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Original Article

Evaluation the Effect of the Ethanolic Extract of *Satureja sahandica* by Montmorillonite Nanoparticles on SARS-CoV2 in Cell Culture by RT-PCR

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ABSTRACT

Background and Aim: The COVID-19 pandemic has highlighted the urgent need for effective antiviral therapies. *Satureja sahandica*, a medicinal herb, is known for its antimicrobial properties and potential antiviral effects. Additionally, montmorillonite nanoparticles can enhance the bioavailability and efficacy of plant extracts. This study aims to evaluate the combined effect of ethanolic extract of *Satureja sahandica* and montmorillonite nanoparticles on SARS-CoV-2 in cell cultures, utilizing Real-Time PCR (RT-PCR) to assess their antiviral activity. **Materials and Methods:** RNA extraction was performed, and samples with low Cycle Threshold (CT) values were used to infect the Vero cell line. Infected cells were subsequently treated with various concentrations of the ethanolic extract *Satureja sahandica*, and cell viability was assessed using the MTT assay. To evaluate the impact of *Satureja sahandica* on the COVID-19 virus, gene expression levels were analyzed through RT- PCR. **Results:** The results obtained from the MTT test indicate that the *Satureja sahandica* extract at a concentration of 1019 µg/mL performed better, with a cell viability percentage of 50%. The results indicated that the average CTs in all untreated cells infected with Covid-19 were 27.81, which after treatment with Montmorillonite nanoparticles and *Satureja sahandica*, the average CT increased to 32.07 ($p<0.0001$). **Conclusion:** The findings of this study suggest that the ethanolic extract of *Satureja sahandica*, particularly when combined with montmorillonite nanoparticles, may offer a promising therapeutic approach against COVID-19. This research highlights the potential of plant-based extracts in antiviral treatment strategies, warranting further investigation into their mechanisms of action and efficacy in clinical settings.

Keywords: Corona Virus, *Satureja sahandica*, Montmorillonite Nanoparticle.

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In this study, after performing Real-time PCR, untreated cells infected with Covid-19 whose CT was less than 25 were placed in one group (Severe) and untreated cells infected with Covid-19 whose CT was higher than 30 were placed in another group (Mild). CT changes in each group were evaluated after treatment with Montmorillonite nanoparticles and *Satureja sahandica*. The mean CT of untreated cells infected with Covid-19 in the Mild group was 32.03, which after treatment with Montmorillonite nanoparticles and *Satureja sahandica*, the mean CT increased to 34.19 ($P<0.0032$). The mean CT of untreated cells infected with Covid-19 in the sever group was 23.39, which after treatment with Montmorillonite nanoparticles and *Satureja sahandica*, the mean CT increased to 29. 94 ($P<0.0032$). The average CTs in all untreated cells infected with Covid-19 were 27.81, which after treatment with Montmorillonite nanoparticles and *Satureja sahandica*, the average CT increased to 32.07 ($P<0.0001$).

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INTRODUCTION

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has posed unprecedented challenges to global health systems. The rapid spread of the virus has highlighted the urgent need for effective antiviral therapies (Zabidi *et al*., 2023). While vaccines have been developed and deployed, the search for antiviral agents remains critical, especially for treating infected individuals and preventing severe disease outcomes (Cheung *et al*., 2023). Natural products, particularly those derived from medicinal plants, have gained attention for their potential therapeutic properties (Dzobo *et al*., 2022). Among these, *Satureja sahandica*, herb known for its antimicrobial and antioxidant effects, has emerged as a candidate for further investigation (Ghasemi *et al*., 2025). *Satureja sahandica*, commonly known as Sahand savory, is a perennial herb belonging to the *Lamiaceae* family (Rezaei *et al*., 2023). It is native to the mountainous regions of Iran and has been traditionally used in folk medicine for various ailments, including respiratory infections, digestive disorders, and inflammatory conditions (Buso *et al*., 2020). Recent studies have indicated that *Satureja sahandica* possesses significant antimicrobial activity against a range of pathogens, including bacteria and fungi (Sharchi *et al*., 2020). The phytochemical composition of this herb includes flavonoids, phenolic acids, and essential oils, which are believed to contribute to its therapeutic effects (Sun *et al*., 2023). The incorporation of nanotechnology in medicine has revolutionized drug delivery systems and therapeutic approaches (Malik *et al*., 2023). Montmorillonite nanoparticles, a type of layered silicate mineral, have shown promise in enhancing the bioavailability and efficacy of various compounds (Park *et al*., 2016). Their unique properties, including high surface area, biocompatibility, and ability to encapsulate bioactive molecules, make them suitable carriers for plant extracts (Karnwal *et al*., 2024). Montmorillonite nanoparticles exhibit significant antibacterial effects due to their unique properties. They interact with bacterial cell membranes, causing structural damage and cell lysis (Arora *et al*., 2020). Additionally, these nanoparticles can release metal ions like silver or copper, enhancing their antibacterial activity. They generate reactive oxygen species (ROS) that damage bacterial components, and disrupt biofilms, making bacteria more susceptible to treatment (Franco *et al*., 2022). Applications include wound healing, where they prevent infections, and coatings for medical devices to reduce infection risks (Deng *et al*., 2022). Their potential in agriculture and food preservation further highlights their versatility as effective antibacterial agents in various fields (Vaou *et al*., 2021). By combining *Satureja sahandica* extract with montmorillonite nanoparticles, it is hypothesized that the antiviral activity against SARS-CoV-2 can be significantly enhanced. This study aims to evaluate the combined effect of ethanolic extract of *Satureja sahandica* and montmorillonite nanoparticles on SARS-CoV-2 in cell cultures, utilizing Real-Time PCR (RT-PCR) to assess their antiviral activity.

MATERIALS AND METHODS

Collection and Extraction of Ethanolic Extract of *Satureja sahandica*

In this phase, the aerial parts of *Satureja sahandica* are collected and dried in the shade. The dried plant is then ground into a fine powder, and ethanolic extract is obtained using a Soxhlet extractor. The resulting extract is concentrated under reduced pressure and stored at -20° C

Montmorillonite Nanoparticles

The nanoparticles were received as a generous gift from Dr. Arjomandi, associate Professor of Azad Islamic republic of Iran branch Bonab.

Sample Preparation of SARS-CoV-2

The SARS-CoV-2 virus samples were obtained from COVID-19 patients visiting Sina Hospital in Maragheh. Upon patient admission, nasopharyngeal swabs were collected following standard clinical protocols to ensure the integrity and viability of the samples. The swabs were placed in Viral Transport Medium (VTM) to preserve the viral RNA and prevent degradation. Samples were labeled with patient identifiers and collected in a sterile environment to minimize contamination. After collection, the samples were transported to the laboratory under cold chain conditions to maintain their viability. In the laboratory, viral RNA was extracted from the samples using a commercial RNA extraction kit, following the manufacturer's instructions. The extracted RNA was then used for further analysis, including RT-PCR, to confirm the presence of SARS-CoV-2 and assess its characteristics for research purposes.

Cell Culture and Viral Infection

Vero cells, derived from the kidney of an African green monkey, are cultured in RPMI1640 supplemented with 10% Fetal Bovine Serum (FBS) and antibiotics. Once the cells reach 80-90% confluency, they are infected with SARS-CoV-2 at a specified Multiplicity of Infection (MOI), determined from preliminary titration studies. After a 2 hr incubation at 37°C with 5% CO2 to facilitate viral entry, the viral inoculum is removed, and the cells are washed with Phosphate-Buffered Saline (PBS). Fresh growth medium is added, and the cells are treated with varying concentrations of ethanolic extract of *Satureja sahandica*, montmorillonite nanoparticles, and their combination. The cells are incubated for 24 to 72 hr, monitored for Cytopathic Effects (CPE), and supernatants are collected for analysis. This approach allows for evaluating the antiviral effects of the treatments against SARS-CoV-2 in a controlled environment.

Evaluation of Antiviral Activity

The antiviral activity is assessed by measuring the level of viral RNA in the cell culture supernatants using RT-PCR. The expression of viral genes is measured, and the efficacy of the treatments is compared with control groups. RNA is extracted from cells infected with the SARS-CoV2 virus using RNA extraction kits. The extracted RNA is then converted into cDNA using reverse transcriptase and specific primers. The produced cDNA serves as a template for PCR, where specific primers for SARS-CoV2 are selected. The PCR reaction includes the following stages: denaturation temperatures, where the temperature rises to 95°C; annealing temperatures, where the temperature decreases to about 50-65°C; and extension temperatures, where the temperature increases to about 72 °C. The PCR products are analyzed using electrophoresis on an agarose gel to check for the presence and quantity of viral RNA in the samples. The results obtained from electrophoresis are analyzed to determine the effect of ethanolic extract and nanoparticles on SARS-CoV2.

MTT assay

The MTT assay is a colorimetric method used to evaluate cell viability. In this process, Vero cells are cultured in 96-well plates and treated with varying concentrations of the desired compounds for 48 hr at 37 ºC. After treatment, MTT solution is added to each well, leading to the production of formazan color upon incubation. Following this, the formazan is dissolved in DMSO, and the color intensity is measured at a wavelength of 570 nm. This absorbance is proportional to the number of viable cells and is used to assess cytotoxic effects and antiviral activity.

Data Analysis

In the present study, data were analyzed at two levels: descriptive and inferential statistics. At the descriptive statistics level, indicators such as mean, median, and standard deviation were used. At the inferential statistics level, the independent t-test method was employed using SPSS v.18 software. GraphPad Prism v.8.1.0 software was used to draw graphs.

RESULTS

Cell viability

To investigate the cytotoxic effect of the *Satureja sahandica* extract and determine the appropriate concentration for the MTT assay, the results obtained from the MTT test indicate that the *Satureja sahandica* extract at a concentration of 1019 µg/mL performed better, with a cell viability percentage of 50%. This suggests a more effective impact of this concentration on the coronavirus. Based on the results of the figure, as the concentration increases, the percentage of cell survival also increases (Figure 1).

Real-Time PCR Reaction

To investigate the effect of *Satureja sahandica* on COVID-19, the expression levels of the RdRp and N genes were examined using the Real-Time PCR method in samples treated with *Satureja sahandica* and control samples. RNase P was used as internal control gene. Amplification Curve of RdRp, N and RNase P were shown in Figure 2.

In this study, after performing Real-time PCR, untreated cells infected with Covid-19 whose CT was less than 25 were placed in one group (Severe) and untreated cells infected with Covid-19 whose CT was higher than 30 were placed in another group (Mild). CT changes in each group were evaluated after treatment with Montmorillonite nanoparticles and *Satureja sahandica*. The mean CT of untreated cells infected with Covid-19 in the Mild group was 32.03, which after treatment with Montmorillonite nanoparticles and *Satureja sahandica*, the mean CT increased to 34.19 ($P<0.0032$). The mean CT of untreated cells infected with Covid-19 in the sever group was 23.39, which after treatment with Montmorillonite nanoparticles and *Satureja sahandica*, the mean CT increased to 29. 94 ($P<0.0032$). The average CTs in all untreated cells infected with Covid-19 were 27.81, which after treatment with Montmorillonite nanoparticles and *Satureja sahandica*, the average CT increased to 32.07 ($P<0.0001$).

Figure 3 shows the comparison of CTs in untreated cells infected with COVID-19 and samples treated with Montmorillonite nanoparticles and *Satureja sahandica* in the Mild, Severe and Average groups. Table 1 shows the details of the comparison of CTs in different groups.

DISCUSSION

The investigation of the research hypotheses indicated that the ethanolic extract of *Satureja sahandica*, combined with montmorillonite nanoparticles, has a significant effect on COVID-19 patients, as assessed by Real-time PCR. The effectiveness of *Satureja sahandica*, in conjunction with montmorillonite, in treating COVID-19 can be attributed to the

Table 1: Details of comparison of CTs in different groups.

Unpaired t test	Mild (CT normal>30)	Sever (CT normal<25)	Average
P value	0.0032	<0.0001	<0.0001
P value summary	**	****	****
Significantly different ($P < 0.05$)?	Yes	Yes	Yes
One- or two-tailed *P* value?	Two-tailed	Two-tailed	Two-tailed
t, d*f*	t=3.172, df=34	t=8.935, df=34	t=4.390, df=70
Mean of column Untreated	32.03	23.39	27.81
Mean of column Treated	34.19	29.94	32.07
Difference between means (Treated - Untreated) ± SEM	2.167 ± 0.6831	6.556 ± 0.7337	4.264 ± 0.9713
95% confidence interval	0.7784 to 3.555	0.7784 to 3.555	2.327 to 6.201
R squared (eta squared)	0.2283	0.2283	0.2159

discovery of antimicrobial, antiviral, and antitumor substances in plants. This resurgence of interest in natural herbal medicines is driven by the side effects and drug resistance associated with chemical medications (Wang *et al.*, 2023). Traditional medicine, particularly Iranian traditional medicine, offers a rich source of medicinal plants with a long history of use for treating various diseases (Buso *et al.*, 2020).

Medicinal plants contain active compounds that maintain a biological equilibrium due to their continuous interaction with other substances, resulting in fewer adverse effects compared to synthetic drugs (El-Saadony *et al.*, 2025). The compounds in these plants exhibit various beneficial effects, including carminative, muscle relaxant, liver protective, anticancer, and antiviral properties (Arulnangai *et al.*, 2025). The *Satureja sahandica*, belonging to the Lamiaceae family, comprises around 200 species and has shown considerable potential in treating infections (Ejaz *et al.*, 2023). Globally, millions have been infected with COVID-19, with a mortality rate of approximately 2.3% among hospitalized patients (Abate *et al.*, 2021). This widespread prevalence underscores the need for effective therapeutic methods to enhance public health (Religioni *et al.*, 2025). Maintaining immune health and identifying immune-boosting treatments are crucial. Unfortunately, no significant advancements have been made in managing COVID-19, with treatments primarily focused on observable symptoms (Monye *et al.*, 2020). While drugs like Remdesivir, Favipiravir, and Chloroquine have been tested, their clinical efficacy has been limited, with toxicity leading to serious side effects (Latarissa *et al.*, 2025). The emergence of viral resistance to current antiviral agents further emphasizes the need for new effective compounds. Consequently, researchers are increasingly turning to medicinal plants recognized for their antiviral properties (Owen *et al.*, 2022). Natural products have historically served as vital sources for drug research and development, particularly for infectious diseases. Many chemical compounds isolated from medicinal plants exhibit antiviral activity (Ahmad *et al.*, 2025). Essential oils from certain Satureja species demonstrate a wide range of biological activities, including antibacterial, antifungal, and antiviral effects (Saghabashi *et al.*, 2025). This study investigated the antiviral activity of the ethanolic extract of *Satureja sahandica* on the coronavirus in 40 samples, both treated and untreated with the extract. The cytotoxicity of the extract was assessed using the MTT method at various concentrations, revealing that the extract caused dose-dependent death of the coronavirus, with 1 mg/mL identified as the effective dose. Following the selection of the effective dose, the expression levels of the RDRP and N genes were examined in treated samples compared to controls. Results showed a significant decrease in viral gene expression in samples treated with the ethanolic extract, indicating its antiviral properties. The findings are consistent with studies demonstrating the antiviral effects of *Satureja sahandica* extract on COVID-19, highlighting its potential to modulate the microbiota and serve as a dietary supplement to enhance immune response (Leka *et al.*, 2022). Additionally, BAŞOĞLU *et al.* reported that L. Satureja acts as a protease inhibitor in COVID-19, significantly reducing the activity of the SARS-CoV-2 protease enzyme (BAŞOĞLU *et al.*, 2022). The present study's MTT test confirmed that the *Satureja sahandica* extract caused the death of the COVID-19 virus, likely through its inhibitory effects on the virus's protease enzyme. Other studies, have identified various compounds with potential therapeutic effects against COVID-19 (Low *et al.*, 2023). Researchers demonstrated that herbal extracts, including Satureja, improved recovery speed and reduced hospitalization time in COVID-19 patients (Demeke *et al.*, 2021). Researcher explored

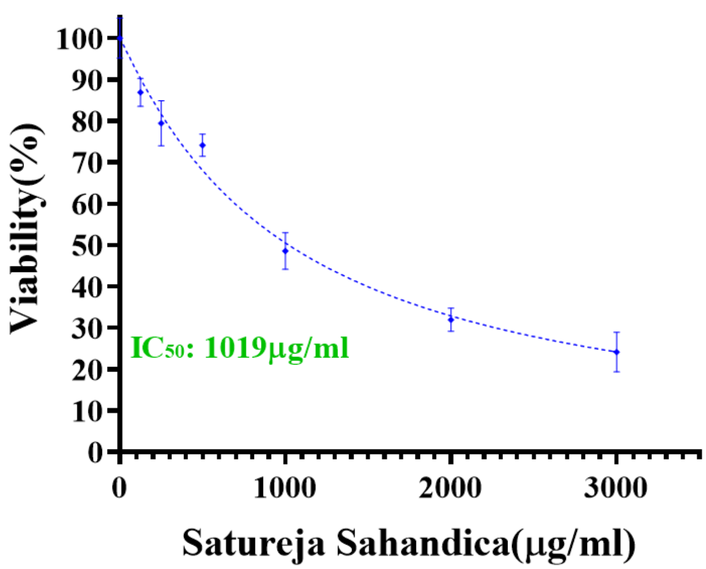
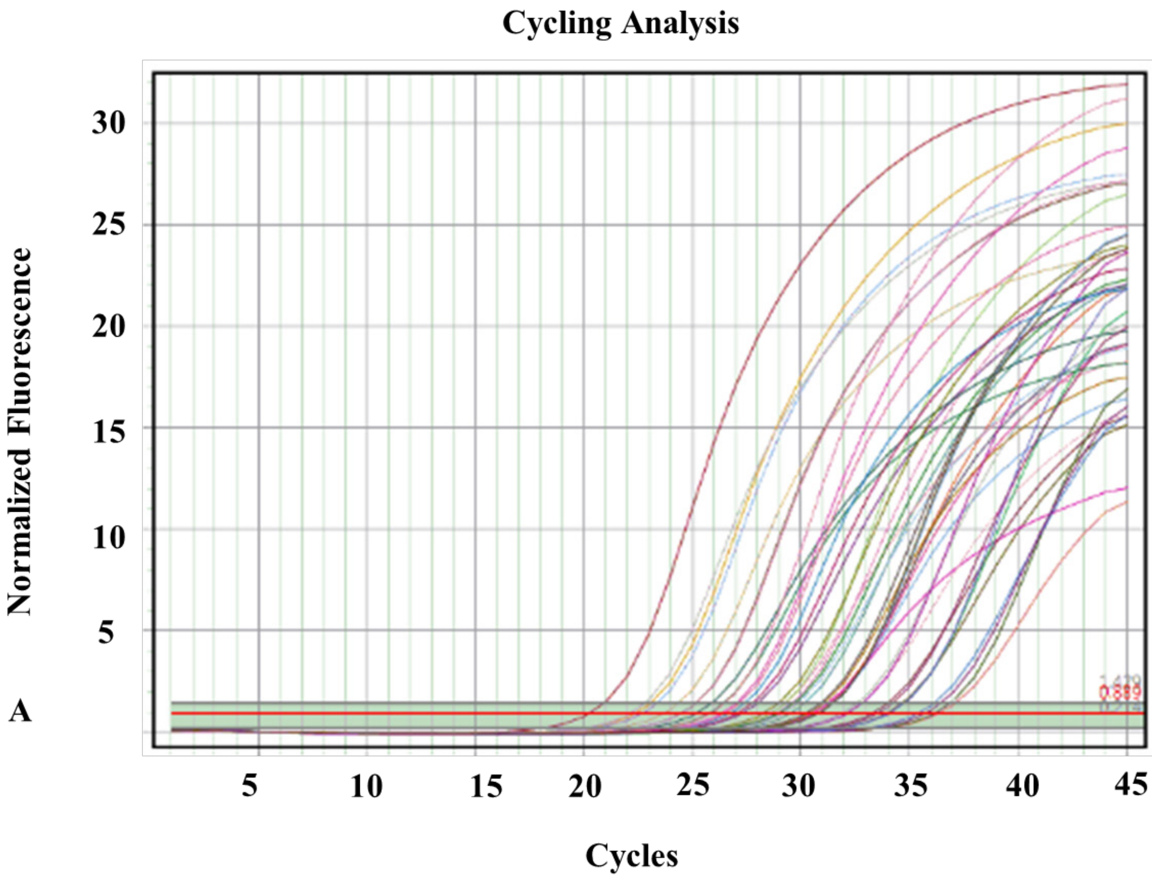


Figure 1: Cell Viability of Vero cells after *Satureja sahandica* treatment.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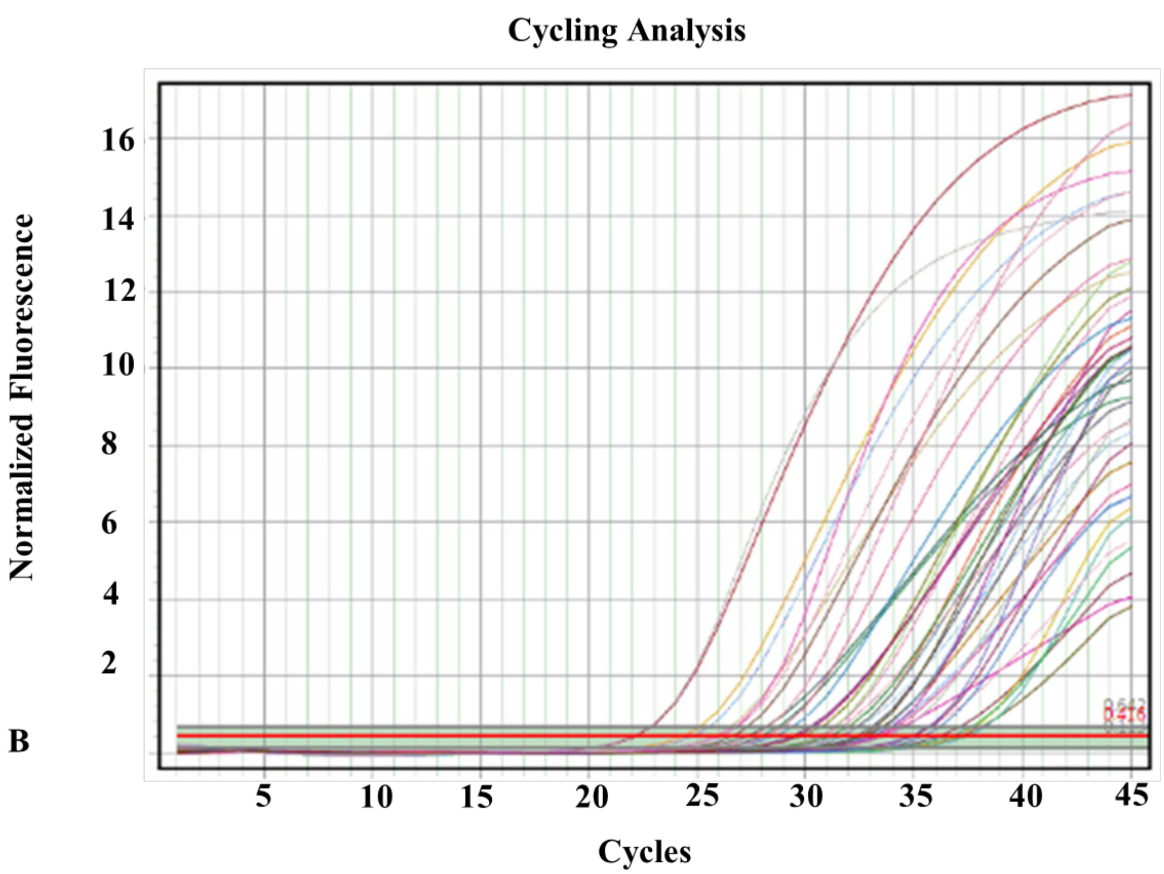
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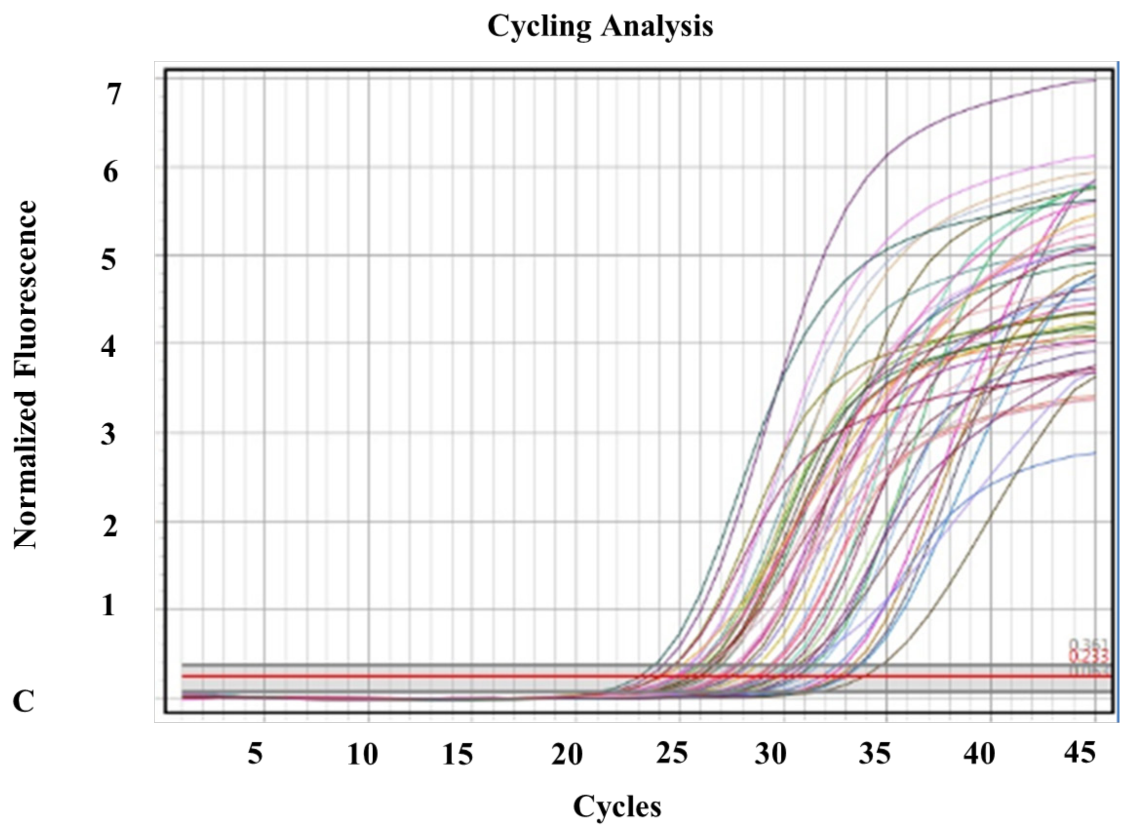
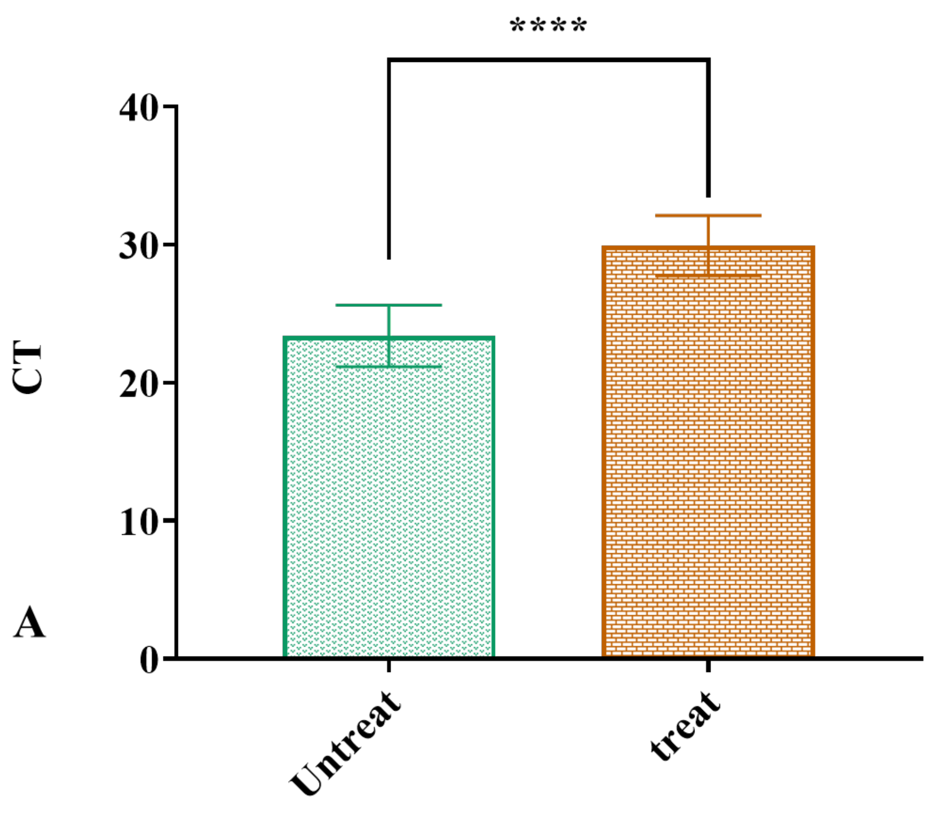
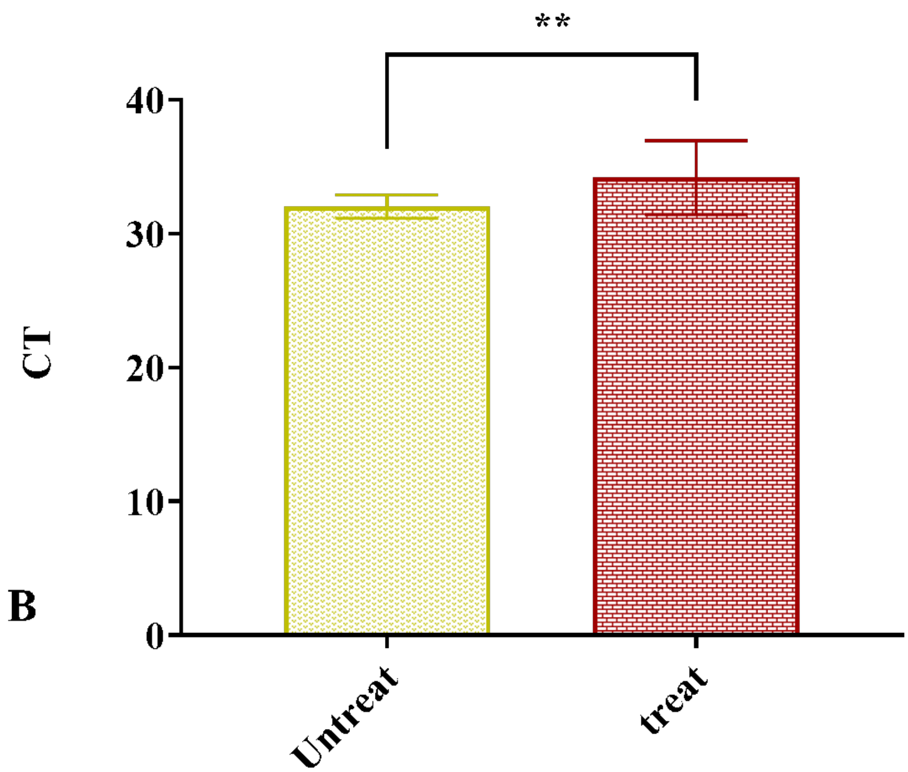


Figure 2: Amplification Curve of RdRp gene of SARS-CoV2 (A), N gene of SARS-CoV2 (B) and RNase P as a internal control gene (C) in Real-Time PCR.





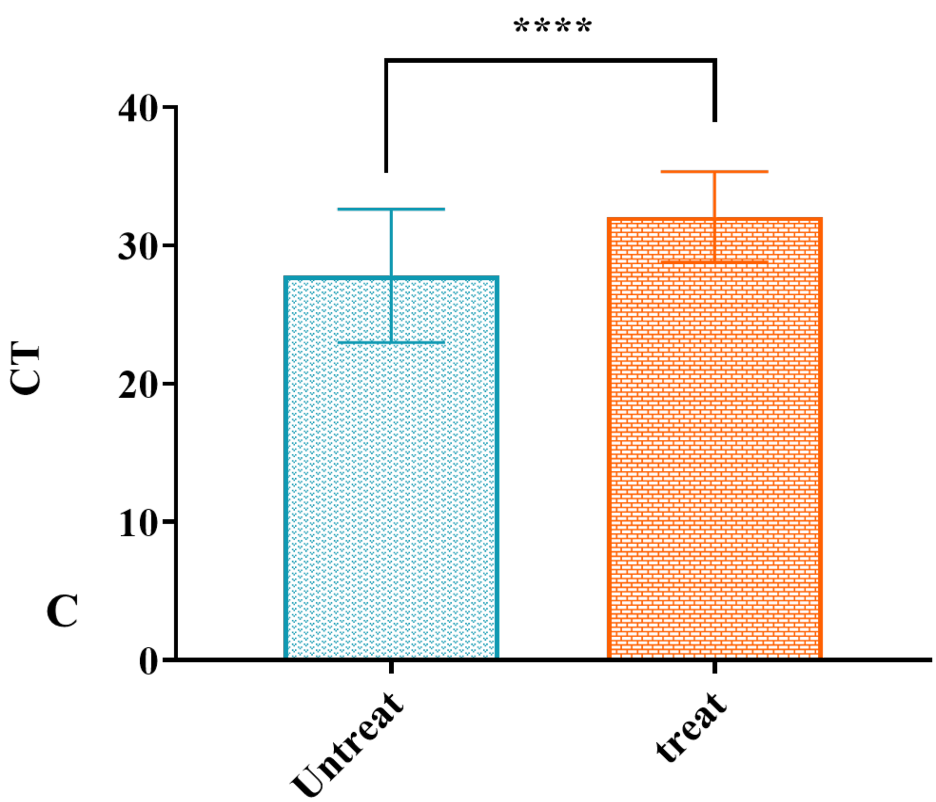


Figure 3: Comparison of CTs in untreated and treated samples with Montmorillonite nanoparticles and *Satureja sahandica*(A: severe group, B: Mild group and C:average group).

various plant-derived compounds that may inhibit COVID-19 infection pathways, identifying several potential therapeutic options (Singh *et al.*, 2023). In conclusion, the compounds present in Satureja and other medicinal plants significantly impact critical functional aspects of COVID-19, leading to viral death and the inhibition of infections. The findings support the use of *Satureja sahandica* as a potential treatment for COVID-19, highlighting the importance of exploring natural products in the ongoing search for effective antiviral therapies.

CONCLUSION

Overall, this study paves the way for exploring *Satureja sahandica* as a viable therapeutic agent against COVID-19, integrating traditional herbal medicine with modern scientific approaches.

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ABBREVIATIONS

COVID-19: Coronavirus disease 2019; **FBS:** Fetal bovine serum; **VTM:** viral transport medium; **CPE:** Cytopathic effects; **PBS:** Phosphate-buffered saline.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

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AUTHOR CONTRIBUTIONS

S.A and, H.S were involved in planning and supervised the work and proof outline. E.N, A. S.A and, H.S drafted the manuscript and designed the figures. H.S was responsible for the substantive review of the article and the project. All authors discussed the results and commented on the manuscript.

DATA AVAILABILITY

The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This project was approved by the university's ethics committee with IR.IAU.TABRIZ.REC.1403.373 The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

SUMMARY

The findings of this study suggest that the ethanolic extract of *Satureja sahandica*, particularly when combined with montmorillonite nanoparticles, may offer a promising therapeutic approach against COVID-19. This research emphasizes the potential of plant-based extracts in antiviral treatment strategies, highlighting the need for further investigation into their mechanisms of action and clinical efficacy. *Satureja sahandica* is known for its diverse biological activities, including antimicrobial and antiviral properties. The addition of montmorillonite nanoparticles may enhance the extract's bioavailability and efficacy, potentially leading to improved therapeutic outcomes. Future research should focus on elucidating the specific mechanisms by which this combination exerts its antiviral effects, including its impact on viral replication and immune modulation. Clinical trials are essential to evaluate the safety and effectiveness of this therapy in humans. Overall, this study paves the way for exploring *Satureja sahandica* as a viable therapeutic agent against COVID-19, integrating traditional herbal medicine with modern scientific approaches.

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