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Original Article

In-vitro for Q-RT-PCR Clinical Evaluation of Oscardia Ledovir Spray Effectiveness on SARS-CoV-2 and Its Effective Variants

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Abstract

Background and Aims: The real target planned is the prevention of COVID-19 using natural treatment tools, other than medical drugs, together with the use of vaccines. Similar to various viruses that lead to upper respiratory diseases, SARS-CoV-2 most frequently enters the body through the nasal cavity and oral cavity. It has been stated that the Oscardia Ledovir Spray can form a mechanical barrier in the mucosa of the nasal and oral cavities, which are the points of entry of the SARS-CoV-2 virus in the body, preventing the bonding of the virus to the receptors, and inhibiting any virus it encounters through direct contact.

Methods: The present study serves as evidence for this treatment. The application of disinfectants in percentage formulations has been officially accepted by the World Health Organization to kill viruses, and this was used to compare its effectiveness with the Oscardia Ledovir Spray. The obtained new sample mixture was placed in a plate. In the course of the study, it was determined that the Oscardia Ledovir Spray has an effect mechanism similar to ethyl alcohol and disinfectants.

Results: Since the Oscardia Ledovir Spray was found to have an effect mechanism similar to ethyl alcohol and disinfectants, this was considered as the preferred treatment approach. The results of the present clinical study revealed that this treatment approach is effective, particularly for SARS-CoV-2.

Conclusions: The Oscardia Ledovir Spray can be considered to provide both prophylactic and therapeutic benefits, thereby contributing to humanity in improving processes that range from simple infections to serious diseases. Furthermore, it was considered that this treatment can be used for both SARS-CoV-2, and viral and bacterial infections.

Keywords: SARS-CoV-2; COVID-19; Omicron; Nasal spray; Oral spray; Disinfectant; Isopropyl alcohol; Ethyl alcohol; Oscardia Ledovir Spray.

Abbreviations: aa, amino acid; ACE2, Angiotensin converting enzyme-2; CT, Cycle terminal domain; CY5, carboxylic acid Cyanine5; DNA, deoxyribonucleic acid; EM, Electron Microscopy Experimental Data Snapshot Resolution; FAM, 6-carboxy-fluorescein; GISAID, Global Initiative on Sharing Avian Influenza Data; HEX: 5′-hexachlorofluorescein-CE phosphoramidite; LSD, Least Significant Difference; LSG105-107del, laparoscopic sleeve gastrectomy 105-107 deletion; NCBI, National Center of Biotechnology Information; NGS, new generation sequencing; NSP6, nonstructural protein-6; mRNA, messenger ribonucleic acid; PDB, protein Data Bank; RBD, receptor-binding domain; Real Time-qPCR, Quantitative real time polymerase chain reaction; RFU, Relative fluorescence units; RNA, ribonucleic acid; RNaseP, Ribonuclease P; ROX, 6-carbocyl-X-Rhoddamine; qPCR, Quantitative real-time polymerase chain reaction; SARS-CoV-2, Severe Acute Respiratory Syndrome-2; vNAT, viral nucleic acid tampon; WHO, World Health Organization.

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Introduction

A number of viruses that can induce infection in the respiratory

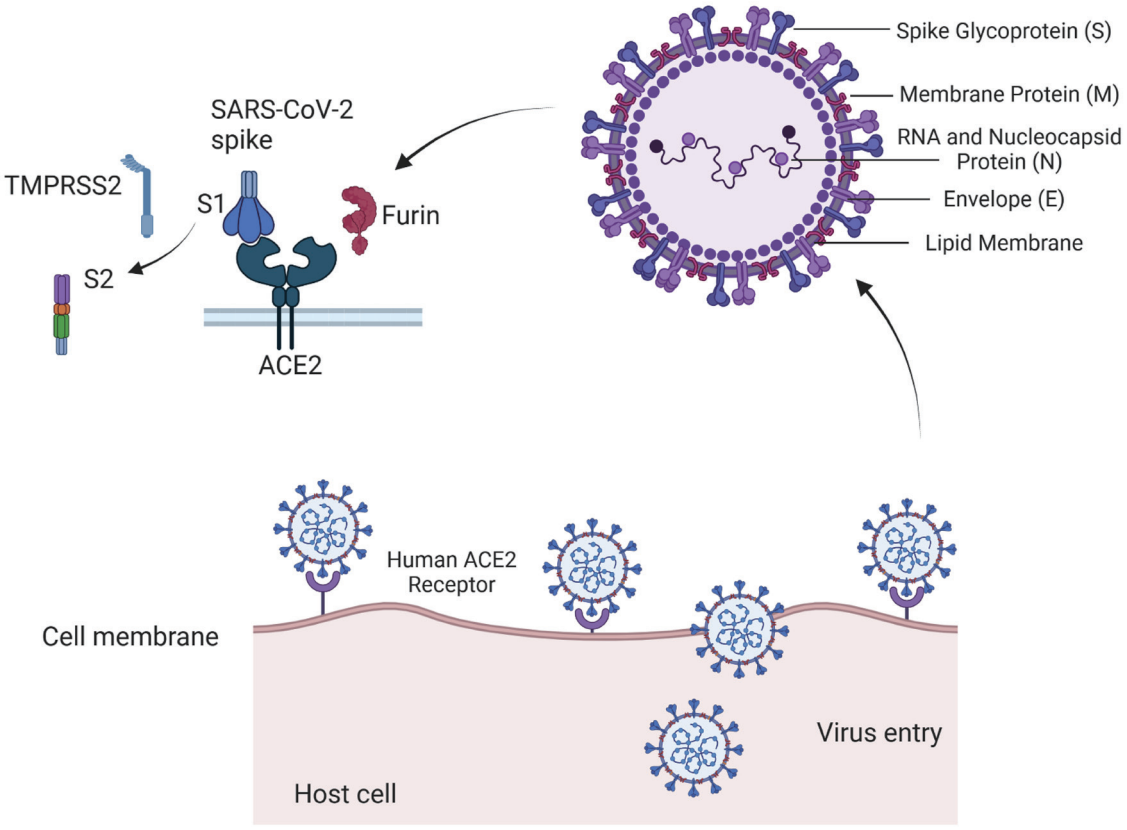

Fig. 1. General structure of SARS-CoV-2, the spike protein and the ACE2 Receptor. ACE2, Angiotensin converting enzyme-2; CD, connector domain; CH, central helix; CT, C-terminal domain; FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; NTD, N terminal area; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD1, subdomain 1; SD2, subdomain 2; TM, transmembrane region. (Designed via BioRender).

tract, including rhinovirus, enterovirus, influenza, adenovirus and coronavirus, have been identified, to date.[1] The virus that manifested in late December 2019 in Wuhan, Hubei, China, which was named, Severe Acute Respiratory Syndrome-2 (SARS-CoV-2), is a member of the Corona virus family.[2,3] The disease was named as, COVID-19, and a pandemic was declared due to this disease in 2020 by the World Health Organization (WHO). This virus was further named as, SARS-CoV-2, by the International Virus Taxonomy Committee.[4] As of 12 May 2022, the WHO has reported 516,922,683 confirmed COVID-19 cases and 6,259,945 deaths caused by COVID-19 worldwide. A total of 11,655,356,423 doses of vaccine have been administered worldwide as of 9 May 2022.[5] SARS-CoV-2 is a type of beta coronavirus and RNA virus with a positive helicoid envelop, and is contagious only in humans and various animals. Its 30 kb genome consist of 14 clear reading frameworks coded to the spike protein (S) and nucleocapsid protein (N). Furthermore, it consists of a small membrane protein (SM) and membrane glycoprotein (M). Viruses that belong to the A sort of beta coronaviruses contain the hemagglutinin-esterase (HE) protein that binds to the membrane. The essentially important part is the spike protein, which contains both the receptor binding domain (RBD) and domains correlated to the fusion where the mutation occurs. This is the most important protein in the process of entry of CoV.[6] The S protein is a glycoprotein in the form of a stick, which ornaments the membrane surface of SARS-CoV-2.[7] The spike protein consists of two sub-units, namely, S1 and S2, which performs a role when the host receptor (ACE2) is encountered. Figure 1 presents RBD.[8,9]

The bat CoV genome exhibits a sequence resemblance with SARS-CoV-2 at a rate of 96%, while the RBD domain of the spike protein exhibits a genomic resemblance of 97.4% with pangolins.[10] SARS-CoV-2 infects a person from another person through droplets, or direct or indirect contact with the mucous membranes in the eyes, nose, or mouth, and through contact with contaminated objects.[11] Based on recent studies, the incubation period was updated as 4–6 days.[12] Individuals within the society, who did not present any symptoms during the period of the disease, were called, asymptomatic. These are among the main reasons for the disease infections. Pre-symptomatic patients were defined as patients who were infected during the early phases of the disease, but did not develop any symptoms.[13] When COVID-19 patients were examined, lower respiratory tract infection findings, such as fever, cough, dyspnea and chest tightness, were observed, in addition to upper respiratory tract infection symptoms, such as sore throat, nasal congestion, nasal flow and anosmia.[14] Anosmia was reported by the European Rhinology Society as one of the most frequently observed symptoms of COVID-19 patients (20–60%). This may arise from viral originated olfactory nerve injury, local inflammation of nasal cavity, or both.[15] The air respired when taking a deep breath initially passes through the nose. A healthy person nasally respires approximately 10,000 liters of air a day. The nasal cavity has two important roles in the body: the first role is heating and moistening the air, and the second role is sending away droplets or foreign particles, such as pathogens that come with the air. Saliva

is the most preferred source for diagnosing COVID-19 through q-RT-PCR tests, due to its high viral load in COVID-19, with an infective copy/ml reaching up to 1.2×10^8. Therefore, nasopharynx swab samples carry higher viral loads, when compared to those detected in the oropharynx, during the q-RT-PCR test.[16] As defined in the literature, certain spray formulations have been investigated under two main categories: those that actively target the virus (*e.g.,* products, such as SaNOtize), and those that passively protect the mucosa against viral contamination (*e.g.,* Taffix and Vicks First Defense). The product named, SaNOtize, was developed in Canada. This is a nasal spray (NONS) with nitric oxide content, and is convenient for individual use. The viral load in infected COVID-19 patients could be mitigated using this spray.[17] Taffix is a product capable of absorbing liquid from the hydroxypropyl methyl cellulose. Its contents create a gel of micron size, and this was tested for SARS-CoV-2, and H1N1 influenza and lentivirus.[18] Remdesivir (an RNA-polymerase inhibitor that binds to viral RNA) and nitrite oxide were also used for COVID-19 treatment without mechanical ventilation.[19] For this reason, it was considered that decreasing the viral load in the nasal region is as important as decreasing the viral load in the oral cavity/oropharynx.

Using the Oscardia Ledovir Spray, positive patient samples were treated with a total volume of 100 µL at 50/50 µL (50% spray/50% sample) in the present study. Then, the obtained mixture was vortexed for 10 seconds for homogenization. Afterwards, the obtained new sample mixture was placed in a plate with 96 cavities, in the order of A1 to H12. Next, using ethyl alcohol (a disinfectant that contains a mixture of 80% ethyl alcohol + 75% isopropyl alcohol), the positive patient samples were treated with a total volume of 100 µL at 50/50 µL ([50% disinfectant [disinfectant containing mixture of 80% ethyl alcohol + 75% isopropyl alcohol]/50% sample), and the obtained mixture was vortexed for 10 seconds for homogenization.

In the present study, the investigators attempted to determine whether it was possible for people to use the Oscardia Ledovir Spray in daily life, in order to protect themselves against infections, such as COVID-19 and the like. The effectiveness of the Oscardia Ledovir Spray was determined, in terms of decreasing the viral load in COVID-19 patients. This would make it easy to manage the disease, relieve the symptoms that bother the patient, such as anosmia, and prevent the progression of the disease, which in turn, prevents severe clinical conditions, such as mechanical ventilation and intubation. Then, its effectiveness was compared to that of the disinfectant that contained ethyl alcohol, which conform with the formulations for definite antiviral properties provided by the WHO through studies, including SARS-CoV-2.[20,21]

The important role of the nasal cavity passage is to filter bacteria or viruses that may expose the sinonasal tracts to high-risk infections. Anosmia is the general symptom that usually manifests in COVID-19 patients before other symptoms (cough, fever, sore throat, etc.), which adversely affects the life quality of patients.[22] Heilmann *et al*. supports the systemic and topical application of corticosteroid for patients with anosmia. Mometazon noted that the use of a nasal spray developed a sense of smell after local application.[23] Furthermore, Vroegop *et al*. reported that a spray formulation would be more effective, when compared to gels or drops, in addition to intranasal corticosteroid use.[24] The infection of COVID-19 and various upper respiratory tract diseases was due to the respiratory droplets and aerosols that diffused from infected individuals in the environment. Thus, agents that can be applied in the nasal cavity or mucosal tissue may be effective in providing protection against initial infections, thereby reducing the spread of the virus.[25] To date, various spray types have been defined as a protective element against bacteria or viruses. Some of these contain decongestant components that are considered to be effective in reducing nasal congestion, such as xylometazoline, tramazoline, or oxymetazoline.[26]

Materials and methods

Sampling, carriage and storage: Nasopharyngeal swab samples were collected from SARS-CoV-2 patients and placed in viral nucleic acid tampon (vNAT) solution tubes by trained personnel of the "Republic of Turkey Ministry of Health, Istanbul Provincial Directorate of Health, Department of Microbiology, Health Institutes of Turkey, COVID-19 Diagnostic Center, Kartal Dr. Lutfi Kirdar City, 34865, Kartal, Turkey", according to the regulations. From these samples, 186 positive patient samples and 93 Omicron positive patient samples were separately and randomly selected, and these were verified using two different kits: the "Bio-Speedy SARS-CoV-2 + Omicron q-RT-PCR" test kit (Bioeksen R&D Technologies, Istanbul, Turkey) and the "DS CORONEX COVID-19 Multiplex Quantitative real-time polymerase chain reaction (real-time-qPCR)" test kit (DS Bio and Nanotechnology Product Tracing and Tracking Co, Ankara, Turkey).

Oscardia Ledovir Spray and ethyl alcohol (80% ethyl alcohol + 75% isopropyl alcohol mixed disinfectant) comparison

Oscardia Ledovir Spray

Oscardia Ledovir Spray is a trademark by Oscardia A.Ş. Turkey, TURKINNOVA Group, Germany and BioMedical Technology GmbH, Germany. This is a class I medical device accepted by the German Medical Device Committee (no: DE/CA61/00189037), and patented by the Turkish Patent Institute and Patent Cooperation Treaty. The Oscardia Ledovir Spray is a nasal spray that contains triacetin, *Aloe barbadensis* fruit extract, *Musa acunminata* fruit extract, *Fragaria vesca* fruit extract, hydroxypropil methylcellulose, Eucalyptus extract, polyethylene glycol 40, Stevia, and water.

The experiments were performed in two different days, with an interval of 72 hours. On the first day, three different positive patient plates were created. The first plate consisted of samples diagnosed with Omicron (93 patients). The sample of a patient was randomly selected and obtained using the "Bio-Speedy SARS-CoV-2 + Omicron q-RT-PCR" from samples diagnosed with Omicron was sequenced, and the sensitivity of the kit was measured. The next-generation sequencing (NGS) result is discussed in detail in the Conclusion section of the manuscript. All samples with Omicron positivity and other positive samples were detected using only the "DS CORONEX COVID-19 Multiplex real-time-qPCR" test kit throughout the entire experiment for the present study. The Omicron kit was only used for studies conducted to detect and sort out patient samples used in routine procedures for sample collection. Furthermore, Omicron positive samples were used for this phase of assessment, because these were sensitive to the kit. The other two plates were prepared using SARS-CoV-2 positive samples. However, the mutation type could not be determined among the samples, because the "DS CORONEX COVID-19 Multiplex real-time-qPCR" test kit was used to determine the positivity of the samples. These samples may contain all mutation types of the COVID-19 disease: Variant of Concern (Alfa [B.1.1.7]), Beta (B.1.351), Gamma (P.1) and Delta (B.1.617.2), and VOI (Epsilon

[B.1.427] and B.1.429); Zeta (S.2); Eta (B.1.525); Teta (S.3); Iota (B.1.526); Kappa (B.1.617.1); Lambda (C.37) and Mu (B.1.621).

Experiment phase

The experiments performed at the first day comprised of three sets of plates, containing a total of 297 patient samples: three plates×93 patient samples. All patient samples were placed in the plate in the same order.

First, the normal samples were routinely placed in the order of the holes on the plates, from A1 to H9, and the results were recorded. Then, 50 µL of Oscardia Ledovir Spray and 50 µL of the patient sample (50% spray/50% sample) were added to the other three plates, and vortexed for 10 seconds for homogenization. Afterwards, the obtained new sample mixture was placed in the order of the holes on the plates, from A1 to H9. Subsequently, 50 µL of disinfectant (80% ethyl alcohol + 75% isopropyl alcohol mixed) and 50 µL of the patient sample were added in the remaining three plates, and vortexed for 10 seconds for homogenization. Then, the obtained new sample mixture was placed in the order of the holes on the plates, from A1 to H9. The positive control was placed in the H10 hole, the negative control was placed in the H11 hole, and the main mixture was placed in the H12 hole for all plates. The H12 hole was used for contamination control. All experiments performed on the first and third days were carried out in the same manner. Thus, a three-plate routine sample study, a 3-plate Oscardia Ledovir Spray study, and a 3-plate ethyl alcohol (80% ethyl alcohol + 75% isopropyl alcohol mixed disinfectant) study were performed on the third day. The samples in all plates were carefully numbered based on the experiments applied on the first day, and these were placed on plates with the same order numbers in the study performed on the third day. All studies were designed and simultaneously performed on the first and third days.

Q-RT-PCR tests

Due to the nucleic acid extraction property of the vNAT solution, in which the samples are added, the extra RNA extraction step was not required. The vNAT solution provides nucleic acid extraction from the nasopharyngeal swab, oropharyngeal swab, and sputum samples. Furthermore, the vNAT reactive disintegrate the virus within five minutes, and releases the nucleic acids. Thus, this allows for the transition from sample to qPCR within five minutes.

Bio-Speedy SARS-CoV-2 + Omicron q-RT-PCR kit and test interpretation

The "Bio-Speedy SARS-CoV-2 + Omicron q-RT-PCR" kit tests the SARS-CoV-2 and SARS-CoV-2 nonstructural protein-6 (NSP6) mutation, and laparoscopic sleeve gastrectomy 105–107 deletion (LSG105-107del) within a period shorter than 30 minutes in a single multiplex reaction. This test is applied by the healthcare service provider on samples obtained from the nasopharyngeal swab, oropharyngeal swab, nasal swab, saliva, and oral swab (saliva swab), which were taken from individuals suspected with COVID-19. The "Bio-Speedy SARS-CoV-2 + Omicron q-RT-PCR" kit targets the Orf1ab and N gen regions common in all SARS-CoV-2 variants, in addition to the NSP6 LSG105-107del mutations. As shown in Table 1, 97% of all SARSCoV-2 variants that contained the NSP6 LSG105-107del mutation were Omicron variants obtained from 1 November 2021 to 5 December 2021, and merely 53% of all SARSCoV-2 variants that contained the S HV69-70del mutation were Omicron variants. The human ribonuclease P (RNaseP) oligo set targets the exon-exon binding in the messenger ribonucleic acid (mRNA), rather than in the human genome. Hence, the sample was used to control the RNA stability, nucleic acid extraction, and inhibition of both qPCR and reverse transcription. Furthermore, the kit contained negative and positive control templates for testing the contamination and qPCR reactive stability.

Table 1. The percentage of variant of concern NSP6 LSG105-107del ve_HV69-70del mutations in all SARS-CoV-2 and worrisome variants collected from 1 November 2021 to 5 December (GISAID database)

NSP6 LSG105107del	S_HV69-70del
538 SARS-CoV-2 (100%)	978 SARS-CoV-2 (100%)
520 Omicron (97%)	515 Omicron (53%)
15 Delta (3%)	291 Delta(30%)
0 Alpha	118 Alpha (12%)
0 Beta	0 Beta
0 Gamma	0 Gamma

NSP6, nonstructural protein-6; LSG105-107del, laparoscopic sleeve gastrectomy; GISAID, Global Initiative on Sharing Avian Influenza Data; SARS-CoV-2, Severe Acute Respiratory Syndrome-2.

The "Bio-Speedy SARS-CoV-2 + Omicron q-RT-PCR" kit, "vNAT Viral Nucleic Acid Tampon (BS-NA-510)" and "vNAT Transfer Tube (BS-NA-513-100)" were used to verify samples obtained from the nasopharyngeal swab, oropharyngeal swab, nasal swab, saliva, and oral swab (saliva swab). These kits were used with the Bio-Rad CFX96 Touch device (Germany) at a qPCR volume of 10 µL.

The oligonucleotides designed for SARS-CoV-2 genomes covered all the main types and significant variants that have recently occurred. The oligonucleotides series was designed to match 100% of all targets in the Global Initiative on Sharing Avian Influenza Data (GISAID) database. The limit of detection of the kit is 500 copies/mL for SARS-CoV-2 and 4,000 copies/mL for NSP6 LSG105-107del. Furthermore, the *in silico* analysis suggested that the oligonucleotide series in the test did not have a cross-reaction with any nucleotide series in the database.

Since the vNAT solution has a nucleic acid extraction property, the extra RNA extraction step was not necessary. A proper strong vortex is sufficient for the RNA extraction step. The real-time PCR kit of the "Bio-Speedy SARS-CoV-2 + Omicron q-RT-PCR" was used. The primaries of the kit were designed based on the protected regions of the ORF1ab and RNaseP genes of SARS-CoV-2. The 6-carboxyfluorescein ([FAM] SARS-CoV-2, ORF1ab + N) and phosphonamidite ([HEX] Human RNase-P mRNA IC]) channel was used for the ORFlab and RNaseP genes, respectively. This kit can also be used for mutation detection in the tetramethyl indo(di)-carbocyanine (Cy5) NSP6 LSG105-107del duct information. According to the kit's protocol, vNAT and 2.5 µL of patient sample were added to 7.5 µL of ready kit mixture, with a total PCR mixture volume of 20 µL (2.5 µL of Cvd + O Oligo Mix + 5 µL of PrimeScript Mix [deoxyribonucleic acid [DNA] polymerase, nucleoside triphosphate mix, and reverse transcriptase]). Then, the enzyme, ribonuclease inhibitor and reaction tampon were obtained. The reaction installation, and qPCR program details and test interpretation are presented in Table 2.

The cycle terminal domain (CT)-FAM ≤33 result was assessed as positive, according to the kit's protocol. Otherwise, the result was assessed as negative. If the CT-carboxylic acid Cyanine5 (Cy5) was ≤33, the difference between CT-Cy5 and CT-FAM was calculated. If the difference was <8, the sample was deemed positive. Otherwise, the sample was deemed negative.

Table 2. Thermal cycle parameters for the RT-PCR amplification and test interpretation

Reaction Setup		q-RT-PCR Program		
Contents	**Reaction**	**Cycle**	**Temperature**	**Time**
2X Prime Script Mix	5 µL	1	52°C	3 minutes
		1	95°C	10 seconds
Cvd + O Oligo Mix	2,5 µL	5	95°C	1 second
			60°C	12 seconds
Template nucleic acid	2,5 µL	35	85°C	1 second
			60°C	1 second
Total reaction volume	10 µL	FAM/HEX/CY5 Cannel		

FAM, 6-Carboxyfluorescein; HEX, phosphoramidite; ROX, 6-carbocyl-X-Rhoddamine; Cy5, carboxylic acid.

Table 3. Product content

Contents	Contents	Cover number	Volume
DS CORONEX COVID-19 Mix E	q-RT-PCR Master mix	1	10×1,250 µL
DS CORONEX COVID-19 PP1	Orf1ab + N and RNase-P genes primer and probe mix	2	5×500 µL
DS CORONEX COVID-19 Kit Positive control	Positive control for Orf1ab N and RNase-P genes	3	200 µL
No-Template Control	No-Template control	4	200 µL

The DS CORONEX COVID-19 Multiplex real-time-qPCR kit and test interpretation

The sample on the first plate used for the experiment (93 patients) comprised of samples assessed within the scope of the protocol, and these were detected to be omicron positive. The DS CORONEX COVID-19 Multiplex real-time-qPCR (Ver. 2.0) kit contains samples, such as nasopharyngeal aspirate/lavage and throat swabs. This is a q-RT-PCR kit with a multiplex base that was realized using labeled oligonucleotides peculiar to the target gene regions of SARS-CoV-2 detected in the samples. The cDNA complementary DNA synthesis and qPCR reaction were realized in the same tube. The "orf1ab", "N" genes, and human RNaseP genes peculiar to SARS-CoV-2 were targeted, as shown in Table 3. Furthermore, the RNase P internal control was used. The limit of detection of the DS CORONEX COVID-19 qPCR kit was determined as 100 lops/ml.

The primaries of the kit were designed based on the protected regions of SARS-CoV-2. The 6′nın ORF1ab and RNaseP genes, and FAM and HEX channel were used for the ORFlab and RNaseP genes, respectively. Merely positive ones were examined for the present study. According to the kit's protocol, 5 µL of patient sample with vNAT was added in 15 µL of ready kit mixture, in order to obtain a total PCR mixture volume of 20 µL (12.5 µL of DS CORONEX COVID-19 Mix E and 2.5 µL of DS CORONEX COVID-19 PP1).

The thermal cycle parameters for the q-RT-PCR amplification are presented in Table 4. The threshold was regulated at 200 using the Bio-Rad CFX96 platform, according to the kit's protocol. Positive results of SARS-CoV-2 were deemed meaningful when sigmoidal with CT values were lower than 33 for the FAM duct, independent from the HEX values. All signals, regardless of whether these were sigmoidal with CT values, were higher than 34 in the FAM duct, and sigmoidal with CT values lower than 35 in the HEX duct were interpreted as negative, according to the kit's protocol. Furthermore, the test contained a SARS-CoV-2 RNaseP region in the HEX duct, and this was protected as the internal control.

The present study determined whether the mixtures that contained the Oscardia Ledovir Spray and ethyl alcohol (80% ethyl alcohol + 75% isopropyl alcohol) mixed disinfectant had negative or misleading effects to the q-RT-PCR device, with HEX and FAM values controlled throughout the entire study. If any of the samples decreases or decays due to a negative factor, the HEX and FAM values from the device used would not give the correct values.

Statistical analysis

SPSS 27.0 (IBM Corporation, Armonk, NY, USA) and PAST 3 (Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. Paleontological statistics) were used for the variable analysis. The Mardia (Dornik and Hansen omnibus) test was used for conformity with normally distributed multi-variable data. The Monte Carlo simulation technique and Wilcoxon signed ranks test were used to compare the two repeated measurements in the Bootstrap results and paired-samples *t*-test dependent quantitative variables. Repeated ANOVA test, which is a parametric analysis, was used to compare the quantitative measurements of three dependent groups. Fisher's least significant difference (LSD) test was used for the post-hoc test, while for the Monte Carlo simulation technique results, Friedman's two-way test (a nonparametric test) was used. The gradual

Table 4. Thermal cycle parameters for the RT-PCR amplification and interpretation of the test

Cycle	Temperature	Time
1	45°C	5 minutes
1	95°C	1 minute
5	95°C	5 seconds
	55°C	10 seconds
35	95°C	5 seconds
	55°C (read)	1 second

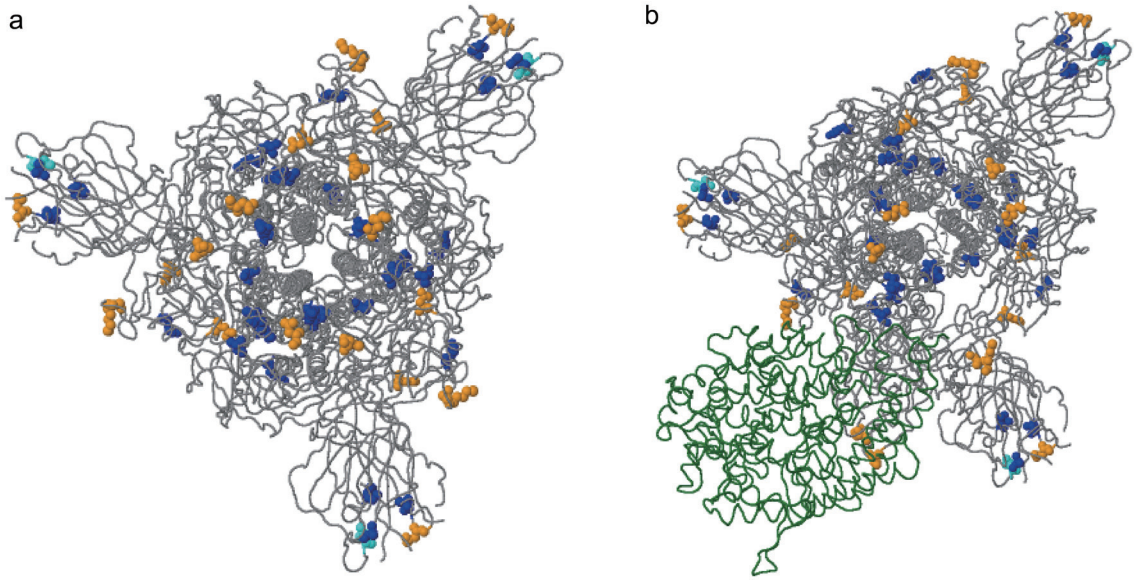

Fig. 2. The 3D structural visualization of the spike glycoprotein with aa changes identified in the query sequences, shown in colored balls. RBD, receptor-binding domain; ACE2, angiotensin converting enzyme-2; PDB, protein data bank; EM, Electron Microscopy Experimental Data Snapshot Resolution; AA, amino acid.

down step comparison test was used for post-hoc analyses. The quantitative variables were presented in the tables as mean (standard deviation) and median (1st quartile/3rd quartile). The variables were analyzed with 95% confidence level, and a *p*-value of <0.05 was considered statistically significant.

Results

The architecture of the effect area of the SARS-CoV-2 spike monomer is presented in Figure 1 as the RBD region**.** The mutant variants and genomes of SARS-CoV-2 in the National Center of Biotechnology Information (NCBI) and GISAID were analyzed by NGS and bioinformatic analysis. For the main application scenario for CoVsurver, the phenotypically or epidemiologically interesting candidate, amino acid (aa), would vary for further research, and this should ideally be combined with experimental tests and verifications of any phenotype stipulated. The comparison result for the reference selection was hCoV-19/Wuhan/WIV04/2019. The protein structure of the swab samples obtained from patients with the mutation is presented in Figure 2. The left column refers to the RBD and protein data bank (PDB: 6acc, Electron Microscopy Experimental Data Snapshot Resolution [EM] 3.6 Angstrom) mutation in the spike glycoprotein in the following conformation. AA % identity: When the value was 98.193′ on the right column, this exhibited (PDB: 6acj, EM 4.2 Angstrom) mutations in the spike glycoprotein complexed with the host cell receptor ACE2 (green line). "#aa IS: 99.450%/# aa changes: 23.

The list of Omicron mutations is presented in the loop/termini region nearest residue, as shown in Table 5.

The NGS was completed in the National Virology Reference Laboratory associated to the Ministry of Republic of Turkey, Directorate of Public Health. The sequence alignments were realized, as shown in Figure 3. The comparison between general SARS-CoV-2 and Omicron mutations were also presented. The FASTA format was created using the NCBI program. Then, all series were aligned to create the mutation changes in the GISIAD program. Dividing the multi-segments, adapters, serial bookshelves and RNA in recombination, and obtaining a genomic series that resembled the capillary electrophorese were parts of the NGS process. NGS is a fast and correct process, and reduces the cost of sequencing. Millions of particles that were generally parallel were aligned. The first plate (93 patients) used in the present study consisted of samples obtained from patients diagnosed with Omicron. The sequencing was performed on the "Bio-Speedy SARS-CoV-2 + Omicron q-RT-PCR" sample obtained from a randomly selected patient, in order to determine the effectiveness of Oscardia Ledovir Spray on variants, and the sensitivity of the kit was measured. The NGS result for all samples was Omicron positive, and the other positive samples were detected throughout the experiment using only the "DS CORONEX COVID-19 Multiplex real-time-qPCR" test kit. That is, NGS was previously performed for one of the samples, in order to compare this with the results for the 93 positive patients detected using the Omicron kit. The results assessed in this segment are presented in the relevant figures and tables. All procedures for the routine sampling were carefully applied, and 93 of the samples were selected from the Omicron positive samples. The general sequencing for SARS-CoV-2 is presented in Figure 3A and C, and in the patient map for Omicron. The "Thermo Fisher Fermentas 219 enzyme region" is presented in Figure 3B and D using the SNAPGENE bioinformatics program.

Plate I consisted of the Omicron variants. When the CT-FAM first-day median values were examined, these were higher ($p < 0.001$) in the Oscardia Ledovir Spray and disinfectant (ethyl alcohol/IPA) groups, when compared to the routine group. No statistically significant difference was detected between the Oscardia Ledovir Spray and disinfectant (ethyl alcohol/IPA) groups ($p = 0.999$). In comparing the third-day CT-FAM values, these were higher in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$), and these were higher in the disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). When the first-day and third-day CT-FAM median val-

Table 5. List of variations displayed in the structure (nearest residue when in the loop/termini region)

Query: hCoV-19/Turkey/HSGM-F10026/2021; EPI_ISL_8082617|2021-12-16; Clade: GRA				
Best reference hit	**% Id**	**% Coverage**	**#Δs**	**List of aa changes**
NSP1 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NSP2 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NSP3 hCoV-19/Wuhan/WIV04/2019	99.9%	99.9%	3	S1265del, L1266I, A1892T
NSP4 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NSP5 hCoV-19/Wuhan/WIV04/2019	99.7%	100%	1	P132H
NSP6 hCoV-19/Wuhan/WIV04/2019	99.7%	99.0%	4	L105del, S106del, G107del, I189V
NSP7 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NSP8 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NSP9 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NSP10 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NSP11 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NSP12 hCoV-19/Wuhan/WIV04/2019	99.9%	99.0%	1	P323L$^{\#o}$
NSP13 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NSP14 hCoV-19/Wuhan/WIV04/2019	99.8%	100%	1	I42V$^{\#o}$
NSP15 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NSP16 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
Spike hCoV-19/Wuhan/WIV04/2019	98.6%	99.6%	23	A67V, H69del$^{\$}$, V70del$^{\$}$, T95I, G142D, V143del$^{\#a}$, Y144del$^{\$\#a}$, Y145del$^{\#a}$, N440K$^{\$\#ao}$, G446S$^{\$\#ra}$, S477N$^{\$\#rao}$, T478K$^{\$\#rao}$, T547K$^{\#o}$, D614G$^{\$\#lo}$, H655Y, N679K, P681H$^{\$}$, N764K$^{\#o}$, D796Y$^{\$\#lo}$, N856K$^{\#o}$, Q954H$^{\#o}$, N969K$^{\#o}$, L981F$^{\#o}$
NS3 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
E hCoV-19/Wuhan/WIV04/2019	98.7%	100%	1	T9I$^{\#o}$
M hCoV-19/Wuhan/WIV04/2019	99.1%	100%	2	Q19E, A63T
NS6 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NS7a hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NS7b hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
NS8 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	No aa changes
N hCoV-19/Wuhan/WIV04/2019	99.3%	99.3%	6	E31del, R32del, S33del, R203K, L382X, P383X

aa, amino acid; NS, nonstructural protein.

ues were compared, the third-day median values are higher, when compared to the first-day median values, in the routine group ($p < 0.001$) and Oscardia Ledovir Spray ($p < 0.001$) group. However, there was no significant difference when compared to the disinfectant (ethyl alcohol/IPA) group ($p = 0.537$) (Table 6).

Plate II + III consisted of mixed variants. When the CT-FAM first-day median values were examined, these are found to be higher in the Oscardia Ledovir Spray group and disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). Furthermore, these were also detected to be higher, when the Oscardia Ledovir Spray group was compared to the disinfectant (ethyl alcohol/IPA) group ($p = 0.005$). For the third-day CT FAM values, these were higher in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$), and these were detected to be higher in the disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). When the first-day and third-day CT-FAM median values were compared, the third-day median values are detected to be higher than the first-day median values in the routine group ($p < 0.001$), Oscardia Ledovir Spray group ($p < 0.001$), and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$) (Figure 4).

For all samples (plate I + II + II), the CT-FAM first-day median values were higher in the Oscardia Ledovir Spray group and disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). Furthermore, these were detected to be higher in the Oscardia Ledovir Spray group, when compared to the disinfectant (ethyl alcohol/IPA) group ($p = 0.038$). For the third-day CT-FAM values, these were higher in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$). Furthermore, these were detected to be higher in the disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). When the

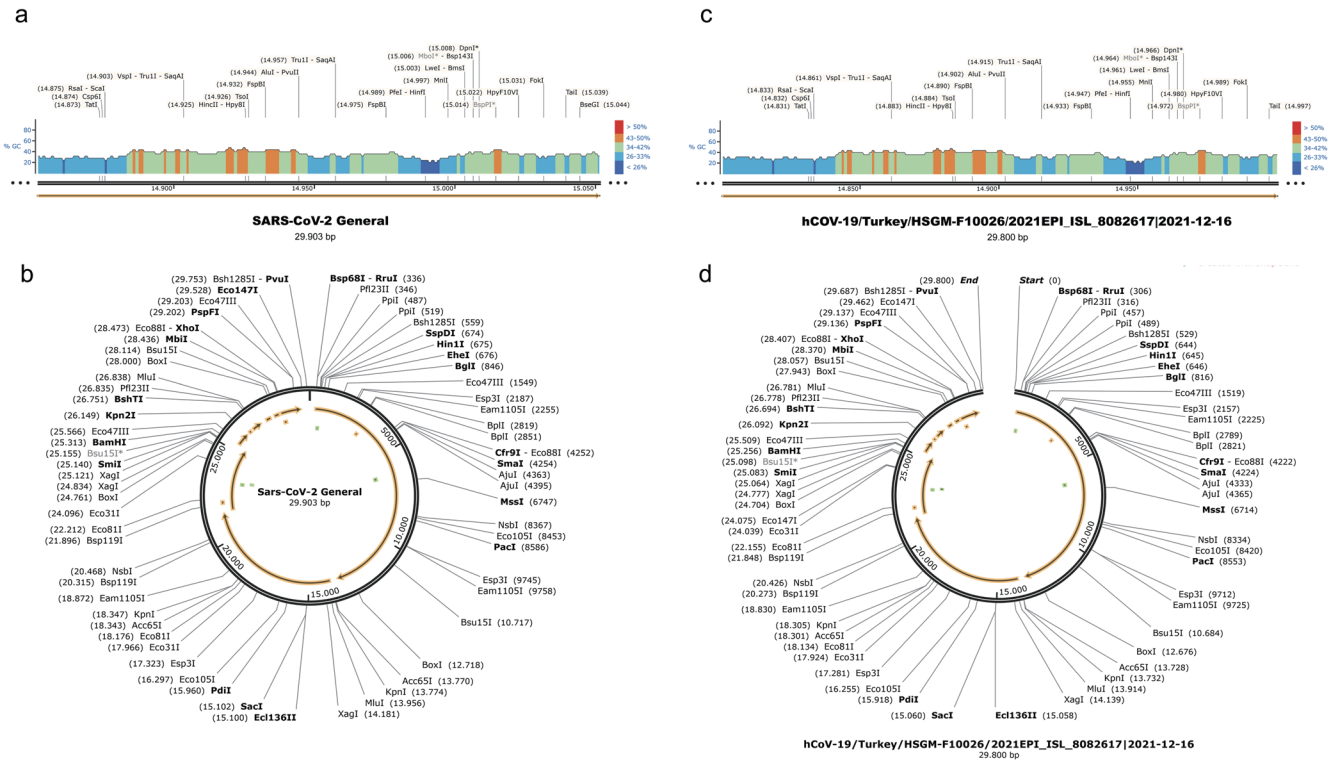

Fig. 3. Sequence alignments for SARS-CoV-2 and the Omicron variant. (A) The SARS-CoV-2 general sequence line map; (B) The SARS-CoV-2 general sequence spiral map; (C) The SARS-CoV-2 Omicron variant sequence line map; (D) The SARS-CoV-2 Omicron variant sequence spiral map. SARS-CoV-2, Severe Acute Respiratory Syndrome-2.

first-day and third-day CT-FAM median values were compared, the third-day median values were detected to be higher than first-day median values in the routine group ($p < 0.001$), Oscardia Ledovir Spray group ($p < 0.001$), and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$) (Table 6 and Figure 4).

When the RFU-FAM first-day median values for plate I that contained samples with the Omicron variant were examined, these were higher in the disinfectant (ethyl alcohol/IPA) group, when compared to the Oscardia Ledovir Spray group and routine group ($p < 0.001$). However, these were detected to be lower in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$). For the third-day RFU-FAM values, these were lower in Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$). Furthermore, no significant difference was detected between the disinfectant (ethyl alcohol/IPA) group and routine group ($p = 0.814$). When the first-day and third-day RFU-FAM median values were compared, the third-day median values are higher than the first-day median values in the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group, while these were detected to be lower in the Oscardia Ledovir Spray group ($p < 0.001$) (Figure 5).

When the RFU-FAM first-day median values for plate II + III that contained samples with mixed variants were examined, the values were lower in the disinfectant (ethyl alcohol/IPA) group and Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$). No significant difference was detected between the Oscardia Ledovir Spray group and disinfectant (ethyl alcohol/IPA) group ($p = 0.999$). For the third-day RFU-FAM values, these were lower in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$), and these were detected to be lower in the disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). When the first-day and third-day RFU-FAM median values were compared, the third-day median values were higher than the first-day median values in the disinfectant (ethyl alcohol/IPA) group. However, these were detected to be lower in the routine group ($p < 0.001$) and Oscardia Ledovir Spray group ($p < 0.001$) (Table 6 and Figure 5).

When the RFU-FAM first-day median values for all samples (plate I + II + II) were examined, these are lower in the disinfectant (ethyl alcohol/IPA) group and Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$). However, no significant difference was detected between the Oscardia Ledovir Spray group and disinfectant (ethyl alcohol/IPA) group ($p = 0.487$). For the third-day RFU-FAM values, these were lower in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$), and these were detected to be lower in the disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). When the first-day and third-day RFU-FAM median values were compared, the third-day median values were higher than the first-day median values in the disinfectant (ethyl alcohol/IPA) group ($p < 0.001$), while these were detected to be lower in the routine group ($p < 0.001$) and Oscardia Ledovir Spray group ($p < 0.001$) (Figure 5).

When the CT-HEX first-day median values for plate I that contained samples with the Omicron variant were examined, these were higher in the Oscardia Ledovir Spray group and disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). However, no statistically significant difference was detected between the Oscardia Ledovir Spray group and disinfectant

Table 6. Pairwise comparison for the routine, ethyl alcohol (disinfectant) and Oscardia Ledovir Spray groups

Plate	Day	Routine	Ethyl alcohol (disinfectant)	Oscardia Ledovir Spray	*p*	Pairwise comparison for the routine, ethyl alcohol (disinfectant), and Oscardia Ledovir Spray groups		
						Routine and ethyl alcohol (disinfectant)	Routine and Oscardia Ledovir Spray	Ethyl alcohol (disinfectant) and Oscardia Ledovir Spray
FAM								
I	CT 1st day	19.58 (18.14/22.45)	23.04 (20.97/26.25)	23.28 (21.55/25.89)	<0.001[f]	<0.001	<0.001	0.999
	CT 3rd day	20.70 (18.76/22.95)	23.31 (20.99/26.18)	26.06 (22.98/28.43)	<0.001[f]	<0.001	<0.001	<0.001
p-value		<0.001[w]	0.537[w]	<0.001[w]				
II + III	CT 1st day	18.76 (16.76/20.41)	20.93 (18.14/23.94)	23.04 (21.03/24.70)	<0.001[f]	<0.001	<0.001	0.005
	CT 3rd day	19.60 (17.32/21.30)	23.07 (20.21/26.28)	25.16 (22.43/27.25)	<0.001[f]	<0.001	<0.001	<0.001
p-value		<0.001[w]	<0.001[w]	<0.001[w]				
I + II + III	CT 1st day	19.12 (17.19/21.10)	21.63 (18.89/25.27)	23.13 (21.19/25.24)	<0.001[f]	<0.001	<0.001	0.038
	CT 3rd day	19.84 (17.99/21.60)	23.21 (20.40/26.28)	25.35 (22.53/27.96)	<0.001[f]	<0.001	<0.001	<0.001
p-value		<0.001[w]	<0.001[w]	<0.001[w]				
I	RFU 1st day	6,649 (5,325/7,849)	2,947 (1,021/4,812)	4,202 (3,114/5,089)	<0.001[f]	<0.001	<0.001	0.005
	RFU 3rd day	7,219 (5,760/8,135)	7,757 (3,227/11,990)	2,978 (2,104/3,955)	<0.001[f]	0.814	<0.001	<0.001
p-value		<0.001[w]	<0.001[w]	<0.001[w]				
II + III	RFU 1st day	9,562.5 (7,645/11,382)	4,367.5 (839/7,990)	4,135 (2,934/5,073)	<0.001[f]	<0.001	<0.001	0.999
	RFU 3rd day	7,305 (6,412/8,501)	5,413.5 (2,476/8,016)	2,667.5 (1,609/3,488)	<0.001[f]	<0.001	<0.001	<0.001
p-value		<0.001[w]		<0.001[w]				
I + II + III	RFU 1st day	8,084 (6,619/10,221)	3,640 (839/6,622)	4,181 (3,034/5,089)	<0.001[f]	<0.001	<0.001	0.487
	RFU 3rd day	7,272.5 (6,209/8,441.5)	5,878 (2,521/8,848)	2,759 (1,690/3,681)	<0.001[f]	<0.001	<0.001	<0.001
p-value		<0.001[w]	<0.001[w]	<0.001[w]				
HEX								
I	CT 1st day	20.17 (19.30/21.37)	21.93 (20.81/23.02)	22.12 (21.10/23.02)	<0.001[f]	<0.001	<0.001	0.999
	CT 3rd day	19.91 (18.73/20.76)	21.19 (20.41/22.34)	26.23 (25.06/28.31)	<0.001[f]	<0.001	<0.001	<0.001
p-value			<0.001[w]	<0.001[w]				
II + III	CT 1st day	20.78 (19.89/21.61)	21.92 (20.49/23.14)	22.65 (21.65/23.77)	<0.001[f]	<0.001	<0.001	<0.001
	CT 3rd day	20.10 (19.25/21.06)	23.39 (21.12/25.23)	25.51 (24.21/27.01)	<0.001[f]	<0.001	<0.001	<0.001
p-value		<0.001[w]	<0.001[w]	<0.001[w]				
I + II + III	CT 1st day	20.56 (19.51/21.58)	21.93 (20.57/23.07)	22.41 (21.48/23.51)	<0.001[f]	<0.001	<0.001	<0.001
	CT 3rd day	20.06 (19.04/21.02)	22.34 (20.69/24.55)	25.75 (24.38/27.21)	<0.001[f]	<0.001	<0.001	<0.001
p-value		<0.001[w]	<0.001[w]	<0.001[w]				

(continued)

Table 6. *(continued)*

Plate	Day	Routine	Ethyl alcohol (disinfectant)	Oscardia Ledovir Spray	*p*	Pairwise comparison for the routine, ethyl alcohol (disinfectant), and Oscardia Ledovir Spray groups		
						Routine and ethyl alcohol (disinfectant)	Routine and Oscardia Ledovir Spray	Ethyl alcohol (disinfectant) and Oscardia Ledovir Spray
I	RFU 1st day	12,103.05 ± 1,592.80	13,076.73 ± 1,909.44	11,806.29 ± 897.74	<0.001[ra]	<0.001	0.075	<0.001
	RFU 3rd day	14,230.46 ± 1,920.12	14,550.15 ± 2,039.35	11,895.25 ± 804.89	<0.001[ra]	0.252	<0.001	<0.001
p-value		0.001[t]	0.001[t]	0.478[t]				
II + III	RFU 1st day	11,689 (10,258/13,811)	13,128 (10,680/15,009.5)	11,784.5 (11,357/12,350)	<0.001[f]	<0.001	0.999	<0.001
	RFU 3rd day	13,392.5 (12,174/14,393.5)	15,181.5 (13,935/16,898)	11,661.5 (11,226/12,199)	<0.001[f]	<0.001	<0.001	<0.001
p-value		<0.001[w]	<0.001[w]	0.296[w]				
I + II + III	RFU 1st day	11,860 (10,623/13,488)	13,044 (11,031/14,717)	11,800 (11,267/12,350)	<0.001[f]	<0.001	0.999	<0.001
	RFU 3rd day	13,550 (12,517/14,717)	14,888 (13,549/16,637)	11,730 (11,270/12,234)	<0.001[f]	<0.001	<0.001	<0.001
p-value		<0.001[w]	<0.001[w]	0.700[w]				

[ra]Repeated Anova (Wilks' Lambda); Post-hoc test: LSD, [f]Friedman test (Monte Carlo); Post-hoc test: Stepwise step-down comparison, [t]Paired Samples *t*-test (Boostrap), [w]Wilcoxon Sign test (Monte Carlo) presented in median (1st quartile/3rd quartile) for non-normally distributed data, and mean ± standard deviation for normal distribution. CT, cycle terminal domain; RFU, relative fluorescence units.

(ethyl alcohol/IPA) group ($p = 0.999$). For the third-day CT FAM values, these were higher in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$), and these were higher in the disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). When the first-day and third-day CT-FAM median values were compared, the third-day median values were higher than the first-day median values in the routine group ($p < 0.001$), disinfectant (ethyl alcohol/IPA) group, and Oscardia Ledovir Spray group ($p < 0.001$) (Table 6 and Figure 4).

When the CT-HEX first-day median values for plate II + III that contained samples with mixed variants were examined, these were higher in the Oscardia Ledovir Spray group and disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$), and these were detected to be higher in the Oscardia Ledovir Spray group, when compared to the disinfectant (ethyl alcohol/IPA) group ($p = 0.001$). For the third-day CT FAM values, these were detected to be higher in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$), and these were detected to be higher in the disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). When the first-day and third-day CT-FAM median values were compared, the third-day median values were detected to be higher than the first-day median values in the routine group ($p < 0.001$), Oscardia Ledovir Spray group ($p < 0.001$), and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$) (Table 6 and Figure 4).

When the CT-FAM first-day median values for all samples (plate I + II + II) were examined, these were higher in the Oscardia Ledovir Spray group and disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$), and these were detected to be higher in the Oscardia Ledovir Spray group, when compared to the disinfectant (ethyl alcohol/IPA) group ($p = 0.001$). For the third-day CT FAM values, these were higher in the Oscardia Ledovir Spray group, routine group ($p < 0.001$), and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$), and these were detected to be higher in the disinfectant (ethyl alcohol/IPA) group, when compared to the routine group ($p < 0.001$). When the first-day and third-day CT-FAM median values were compared, the third-day median values were detected to be higher than the first-day median values in the routine group ($p < 0.001$), Oscardia Ledovir Spray group ($p < 0.001$), and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$) (Figure 4).

When the RFU-HEX first-day median values for plate I that contained samples with Omicron variants were examined, these were detected to be lower in the Oscardia Ledovir Spray group, when compared to the disinfectant (ethyl alcohol/IPA) group ($p < 0.001$). However, no significant difference was detected between the Oscardia Ledovir Spray group and routine group ($p = 0.075$), and these values were detected to be lower in the routine group, when compared to the disinfectant (ethyl alcohol/IPA) group ($p < 0.001$). For the third-day RFU-HEX values, these were lower in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$). However, no significant difference was detected between the disinfectant (ethyl alcohol/IPA) group and routine group ($p = 0.252$). When the first-day and third-day RFU-FAM median values were compared, the third-day median values were higher than the first-day median values in the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group. However, no significant difference was detected when these were compared to the Oscardia Ledovir Spray group ($p = 0.478$) (Table 6 and Figure 5).

When the RFU-HEX first-day median values for plate II + III that

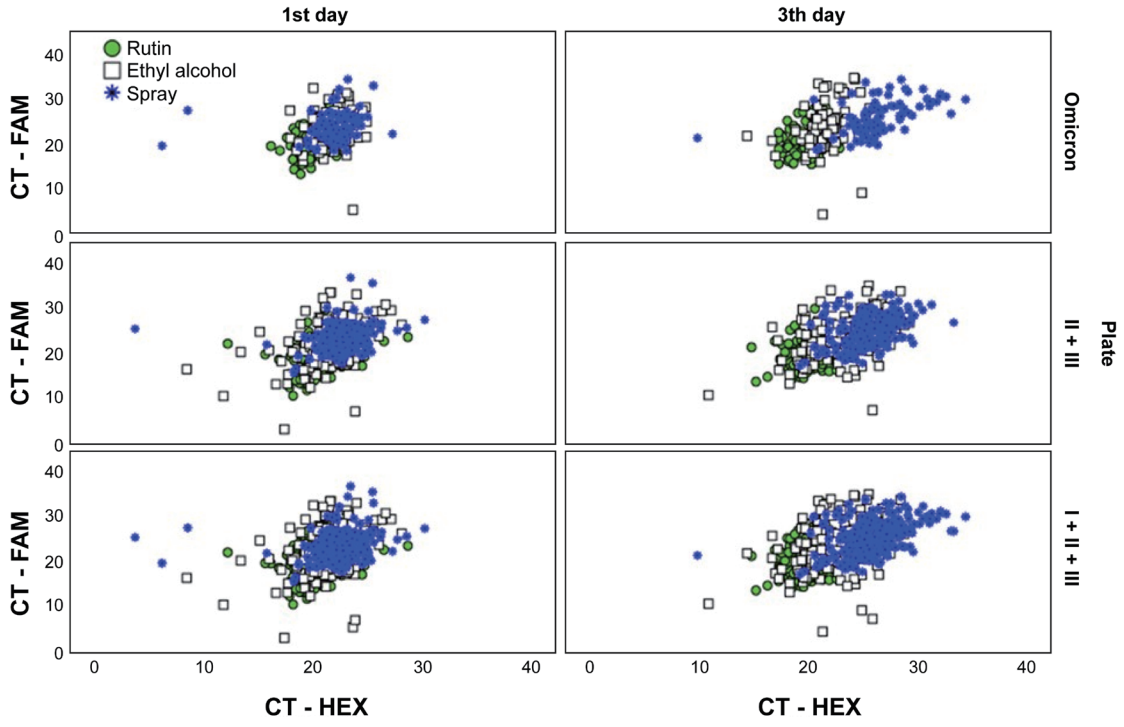

Fig. 4. Comparison of CT values for the routine, ethyl alcohol (disinfectant), and Oscardia Ledovir Spray groups (based on the FAM-HEX channel). CT, cycle terminal domain; FAM, 6-Carboxyfluorescein; HEX, phosphoramidite.

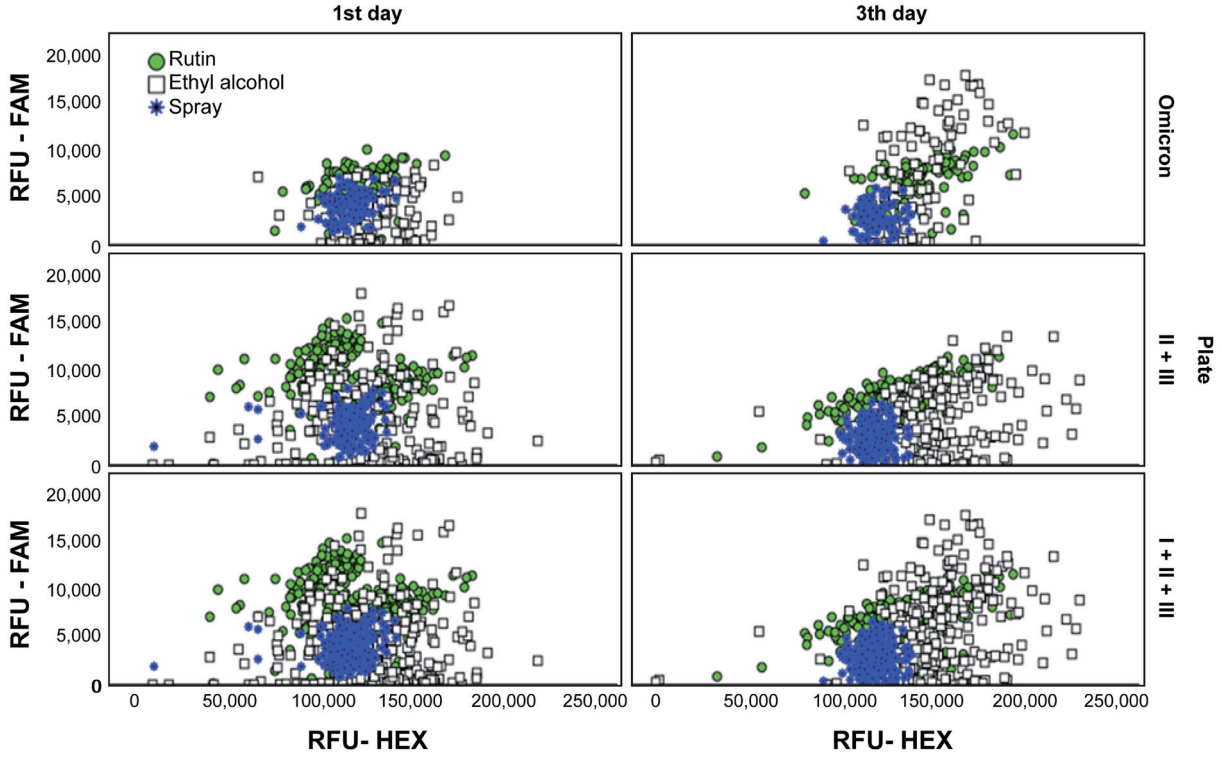

Fig. 5. Comparison of RFU values for the routine, ethyl alcohol (disinfectant), and Oscardia Ledovir Spray groups (based on the FAM-HEX channel). RFU, relative fluorescence units; FAM, 6-Carboxyfluorescein; HEX, phosphoramidite.

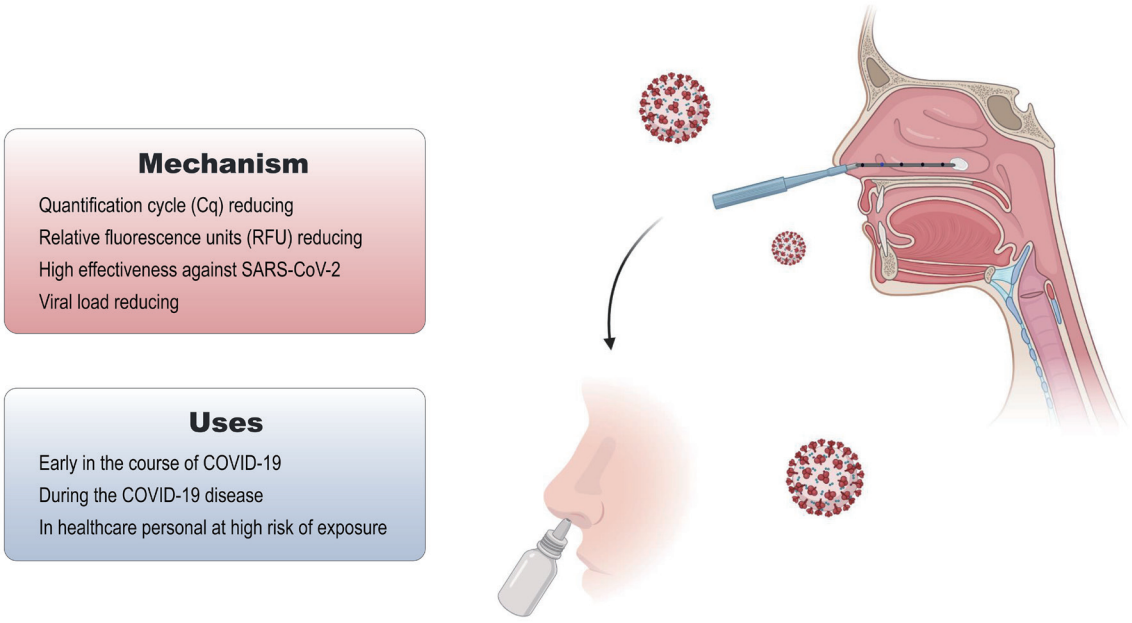

Fig. 6. General mechanism of the Oscardia Ledovir Spray and its usage (Designed via BioRender).

contained samples with mixed variants were examined, these were lower in the Oscardia Ledovir Spray group and routine group, when compared to the disinfectant (ethyl alcohol/IPA) group ($p < 0.001$). However, no significant difference was detected between the Oscardia Ledovir Spray group and routine group ($p = 0.999$). For the third-day RFU-HEX values, these were lower in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$), and these were detected to be lower in the routine group, when compared to the disinfectant (ethyl alcohol/IPA) group ($p < 0.001$). When the first-day and third-day RFU-FAM median values were compared, the third-day median values were higher than the first-day median values in the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group. However, no significant difference was detected in the Oscardia Ledovir Spray group ($p = 0.296$) (Table 6 and Figure 5).

When the RFU-HEX first-day median values for all samples (plate I + II + II) were examined, these were lower in the Oscardia Ledovir Spray group and routine group, when compared to the disinfectant (ethyl alcohol/IPA) group ($p < 0.001$). However, no significant difference was detected between the Oscardia Ledovir Spray group and routine group ($p = 0.999$). For the third-day RFU-HEX values, these were lower in the Oscardia Ledovir Spray group, when compared to the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group ($p < 0.001$), and these were detected to be lower in the routine group, when compared to the disinfectant (ethyl alcohol/IPA) group ($p < 0.001$). When the first-day and third-day RFU-FAM median values were compared, the third-day median values were higher than the first-day median values in the routine group ($p < 0.001$) and disinfectant (ethyl alcohol/IPA) group. However, no significant difference was detected in the Oscardia Ledovir Spray group ($p = 0.700$) (Table 6 and Figure 5).

Discussion

The results obtained in the present study and the statistical data show that ethyl alcohol (80% ethyl alcohol + 75% isopropyl alcohol mixed disinfectant) disintegrates the lipoproteins in the external surface structure of SARS-CoV-2, preventing the virus from reproducing. A number of studies have been conducted on the effectiveness of ethanol in inhibiting the reproductive characteristics of the virus. Furthermore, various products, such as disinfectants or surface cleaners, have been designed for this purpose. For studies conducted on the first day of the Oscardia Ledovir Spray, it could be clearly observed that there was a significant increase in CT values and a serious suppression in RFU values, both in the positive patient samples that containing the Omicron variants and in all other positive patient samples, when compared to the routinely checked samples with the Omicron variant and other COVID-19 variants, and samples with the Omicron variant, which were treated with ethyl alcohol (80% ethyl alcohol + 75% isopropyl alcohol containing disinfectant). For the CT and RFU values, and statistical values for studies conducted on the first day and third day (Figures 4 and 5), it can be considered that the Oscardia Ledovir Spray prevents the reproduction of SARS-CoV-2, similar to ethyl alcohol (80% ethyl alcohol + 75% isopropyl alcohol containing disinfectant), by destroying the lipoprotein layer and genetic material (RNA structure) of the contacted SARS-CoV-2. Furthermore, based on the results for studies conducted on the third day, the effect of stopping the virus production and the definite reduction in replication power increasingly continued within a period of 72 hours.[27]

Elif *et al*. determined how long the positivity of COVID-19 disease could last in the viral transport medium solution at different temperatures (+4°C and -20°C). At the end of 10 days, the positivity was rejected based on the research.[28]

Evidence through the results of clinical studies in all sectors of society, particularly health personnel, can help in the establishment of good measures against disease transfer among individuals. The Oscardia Ledovir Spray can be used both prophylactically and during the disease, due to its effect mechanisms (Figure 6). Furthermore, its early use for COVID-19 patients can reduce its virulence, enabling the patient to easily go through the disease.[29–33]

Future directions

In the present clinical study, it was found that the Oscardia Ledovir Spray, which is clearly effective on structurally strong viruses, such as SARS-CoV-2, can be used both for the treatment of various viruses, such as other corona viruses, influenza viruses, rhinovirus, and similar viral and bacterial pathogens. The investigators consider that this can provide both prophylactic and therapeutic benefits, contributing to humanity in processes that range from simple infections to serious diseases.

A new clinical trial has been planned and initiated for the clinical effectivity and probable adverse effect of the Oscardia Ledovir Spray on volunteers. The present study protocol was reviewed and approved on 28 September 2022 by the Ethics committee, with meeting number 10 of the Uskudar University, Istanbul, Turkey (Approval date: 30 September 2022; Subject no: 61351342/Eylül 2022-26). After the evaluation of the data, the results will be shared with the scientific world in a new article.

Conclusions

The prophylactic use of the Oscardia Ledovir Spray prevents the reproduction of the virus through a mechanism that resembles the effect mechanism of ethyl alcohol (80% ethyl alcohol + 75% isopropyl alcohol containing disinfectant).

The basic mechanism of the antiviral activities of the Oscardia Ledovir Spray as an antiviral agent includes the following: (a) due to its barrier effect, it prevents the binding of the virus to the host cells, and protects the host receptors against the virus; (b) it inhibits the lipoprotein layer and genetic materials of the virus, and stops the viral replication through contact effect.

Considering that the Oscardia Ledovir Spray has an effective mechanism similar to ethyl alcohol and disinfectants, this can be applied for SARS-CoV-2, as well as for viral, bacterial and infection cases. Due to its similar effect mechanism, the Oscardia Ledovir Spray can be used as an alternative when ethyl alcohol or disinfectants with similar content cannot be used for some reason.

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Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

MSK and YA developed the protocol, determined the method, conducted the experiments, and analyzed the data; YA created the administrative process and vouched for the entire experimental process; MSK vouched for the entire experimental process and the products used; NPC wrote and revised the article; YU revised the article and vouched for it; SDT, HS, NH, NU and GKG provided support on the academic consultancy for the research process and vouched for it.

Statement of ethics

The research was ethically conducted in accordance with the World Medical Association Declaration of Helsinki. The study protocol was reviewed and approved by the Republic of Turkey, Ministry of Health, COVID-19 Scientific Research Studies (Approval no: YakupArtik-2022-02-26T01_50_43), and the Ethics Committee of Uskudar University.

Data sharing statement

All data generated and analyzed in the study are available from the corresponding author upon request.

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