression by S. surattense Extracts*

| Treatment | Concentration | NS3 Expression (%) ± SD | % Inhibition vs Control |
|---|---|---|---|
| Leaf Extract | 52.5 µg/mL | 38.6 ± 2.0 | 61.4 |
| Leaf Extract | 105 µg/mL | 14.3 ± 1.2 | 85.7 |
| Flower Extract | 52.5 µg/mL | 60.8 ± 2.3 | 39.2 |
| Flower Extract | 105 µg/mL | 41.9 ± 1.8 | 58.1 |
| Daclatasvir | 105 nM | 27.2 ± 1.5 | 72.8 |
| Control (untreated) | — | 100 ± 0 | — |

In general, at least S. surattense leaf extract has been shown to inhibit HCV-3a replication and NS3 expression in a dose-dependent manner and was superior to flower extract and matched the antiviral activity of daclatasvir at higher concentrations. These results suggest that phytochemicals extracted out of leaves can be valuable precursors in the design of new anti-HCV products.

## DISCUSSION

The current paper shows that leaf extract of Solanum surattense has powerful inhibitory activities in the replication of HCV-3a virus and the expression of NS3 protein in the Huh-7 hepatoma cell line, which clearly indicates that the extract has a potential development as a novel antiviral agent. The dose-dependent decrease in viral replication including a 65.1 percent and an 88.2 percent inhibition rate at 52.5 µg / mL and 105 µg / mL respectively supports the effectiveness of the leaf-derived phytochemicals. Interestingly, these effects were similar with the clinically approved direct-acting antiviral daclatasvir, which indicated that the bioactive constituents of S. surattense could be associated with various stages of the HCV life cycle. Flower extracts (moderation), exhibited less antiviral activity, and the antiviral activity is presumably concentrated in the leaves, which may have more or less of particular secondary metabolites (also alkaloids, flavonoids, and saponins) which inhibit viral replication.

Such results are especially important in light of the increasing resistance to the currently used DAAs, largely due to its resistance-associated substitutions (RASs). The S. surattense leaf extract has the potential to inhibit the replication of HCV even at non-cytotoxic doses, and this capacity is important in supporting the further development of the extract as an alternative or complementary therapy--a concept especially open to consideration in resource-limited areas that are desperately in need of cost-effective therapies. Mechanistically, the NS3 inhibition, which is a vital viral protease that is linked to polyprotein processing and replication complex formation inhibits, implies that the extract can interfere with vital enzymatic activities of the HCV, hence limiting viral replication. To clarify the therapeutic potential and molecular targets of these phytochemicals, future research on bioassay-based fractionation, active compounds discovery, and in vivo effect determination of these phytochemicals will be required.

Altogether, the current work forecasts S. surattense as a candidate agent of anti-HCV drug development, which can provide innovative plant-based intervention against chronic liver disease. Combined with low cytotoxicity the strong antiviral effect makes leaf extract an excellent lead to develop new therapeutics effective and affordable in the face of a global emergency in treating HCV.

## CONCLUSION

The present research defines Solanum surattense leaf extract as a beneficial inhibitor of HCV-3a replication and the expression of NS3 protein in Huh-7 cells and exhibits dose-dependent antiviral activity to daclatasvir, which is an approved therapeutic agent. The high leaf activity compared to those of flowers suggests that individual bioactive, phytochemicals, especially flavonoids, can mediate these effects by disrupting critical viral, enzyme activities and replication cycles. Notably, the concentrations needed to be effective were not cytotoxic, which indicates a positive therapeutic index. The data substantiates the possibility of S. surattense as an alternative or a supplement to the existing DAAs as the new, plant-based anti-HCV agent. To convert this ethnomedicinal plant into a clinically relevant antiviral treatment, further research, such as bioassay-guided fractionation, characterizing of compounds, and in vivo confirmation, is necessary to enable its use as a primary antiviral treatment in resource-limited areas with high HCV prevalence and antiviral resistance increases

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