



People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
Abou Bekr Belkaid University of Tlemcen
Faculty of Natural and Life Sciences and Earth and Universe Sciences
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THESIS

Submitted in fulfilment of the requirements for the Degree of

Doctorate in Microbiology

Registered under Ministerial Decree No. 1275 relating to Innovative Projects (**Patent**)

Presented by

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In Silico Design of a Nanobiotherapeutic Approach Based on Phytomolecules and Colloidal Metals Targeting SARS-CoV-2 with Epidemiological Insights into COVID-19

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Academic Year 2025-2026

Author's Declaration

I hereby certify that the work presented in this dissertation has been conducted in full compliance with the University's Regulations and Code of Practice for Research Degree Programs. This dissertation has not been submitted, in whole or in part, for any other academic qualification or award. Unless explicitly referenced within the text, the content represents my own independent work. Contributions from collaboration or assistance by others are properly acknowledged. All views and interpretations expressed here are solely those of the author.

20/01/2026

A handwritten signature in black ink, reading "Ilyes Zatta", is enclosed within a thin, hand-drawn oval border. The signature is written in a cursive style.

Author's Biographical Sketch

Ilyes Zatla is a microbiologist passionate about studying viruses, especially coronaviruses, and developing innovative antiviral strategies. He earned his Bachelor's degree in Molecular Biology and his Master's degree in Fundamental Microbiology from the University of Abou Bekr Belkaid Tlemcen, Algeria.

Throughout his academic career, Ilyes has been deeply involved in virology and infectious disease research, gaining expertise in epidemiology, population genetics, and mechanisms of antimicrobial resistance. His work later expanded to include nanotechnology-based antiviral strategies, emphasizing the potential of natural compounds and nanoparticles to inhibit SARS-CoV-2 and other emerging viral threats.

Alongside his laboratory and computational research, Zatla has actively contributed to the scientific community by presenting his findings at international, indexed conferences and publishing in MDPI, De Gruyter and Bentham Science journals. His multidisciplinary approach combines microbiology, virology, and nanomedicine, showing his commitment to developing effective antimicrobial therapeutics.

Driven by his fascination with viruses and their intricate mechanisms, Mr. Zatla Ilyes aims to explore the intersection of virology, nanomedicine, and bioinformatics to advance next-generation antiviral therapies. His long-term goal is to advance nanotechnology-driven drug-delivery systems and to help develop innovative strategies to fight infectious diseases worldwide.

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Deity Divine

*Oh God, in the shadows of your love I lay, and by your rules I humbly obey.
Beauty of science, your work displayed. Marvels to behold, in wonder I sway.
Symphony of life, electrifying my way. Mysteries of time, guiding my soul astray.*

*Oh God, grant me knowledge, grant me wisdom, make my vision clear.
I did my best with what I had, and with what I know, without any fear.*

*In the radiance of daylights and the gloom of nights, your presence stays.
With me, bound to Thee, till the end of my days.*

- Ilyes Zatla

Dedication

To my parents.

Our Genesis — a Dual Origin: Divinely Bestowed, Parentally Nurtured.

*To my Mother, for her Appreciation & Enlightenment. To my Father, for his Moral & Financial
Support.*

Love Shared. Moments Lived. Life Embraced.

Acknowledgments

With deep respect and appreciation, I thank my supervisor, Mrs. Boublenza Lamia, Professor at the University of Tlemcen, for their mentorship, guidance, and educational outreach.

I would like to express my sincere gratitude to Mrs. Hassaine Hafida, Professor at the University of Tlemcen, President of the Jury, for the honour of presiding over the defence and for their thoughtful encouragement.

I would like to warmly thank the Examiners: Mrs. Abdelli Imane, Professor at the Higher School of Applied Sciences of Tlemcen; Mrs. Belhadj Amina, Associate Professor at the University of Oran; and Miss. Nouari Wafa, Associate Professor at the University of Tlemcen, for their rigorous evaluation, constructive remarks, and valuable suggestions that helped refine and strengthen this thesis.

I wish to extend my sincere thanks to Mrs. Meziane Hassiba, Professor at the University of Tlemcen, for overseeing the 1275 project and the related patent, for their insightful advice and collaboration.

I owe special thanks to Mrs. Lemerini Wafaa, Ms. Triqui Chahinez, and Mrs. Bellifa Samia, Associate Professors at the University of Tlemcen, who have paved the way for me throughout this academic journey. Their confidence in my abilities and willingness to place me at the forefront of valuable academic and professional experiences have had a profound impact on my growth.

I gratefully acknowledge the professors who hosted and supervised me during my short internships abroad. Their guidance and openness to academic exchange played an important role in shaping my ideas within a respectful and professional environment.

Mr. Ghassan Dbaiibo, Professor at the American University of Beirut, Lebanon.

Mr. Yassine Hadi, Professor at Qatar University, Qatar.

Mr. Emrah Eroğlu, Professor at Istanbul Medipol University, Turkey.

Mr. Jorge Gonçalves, Professor at the University of Porto, Portugal.

Mr. Enrico Palermo, Professor at the Institut Pasteur in Italy, Cenci Bolognetti Foundation, Italy.

I am profoundly grateful to all my teachers — from my earliest days in kindergarten to my university years — whose guidance, high expectations, and, at times, difficult lessons shaped my curiosity, discipline, and inner strength. Both support and adversity played decisive roles in shaping my resilience and deepening my commitment to learning. I am especially thankful to the professors who not only taught me once but also later welcomed me as a colleague, allowing me to teach, present, and contribute meaningfully alongside them.

“To each of you, I offer my sincere thanks and utmost respect”

Abstract

The global COVID-19 pandemic has highlighted the urgent need to develop both innovative antiviral therapies and effective public health strategies. This thesis adopts a dual approach to address this challenge: (I) an epidemiological evaluation of the COVID-19 vaccination campaign conducted in Tlemcen, Algeria, between 2020 and 2022, and (II) an *in silico* investigation of novel antiviral formulations based on natural compounds supported by nanobiotechnological tools.

The first part relies on a retrospective analysis of regional vaccination data, focusing on five major vaccines: *Sputnik V*, *AstraZeneca*, *Sinovac*, *Sinopharm*, and *Janssen*. Descriptive statistical methods were applied to assess vaccination coverage, demographic trends, and logistical management. The second part consists of an *in silico* study using molecular modelling techniques, including molecular docking and molecular dynamics simulations, to evaluate the interactions of gold and silver nanoparticles conjugated to β -escin and Rutin with key SARS-CoV-2 target proteins.

The epidemiological analysis revealed marked disparities in vaccination coverage, particularly among younger populations, as well as challenges related to vaccine hesitancy, logistics, and stock management. The *in silico* findings demonstrated strong, stable interactions between the nanobiological conjugates and viral proteins, suggesting promising antiviral activity and potential immunomodulatory effects.

These findings underscore the need to enhance communication and organisation in vaccination campaigns while highlighting the potential of nanobiotechnological approaches combining natural compounds and colloidal metals for the development of innovative antiviral formulations, particularly for nasal prophylactic applications. This integrated framework contributes to both pandemic preparedness and future therapeutic development.

Keywords: SARS-CoV-2, COVID-19, Nanoparticles, Phytomolecules, *In Silico* Analysis, Public Health.

Résumé

La pandémie mondiale de COVID-19 a mis en évidence la nécessité urgente de développer des thérapies antivirales innovantes et d'optimiser les stratégies de santé publique. Cette thèse adopte une approche double pour répondre à cette problématique : (I) une évaluation épidémiologique de la campagne de vaccination contre la COVID-19 dans la wilaya de Tlemcen (Algérie) entre 2020 et 2022, et (II) une étude *in silico* explorant de nouvelles formulations antivirales à base de composés naturels et de nanotechnologies.

La première partie repose sur une analyse rétrospective des données régionales de vaccination, portant sur cinq vaccins majeurs : *Sputnik V*, *AstraZeneca*, *Sinovac*, *Sinopharm*, et *Janssen*. Des analyses statistiques descriptives ont permis d'évaluer la couverture vaccinale, les tendances démographiques et la gestion logistique des campagnes. La seconde partie est une investigation *in silico* utilisant la modélisation moléculaire pour évaluer l'interaction entre des nanoparticules d'or et d'argent, conjuguées à la β -escine et à la rutine, et les protéines cibles du SARS-CoV-2.

Les résultats de l'étude épidémiologique révèlent des disparités marquées dans la couverture vaccinale, notamment chez les jeunes, ainsi que des difficultés liées à l'hésitation vaccinale et à la logistique. Les résultats *in silico* montrent des interactions fortes et stables entre les complexes nano-biologiques et les protéines virales, suggérant une activité antivirale prometteuse et un potentiel d'effet immunomodulateur.

Ces travaux mettent en lumière, d'une part, la nécessité de renforcer la communication et la planification des campagnes de vaccination, et, d'autre part, le potentiel des approches nano-biotechnologiques combinant des composés naturels et des métaux colloïdaux pour le développement de formulations antivirales innovantes, notamment à usage prophylactique nasal. Cette approche intégrée apporte une contribution complémentaire à la préparation face aux pandémies futures.

Mots-clés: SARS-CoV-2, COVID-19, Nanoparticules, Phytomolécules, Analyse *In Silico*, Santé Publique.

الملخص

لقد سلّطت جائحة كوفيد-19 العالمية الضوء على الحاجة الملحة لتطوير علاجات مضادة للفيروسات مبتكرة واستراتيجيات فعالة للصحة العامة. تتبنى هذه الأطروحة نهجًا مزدوجًا لمواجهة هذا التحدي: (I) تقييم وبائي لحملة التطعيم ضد كوفيد-19 التي أُجريت في تلمسان، الجزائر، بين عامي 2020 و2022، و(II) دراسة حاسوبية لتركيبات جديدة مضادة للفيروسات مبنية على مركبات طبيعية مدعومة بأدوات التكنولوجيا الحيوية النانوية.

يعتمد الجزء الأول على تحليل رجعي لبيانات التطعيم الإقليمية، مع التركيز على خمس لقاحات رئيسية: سبوتنيك V، أسترازينيكا، سينوفاك، سينوفارم، وجانسين. طُبقت أساليب إحصائية وصفية لتقييم تغطية التطعيم، والاتجاهات الديموغرافية، والإدارة اللوجستية. يتكون الجزء الثاني من دراسة حاسوبية باستخدام تقنيات النمذجة الجزيئية، بما في ذلك الالتحام الجزيئي ومحاكاة الديناميكيات الجزيئية، لتقييم تفاعل الجسيمات النانوية الذهبية والفضية والمقترنة بـ β -escin وRutin ضد بروتينات هدف SARS-CoV-2 الرئيسية.

كشف التحليل الوبائي عن تفاوتات ملحوظة في تغطية التطعيم، لا سيما بين الفئات السكانية الأصغر سنًا، بالإضافة إلى تحديات تتعلق بالتردد في تلقي اللقاح، واللوجستيات، وإدارة المخزون. أظهرت النتائج الحاسوبية تفاعلات قوية ومستقرة بين المقترنات النانوية الحيوية والبروتينات الفيروسية، مما يشير إلى نشاط مضاد للفيروسات واعد وتأثيرات مُعدّلة للمناعة.

تؤكد هذه النتائج على الحاجة إلى تعزيز التواصل والتنظيم في حملات التطعيم، مع تسليط الضوء على إمكانات مناهج التكنولوجيا الحيوية النانوية التي تجمع بين المركبات الطبيعية والمعادن الغروية لتطوير تركيبات مُبتكرة مضادة للفيروسات، لا سيما لتطبيقات الوقاية الأنفية. يُسهم هذا الإطار المتكامل في كل من التأهب للجائحة وتطوير العلاجات المستقبلية.

الكلمات المفتاحية: SARS-CoV-2، كوفيد-19، الجسيمات النانوية، الجزيئات النباتية، تحليل حاسوبي، الصحة العامة.

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List of Abbreviations

ACE2	Angiotensin-Converting Enzyme 2
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
AgNPs	Silver Nanoparticles
ARDS	Acute Respiratory Distress Syndrome
AuNPs	Gold Nanoparticles
β-Escin	Beta-Escin
COVID-19	Coronavirus Disease 2019
Ct	Cycle threshold
CT	Computed Tomography
DFT	Density Functional Theory
EC50	Half-maximal Effective Concentration
EMMPRIN	Extracellular Matrix Metalloproteinase Inducer
EPSP	Public Local Health Care Establishment
GDP	Gross Domestic Product
H-bond	Hydrogen Bond
HCovs	Human Coronaviruses
IC50	Half-maximal Inhibitory Concentration
IFNs	Type I Interferons
IL	Interleukins
Kd	Dissociation Constant
LNPs	Lipid Nanoparticles
LSPR	Localized Surface Plasmon Resonance

MD	Molecular Dynamics
MHC	Major Histocompatibility Complex
MM-PBSA	Molecular Mechanics Poisson-Boltzmann Surface Area
MM/GBSA	Molecular Mechanics Generalized Born Surface Area
Mpox	Monkeypox Disease
Mpro	Main Protease
NK	Natural killer
NPs	Nanoparticles
NRP1	Neuropilin-1
NSP	Non-Structural Protein
PBSA	Poisson-Boltzmann Surface Area
PDB	Protein Data Bank
PTSD	Post-Traumatic Stress Disorder
PoGNPs	Porous Gold Nanoparticles
QSAR	Quantitative Structure-Activity Relationship
RBD	Receptor-Binding Domain
RdRp	RNA-dependent RNA Polymerase
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
ROS	Reactive Oxygen Species
RT-PCR	Real-time Reverse Transcription Polymerase Chain Reaction
S Protein	Spike Protein
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
sIgA	Secretory IgA

List of Abbreviations

TNFs	Tumour Necrosis Factors
TMPRSS2	Transmembrane Serine Protease 2
VOC	Variant of Concern
VOI	Variant of Interest
WHO	World Health Organisation



Introduction

Introduction

The COVID-19 (Coronavirus Disease 2019) pandemic, caused by the novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has presented unprecedented challenges to global health, economic stability, and public policy since it emerged in late 2019 [(Abodunrin *et al.*, 2020); (Onyeaka *et al.*, 2021); (Filip *et al.*, 2022); (Maqbool *et al.*, 2023); (Cati, 2024)]. As of 2025, it has led to hundreds of millions of confirmed cases and millions of deaths worldwide, along with significant disruptions to healthcare systems, trade, education, and daily life [(Benach & Muntaner, 2025); (Chen *et al.*, 2025); (Lippi *et al.*, 2025); (Puigdemívol-Sánchez *et al.*, 2025)].

The swift development and deployment of the viral disease vaccines mark a significant scientific milestone. However, the ongoing emergence of viral variants with higher transmissibility and partial immune escape, along with waning immunity after vaccination and persistent vaccine hesitancy, continues to hinder global efforts to control the virus [(Gong *et al.*, 2022); (Firouzabadi *et al.*, 2023); (Khoury *et al.*, 2023); (Saad-Roy *et al.*, 2024); (Wang & Huang, 2024); (Prasad & Makary, 2025)]. These issues highlight the urgent need for additional preventive and therapeutic strategies that can work together with vaccination campaigns to provide lasting protection and prepare for future viral threats [(Fries *et al.*, 2021); (Ghattas *et al.*, 2021); (Firdaus *et al.*, 2025); (Goswami *et al.*, 2025); (Navabhatra *et al.*, 2025)].

Nanobiotechnology and natural bioactive compounds are promising options for developing new antiviral solutions. Metallic nanoparticles, especially gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs), have unique physicochemical and antimicrobial properties that can be tailored for drug delivery, viral inhibition, and immune modulation. At the same time, plant-derived biomolecules such as Beta-Escin (β -Escin), a triterpenoid saponin from horse chestnut seeds, and Rutin, a polyphenolic flavonoid glycoside found in many medicinal plants, show significant anti-inflammatory and antiviral effects. Combining these nanoscale and natural bioactive strategies may open new avenues for broad-spectrum antiviral therapies that are both effective and safe.

At the same time, understanding the epidemiological dynamics of COVID-19 remains essential for optimizing vaccination campaigns and public health strategies. Local investigations,

especially in developing and middle-income countries such as Algeria, provide valuable insights into vaccine uptake, distribution challenges, and behavioural factors that influence immunization success. Such data-driven assessments are crucial for enhancing pandemic response and strengthening health system resilience.

In this context, the present thesis integrates two complementary lines of investigation:

- An epidemiological analysis of the COVID-19 vaccination campaign in Tlemcen, Algeria, focusing on coverage, logistics, and behavioural factors influencing vaccine acceptance.
- A nanobiotechnological investigation exploring conjugates of natural biomolecules with gold and silver nanoparticles as potential antiviral candidates against the novel coronavirus.

By bridging population-level epidemiological insights with molecular-level antiviral design, this thesis aims to provide a holistic framework for pandemic management, one that integrates public health surveillance with nanobiotechnological innovation to support the development of adaptive strategies uniting prevention, preparedness, and therapeutic response against current and future viral threats.

In alignment with these objectives, it is hypothesized that (i) key determinants influencing vaccination uptake in Tlemcen can be identified through contextual epidemiological analysis, and (ii) biosynthesized Au–Ag nanoconjugates with β -Escin and Rutin possess inhibitory potential against major SARS-CoV-2 targets, thereby supporting their consideration as prospective antiviral candidates.

Chapter 1:

State of the Art

State of the Art

Biology is a vast and multifaceted discipline encompassing the mechanisms that govern life, from molecular processes to complex ecological systems. Within this broad field, microbiology holds a central and indispensable position, offering critical insights into microbial diversity, host interactions, and biotechnological applications. Microorganisms (e.g., bacteria, viruses, fungi, and archaea) play fundamental roles in maintaining ecological balance and driving biomedical innovation. Modern microbiology extends far beyond the identification of microbial species; it integrates genomics, metabolic pathway analysis, virulence mechanisms, and host–pathogen interactions, all of which shape advancements in medicine and biotechnology [(Russel *et al.*, 2020); (Campbell *et al.*, 2024); (CAN group, 2025)].

A major contribution of microbiology to modern biomedical science lies in its central role in infectious disease research and antimicrobial development. The emergence and resurgence of pathogens such as SARS-CoV-2, Monkeypox virus, *Vibrio cholerae*, *Neisseria gonorrhoeae*, and *Candida auris* continue to challenge global health systems, emphasizing the need for sustained innovation in therapeutic strategies. In response, microbiologists are increasingly exploring advanced nanotechnological approaches, bioactive natural compounds, and other integrative interventions that offer enhanced specificity and efficacy against evolving pathogens [(Boyd, 2023); (Mong, 2024); (Awasthi, 2025)]. Collectively, these developments highlight microbiology’s vital role in shaping the next generation of antimicrobial and antiviral therapies.

1. SARS-CoV-2 Virus: Biological and Epidemiological Overview

1.1. Emergence and Global Spread

The novel coronavirus was first identified in December 2019 in Wuhan, Hubei Province, China, following a cluster of unexplained pneumonia cases epidemiologically linked to the Huanan seafood market. Local public health authorities initiated an investigation, and the World Health Organisation (WHO) was officially notified. Within weeks, the virus spread worldwide, prompting the WHO to declare COVID-19 a pandemic on March 11, 2020. Despite the rapid implementation of public health interventions, the virus continued to spread extensively across continents [(Mohan & Nambiar, 2020); (Kumar *et al.*, 2021); (Worobey *et al.*, 2022); (Zatla *et al.*, 2022b)]. Although mass vaccination campaigns began in early 2021, global case numbers and mortality

continued to rise. By November 2021, the pandemic had resulted in more than 250 million confirmed infections and 5 million deaths. As of August 2024, cumulative estimates exceed 775 million confirmed cases and 7 million deaths worldwide, underscoring the sustained impact of this pathogen on global health (Maison *et al.*, 2025).

1.2. Structure and Genome Organisation

SARS-CoV-2 is a novel virus belonging to the family Coronaviridae, genus Betacoronavirus, within the subfamily Orthocoronavirinae. It is an enveloped, positive-sense, single-stranded RNA virus with a genome of approximately 29.9 kilobases, making it one of the largest RNA viral genomes known. Virions are spherical to pleomorphic, typically measuring 80–120 nm in diameter. Their distinctive crown-like morphology is created by the protruding spike (S) glycoproteins, a hallmark of coronaviruses (Figure 1).

The viral genome encodes four major structural proteins: the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins, as well as numerous non-structural and accessory proteins (nsps). These components orchestrate viral entry, replication, assembly, and mechanisms of immune evasion, forming the basis of the virus's pathogenicity (Figure 2) [(Hasöksüz *et al.*, 2020); (Creesh *et al.*, 2021); (Zatla *et al.*, 2021a, 2021b); (Lamers & Haagmans, 2022); (Markov *et al.*, 2023); (Steiner *et al.*, 2024); (Yuan & Wilson, 2025)].

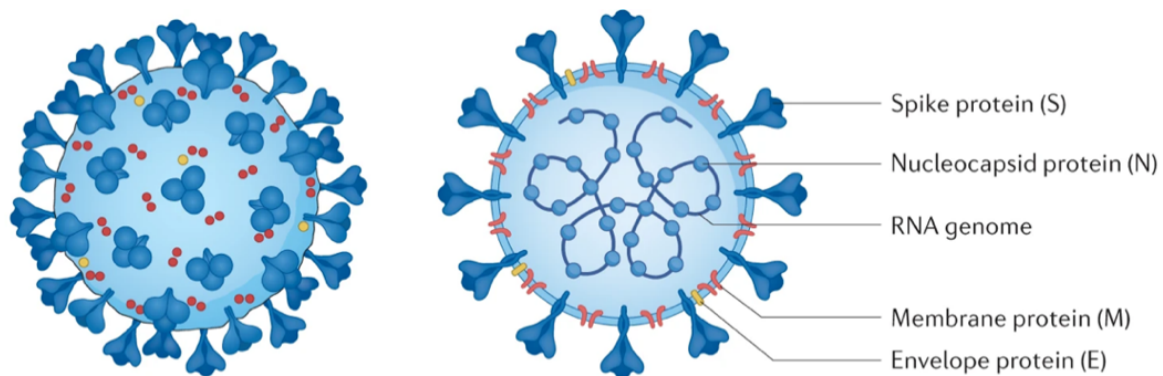


Figure 1. SARS-CoV-2 Structural Proteins

spike protein (S), nucleocapsid protein (N), membrane protein (M) and envelope protein (E) (Reproduced from Lamers & Haagmans, 2022).

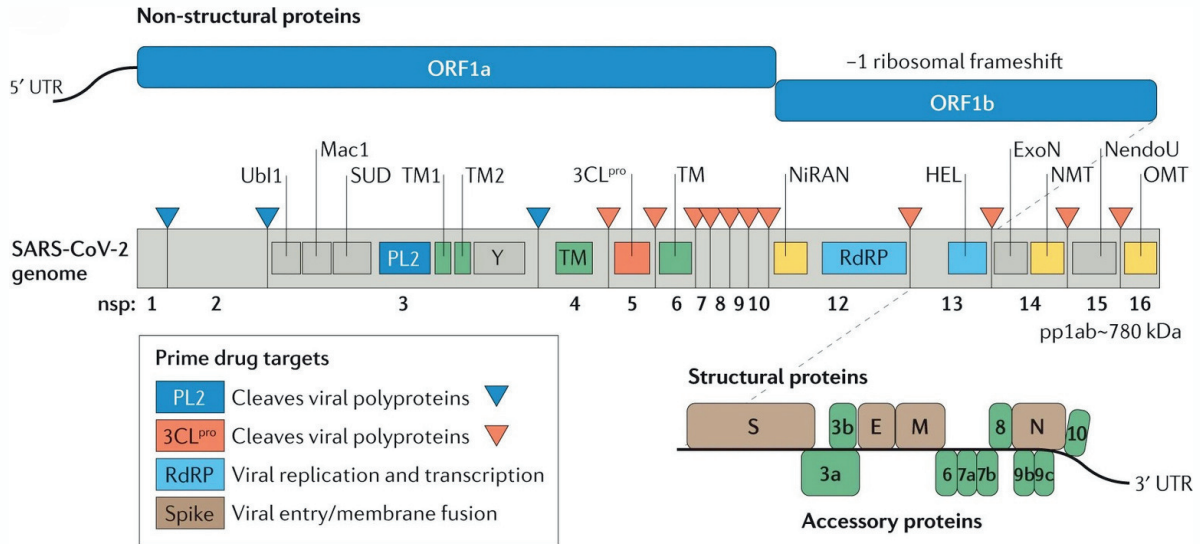


Figure 2. SARS-CoV-2 Genome Organisation

The first part of the SARS-CoV-2 genomic RNA, encoding non-structural proteins (NSPs). The second part of the RNA genome encodes mainly four structural proteins. Functional domains are shown in rectangles, in addition to several accessory proteins (Adapted from Yang & Rao, 2021).

1.3. Emergence and Classification of Viral Variants

Since its emergence, the viral pathogen has accumulated genetic mutations, leading to the formation of multiple variants with distinct epidemiological characteristics. The WHO established two principal classification categories (**Table 1**):

- Variants of Interest (VOIs), including Eta, Iota, Kappa, Lambda, and Mu, exhibited mutations potentially affecting viral behaviour, such as transmissibility or immune escape, though their global impact remained limited or inconclusive.
- Variants of Concern (VOCs), notably Alpha, Beta, Gamma, Delta, and Omicron, demonstrated increased transmissibility, immune evasion, and/or heightened disease severity. These variants significantly shaped the pandemic trajectory and influenced vaccine strategies and public health responses.

SARS-CoV-2's ongoing genetic diversification highlights its adaptability and continues to challenge surveillance, therapeutic development, and global pandemic control efforts [(Chakraborty *et al.*, 2021); (Choi & Smith, 2021); (Tamesh *et al.*, 2021); (Thye *et al.*, 2021); (Erkihun *et al.*, 2024)].

Table 1: WHO Classification of SARS-CoV-2 Variants (*Reproduced from Mistry et al., 2022*).

WHO Classification	WHO Label (Present Name)	PANGO Lineage Name	Earliest Detection
VOC	Alpha	B.1.1.7	United Kingdom, Sep-2020
	Beta	B.1.351	South Africa, May-2020
	Gamma	P.1	Brazil, Nov-2020
	Delta	B.1.617.2	India, Oct-2020
	Omicron	B.1.1529	South Africa, November 2021
VOI	Epsilon	B.1.427/B.1.429	United States of America, Mar-2020
	Zeta	P.2	Brazil, Apr-2020
	Eta	B.1.525	Multiple countries, Dec-2020
	Theta	P.3	Philippines, Jan-2021
	Iota	B.1.526	United States of America, Nov-2020
	Kappa	B.1.617.1	India, Oct-2020

*Pango Lineage Nomenclature: provisional rules for naming recombinant lineages

1.4. Life Cycle and Pathogenesis of SARS-CoV-2

The infectious agent primarily targets the respiratory tract but exhibits broad organotropism, affecting the cardiovascular, neurological, renal, and gastrointestinal systems. Infection begins when the viral spike (S) glycoprotein binds to host cell receptors to initiate entry. The S protein consists of two functional subunits (**Figure 3**): S1, responsible for receptor recognition, and S2, which mediates fusion between the viral envelope and the host cell membrane. The major entry receptor is angiotensin-converting enzyme 2 (ACE2), whose distribution across multiple tissues partly accounts for the virus's systemic manifestations [(Harrison *et al.*, 2020); (Zatla *et al.*, 2022a, 2022b)].

Efficient viral entry requires proteolytic priming of the S protein at two cleavage sites. The S1/S2 junction is typically cleaved by furin-like proteases, whereas cleavage at the S2' site is predominantly mediated by TMPRSS2, which facilitates direct membrane fusion and enhances infectivity. In addition to ACE2 and TMPRSS2, several alternative or auxiliary host factors have been proposed. CD147 (Basigin/EMMPRIN) has been suggested as an alternative receptor, although supporting evidence remains debated [(Wang *et al.*, 2020); (Fenizia *et al.*, 2021)]. Neuropilin-1 (NRP1) may act as a co-factor enhancing entry, particularly in tissues with low ACE2 expression [(Cantuti-Castelvetri *et al.*, 2021); (Gudowska-Sawczuk & Mroczko, 2021)].

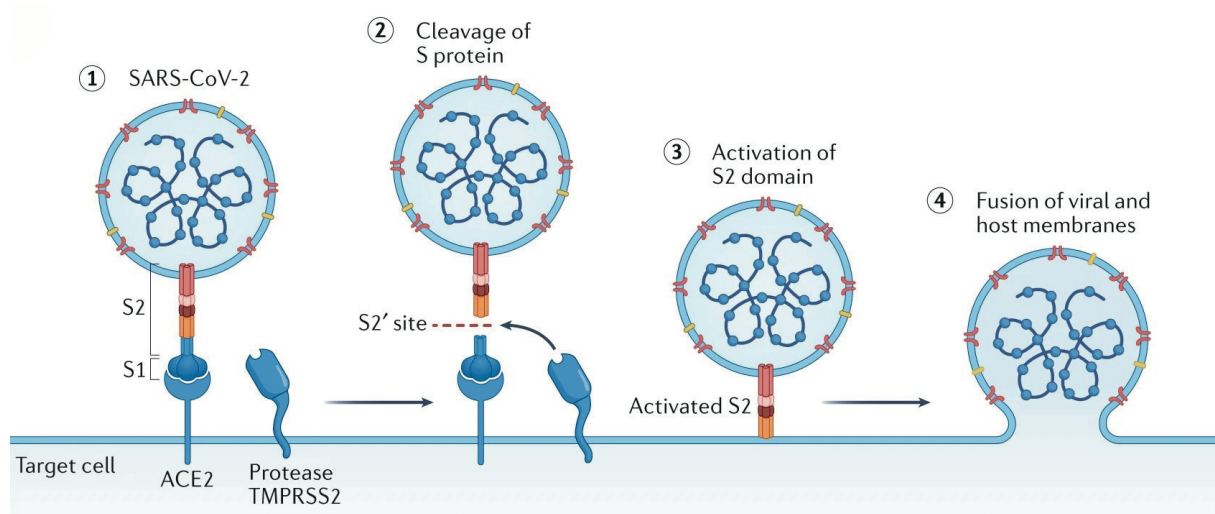


Figure 3. Mechanism of SARS-CoV-2 Cell Entry via the Spike (S) Protein

The S protein attaches to the receptor angiotensin-converting enzyme 2 (ACE2) on the host cell using the S1 domain (stage 1). This allows TMPRSS2 to cleave the S protein (stage 2), thereby activating the S2 domain for fusion (stage 3). Activated S2 fuses viral and host lipid bilayers, leading to deposition of the viral positive-sense, single-stranded RNA genome into the host cell (stage 4) (Reproduced from Lamers & Haagmans, 2022).

Once fusion occurs, the viral genome is released into the cytoplasm, where it undergoes translation, polyprotein cleavage, replication via the viral RNA-dependent RNA polymerase (RdRp), and assembly of new virions (**Figure 4**). Collectively, the entry receptors, proteases, and replication complexes described here define a set of rational targets for direct-acting antivirals and for nanobiotherapeutic systems designed to interfere with viral attachment, fusion, or replication.

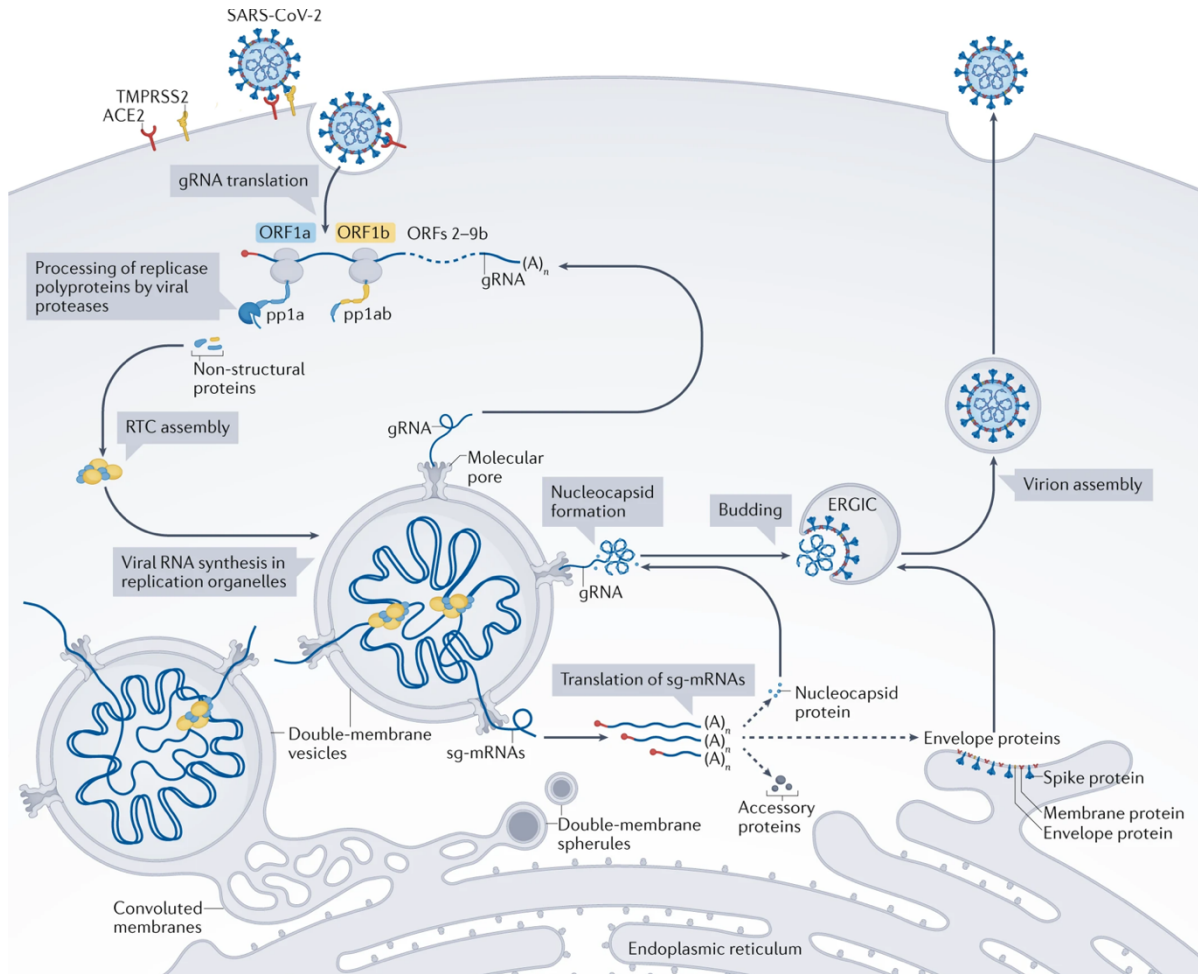


Figure 4. SARS-CoV-2 Infection Cycle and Replication Mechanism

After entry, the viral genomic RNA (gRNA) is released and translated into two replicase polyproteins (pp1a and pp1ab), which are cleaved by viral proteases into non-structural proteins that form replication–transcription complexes (RTCs). These complexes drive RNA synthesis inside virus-induced vesicles, generating genomic and subgenomic RNAs (sg-mRNAs). The sg-mRNAs encode structural (S, M, E, N) and accessory proteins. Genomic RNA is either replicated or packaged with nucleocapsid (N) protein. Viral assembly occurs in the ER–Golgi intermediate compartment (ERGIC), where virions acquire their envelope. (Adapted from Malone et al., 2022).

2. Host Immune Responses and the Pathophysiology of Long COVID

2.1. Acute Infection and Immune Activation

The host immune response to the respiratory virus involves a tightly orchestrated combination of innate and adaptive mechanisms. The innate immune system recognises viral components and rapidly induces type I Interferons (IFNs) and proinflammatory cytokines,

activating dendritic cells, macrophages, and natural killer cells (NK). These early events restrict viral replication and initiate antigen presentation.

Subsequently, the adaptive immune response is activated. CD4⁺ and CD8⁺ T cells coordinate viral clearance, while B cells produce neutralizing antibodies, particularly against the receptor-binding domain (RBD) of the spike protein (**Figure 5**). In most individuals, this coordinated response leads to resolution of infection [(Jackson *et al.*, 2022); (Lamers & Haagmans, 2022); (Malone *et al.*, 2022); (Teixeira *et al.*, 2022)].

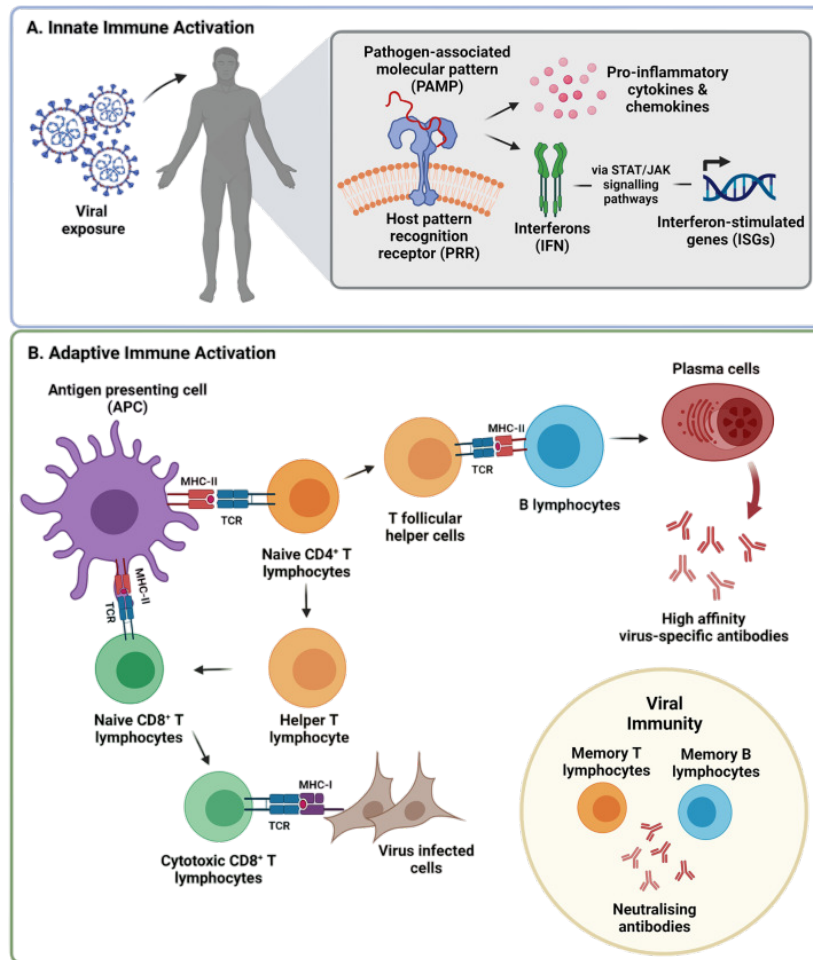


Figure 5. Two Branches of the Immune System Activated During Viral Infection, Followed by the Development of Viral Immunity

*(A) The innate immune response is activated within hours of viral exposure as the body's first line of defense that releases a series of anti-viral molecules at the site of infection. (B) An adaptive immune response is primed by components of the innate immune system and initiates pathogen-specific cellular and humoral immune responses days after infection (Adapted from Mistey *et al.*, 2022).*

2.2. Immune Dysregulation and Cytokine Storm

In severe coronavirus disease, this immune response becomes dysregulated, resulting in a cytokine storm characterised by excessive production of Interleukins (IL-6, IL-1 β), Tumour Necrosis Factor (TNF- α), and other inflammatory mediators. This hyperinflammatory state disrupts endothelial integrity, increases vascular permeability, and promotes massive infiltration of immune cells into pulmonary tissues. The consequences include acute respiratory distress syndrome (ARDS), multi-organ failure, and high mortality (**Figure 6**) [(Li *et al.*, 2022); (Montazersaheb *et al.*, 2022)].

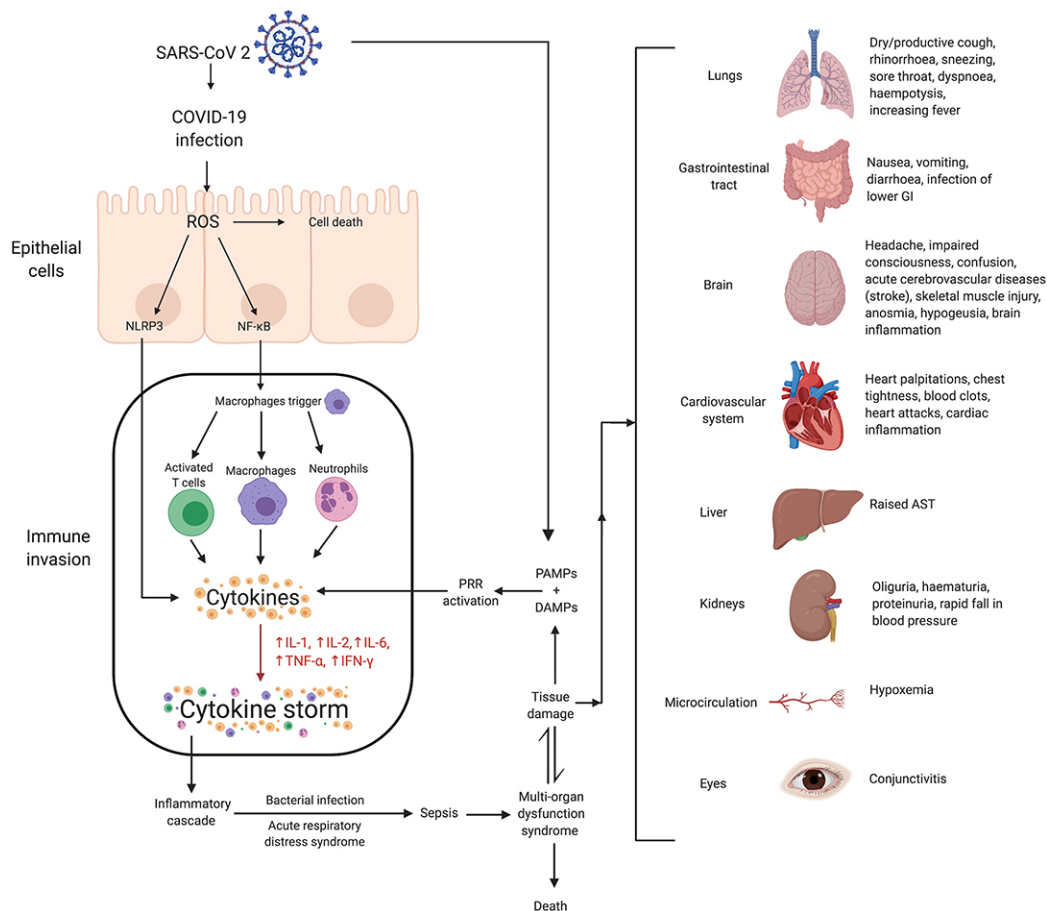


Figure 6. Mechanisms of SARS-CoV-2-Induced Cytokine Storm and Systemic Damage

*SARS-CoV-2 infection can trigger an excessive inflammatory response where reactive oxygen species (ROS) from epithelial cells activate the NLRP3 inflammasome and NF-κB signaling. This leads to overproduction of cytokines, a “cytokine storm” which contributes to acute respiratory distress, sepsis, and multiple organ dysfunction. The lower gastrointestinal tract, rich in ACE2 receptors, is especially affected, causing diarrhea in about 20% of cases (Adapted from Bhaskar *et al.*, 2020).*

2.3. Viral Immune Evasion Mechanisms

The studied virus possesses several strategies that compromise host immunity. These include:

- Suppression of type I IFN signalling, which reduces effective early antiviral responses.
- Downregulation of antigen presentation, particularly via interference with Major Histocompatibility Complex (MHC-I/II) pathways.
- Mutation-driven escape from neutralizing antibodies, particularly in emerging variants.
- Syncytia formation, enabling direct cell-to-cell viral spread without exposure to antibodies.

These mechanisms contribute to viral persistence, severity of disease, and challenges in vaccine and therapeutic efficacy [(Yang *et al.*, 2020); (Sette & Crotty, 2021); (Li *et al.*, 2022); (Lasrado & Barouch, 2023); (Sievers *et al.*, 2024)].

2.4. Long COVID: A Persistent Post-Viral Syndrome

While most individuals recover within weeks, a substantial number develop Long COVID, characterised by persistent or recurrent symptoms lasting months beyond acute infection. Reported manifestations include fatigue, dyspnoea, cognitive impairment, anosmia, chest pain, myalgia, mood disturbances, and cardiovascular abnormalities (**Figure 7**). Notably, Long COVID affects individuals regardless of the severity of the initial infection, including previously healthy individuals [(Raveendran *et al.*, 2021); (Cutler, 2022); (Altmann *et al.*, 2023); (Bohmwald *et al.*, 2024)].

Emerging hypotheses propose several potential mechanisms:

- Viral persistence in reservoirs.
- Chronic immune activation or dysregulation.
- Endothelial dysfunction and microvascular injury.
- Micro-clot formation impairing tissue oxygenation.

Combined, these contribute to substantial morbidity and impose a significant burden on healthcare systems and workforce productivity. Addressing Long COVID requires multidisciplinary research, targeted long-term therapies, and structured support networks for affected individuals. It also strengthens the rationale for antiviral strategies that simultaneously

inhibit viral replication and modulate inflammation and oxidative stress, such as those based on bioactive phytochemicals integrated into nanobiotherapeutic platforms.

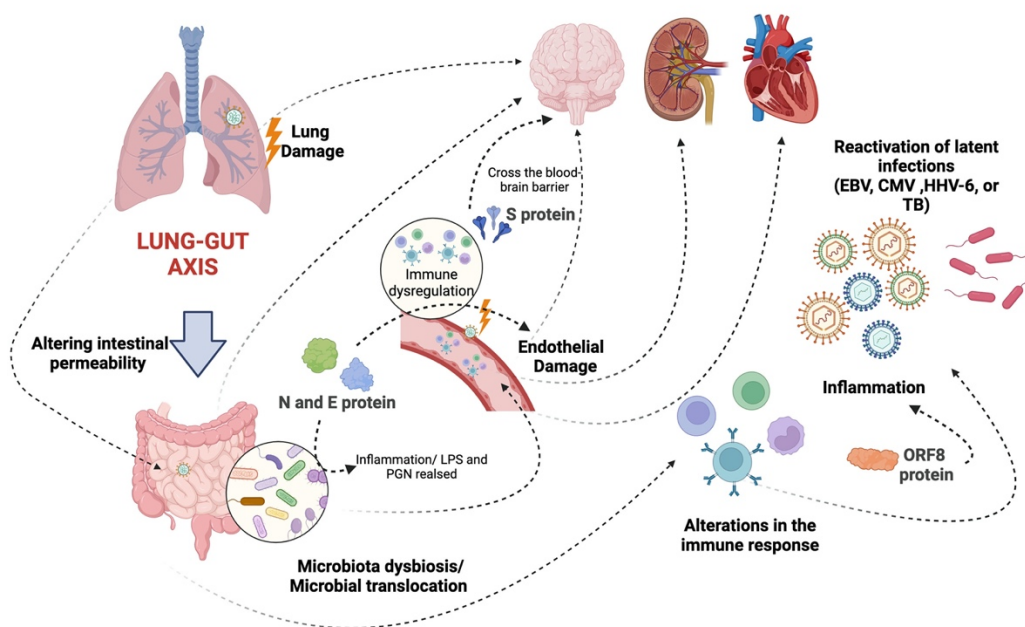


Figure 7. Proposed Mechanisms Underlying Long COVID Pathologies

(Adapted from Bohmwald *et al.*, 2024).

3. The COVID-19 Pandemic: Biomedical and Public Health Perspectives

3.1. Integrative Scientific Approaches During the Pandemic

The viral disease pandemic underscored the need for integrative, interdisciplinary strategies that combine microbiological, immunological, computational, and clinical methodologies to address emerging viral threats. A comprehensive understanding of viral pathogenesis, host immune dynamics, and mechanisms of antimicrobial resistance has been essential for developing effective therapeutic and preventive responses to infectious diseases, particularly those of viral origin [(Zatla *et al.*, 2021c, 2022a, 2022b); [(Chatterjee *et al.*, 2024)].

3.2. Global Emergence and Impact of SARS-CoV-2

The emergence of SARS-CoV-2 rapidly transformed a localized outbreak into a global health crisis of unprecedented scale. The virus exerted immense pressure on healthcare infrastructures, disrupted international economies, and reshaped societal routines. Conventional public health measures, such as lockdowns, travel restrictions, and institutional closures, proved

effective in slowing viral transmission but also revealed their limitations. These interventions, though necessary, imposed substantial social and psychological burdens, particularly among vulnerable populations including the elderly, individuals with chronic illnesses, frontline workers, and socioeconomically disadvantaged communities [(Adamczak *et al.*, 2022); (Zatla *et al.*, 2022b, 2023a); (Omotayo *et al.*, 2024)].

3.3. Public Health Measures and Confinement Policies

To interrupt transmission chains, governments worldwide implemented large-scale quarantines and stay-at-home mandates. These measures significantly altered daily life, reshaped social dynamics, and accelerated digital transitions in education and work. However, they also intensified pre-existing socioeconomic inequalities and heightened stress across all demographic groups. Essential workers remained exposed to elevated risks, while many households faced income instability, educational disruptions, and reduced social support. Public adherence to restrictions varied, often influenced by pandemic fatigue, misinformation, and cultural or socioeconomic factors [(Taghrir *et al.*, 2020); (Memon *et al.*, 2021); (Zatla *et al.*, 2022b)].

In parallel, the pandemic reinforced the importance of basic hygiene and protective practices, including regular handwashing, use of sanitizers, mask-wearing, and physical distancing. These implementations, heavily promoted through national health campaigns and digital communication platforms, proved essential for limiting viral spread [(Wróblewski *et al.*, 2020); (Finlay *et al.*, 2021); (Hu *et al.*, 2022); (Ali *et al.*, 2023); (Zatla *et al.*, 2023b)]. Nevertheless, disparities in access to clean water, protective equipment, and reliable information created challenges for equitable adoption of these measures. Furthermore, misinformation and social stigma often undermined compliance and contributed to scepticism toward public health guidance [(Haque *et al.*, 2020); (Bloomfield & Ackerley, 2023); (Mathew *et al.*, 2025)].

3.4. Psychological and Social Consequences of the Pandemic

As the pandemic progressed, its psychological repercussions became increasingly evident. Social isolation, bereavement, economic instability, and fear of infection contributed to a global rise in anxiety, depression, and stress-related disorders. Several studies reported a two- to threefold increase in mental health symptoms relative to pre-pandemic levels. Young adults, caregivers, healthcare workers, and individuals living alone were particularly vulnerable. Health systems, already strained by clinical demands, often lacked sufficient resources to address these growing

mental health needs. In many regions, access to psychological support remained limited due to stigma, infrastructural barriers, or insufficient investment, creating a parallel and often underrecognized public health challenge [(Mukhtar, 2020); (Gruber *et al.*, 2021); (Taylor, 2022); (Zatla *et al.*, 2023b)].

4. Vaccination Strategies and Their Limitations

The rapid development and deployment of COVID-19 vaccines were among the most significant scientific achievements of the pandemic. Within an unprecedented timeframe, multiple vaccine candidates progressed from laboratory research to emergency use authorisation, offering the first major global opportunity to curb transmission and reduce morbidity and mortality. However, the real-world impact of vaccination strategies varied considerably across regions, shaped by disparities in vaccine supply, logistical capabilities, political governance, and socio-cultural attitudes toward immunisation. In many settings, vaccine hesitancy emerged as a critical barrier to widespread uptake, driven by misinformation, distrust in authorities, perceived risks, and inadequate community-centred communication. Effectively addressing such challenges required not only the dissemination of evidence-based information but also targeted interventions tailored to specific social contexts [(Quinn *et al.*, 2020); (Fortner & Schumacher, 2021); (Acosta-Coley *et al.*, 2022); (Zatla *et al.*, 2023c); (Prosser *et al.*, 2025)].

4.1. Global Vaccine Development and Deployment

In response to the urgent need for protection against this novel beta-coronavirus, the global scientific community mobilised one of the fastest and most collaborative vaccine development efforts in history. Only months after the virus was identified, multiple vaccine candidates had entered clinical trials. By late 2020, the Pfizer-BioNTech and Moderna vaccines, which were both developed using innovative mRNA platforms, became the first to receive emergency use authorisation. These breakthroughs were followed by vaccines based on more traditional technologies, including adenoviral vector platforms (AstraZeneca, Sputnik V, Janssen) and inactivated virus approaches (Sinopharm, Sinovac), as summarised in **Table 2**.

Beyond its scientific importance, the “vaccine race” reflected geopolitical dynamics and highlighted unequal global access to life-saving technologies, with low- and middle-income countries often facing delays in supply and distribution [(Corey *et al.*, 2020); (Excler *et al.*, 2021); (Abdaal *et al.*, 2024); (Allan *et al.*, 2025)].

Table 2: Comparative Overview of SARS-CoV-2 Vaccine Types and Development Platforms*(Adapted from Acosta-Coley et al., 2022).*

Vaccine Type	Vaccine Subtype	Description	Example (Manufacturer / Vaccine Name)
Whole virus-vaccine	Inactivated	Utilizes a pathogenic virus that has been chemically or physically inactivated, rendering it incapable of replication while maintaining its antigenic properties to elicit an immune response.	-BBIBP-CorV (Sinopharm) -CoronaVac (Sinovac)
	Live Attenuated	Employs a weakened form of the virus achieved through deletion or modification of non-structural and essential genes. The attenuated virus replicates poorly in the host, stimulating both humoral and cellular immunity without causing disease.	-COVI-VAC (Codagenix / Serum Institute of India)
Viral vector-vaccine	Replicating	Based on a genetically engineered viral vector distinct from the target pathogen, capable of limited replication within host cells to express the target antigen, thereby inducing a robust immune response.	-rVSV-SARS-CoV-2 (Merck)
	Non-Replicating	Involves a modified viral vector rendered replication-deficient through deletion of essential genes. It delivers genetic material encoding target antigens without propagating within host cells.	-Oxford–AstraZeneca (ChAdOx1 nCoV-19) -Sputnik V (Gam-COVID-Vac) -Johnson & Johnson (Ad26.COV.S)
Protein-based vaccine	Virus-Like Particles	Composed of self-assembled viral structural proteins that mimic the morphology of the native virus but lack nucleic acids, thus incapable of replication. They induce strong antigen-specific immune responses.	Medicago CoVLP (Medicago Inc.)
	Recombinant Protein	Consists of purified viral proteins produced through recombinant DNA technology. These vaccines are inherently safe as they contain no infectious viral material.	NVX-CoV2373 (Novavax)
Nucleic acid vaccine	DNA Vaccine	Incorporates plasmid DNA encoding viral antigens, often delivered via nanoparticles or electroporation. Once inside the host cell nucleus, the DNA is transcribed into mRNA, which is subsequently translated into viral proteins that trigger an adaptive immune response.	ZyCoV-D (Zydus Cadila, India)
	RNA Vaccine	Comprises synthetic messenger RNA encoding the viral spike (S) protein, encapsulated within lipid nanoparticles (LNPs) for protection and delivery. Upon cellular uptake, the mRNA is translated by ribosomes into antigenic proteins that stimulate immunity.	-BNT162b2 (Pfizer–BioNTech) -mRNA-1273 (Moderna)

4.2. The Rise of mRNA Vaccines

Among the most transformative outcomes of the pandemic was the successful large-scale application of messenger RNA (mRNA) technology for human vaccination. Unlike conventional vaccines that rely on live-attenuated or inactivated viral particles, mRNA vaccines deliver genetic instructions encoding the viral spike protein, prompting host cells to generate the antigen and trigger a robust immune response. The Pfizer-BioNTech and Moderna vaccines demonstrated high efficacy and could be rapidly updated to address emerging viral variants. Despite initial scepticism due to their novelty, mRNA vaccines have reshaped the future landscape of vaccinology, opening pathways for rapid responses to future outbreaks and for potential applications in oncology and other infectious diseases [(Park *et al.*, 2021); (Uddin & Roni, 2021); (Fang *et al.*, 2022); (Kumar *et al.*, 2022); (Leong *et al.*, 2025)].

4.3. Public Concerns, Safety Perceptions, and Misinformation

While vaccine development progressed at a remarkable speed, public concerns regarding safety remained widespread, particularly in the early stages of rollout. The novelty of mRNA technology, the accelerated timelines of clinical trials, and the unprecedented scale of regulatory approvals fuelled scepticism among certain populations. Social media played a complex role, facilitating the dissemination of scientific updates while enabling the rapid spread of misinformation. False claims related to infertility, microchips, genetic alteration, and mortality amplified fear and distrust. Although rare adverse events such as vaccine-associated blood clots (AstraZeneca, Janssen) or myocarditis (mRNA vaccines) received intense media attention, extensive epidemiological data confirmed favourable benefit-risk profiles across all approved vaccines [(Razai *et al.*, 2021); (Sallam, 2021); (Soares *et al.*, 2021); (Troiano & Nardi, 2021)].

4.4. Vaccine Hesitancy: A Persistent Global Challenge

Vaccine hesitancy proved to be one of the most formidable obstacles to achieving population-level immunity. Importantly, hesitancy was not merely a product of misinformation but reflected deeper socio-cultural and historical factors. In some communities, past experiences with medical exploitation, limited healthcare access, and political marginalisation shaped perceptions of institutional trust. Among younger populations, hesitancy was often driven by a sense of low personal risk and by social media narratives. Overcoming hesitancy required multidimensional strategies, including transparent communication, culturally sensitive awareness

campaigns, involvement of community leaders, and equitable access to both vaccines and reliable information. Only such integrated approaches can support strong public confidence and sustained vaccination uptake [(Kricorian *et al.*, 2022); (Zatla *et al.*, 2023c)]. Nonetheless, the persistence of immune-evasive variants, heterogeneous coverage driven by hesitancy, and concerns about waning immunity indicate that vaccines alone are unlikely to fully control SARS-CoV-2, underscoring the need for complementary, easily deployable intranasal nanobiotherapeutic antivirals that can reduce transmission and disease burden independently of systemic immunisation status.

5. Natural Bioactive Compounds

Nature has long served as a rich source of pharmacologically active compounds, many of which have shaped the foundations of modern therapeutics. Microbial- and plant-derived secondary metabolites possess diverse biological functions, including potent antibacterial, antifungal, and antiviral properties. Advances in natural product chemistry, combined with virology, structural biology, and computational modelling, have facilitated the identification of numerous bioactive molecules capable of disrupting viral entry, replication, or assembly [(Aberra, 2006); (Kasprzyk-Hordern, 2010); (Velu *et al.*, 2018); (Liu, 2025)].

Recent investigations have highlighted the antiviral potential of several plant-derived compounds, particularly saponins and flavonoids. Among these, β -escin and rutin have attracted considerable attention for their synergistic pharmacological effects against human coronaviruses (hCoVs), including SARS-CoV-2. These compounds exhibit diverse mechanisms of action, such as modulation of immune responses, disruption of viral envelope integrity, and inhibition of essential viral enzymes. Their ability to interfere with multiple stages of the viral life cycle, combined with favourable biocompatibility and low cytotoxicity, positions them as attractive candidates for antiviral drug development and nanobiotechnological applications. The exploration of such natural antivirals is particularly relevant in confronting emerging viral threats, where conventional antiviral therapies may be insufficient or limited [(Prasad *et al.*, 2020); (Thomas *et al.*, 2021); (de Oliveira *et al.*, 2022); (Giordano *et al.*, 2023); (Chebaibi *et al.*, 2024); (Zahmanova *et al.*, 2025)].

5.1. Pharmacological Properties

5.1.1. β -Escin

Beta-Escin is a triterpenoid saponin predominantly extracted from the seeds of the horse chestnut tree (*Aesculus hippocastanum*), a deciduous tree native to the Balkan Peninsula and now widely cultivated across Europe and parts of Asia. This species, belonging to the family Sapindaceae, is distinguished by its large palmate leaves composed of five to seven toothed leaflets and by its prominent upright clusters of white flowers marked with red or yellow spots. The characteristic seeds, which are smooth, glossy brown nuts encased in spiny green capsules, represent the primary source of β -escin, although smaller quantities may also be found in the bark and flowers [(Salinas *et al.*, 2019); (Çelik & Aslantürk, 2023)].

Chemically, the molecule comprises a mixture of structurally related saponins, such as escin Ia/Ib and escin IIa/IIb, with β -escin being the most biologically active isomer. Its amphiphilic structure consists of a hydrophobic aglycone (escigenin or protoaescigenin) linked to sugar moieties (typically glucose and rhamnose) conferring the molecule with surface-active properties and a high affinity for lipid membranes [(Boboev *et al.*, 2024); (Shah *et al.*, 2024); (Yan *et al.*, 2024)]. These structural features underlie its wide pharmacological spectrum.

Traditionally, the phytomolecule has been recognized for its anti-inflammatory, venotonic, and vasoprotective activities, forming the basis of its use in managing chronic venous insufficiency and related vascular disorders. More recently, its antiviral and antitumoral properties have stimulated scientific interest, particularly in nanomedicine [(Michelini *et al.*, 2018); (Sikorska *et al.*, 2021); (Akar *et al.*, 2022); (Gözcü, 2022); (Broni *et al.*, 2023); (Wang *et al.*, 2023); (Fazliev *et al.*, 2025); (Petkov *et al.*, 2025)].

5.1.2. Rutin

Rutin (rutoside or quercetin-3-O-rutinoside) is a polyphenolic flavonoid glycoside present in numerous plant species, and is particularly abundant in the leaves, flowers, and fruits of the wild cherry tree (*Prunus avium*). A member of the Rosaceae family, *P. avium* is native to Europe and Western Asia and is characterized by its tall stature, smooth reddish-brown bark, serrated, ovate leaves, and fragrant white blossoms that appear in spring. Its sweet red cherries, widely consumed

for their nutritional value, are rich in flavonoids, including rutin, which accumulates notably in the fruit skin, leaves, and seeds [(Nunes *et al.*, 2022)].

The plant compound consists of the aglycone quercetin conjugated to the disaccharide rutinose (rhamnose + glucose), a structural modification that enhances water solubility and improves pharmacokinetic behaviour compared with quercetin alone [(Bose *et al.*, 2017); (Ortega-Vidal *et al.*, 2021)]. This configuration supports a wide range of biological effects.

The phytochemical is best known for its potent antioxidant activity, which stems from its ability to scavenge reactive oxygen species and stabilize free radicals. Additionally, rutin exhibits notable anti-inflammatory, anti-thrombotic, vasoprotective, neuroprotective, and emerging antiviral properties. Its ability to interact with lipid membranes and metal ions has led to its incorporation into various nano-formulations designed for targeted drug delivery, including investigational applications against viral pathogens [(Yook *et al.*, 2010); (Al-Zahrani, 2020); (Mazik, 2022); (Mane *et al.*, 2024)].

6. Colloidal Metals for Antiviral Applications

Nanotechnology has transformed antiviral research by enabling the development of nanoscale materials with tailored physicochemical and biological properties. Nanoparticle-based formulations offer several advantages over conventional therapeutics, including enhanced solubility, improved pharmacokinetics, targeted delivery, and sustained release, features that collectively increase therapeutic efficacy while minimising systemic toxicity [(Medhi *et al.*, 2020); (Abate *et al.*, 2022); (Kumar & Parashar, 2025); (Mishra, 2025)]. Among the diverse types of engineered nanostructures, AuNPs and AgNPs are particularly prominent due to their intrinsic antimicrobial and antiviral activities. These effects arise from multiple mechanisms: inhibition of viral proteins involved in entry and replication, disruption of viral envelopes or capsids, interference with host–virus interactions, and modulation of host immune responses [(Itani *et al.*, 2020); (Sarkar *et al.*, 2022); (Bhatti & DeLong, 2023); (Khedr *et al.*, 2023)].

Synthesis of colloidal metals can be achieved through chemical, physical, or biological approaches, as summarised in **Figure 8**.

- Chemical synthesis relies on reduction or precipitation via chemical reagents. It allows tight control over nanoparticle size and morphology, but may produce toxic by-products that require extensive purification.
- Physical methods, including laser ablation, evaporation–condensation, and high-energy milling, produce highly pure nanoparticles but demand specialised equipment and substantial energy input.
- Biological (green) synthesis uses plant extracts, microorganisms, or biomolecules as reducing and stabilising agents. This method is eco-friendly, cost-effective, and generates biocompatible nanoparticles with low toxicity. Its limitations include broader size distributions and reproducibility challenges [(Roy *et al.*, 2021); (Zahoor *et al.*, 2021); (Khan *et al.*, 2022a); (Patil *et al.*, 2023); (Srinivasan & Rana, 2024)]

The growing interest in biosynthetic routes aligns with the broader shift toward sustainable nanomedicine and supports the integration of natural antiviral compounds into nanoparticle platforms.

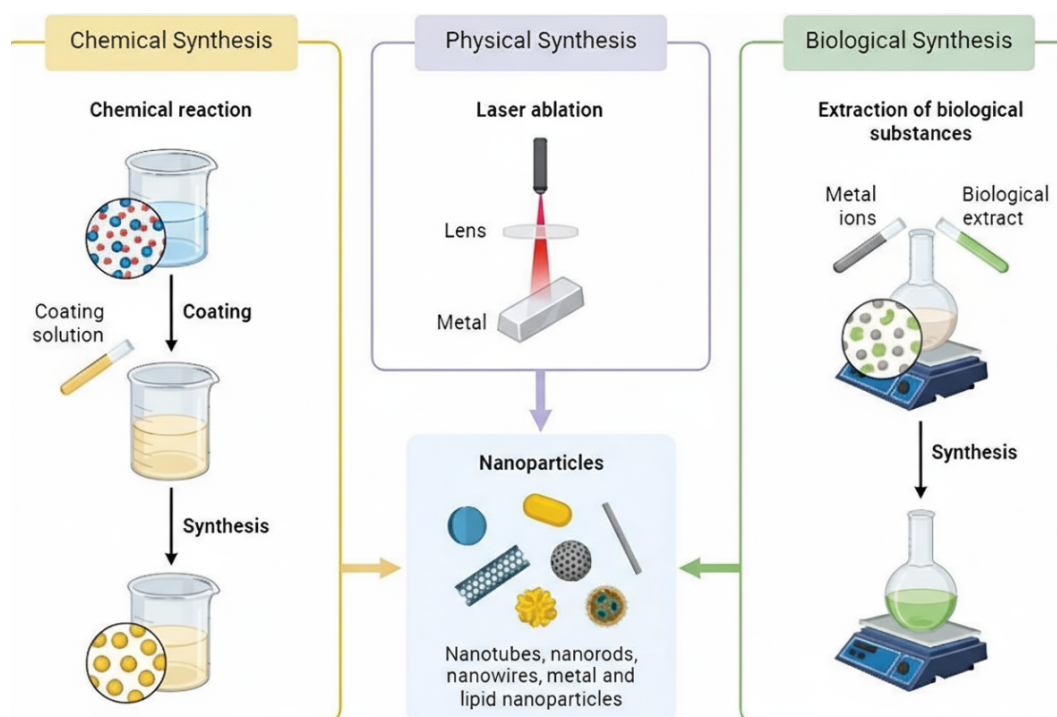


Figure 8. Different Synthesis Methods of Nanoparticles

(Reproduced from Srinivasan & Rana, 2024).

6.1. Colloidal Gold

Colloidal gold is composed of gold nanoparticles (1–100 nm) suspended in aqueous media. These particles exhibit distinct optical behaviour due to localised surface plasmon resonance (LSPR), the collective oscillation of surface electrons when excited by light. LSPR confers strong absorption and scattering properties, producing a characteristic ruby-red to purple colouration that depends on nanoparticle size and shape. Beyond their optical features, AuNPs offer exceptional chemical stability, tunable surface chemistry, and biocompatibility, which have made them indispensable in biosensing, imaging, diagnostics, and photothermal therapy [(Gnanaraj *et al.*, 2024); (Teimouri *et al.*, 2025)].

In biomedical applications, AuNPs are particularly valued for their versatility in surface functionalization. Their surfaces can be conjugated with drugs, peptides, antibodies, or phytochemicals to achieve targeted delivery, improved bioavailability, and controlled therapeutic release. Increasing evidence indicates that gold nanoparticles also possess direct antiviral activity, which can be greatly enhanced when combined with natural bioactive molecules. In particular, AuNP–AuNP-phytochemical conjugates have shown promising inhibition of respiratory viruses, including SARS-CoV-2, via mechanisms such as protein binding interference, membrane disruption, and inhibition of viral entry pathways. These properties make colloidal gold especially suitable for nasal or mucosal nanotherapeutic systems, where localised delivery can maximise antiviral activity while limiting systemic exposure [(Babaei *et al.*, 2021); (Chaika *et al.*, 2022); (Li *et al.*, 2023); (Salesa *et al.*, 2023); (Behera, 2024); (Bhebhe *et al.*, 2024); (Naghib *et al.*, 2024); (Dehesh & Dehesh, 2025)].

6.2. Colloidal Silver

Colloidal silver consists of silver nanoparticles (1–100 nm) dispersed in purified water. Similar to AuNPs, AgNPs display distinctive LSPR-related optical properties, typically a yellowish-brown colouration, and exhibit high electrical conductivity and strong surface reactivity. These features, combined with their large surface area, enable efficient interaction with microbial membranes, proteins, and nucleic acids [(Luceri *et al.*, 2023); (Sabaghian, 2024)].

Silver nanoparticles are widely recognised for their broad-spectrum antimicrobial activity, encompassing antibacterial, antifungal, and antiviral effects. Their antiviral mechanisms include disrupting viral envelopes or capsids, generating reactive oxygen species (ROS) that impair viral

structures, inhibiting viral replication machinery, and interfering with host cell receptor binding. When conjugated to natural bioactive molecules such as flavonoids or saponins, AgNPs often exhibit synergistic antiviral activity, enhancing both stability and biological function. Their favourable biocompatibility at controlled doses and flexible surface chemistry makes AgNPs valuable components of next-generation antiviral nanoplatforms, particularly for respiratory and mucosal delivery systems targeting pathogens like influenza viruses and SARS-CoV-2 [(Doszpoly *et al.*, 2023); (Naumenko *et al.*, 2023); (Agnol *et al.*, 2024); (Al-Radadi & Abu-Dief, 2024); (Martín-Faivre *et al.*, 2024); (de Souza *et al.*, 2025)].

6.3. Biosynthesis of Gold and Silver Nanoparticles

Biological or green synthesis has emerged as a sustainable and biocompatible alternative that aligns with modern eco-friendly nanotechnological practices. This method utilises plant extracts, microbial cultures, or purified natural compounds as both reducing and capping agents, eliminating toxic chemicals and enabling nanoparticle formation under mild reaction conditions [(Alaqad & Saleh, 2016); (Yaqoob *et al.*, 2020); (Hammami & Alabdallah, 2021); (Nguyen *et al.*, 2023); (Patil *et al.*, 2023)].

Plant-derived phytochemicals, including polyphenols, flavonoids, terpenoids, and saponins, play a pivotal role in controlling nanoparticle size, shape, and surface stability. Within this context, β -escin and rutin have proven highly effective as dual reducing–stabilizing agents due to their intrinsic antioxidant, surfactant, and metal-ion–binding properties. Biosynthesis using these compounds yields nanoparticles that not only exhibit favourable physicochemical characteristics but also retain the inherent antiviral and antioxidant activities of the biomolecules used in their production [(Saravanan *et al.*, 2021); (Arjun *et al.*, 2022); (Barabadi *et al.*, 2022); (Karthik *et al.*, 2022); (Al-Askar *et al.*, 2023); (Elnosary *et al.*, 2023); (Parvez & Ahmed, 2023); (Abdel-Rahman *et al.*, 2024); (El Bagoury *et al.*, 2025)].

This integrated biosynthetic strategy generates multifunctional nanostructures that inhibit viral entry, block replication, modulate immune pathways, and mitigate oxidative stress. The synergy between the metal core and the phytochemical shell exemplifies the emerging paradigm of nature-inspired nanomedicine, offering promising antiviral solutions, particularly for respiratory viruses such as coronaviruses [(Prasad *et al.*, 2020); (Duarte & Takahashi, 2022)].

7. Computational and Nanobiological Approaches

Computational modelling has become an essential component of modern antiviral research, enabling the rapid identification and characterisation of molecules with potential therapeutic activity against SARS-CoV-2. Molecular docking and molecular dynamics (MD) simulations have played a particularly important role in predicting the interactions between natural bioactive compounds and viral proteins, allowing researchers to prioritise the most promising candidates for further *in vitro* and *in vivo* evaluation.

Within this context, computational studies on biosynthesized silver nanoparticles have indicated possible binding to the receptor-binding motif of SARS-CoV-2, supporting a potential mechanism for inhibition of viral entry, while broader reviews also highlight gold and silver nanoparticles as promising antiviral platforms [(Ayipo *et al.*, 2022); (Daoudi *et al.*, 2024); (Kar *et al.*, 2024)].

In contrast to nanoparticles and several extensively studied phytocompounds, direct *in silico* investigations of β -escin against SARS-CoV-2 remain limited. Nevertheless, the molecule has shown promising antiviral and anti-inflammatory evidence from review and laboratory studies, including inhibition of viral binding, replication, and virion stability in other viral models [(Michelini *et al.*, 2018); (Hazman *et al.*, 2023); (Lai *et al.*, 2023); (Peñaranda Figueredo *et al.*, 2024)].

Conversely, Rutin has demonstrated strong *in silico* binding affinity for essential viral targets, including the main protease (Mpro) and the S protein, indicating a dual potential to inhibit viral entry and replication. Beyond its predicted protein–ligand interactions, rutin is widely recognised for its biological activities that may contribute to mitigating SARS-CoV-2 infection and its associated inflammatory responses [(Agrawal *et al.*, 2021); (Ibrahim *et al.*, 2021); (Rahman *et al.*, 2021)].

7.1. Integration of Bioactive Phytomolecules with Noble Metal Nanoparticles

The combination of bioactive natural compounds with metallic nanocarriers represents a promising frontier in nanobiotechnology-driven antiviral therapy [(Wu *et al.*, 2020); (Patel *et al.*, 2024)]. The convergence of β -Escin and Rutin with AuNPs and AgNPs enables the development

of multifunctional nanosystems with enhanced stability, bioavailability, and targeted delivery capabilities.

Each constituent contributes unique bio-pharmacological features:

- β -Escin: membrane-active saponin capable of disrupting viral envelopes, modulating inflammatory cascades, and stabilizing nanoparticles.
- Rutin: polyphenolic antioxidant with protein-binding potential, capable of enhancing nanoparticle biocompatibility and antiviral specificity.
- Gold and silver nanoparticles exhibit intrinsic antiviral, antibacterial, and immunomodulatory effects, and their surfaces enable efficient conjugation with natural molecules.

When combined, these elements form a synergistic nanoplatform capable of:

- Blocking viral attachment and membrane fusion.
- Destabilizing viral envelopes.
- Inhibiting replication-associated enzymatic functions.
- Reducing oxidative stress.
- Modulating immune responses and improving pharmacokinetic behaviour through nano-enabled delivery.

7.2. Translational Perspectives and Therapeutic Potential

Computational analyses further suggest that these nanoparticles, particularly when functionalized with phytochemicals, interact strongly with viral protein complexes and may interfere with their structural integrity, thereby limiting infectivity and replication. Such nanobiological approaches align with growing interest in targeted nano-delivery systems for antiviral therapy, a direction supported by earlier foundational work demonstrating the ability of nanoparticles to enhance the pharmacological performance of antiviral agents [(Amini, 2019); (Rai *et al.*, 2014, 2015)].

8. Mucosal and Nasal Antiviral Delivery Systems

The growing interest in mucosal vaccines and intranasal antiviral therapeutics reflects a strategic shift toward immune defence approaches that better mimic the natural route of respiratory viral infections. As the nasal cavity represents a primary entry site for pathogens such as

coronaviruses and influenza viruses, it provides an optimal target for localized antiviral intervention [(Li *et al.*, 2020); (Lavelle & Ward, 2022)]. Consequently, nasal spray-based systems have emerged as promising prophylactic and therapeutic tools due to their rapid onset of action, high local drug concentrations, and non-invasive administration [(Eccles, 2021); (Hoffmann, 2023); (Hickman & Moutsopoulos, 2024); (Pagni *et al.*, 2026)]. In addition, intranasal delivery bypasses hepatic first-pass metabolism, improving bioavailability while reducing systemic toxicity.

A major advantage of intranasal formulations is their ability to stimulate both systemic and mucosal immunity. Unlike injectable vaccines that mainly induce systemic IgG responses, intranasal delivery promotes the production of secretory IgA (sIgA), which plays a critical role in neutralising viruses at the site of entry and limiting transmission [(Yusuf & Kett, 2017); (Travis, 2020); (Tiboni *et al.*, 2021); (Hartwell *et al.*, 2022)]. The large surface area, rich vasculature, and high permeability of the nasal epithelium further enhance drug absorption and therapeutic efficiency [(Adekiya *et al.*, 2023); (Žigayová *et al.*, 2023)]. Moreover, intranasal products offer practical advantages including ease of self-administration, improved patient compliance, and reduced dependence on healthcare personnel during large-scale vaccination or treatment campaigns [(Higgins *et al.*, 2020); (Tiwari *et al.*, 2022); (Affrald & Narayan, 2024); (Bai *et al.*, 2024)].

Despite significant progress in vaccines and antiviral therapies, respiratory pathogens such as SARS, MERS, and influenza viruses continue to pose major global health threats due to viral mutation, reduced vaccine efficacy, antiviral resistance, and treatment-associated side effects [(Higgins *et al.*, 2020); (Moreno-Fierros *et al.*, 2020); (Afkhami *et al.*, 2022)]. In this context, nanotechnology has emerged as a promising strategy for the development of next-generation antiviral platforms. Metal-based nanoparticles, particularly gold and silver nanostructures, exhibit intrinsic antiviral, anti-inflammatory, and immunomodulatory properties. Furthermore, their combination with natural bioactive compounds enables the design of multifunctional intranasal nano-bio systems with enhanced stability, targeted delivery, and improved therapeutic potential [(Hoseini-Tavassol *et al.*, 2021); (Kramer *et al.*, 2021); (Khan *et al.*, 2022b); (Meister *et al.*, 2022); (Xi, 2022); (Chavda *et al.*, 2023)]. Such hybrid nano-bio formulations represent a promising frontier in the development of next-generation prophylactic and therapeutic strategies for respiratory viral infections.

Chapter 11:

Methodology

Methodology

The COVID-19 pandemic has highlighted the intrinsic link between population-level dynamics and the molecular mechanisms of viral infection, underscoring the need for integrated methodological approaches. Accordingly, this article-based thesis is structured around a dual-axis methodological framework combining epidemiological analysis (Axis I) with nanobiotechnological antiviral development (Axis II).

Axis I addresses population-level vulnerabilities and systemic challenges, thereby contextualizing public health needs, while Axis II focuses on molecular-level antiviral innovation through natural bioactive compounds, green synthesis strategies, and computational design. Together, these complementary axes establish a coherent and multidimensional methodology that integrates public health evaluation with molecular antiviral research. General methodological principles are outlined in the following sections, with detailed procedures reported in the individual articles and their associated summaries.

○ **Axis I: COVID-19 Epidemiological and Vaccination Insights in Tlemcen**

1. Study Design

Axis I employs a retrospective, descriptive, and analytical epidemiological design, grounded in real-world health surveillance data collected in Tlemcen during the COVID-19 pandemic. The aim was to delineate the clinical and diagnostic characteristics of confirmed cases and evaluate the performance of the vaccination system.

2. Data Sources

2.1. Two complementary datasets underpin Axis I:

- ◆ Diagnostic and Clinical Surveillance Data Used in *“Profiling COVID-19 Cases in Tlemcen, Algeria: PCR and CT Imaging Insights”*

This dataset includes:

- RT-PCR testing records
- CT imaging reports
- Basic clinical profiles

- ◆ Vaccination Monitoring and Inventory Records used in the article “*Assessment of COVID-19 vaccination performance in Tlemcen: Insights into vaccine inventory management*”

These data include:

- Daily vaccine stock records
- Vaccine consumption logs
- Vaccine wastage reports
- Vaccine uptake indicators from target populations
- Regional supply chain inventory and distribution logs

2.2. Variables and Measures

The combined datasets allowed assessment of:

- RT-PCR positivity profiles
- CT severity classifications
- Temporal patterns of confirmed COVID-19 cases
- Vaccine availability, distribution efficiency, and wastage
- Vaccination uptake trends and determinants across demographic groups.

2.3. Analytical Approach

Diagnostic profiles were analysed via frequency distributions, severity scoring, and correlation patterns between PCR and CT outcomes. Descriptive statistics were employed to quantify trends in vaccine utilization, wastage, and case distribution. Comparative analyses (e.g., between periods of high vs. low vaccine consumption) were conducted when appropriate.

3. Ethical Considerations

All epidemiological data were anonymized and handled according to national regulations. No personal identifiers were included, and all data were obtained through authorized health system channels.

- **Axis II: SARS-CoV-2 Antiviral Computational Investigation**

1. Conceptual Scientific Approach

Axis II integrates green nanotechnology, natural bioactive compounds, and computational antiviral modelling to investigate potential inhibitory strategies against SARS-CoV-2. This axis is based on the following articles:

- *“Battling COVID-19 Leveraging Nanobiotechnology: Gold and Silver Nanoparticle–B-escin Conjugates as SARS-CoV-2 Inhibitors”*
- *“Green-Synthesized Colloidal Metal-B-escin Bioconjugates for Nasal Prophylaxis and Treatment: A Hypothetical Approach Against SARS-CoV-2 Variants”*
- *“Enhanced Nanobiotechnological Strategy Against SARS-CoV-2: Synergistic Potential of Rutin”*

2. Nanoparticles and Phytomolecules

AuNPs and AgNPs were selected for their reported antiviral properties and their capacity to serve as carriers for bioactive compounds, thereby enhancing molecular stability, bioavailability, and targeted delivery. β -Escin and rutin were employed as bioactive ligands due to their potential antiviral and immunomodulatory activities.

The methodological framework focused on the conceptual design and computational evaluation of nanoparticle–ligand bioconjugates. Experimental and physicochemical considerations described in the associated articles included:

- Green synthesis strategies for nanoparticle preparation
- Ligand conjugation approaches
- Predicted physicochemical characteristics, including particle size, surface charge, colloidal stability, and functional group interactions
- Literature-supported characterization principles involving UV–Vis spectroscopy, FTIR, TEM/SEM, and DLS analyses

Altogether, these parameters established a coherent framework linking nanoparticle engineering, bioactive molecule functionality, and antiviral potential.

conjugates.

3. Computational Antiviral Evaluation

Computational approaches were employed to investigate the antiviral potential of the proposed nanobioconjugates against major SARS-CoV-2 targets, including the spike glycoprotein (S), main protease (Mpro), and RNA-dependent RNA polymerase (RdRp).

The *in silico* workflow integrated multiple stages of molecular modelling and analysis:

- Ligand and structure preparation: Bioactive ligands were drawn and optimized using *ChemDraw*, with molecular structures retrieved from PubChem and Protein Data Bank (PDB) repositories.
- Molecular docking: Protein–ligand interactions were evaluated using *AutoDock Vina* and *HDOCK* to estimate binding affinity and predict interaction modes.
- Structural visualization and interaction analysis: Docking poses and molecular interactions were analysed using *PyMOL* and *Discovery Studio Visualizer*.
- Molecular dynamics simulations: Stability and dynamic behaviour of selected complexes were assessed using *GROMACS* to evaluate conformational stability over time.
- Drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity)) prediction: Pharmacokinetic and toxicity profiles were evaluated using web-based tools such as *SwissADME* and *pkCSM*.

These integrated computational tools enabled a multi-level assessment of binding affinity, structural stability, and pharmacological potential of the proposed nanobioconjugates.



Research Articles

Axis 1: COVID-19

Epidemiological and Vaccination

Insights in Tlemcen

Profiling COVID-19 Cases in Tlemcen, Algeria: PCR and CT Imaging Insights

Ilyes Zatla, Lamia Boublenza, Wafaa Lemerini, Chahinez Triqui, and Nabahte Selka

Zatla, I., Boublenza, L., Lemerini, W., Triqui, C., & Selka, N. (2025). Profiling COVID-19 Cases in Tlemcen, Algeria: PCR and CT Imaging Insights. *COVID*, 5(9), 149.

DOI: 10.3390/covid5090149



○ **Objective**

This article examined the clinical, diagnostic, and epidemiological profiles of COVID-19 cases in Tlemcen, Algeria, during 2021. A total of 68,745 confirmed active cases were documented through surveillance records; individuals were assessed at the Public Local Health Care Establishment (EPSP) and the University Hospital of Tlemcen.

○ **Method**

This retrospective study used clinical and diagnostic records of patients suspected or confirmed to have the disease in 2021. Data were collected from hospital archives and included results from real-time reverse transcription polymerase chain reaction (RT-PCR) assays and chest computed tomography (CT) imaging. Nasopharyngeal and oropharyngeal swab samples had been previously analysed by RT-PCR to detect SARS-CoV-2 RNA, with cycle threshold (Ct) values recorded to estimate viral load and categorize infection severity. In parallel, CT scan reports were reviewed and interpreted according to standard radiological criteria for the viral illness, including ground-glass opacities, consolidations, and bilateral distribution. The integration of molecular and imaging data enabled a comparative assessment of diagnostic modalities and provided a comprehensive overview of disease patterns within the study region.

○ **Results**

Clinical assessment revealed that fever, cough, and shortness of breath were the most frequently reported symptoms, with a subset of patients progressing to acute respiratory distress syndrome (ARDS). PCR testing showed cycle threshold (Ct) values ranging from 15 to 35, with the majority of patients exhibiting moderate viral loads (Ct values between 20 and 30). CT imaging consistently identified pulmonary abnormalities, most notably ground-glass opacities, followed by consolidation, and allowed disease severity to be classified into mild, moderate, and severe categories. These imaging findings underscored CT's importance in evaluating the extent of lung involvement and complementing molecular diagnostics.

At the epidemiological level, cumulative case counts demonstrated a steady upward trend throughout 2021, peaking in July before gradually declining toward the year's end. Comparative analysis of diagnostic modalities indicated that CT imaging consistently reported slightly higher case counts than PCR testing, although statistical analysis revealed no significant difference in

daily detection rates between the two methods. This discrepancy likely reflects CT scans' greater sensitivity in detecting early or subclinical pulmonary lesions that may not yet yield a positive PCR result, particularly in cases of low viral load or improper sampling. Therefore, reliance on PCR testing alone could miss certain active infections, leading to a potential underestimation of the actual infection burden

Geographical distribution across communes highlighted disparities in case increases, with denser areas such as Tlemcen, Mansourah, and Chetouane showing the greatest rises, while smaller communes reported minimal increases. These differences likely reflected variations in population density, access to diagnostic services, public health responses, and vaccination coverage. Importantly, the observed discrepancies between PCR and CT results underscore the need for multimodal diagnostic strategies to reduce false negatives and improve case detection, particularly in regions with limited access to testing.

- **Conclusion**

The findings illustrate sustained community transmission in Tlemcen during 2021, placing considerable strain on local health services. They further demonstrate that combining PCR with CT imaging provides a more comprehensive framework for monitoring disease progression, assessing severity, and managing outbreaks. Despite limitations such as its retrospective design, reliance on aggregate data, and regional scope, the study contributes valuable insights into the dynamics of COVID-19 in Algeria. It also underscores the critical importance of integrating epidemiological surveillance with diverse diagnostic modalities to enhance preparedness for future pandemics.

- **Limitations**

This study is limited by its retrospective design and dependence on previously recorded clinical and diagnostic data, which may contain inconsistencies or incomplete entries. The focus on a single region (Tlemcen) restricts the generalizability of findings to broader national trends. The absence of serological data, genomic sequencing, and individual vaccination status reduces the ability to contextualize disease severity or variant circulation. Additionally, variability in sampling quality, Ct value reporting, and CT interpretation across facilities may have introduced diagnostic bias.

- **Perspectives**

Future work should prioritize prospective, multi-centre investigations integrating PCR, CT, antigen testing, serology, and genomic surveillance to provide a more comprehensive understanding of COVID-19 dynamics in Algeria. Incorporating individual-level clinical follow-up, comorbidity profiles, and vaccination data would enhance risk stratification and diagnostic evaluation. Expanding standardized diagnostic protocols and digital reporting systems across regions would improve surveillance accuracy and strengthen preparedness for emerging respiratory outbreaks.



Brief Report

Profiling COVID-19 Cases in Tlemcen, Algeria: PCR and CT Imaging Insights

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Abstract

The COVID-19 pandemic caused by SARS-CoV-2 has significantly impacted public health worldwide. This study aimed to assess the clinical and diagnostic features of COVID-19 cases in the Tlemcen region, Algeria, and analyze epidemiological trends from January to December 2021. This retrospective study included 68,745 confirmed snapshot active COVID-19 cases from the Public Local Health Care Establishment (EPSP)—University Hospital of Tlemcen. Patients underwent PCR testing and chest CT imaging for clinical evaluation. Data on symptoms, PCR cycle threshold (Ct) values, and CT imaging findings were collected, and statistical analysis was performed to examine the patient's viral load and lung involvement data. Among 488 confirmed cases, common symptoms included fever, cough, and shortness of breath. PCR Ct values ranged from 15 to 35, and CT imaging revealed widespread lung involvement, with ground-glass opacities being the predominant feature. Epidemiological trends showed a consistent increase in cumulative cases, highlighting sustained transmission throughout the study period. Over the study period, epidemiological surveillance recorded a progressive rise in daily cases, peaking in July with 72 cases, followed by a gradual decline toward the end of the year. The findings underscore the utility of PCR Ct values and CT imaging in evaluating disease severity and monitoring regional case progression. The upward trend in cumulative cases emphasizes the need for ongoing public health measures and diagnostic strategies to manage future outbreaks effectively.

Keywords: SARS-CoV-2; COVID-19; epidemiology; PCR; CT scan; public health; Algeria



Academic Editor: Anna Puigdemol· Sánchez

Received: 14 August 2025

Revised: 30 August 2025

Accepted: 2 September 2025

Published: 5 September 2025

Citation: Zatla, I.; Boublenza, L.; Lemerini, W.; Triqui, C.; Selka, N. Profiling COVID-19 Cases in Tlemcen, Algeria: PCR and CT Imaging Insights. *COVID* **2025**, *5*, 149. <https://doi.org/10.3390/covid5090149>

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1. Introduction

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has led to an unprecedented global health crisis, resulting in millions of cases and fatalities worldwide. The virus has exhibited remarkable variability in its clinical presentation and progression, influenced by factors such as age, underlying health conditions, and geographical location. In Algeria, as in many parts of the world, the pandemic has had significant regional differences in terms of disease severity, transmission dynamics, and healthcare system responses [1–3].

Tlemcen, a city in northwestern Algeria, has seen its share of COVID-19 cases throughout the pandemic. Given the importance of accurate and early diagnosis for effective clinical management, integrating different diagnostic tools has become a focal point of research. Polymerase Chain Reaction (PCR) testing and Computed Tomography (CT) imaging have been at the forefront of the diagnostic strategies for COVID-19. PCR, known for its ability to detect the viral genome, has been the gold standard for diagnosing COVID-19, while CT imaging has proven invaluable in assessing lung involvement and disease severity [4–6].

The aim of this study is to analyze the profiles of COVID-19 cases in Tlemcen during 2021, with a particular focus on the role of PCR testing and CT imaging in diagnosis and disease monitoring. By reviewing data from confirmed COVID-19 cases, this study seeks to offer insights into the clinical characteristics of the disease in the region, highlighting the importance of combining these diagnostic tools to assess the progression and severity of the disease. Furthermore, the study aims to contribute to the broader understanding of the epidemiology of COVID-19 in Algeria, providing valuable information for both healthcare providers and public health officials.

2. Materials and Methods

2.1. Study Design and Population

This retrospective study was conducted in the Tlemcen region, Algeria, using data from confirmed COVID-19 cases reported between January and December 2021. Data were obtained from the public health database at the Public Local Health Care Establishment and from hospital records. The study included patients who were assessed for COVID-19 using either PCR testing, CT imaging, or both, depending on availability and clinical judgment.

Datasets

This study draws upon two complementary datasets.

Patient-level cohort: We included 488 patients admitted to University Hospital Center of Tlemcen. All patients underwent both PCR testing and chest CT imaging for clinical assessment. For each patient, demographic characteristics, symptom onset, cycle threshold (Ct) values, severity classification, were recorded.

Population-level surveillance: In parallel, we obtained cumulative counts of PCR+ and CT+ cases reported daily by communes in the Tlemcen region during the same period. These data were derived from official municipal surveillance registries and represent aggregated case counts, not individual-level records.

Individual-level demographic and clinical variables (e.g., age, sex, comorbidities, and outcomes) were not accessible in this dataset due to data protection regulations and absence of patient consent. Consequently, our analyses were limited to aggregate counts and diagnostic modality comparisons.

2.2. Data Collection

The following data were collected from medical records:

- Clinical Presentation: Symptoms such as fever, cough, shortness of breath, and fatigue;
- PCR Results: Cycle threshold (Ct) values for SARS-CoV-2 RNA detection;
- CT Imaging Findings: Degree of lung involvement, including the presence of ground-glass opacities (GGO), consolidation, and other abnormalities consistent with COVID-19-related pneumonia.

2.3. Diagnostic Methods

- PCR Testing: Nasopharyngeal swabs were collected from patients and processed according to the standard procedures using commercially available kits (e.g., RT-PCR kits). Ct values were recorded as a measure of viral load;
- CT Imaging: Chest CT scans were performed for all patients, with findings classified based on the extent of lung involvement. The images were evaluated by trained radiologists for common COVID-19 features, such as GGO (ground-glass opacities) and consolidation.

2.4. Statistical Analysis

Data were analyzed using SPSS software (version 26). Descriptive statistics were used to summarize demographic characteristics, clinical symptoms, PCR results, and CT imaging findings.

Statistical analyses were performed using daily detection numbers derived from cumulative counts of PCR and CT-positive cases. Daily values were obtained by calculating the difference between two consecutive cumulative entries. A Student's *t*-test (Welch's correction for unequal variances) was applied to compare daily detection numbers between PCR and CT imaging. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

A total of 488 confirmed COVID-19 cases were included in this study. Clinical symptoms were reported as fever, cough, shortness of breath, and ARDS (acute respiratory distress syndrome).

3.1. PCR Results

The PCR testing revealed varying viral loads among the patients, with Ct values ranging from 15 to 35. Most patients had Ct values between 20 and 30, suggesting moderate viral loads.

3.2. CT Imaging Findings

CT scans revealed evidence of lung involvement, with ground-glass opacities being the most common finding, followed by consolidation. The severity of lung involvement was classified into mild, moderate, and severe categories as follows:

Table 1 presents the cumulative number of confirmed PCR+ (positive for COVID-19 via PCR testing) and CT+ (positive for COVID-19 via CT imaging) cases in the communes of Tlemcen from 6 January 2021 to 29 December 2021. Each row represents data from a specific date, showing the total number of PCR+ and CT+ cases reported for that day, as well as the combined total. This cumulative data highlights the trend of COVID-19 case progression over the course of the year, illustrating the increasing number of confirmed cases as the pandemic evolved in the region. The table provides a comprehensive view of the overall case burden, although it does not capture daily new case counts, making it challenging to analyze short-term fluctuations.

When comparing the daily detection numbers between PCR and CT imaging using a Student's *t*-test, no statistically significant difference was observed ($t = -0.48$, $p = 0.63$). This finding suggests that although CT consistently yielded slightly higher cumulative counts than PCR, the daily detection variations between the two methods were not significantly different. When deriving daily detection numbers from cumulative counts, some negative values were observed due to retrospective corrections in reporting. For consistency, these values were adjusted to zero before statistical analysis.

Table 1. Snapshot active and daily COVID-19 Cases (PCR+ and CT+) in Wilaya of Tlemcen from January to December 2021.

Date	Snapshot Active PCR+ Cases	Snapshot Active CT+ Cases	PCR Daily Cases	CT Daily Cases
6 January 2021	1090	1254	44	3
13 January 2021	1134	1257	18	26
20 January 2021	1152	1283	24	40
3 February 2021	1176	1323	8	46
17 February 2021	1184	1369	5	10
24 February 2021	1189	1379	1	14
3 March 2021	1190	1393	9	10
10 March 2021	1199	1403	2	10
24 March 2021	1201	1413	3	5
31 March 2021	1204	1418	2	5
7 April 2021	1206	1423	4	15
28 April 2021	1210	1438	0	1
5 May 2021	1210	1439	1	2
12 May 2021	1211	1441	2	2
19 May 2021	1213	1443	1	1
27 May 2021	1214	1444	2	3
3 June 2021	1216	1447	11	8
23 June 2021	1227	1455	0	16
1 July 2021	1214	1471	50	22
10 November 2021	1264	1493	0	4
17 November 2021	1264	1497	11	1
24 November 2021	1275	1498	26	18
8 December 2021	1301	1516	5	1
15 December 2021	1306	1517	12	14
22 December 2021	1318	1531	0	0
29 December 2021	1311	1521	0	0

Figure 1 illustrates data on PCR+ and CT+ COVID-19 cases specifically in Tlemcen commune only, with a general upward trend in the number of cases over the studied period. The cumulative number of PCR+ cases increased from 3376 on January 2021, to 5236 on December 2021, while the cumulative number of CT+ cases rose from 3794 to 6085 during the same time frame. It is important to note that these data do not provide information on the number of new cases for each date, but rather the cumulative total since the onset of the epidemic. As such, analyzing short-term fluctuations in case numbers is challenging.

The data in this figure highlight daily variations in both PCR+ and CT+ detections, with CT+ cases consistently exceeding PCR+ cases. This trend suggests that CT imaging often identifies additional cases beyond PCR testing, underlining the importance of multi-method diagnostic strategies. More importantly, the daily fluctuations provide insights into healthcare system burdens, since sudden peaks in detections can significantly strain local resources. The steady increase throughout 2021 may reflect ongoing transmission despite public health efforts, potentially influenced by factors such as new variants, fluctuating testing availability, and vaccination campaigns. The slight acceleration in cases after November 2021 suggests a possible late-year resurgence or winter weather involvement, underscoring the need for sustained surveillance and adaptive public health measures to control the spread of infections effectively.

Table 2's data represent cumulative COVID-19 cases (PCR+ and CT+) in different communes of Tlemcen from January 2021, to December 2021, providing a comparison of cases at two different dates, showing the increase over the year.

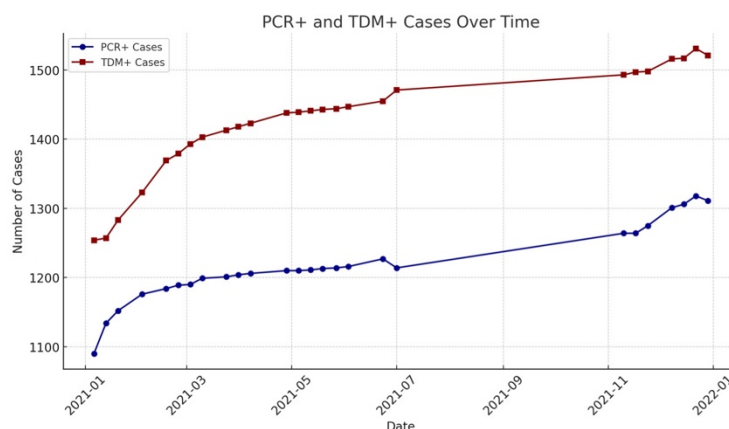


Figure 1. Snapshot active COVID-19 Cases (PCR+ and CT+) in Tlemcen.

Table 2. Cumulative and incremental (annual change) detection numbers of COVID-19 cases by PCR and CT in major communes of Tlemcen, Algeria (2021).

Commune	PCR+ (6 January 2021)	PCR+ (29 December 2021)	Increase	CT+ (6 January 2021)	CT+ (29 December 2021)	Increase
Tlemcen	590	740	+150	708	833	+125
Mansourah	219	235	+16	217	276	+59
Chetouane	98	119	+21	129	169	+40
Sabra	72	80	+8	61	87	+26
Terny	6	7	+1	10	10	0
Ain Ghoraba	3	4	+1	11	12	+1
Amieur	8	9	+1	13	20	+7
Ain Fezza	12	20	+8	23	24	+1
Bouhlou	2	3	+1	5	4	−1 *
Beni Mester	80	94	+14	77	86	+9

* Note: The small negative value in Bouhlou CT likely reflects retrospective data correction.

The data shows the cumulative increase in COVID-19 cases (PCR+ and CT+) across different communes in Tlemcen from January 2021 to December 2021. The overall trend indicates a continuous rise in cases, with the highest increases observed in Tlemcen, Mansourah, and Chetouane, likely due to higher population density and transmission rates. In contrast, smaller communes such as Bouhlou and Terny experienced only minor increases, possibly due to lower population density or effective local containment measures. The comparison between PCR+ and CT+ cases highlights differences in testing practices, as some communes reported more CT+ cases, suggesting either limited access to PCR testing or greater reliance on imaging for diagnosis. Several factors may have influenced these trends, including the unavailability of testing, the emergence of new variants, changes in public health measures, and vaccination campaigns, which may have mitigated severe cases despite rising infections. Understanding these patterns is crucial for assessing the effectiveness of interventions and guiding future public health strategies.

4. Discussion

The COVID-19 pandemic has significantly impacted global healthcare systems, with outcomes varying widely across regions. Algeria, classified as a high-risk country by the World Health Organization (WHO), has faced substantial challenges in managing

the pandemic, particularly in regions like Tlemcen. However, similar to other African nations, Algeria exhibited lower case fatality rates (CFR) compared to European and North American countries. Studies suggest that factors such as high sunlight exposure, lower population density, and regional public health responses may contribute to these differences [7–10].

Tlemcen, a region with a population of approximately 150,000, experienced considerable hospital admissions during 2021, emphasizing the need for detailed regional data to guide public health strategies. Despite the notable case burden, consistent and comprehensive data on admissions, recoveries, and mortality remain scarce, complicating response efforts. This study addresses this gap by analyzing diagnostic data and epidemiological trends from Tlemcen to better understand local transmission and disease progression.

A significant portion of the 197,558 confirmed cases in Tlemcen presented with classical COVID-19 symptoms such as fever, cough, and shortness of breath, consistent with global reports of respiratory manifestations in COVID-19 patients.

PCR testing revealed cycle threshold (Ct) values ranging from 15 to 35, with most cases showing moderate viral loads (Ct 20–30). Ct values, an inverse measure of viral load, have been correlated with disease severity in multiple studies. Although causation cannot be established from this data, the observed Ct patterns suggest a potential link between higher viral loads and adverse clinical outcomes, warranting further investigation. Chest CT imaging, a key diagnostic tool in this study, revealed widespread lung involvement, with ground-glass opacities (GGO) as the predominant finding. These imaging features are characteristic of COVID-19-related pneumonia and have been strongly associated with disease severity and progression. Classifying lung involvement into mild, moderate, and severe categories underscores the utility of CT imaging in guiding clinical decision-making, particularly in resource-limited settings where molecular diagnostics may be inaccessible [11–14]. Cumulative case trends for PCR-positive (PCR+) and CT-positive (CT+) cases exhibited a steady increase throughout 2021, indicative of sustained community transmission. These findings highlight the strain on the local healthcare system and underscore the importance of continued public health interventions, including vaccination campaigns, testing, and community education, to curb transmission.

The importance of integrating PCR and CT imaging in COVID-19 management is evident when comparing the Tlemcen experience to other regions. For instance, during the early stages of the pandemic, La Paz University Hospital in Madrid, Spain, managed over 3100 COVID-19 patients between February and April 2020, highlighting the role of multi-modal diagnostics in responding to high caseloads [14]. In contrast, hospitals in Brazil experienced systemic collapse during the first wave due to overwhelming patient numbers, illustrating the importance of robust healthcare planning and preparedness [15]. While Algeria in general [16–18] and Tlemcen specifically did not face such catastrophic scenarios, careful management was crucial to prevent overwhelming local healthcare services.

Our findings further emphasize the well-recognized limitation of RT-PCR testing; despite being regarded as the diagnostic gold standard, PCR is prone to false negatives due to factors such as sample quality, timing of collection, and the inherent sensitivity of reverse transcriptase to external disturbances. It is widely acknowledged that repeated PCR testing is sometimes necessary to confirm infection, and many patients with clear clinical symptoms may initially test negative. This highlights the concept of ‘*probatio diabolica*’ (devil’s proof), where proving the absence of infection is nearly impossible.

In this context, the higher number of CT+ cases compared to PCR+ in several communes underscores the value of complementary diagnostic approaches. While a positive result from any method confirms infection, reliance on PCR alone may underestimate the true number of cases. For future outbreak management, combining PCR with imaging or

other rapid diagnostic methods could reduce false negatives and provide a more accurate reflection of disease burden.

This study has several limitations. Its retrospective design, reliant on secondary medical records, may introduce biases or incomplete data, especially concerning post-discharge follow-up information. Another limitation of our study is the absence of cycle threshold (Ct) data, which could have provided deeper insights into viral load distribution and its potential link to the observed lower case fatality rates. Additionally, as the study is confined to a single region, the findings may not be generalizable to other parts of Algeria or similar settings globally.

Future research should prioritize larger, multi-center studies to validate these findings and explore variations in COVID-19 impact across regions, and incorporating Ct values will be essential to better contextualize our findings. Furthermore, investigating the long-term effects of COVID-19, including post-acute sequelae (commonly known as “long COVID”), is critical for understanding recovery trajectories and informing future public health strategies.

5. Conclusions

This study provides a comprehensive analysis of COVID-19 cases in the Tlemcen region during 2021, highlighting key clinical and diagnostic findings. The correlation between PCR Ct values and CT imaging underscores the importance of integrated diagnostic approaches in managing COVID-19. Additionally, the upward trend in cumulative cases emphasizes the need for sustained public health measures to mitigate the impact of future outbreaks. Future research should focus on longitudinal studies incorporating individual-level data to explore the interplay between viral load, clinical outcomes, and population-level interventions.

Author Contributions: Conceptualization, I.Z.; methodology, I.Z.; validation, W.L. and C.T.; formal analysis, I.Z.; investigation, W.L.; resources, N.S.; data curation, I.Z.; writing—original draft preparation, I.Z.; writing—review and editing, I.Z.; visualization, I.Z.; supervision, L.B. and N.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study due to the use of fully anonymized, aggregated retrospective data (total case counts per general geographic area, stratified by PCR confirmation or CT value ranges), in accordance with EPSP policy.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Conflicts of Interest: The authors declare no conflicts of interest.

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**Assessment of COVID-19 Vaccination Performance in Tlemcen: Insights into Vaccine
Inventory Management**

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Zatla, I., Boublenza, L., Lemerini, W., Bahar, D., Dahmani, B., Khouani, A., Selka, N.
(2025). Assessment of COVID-19 vaccination performance in Tlemcen: Insights into vaccine
inventory management. *Microbes and Infectious Diseases*, 6(3), 1036-1043.

DOI: 10.21608/mid.2025.307979.2112



○ **Objective**

In this study, we conducted a comprehensive epidemiological analysis of the COVID-19 vaccination campaign in Tlemcen, Algeria, from 2021 to 2022, with a particular focus on vaccine distribution, uptake patterns, and inventory management. Our aim was to identify key strengths and inefficiencies in the local vaccination strategy to inform future public health initiatives, especially in resource-limited settings.

○ **Method**

We adopted a descriptive, retrospective approach using real-world data sourced from official public health records. The data were analysed using Microsoft Excel and SPSS to ensure a systematic, structured evaluation. Descriptive statistics, including means, standard deviations, and percentages, were used to capture trends in vaccine uptake and wastage across multiple vaccine types.

○ **Results**

Our results revealed several important insights. Sinovac showed the highest uptake, potentially reflecting strong public trust or effective logistics. Sputnik demonstrated the lowest wastage rate, indicating sound stock and inventory control. Janssen appeared particularly popular among younger age groups, suggesting age-based preferences that could help shape targeted campaigns. Furthermore, individuals with comorbidities exhibited high vaccine response rates, particularly with Sinovac, reinforcing the importance of prioritizing high-risk populations in future planning. Despite these successes, our data highlighted significant losses in AstraZeneca stock due to expirations, indicating weaknesses in inventory management and forecasting. These findings underscore the need for improved coordination between supply chain operations and vaccine demand.

○ **Conclusion**

In conclusion, our analysis presents a detailed, data-driven overview of the COVID-19 vaccination campaign in Tlemcen. The study's strengths lie in its rigorous data collection, clear presentation of results, and relevant public health implications. By expanding the statistical depth, addressing logistical and behavioural factors, and integrating longitudinal perspectives, this research has the potential to significantly enhance vaccination strategies at both national and

regional levels. Continued investigation into vaccine distribution, public perception, and system-level challenges will be essential for preparing effective responses to future health crises.

- **Limitations**

While our descriptive analysis provided valuable baseline insights, we acknowledge that the study could be strengthened by the inclusion of inferential statistical methods such as chi-square tests or logistic regression. These analyses would help establish statistical significance for observed differences in vaccine preferences and wastage rates. Additionally, exploring correlations between vaccine uptake and demographic variables, such as age, comorbidity status, and socioeconomic background, would provide a deeper understanding of vaccination behaviour. We also noted the lack of an in-depth exploration of supply chain challenges contributing to vaccine wastage. A detailed assessment of storage conditions, cold chain reliability, and logistics could lead to actionable improvements. Moreover, the study relied primarily on static data through 2022. Incorporating longitudinal data, including booster uptake trends and seasonal variation in vaccine administration (e.g., during pandemic waves), would offer a more dynamic and predictive perspective.

- **Perspectives**

Future research should investigate the psychological, social, and economic factors influencing vaccine hesitancy in the Algerian context. This includes assessing the impact of misinformation, media coverage, and public health messaging. The role of healthcare workers in promoting vaccine acceptance should also be explored, along with the effectiveness of community-driven initiatives, particularly among vulnerable or hesitant groups. To build on this study, we propose additional comparative analyses with data from other North African or Middle Eastern countries. This would contextualize Tlemcen's experience and reveal regional best practices. Post-vaccination outcomes, such as adverse effects and breakthrough infections, should also be evaluated to assess long-term vaccine efficacy and safety. Furthermore, we recommend incorporating emerging tools such as machine learning to predict vaccine demand, identify at-risk populations, and optimize distribution strategies.



Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

Original article

Assessment of COVID-19 vaccination performance in Tlemcen: Insights into vaccine inventory management

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ARTICLE INFO

Article history:

Received 28 July 2024

Received in revised form 24 January 2025

Accepted 27 January 2025

Keywords:

SARS-CoV-2

COVID-19

Epidemiology

Vaccines

Public Health

Algeria

ABSTRACT

Background: The global response to the COVID-19 pandemic has been characterized by unprecedented vaccination efforts aimed at mitigating the spread of the virus and curbing its impact on public health. **Methods:** This study presents a comprehensive analysis of the vaccination campaign in Tlemcen, Algeria, focusing on vaccine distribution, utilization, and demographic trends. Data from the Public Health Establishment near Tlemcen were analyzed using Excel and SPSS to assess the distribution and management of five distinct vaccine brands: Sputnik, AstraZeneca, Sinopharm, Sinovac, and Janssen. Statistical analysis included the calculation of means, standard deviations, and percentages to evaluate vaccine distribution, uptake, and wastage. **Results:** The findings revealed varying levels of efficiency and challenges in vaccine management across different brands. Specifically, Sinovac had the highest administered doses at 93,908, representing 91.94% of its total imported vaccines. Sputnik had the lowest wastage rate, with no expired vaccines and only 10.35% lost doses. Age-specific trends in vaccine uptake highlighted Sinovac's prominence among the 50-64 and 65+ age groups, while Janssen was more popular in the 30-49 age group. Additionally, individuals with comorbidities showed a higher vaccine response to Sinovac, suggesting the need for tailored vaccination strategies for this subgroup. **Conclusion:** These findings provide valuable insights into the dynamics of the vaccination campaign in Tlemcen and contribute to ongoing efforts to optimize vaccination strategies and mitigate the impact of COVID-19.

Introduction

The COVID-19 pandemic has prompted an unparalleled global vaccination campaign, marking a pivotal moment in contemporary history. As the world grappled with the unanticipated onslaught of the pandemic, its toll surpassed what many had deemed conceivable (1, 2). With the identification of the causative agent and the decoding of its genetic

blueprint, efforts to develop a vaccine against SARS-CoV-2 commenced as early as January 2020. Multiple entities embarked on simultaneous vaccine development endeavors, employing a diverse array of vaccine platforms (3). Notable vaccines developed during this period include Sinovac and Sinopharm from China, Sputnik V from Russia, Janssen (Johnson & Johnson) from the United

DOI: 10.21608/MID.2025.307979.2112

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States, and AstraZeneca from the United Kingdom. These vaccines utilized various technological approaches, such as inactivated viruses, adenovirus vectors, and recombinant protein platforms, reflecting the rapid and collaborative response to the pandemic.

While the precise magnitude of infections and fatalities remains elusive, COVID-19 has exacted a staggering toll, claiming at least seven million lives by May 5, 2023, prompting the World Health Organization (WHO) to rescind its declaration of a global health emergency (4). Vaccination emerged as a pivotal milestone in the pandemic narrative, with a staggering 13.3 billion doses administered worldwide. Presently, WHO reports that 89% of healthcare workers and 82% of individuals over 60 years have completed their primary course of COVID-19 vaccination, though coverage within these priority groups varies significantly across regions (5). Despite the WHO's proclamation of the pandemic's conclusion, vaccination efforts persist as new cases or reinfections continue to be reported, albeit at reduced rates (6).

At the outset of the COVID-19 crisis, Algeria found itself ill-equipped to confront the escalating threat, lacking both a coherent containment strategy and adequate medical infrastructure to manage the surge in cases (7, 8). Notably, Tlemcen, a province within Algeria, has actively engaged in vaccination campaigns, targeting individuals across diverse age groups and health conditions. This study endeavors to provide a comprehensive analysis of vaccination data in Tlemcen, from the start of the outbreak till the end of the year 2022, encompassing the distribution of five distinct vaccines and their administration for three doses.

Materials and Methods

This study is a descriptive, retrospective analysis of the vaccination campaign management at the University Hospital of Tlemcen during the COVID-19 pandemic.

Data collection

The data for this study were obtained from the Public Health Establishment near Tlemcen (EPSP) and encompassed records from the onset of the COVID-19 vaccination campaign in University Hospital of Tlemcen through December 2022. Data sources included vaccination logs, inventory records, and demographic information of vaccine

recipients. The primary variables collected comprised:

1. Vaccine Administration Data
 - Total doses administered for each vaccine type.
 - Distribution of first, second, and third doses across different age groups.
2. Demographic Data
 - Age and gender of vaccine recipients.
 - Presence or absence of comorbidities.
3. Vaccine Inventory and Utilization
 - Quantities of each vaccine type received, administered, or wasted.
 - Expired doses and wastage rates for each vaccine.

Data were collated by healthcare professionals at the EPSP and ethical approval for the use of anonymized data was obtained from relevant authorities. All records were anonymized prior to analysis to ensure privacy and confidentiality.

Data Analysis

The collected data underwent rigorous statistical analysis using SPSS software to unveil key insights and trends. Descriptive statistics, including mean, median, standard deviation, and frequency distributions, were computed to characterize the distribution of vaccinated individuals with either Sinovac, Sinopharm, Sputnik V, Janssen, or AstraZeneca across age groups and to quantify the prevalence of underlying morbidities within each cohort.

To ensure the robustness of findings, subgroup analyses have been conducted to investigate the consistency of results across different demographic strata or vaccination scenarios. The significance level was set at $p < 0.05$ to determine statistical significance in all analyses.

Results

Vaccine Distribution and Management

Table 1 provides a comprehensive overview of vaccine distribution, utilization, and management across five distinct vaccine brands deployed in Tlemcen's vaccination campaign, namely Sputnik, AstraZeneca, Sinopharm, Sinovac, and Janssen. This table encapsulates mean values for

imported, administered, lost, expired, and remaining vaccine doses, offering valuable insights into the efficiency and challenges of vaccine management.

Table 2 provides an overview of the vaccine doses administered at the University Hospital of Tlemcen. It includes the total number of first, second, and third doses administered to individuals, reflecting the hospital's vaccination efforts.

Health personnel and patients

The group statistics in Table 3 compares mean values, standard deviations, and standard error means for health and vaccinated personnel across different iterations of Sputnik, AstraZeneca, Sinopharm, Sinovac, and Janssen vaccines.

Age-Based Vaccine Distribution

The bar chart (Figure 1) depicting mean values of different vaccines across various age groups elucidates intriguing trends in vaccine uptake. Sinovac stands out prominently, exhibiting significantly higher mean values in the 50-64 and 64 or more age brackets, suggesting either enhanced

efficacy or greater utilization in these age cohorts. Conversely, other vaccines display relatively consistent mean values across age groups, with minor fluctuations. Notably, Janssen displays a marginally higher mean value in the 30-49 age group compared to others.

Impact of Comorbidities on Vaccine Response

The bar chart (Figure 2) comparing mean values of a specific metric among individuals with and without comorbidities unveils intriguing insights into vaccine response dynamics. Sinovac emerges as a standout performer, registering significantly higher mean values among individuals with comorbidities, indicative of a pronounced effect or correlation with this subgroup. In contrast, mean values for individuals without comorbidities remain relatively consistent across all vaccines, underscoring the potential interaction between comorbidities and vaccine response, particularly in the context of Sinovac.

Table 1. Vaccine inventory at the University Hospital of Tlemcen.

Vaccines					
Vaccine Name	Imported vaccines	Administered vaccines	Lost vaccines	Expired vaccines	Remaining vaccines
Sputnik	7910	4352	819	2739	0
AstraZeneca	26100	9918	8576	7606	0
Sinopharm	5200	5192	8	0	0
Sinovac	102140	93908	3767	0	4465
Janssen	18605	5511	2534	9035	1525

Table 2. Administered vaccine doses at the University Hospital of Tlemcen

Total administered 1 st dose	66841
Total administered 2 nd dose	46736
Total administered 3 rd dose	5304
Total doses	118,881

Table 3. Vaccinated groups statistics at the University Hospital of Tlemcen.

Vaccines	Doses	Group Statistics			
		group	Mean	Std. Deviation	Std. Error Mean
Sputnik	1	Health personnel	17.6250	27.28651	9.64724
		Vaccinated individuals	260.6250	174.43455	61.67193
	2	Health personnel	17.6250	27.28651	9.64724
		Vaccinated individuals	236.7500	140.55375	49.69326
	3	Health personnel	00	00	00
		Vaccinated individuals	11.3750	5.97465	2.11236
AstraZeneca	1	Health personnel	0.7500	2.12132	0.75000
		Vaccinated individuals	597.2500	626.42654	221.47523
	2	Health personnel	0.7500	2.12132	0.75000
		Vaccinated individuals	561.5000	585.00183	206.82938
	3	Health personnel	00	00	00
		Vaccinated individuals	79.5000	85.03109	30.06303
Sinopharm	1	Health personnel	16.1250	22.66802	8.01436
		Vaccinated individuals	308.8750	148.64765	52.55488
	2	Health personnel	16.0000	22.39260	7.91698
		Vaccinated individuals	306.7500	148.23509	52.40902
	3	Health personnel	00	00	00
		Vaccinated individuals	1.2500	1.90863	.67480
Sinovac	1	Health personnel	197.7500	191.29390	67.63261
		Vaccinated individuals	6472.5000	3078.98425	1088.58532
	2	Health personnel	155.6250	147.66655	52.20801
		Vaccinated individuals	4345.2500	2677.60153	946.67510
	3	Health personnel	8.3750	17.10420	6.04725
		Vaccinated individuals	357.2500	129.12757	45.65349
Janssen	1	Health personnel	9.6250	9.51596	3.36440
		Vaccinated individuals	474.0000	264.30394	93.44555
	2	Health personnel	00	00	00
		Vaccinated individuals	00	00	00
	3	Health personnel	13.1250	23.01203	8.13598
		Vaccinated individuals	192.1250	52.91621	18.70870

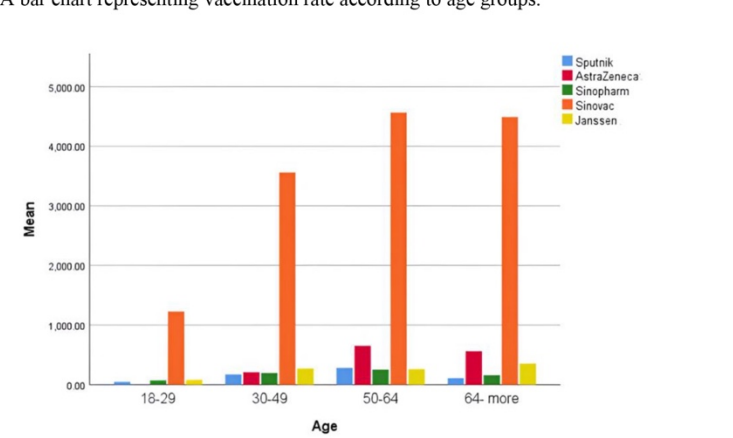
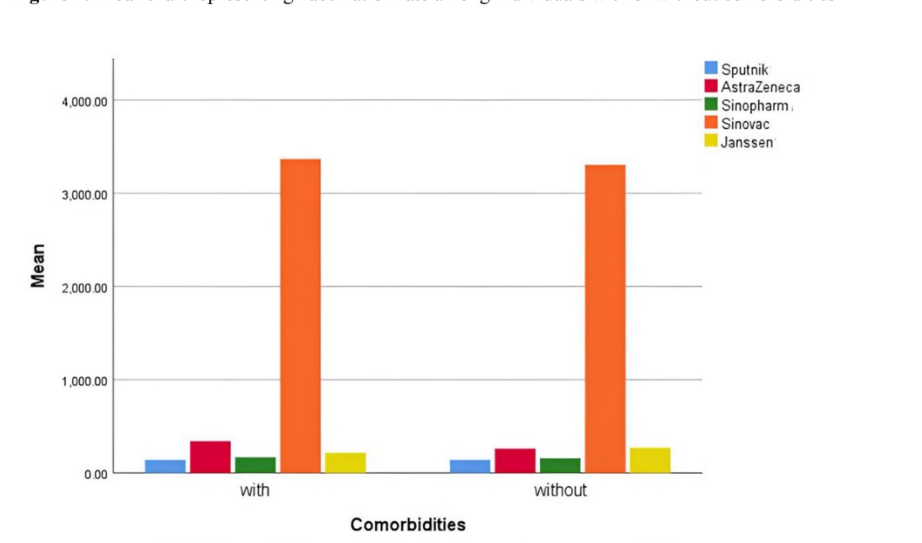
Figure 1. A bar chart representing vaccination rate according to age groups.

Figure 2. A bar chart representing vaccination rate among individuals with or without comorbidities

Discussion

The ongoing COVID-19 pandemic has necessitated robust vaccination campaigns worldwide to curtail the spread of the virus and mitigate its public health impact. Our study provides a descriptive analysis of the vaccination campaign in Tlemcen, Algeria, shedding light on local vaccination trends and contributing to understanding broader global vaccination efforts. The analysis of vaccine distribution, utilization, and demographic trends in Tlemcen reveals insights that resonate with broader global vaccination challenges and strategies.

As of December 31, 2023, the World Health Organization (WHO) reported that 67% of the global population had completed the primary series of a COVID-19 vaccine. Sinovac and Sinopharm, developed in China, have been particularly prominent in low- and middle-income countries due to their easier storage requirements, with significant use in places like Algeria. Sputnik V, from Russia, has also been deployed in various countries, including Algeria. The Janssen vaccine, a single-dose option from the U.S., has been used worldwide, particularly in Africa, to increase vaccination coverage. Meanwhile, the AstraZeneca vaccine, developed in partnership between the U.K. and Sweden, has been a cornerstone of the global vaccination effort, especially through the COVAX initiative, and has been distributed across many African nations. These vaccines have been integral

to the global response to COVID-19, with the WHO closely monitoring their distribution to ensure broad and equitable access (9).

Sinovac emerges as the frontrunner in both imported and administered vaccine doses, boasting a substantial surplus of remaining vaccines (4,465), indicative of efficient utilization with minimal wastage. Conversely, AstraZeneca exhibits significant losses, with notable numbers of both lost (8,576) and expired (7,606) vaccine doses, hinting at potential issues in distribution or storage protocols. Sinopharm demonstrates commendable efficiency, with nearly all imported doses administered and minimal losses recorded. However, both Sputnik and Janssen register noteworthy numbers of expired vaccine doses, hinting at potential overestimation of demand or logistical inefficiencies. Comparable findings in other regions of Algeria suggest that vaccine booster acceptance remains moderate, highlighting the critical role of targeted communication to address public concerns and ensure continued booster uptake (10, 11).

The age-specific and comorbidity-based variations in vaccine uptake and response observed in this study reflect the universal need for tailored vaccination approaches. These results highlight the critical importance of optimizing vaccine distribution and administration strategies to enhance vaccine accessibility and efficacy, particularly for vulnerable populations with underlying health

conditions. Studies conducted in Algeria reinforce the importance of vaccine equity and engagement strategies to overcome hesitancy and ensure widespread uptake. For instance, determinants of vaccine acceptance have been analyzed in population-based studies, revealing challenges such as hesitancy, resource limitations, and misconceptions about vaccine safety and efficacy (12).

There has been a notable rise in the acceptance of COVID-19 vaccines, particularly among healthcare workers, compared to earlier studies. However, the overall acceptance rate among the general population remains low (13). The adoption of pandemic-related vaccinations is a positive step towards restoring the global economy to its previous state. Evidence of stock market recovery post-COVID-19 vaccination implementation indicates the effectiveness of government interventions in controlling the virus's spread and severity (14). Our findings underscore the multifaceted nature of vaccine distribution and storage, with each vaccine brand presenting unique challenges and opportunities for optimization. Addressing these discrepancies is crucial for enhancing vaccine accessibility and efficacy.

Approximately two-thirds of Algerians are unlikely to engage in COVID-19 vaccine uptake, positioning them as one of the least receptive populations to voluntary vaccination in the MENA (Middle East North Africa) region. This lack of engagement likely contributes to the slow increase in the vaccination rate, posing significant public health concerns (15). Despite Algeria boasting one of Africa's top healthcare systems, it falls short of the standards set by wealthier countries. The country received the first 50,000 doses of the Russian vaccine Sputnik V on January 29, 2021, prioritizing distribution to healthcare workers, vulnerable individuals, and police officers, and with a population of approximately 44 million, this initial distribution marks the beginning of a massive vaccination campaign requiring substantial resources (16). Our observations underscore the potential influence of age on vaccine preferences and efficacy, with Sinovac demonstrating heightened effectiveness among older populations. Further research is warranted to elucidate the underlying mechanisms driving these age-specific trends and optimize vaccine distribution strategies accordingly.

For all vaccines listed in our study indicate that there are no statistically significant differences between group means for any of the vaccines. This suggests that, within each vaccine iteration, the different conditions or treatments being compared do not lead to different outcomes. Also, the mean values for vaccinated personnel are significantly higher than for health personnel in nearly all categories, which indicates a substantial difference in the sample measurements between health and vaccinated personnel. Overall, the data reflects substantial differences in the responses of health versus vaccinated personnel to various vaccine iterations, with considerable variability in some groups. Furthermore, the mean differences are negative across all groups, indicating that the mean value for the first group is lower than for the second group in all cases.

For most vaccines, the mean values for those with comorbidities are slightly higher than for those without, suggesting that comorbidities may have a mild effect on the outcomes measured. However, for Sinovac, the mean value is exceptionally high for both groups, indicating a significant outcome associated with this vaccine regardless of comorbidities. These results underscore the importance of considering comorbidities when evaluating vaccine effects but also highlights that the magnitude of the effect varies significantly depending on the vaccine type.

This study also provides the first insights into the attitudes of the Algerian public of Tlemcen towards COVID-19 vaccine boosters. The acceptance rate of boosters remains moderate, lower than those reported in developed countries. Specific groups with the highest acceptance rates include males, individuals over 60 years old, and those with chronic diseases, while healthcare professionals and postgraduate students exhibit higher hesitancy. Psychological factors related to vaccine effectiveness and safety play a significant role in booster acceptance (17). Additionally, this study confirms the short-term safety of adenoviral-based and inactivated-virus booster doses used in Algeria. Side effects following such boosters, including injection site pains and mild systemic reactions like fever and fatigue, were generally brief and less severe compared to those following initial doses. Notably, individuals who received inactivated-virus or adenoviral-based vaccines for their primer-booster doses experienced more severe side effects (18).

To date, monitoring of the effects of COVID-19 has rested on several epidemiologic and clinical measures, which have shaped the recommended or mandated protective actions. Most commonly, these measures have included estimated rates of COVID-19 cases, hospitalizations, and deaths; monitoring has also been conducted of circulating SARS-CoV-2 variants and their susceptibility to available vaccines and treatments (19). Vaccine hesitancy is an increasingly pervasive challenge across the world and the saving of more than 19 million lives by the unprecedented rapidity of development and roll-out of COVID-19 vaccines is an extraordinary global health feat. Nonetheless, millions of additional lives could have been saved by more equitable distribution of vaccines (20). The discussion extends beyond the local context to highlight broader implications and challenges related to COVID-19 vaccine acceptance and coverage. Despite limitations, the study confirms the short-term safety of booster doses used in Algeria, emphasizing the need for targeted communication and engagement efforts to address psychological factors and ensure vaccine safety. Furthermore, these observations align with national findings on vaccine hesitancy and resource allocation, highlighting the need for innovative strategies to improve public trust and ensure equitable vaccine distribution (21).

Conclusion

This study highlights the dynamic interplay between vaccine distribution, age demographics, and comorbidity status in shaping vaccination outcomes in Tlemcen. Addressing the observed discrepancies in vaccine utilization and response is paramount for optimizing vaccination campaigns and mitigating the impact of COVID-19. Future research endeavors should focus on elucidating the underlying mechanisms driving age-comorbidity-specific vaccine responses to inform targeted vaccination strategies and enhance public health outcomes.

Acknowledgments

We would like to express our gratitude to Professor Latifa Hennaoui, MD, at Dr. Tidjani Damerdjil University Hospital, Department of Epidemiology, Tlemcen, Algeria, for her invaluable support in facilitating the research process.

Authors' contributions

All authors contributed equally to the work.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Funding

This research received no external funding.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon request.

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*Axis II: SARS-CoV-2
Antiviral Computational
Investigation*

**Battling COVID-19 Leveraging Nanobiotechnology: Gold and Silver Nanoparticle–B-escin
Conjugates as SARS-CoV-2 Inhibitors**

Ilyes Zatla and Lamia Boublenza

Zatla, I. and Boublenza, L. (2025) Battling COVID-19 leveraging nanobiotechnology:
Gold and silver nanoparticle–B-escin conjugates as SARS-CoV-2 inhibitors. *Open Life Sciences*,
20(1), 20221047.

DOI: 10.1515/biol-2022-1047



○ Objective

This paper presents a computational evaluation of gold and silver nanoparticles conjugated to β -escin as potential inhibitors of SARS-CoV-2. By combining the unique antiviral properties of metallic nanoparticles with the bioactivity of β -escin, the research explores a novel nanobiotechnological strategy to block viral entry, replication, and key enzymatic functions. Our primary objective was to evaluate the potential of these nanobiotechnological agents to disrupt the virus by targeting key viral proteins, including the spike glycoprotein, Mpro, and RdRp, using a combination of molecular docking, computational modelling, and structural analysis with advanced bioinformatics tools.

○ Methods

The study used crystal structures of five SARS-CoV-2 S protein variants (Alpha, Beta, Delta, Omicron, Gamma) and the human ACE2 receptor obtained from the PDB as the basis for all interaction studies. Molecular docking on the HDock server was first performed to evaluate the binding affinity and conformational stability of each spike RBD–ACE2 complex. Subsequently, AuNPs and AgNPs were modelled, energy-minimized with AutoDock 4.2, and docked onto the preformed ACE2–spike complexes to assess their capacity to disrupt virus–receptor binding. To examine potential effects on viral replication, both nanoparticles were also docked to the Mpro and the RdRp as additional antiviral targets. Finally, β -escin was docked onto the nanoparticle–ACE2–spike assemblies to probe its role in strengthening nanoparticle–protein interactions, and all complexes were visualized and inspected in PyMOL and UCSF Chimera to analyse binding modes and structural stability.

○ Results

All spike variants bound strongly to ACE2, with Omicron and Delta showing the most favourable binding energies, consistent with their higher transmissibility, and Gamma showing the weakest interaction. Both AuNPs and AgNPs engaged all spike variants, with AuNPs displaying slightly stronger spike-surface affinities, whereas AgNPs interacted more strongly with RdRp and slightly better with Mpro, indicating a greater capacity to inhibit key enzymes involved in viral replication. Conjugation with β -escin further stabilized nanoparticle–protein assemblies, particularly for AgNP–ACE2–spike Alpha and AuNP–ACE2–spike Beta, through extensive hydrogen bonding and van der Waals contacts. Structural inspection showed that β -escin

preferentially localizes at the ACE2–spike interface, forming multiple hydrogen bonds, and binds an allosteric pocket on RdRp, suggesting a mechanism for disrupting receptor engagement and impairing RNA synthesis.

○ **Conclusion**

Collectively, these results propose a multi-target antiviral mechanism for the AuNP/AgNP– β -escin conjugates, involving three complementary actions:

- Blocking viral entry through nanoparticle binding to the spike glycoprotein, preventing ACE2-mediated attachment.
- Inhibiting proteolytic cleavage by interacting with Mpro, thereby disrupting viral polyprotein processing.
- Suppressing viral replication by targeting RdRp, particularly through the enhanced binding behaviour of AgNPs.

Additionally, the amphipathic nature of β -escin may contribute to destabilization of the viral envelope, further reducing infectivity and reinforcing the proposed broad-spectrum antiviral potential of these nanobioconjugates.

○ **Limitations**

While our computational results provide strong evidence for the antiviral efficacy of NP– β -escin conjugates, it is essential to recognize the limitations of *in silico* studies. Experimental validation through *in vitro* assays using SARS-CoV-2-infected cell lines and *in vivo* models is necessary to confirm these findings. Further research should focus on cytotoxicity assessment, nanoparticle biocompatibility, and pharmacokinetic studies to evaluate their clinical applicability. Additionally, controlled drug-delivery systems, such as nasal formulations, could enhance therapeutic efficacy by targeting the upper respiratory tract, a primary site of coronavirus infection.

○ **Perspectives**

Furthermore, future research should explore the pharmacodynamics and pharmacokinetics of these nanoparticle formulations to optimize their bioavailability and targeted delivery. Nanoparticle functionalization using ligands or polymeric coatings (e.g., thiol groups) may enhance stability and reduce cytotoxicity. Additionally, large-scale computational simulations,

including molecular dynamics studies, could provide deeper insights into the conformational flexibility of NP–protein interactions.

Research Article

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Battling COVID-19 leveraging nanobiotechnology: Gold and silver nanoparticle–B-escin conjugates as SARS-CoV-2 inhibitors

<https://doi.org/10.1515/biol-2022-1047>

received September 04, 2024; accepted December 17, 2024

Abstract: The COVID-19 pandemic, an unprecedented global health crisis, has thrust humanity into a relentless battle with a variety of treatments and vaccines against the SARS-CoV-2 virus. Recent developments in nanotechnology have garnered significant interest in the application of metallic nanoparticles (NPs); specifically, silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) have demonstrated antimicrobial and antiviral properties. This study investigates the molecular interactions between the receptor binding domains of five SARS-CoV-2 spike protein variants (Alpha, Beta, Delta, Omicron, and Gamma) and the angiotensin-converting enzyme 2 (ACE2) receptor, followed by the docking of AuNPs and AgNPs and the natural compound Beta-escin onto these complexes. As well as the inspection of both NPs against the virus main protease (Mpro) and RNA-dependent RNA polymerase (RdRp). Comprehensive computational simulations utilizing Autodock 4.2 and HDock server were employed to evaluate the binding affinities of these NPs toward key viral targets, SARS-CoV-2 Mpro, RdRp, and the spike glycoprotein. The results revealed that both AgNPs and AuNPs exhibited successful binding to the active pockets of SARS-CoV-2 Mpro, with slightly varying binding energies. In contrast, for RdRp, AgNPs demonstrated superior binding affinity compared to AuNPs, with differences in the residues involved in the binding pocket. AuNPs exhibited stronger binding affinities in the spike protein pocket. We also determined robust

binding affinities between ACE2 and the spike variants, with the Omicron variant exhibiting the highest affinity. Subsequent docking of AuNPs and AgNPs revealed strong interactions with all ACE2–spike complexes, with AuNPs showing slightly higher affinities. The findings contribute to a deeper understanding of the interactions between NPs and viral proteins, shedding light on their mechanisms of action and their potential to offer innovative solutions for combating infectious diseases, particularly those caused by SARS-CoV-2.

Keywords: SARS-CoV-2, COVID-19, antivirals, nanoparticles, biomolecules, molecular dynamics

1 Introduction

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has precipitated a global health crisis, necessitating the rapid development of effective therapeutic and diagnostic strategies [1]. Central to the virus's pathogenicity is the spike protein, which facilitates viral entry by binding to the angiotensin-converting enzyme 2 (ACE2) receptor on host cells. Variants of concern such as Alpha, Beta, Delta, Omicron, and Gamma have emerged, characterized by mutations in the spike protein that enhance transmissibility and potentially alter vaccine efficacy [2]. Understanding the molecular interactions between these spike protein variants and the ACE2 receptor is critical for developing targeted interventions. Molecular docking, a computational method that predicts the preferred orientation of one molecule to a second, provides valuable insights into these interactions [3]. Also, among the various targets within the realm of coronaviruses, particular focus has been placed on the main protease (Mpro), RNA-dependent RNA polymerase (RdRp), and the spike protein [4].

These metallic nanoparticles (NPs), notably silver and gold, have emerged as highly promising agents due to their demonstrated effectiveness against a spectrum of

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Table 1: HDock results of docking ACE2 as a ligand on the five spike receptor variants

Complex	ACE2-spike Alpha variant	ACE2-spike Beta variant	ACE2-spike Delta variant	ACE2-spike Omicron variant	ACE2-spike Gamma variant
Docking score (kcal/mol)	−298.59	−281.90	−333.36	−359.33	−269.59
Confidence score	0.9513	0.9333	0.9751	0.9850	0.9162
Ligand rmsd (Å)	1.19	84.66	0.80	0.88	47.17

microorganisms, including bacteria, viruses, and other eukaryotic entities. This heightened interest among researchers is a response to the escalating problem of microbial resistance and the development of resilient strains [5,6]. Silver nanoparticles (AgNPs) exhibit remarkable antimicrobial properties attributed to their distinctive physiochemical attributes and superior biological functionalities [7]. They also possess antiviral properties, with reports indicating that AgNPs synthesized in Hepes buffer can impede the replication of HIV-1, displaying anti-human immunodeficiency virus (HIV) activity, in addition to inhibiting hepatitis B virus and herpes simplex virus [8,9]. Gold nanoparticles (AuNPs) have garnered significant attention in various domains, including industry and nanomedicine, owing to their exceptional electrical, optical, mechanical, and biological attributes [10,11]. The antiviral properties of AuNPs have been supported by research studies, with their minute particle size, low toxicity, non-immunogenicity, and capacity to bind biological ligands rendering them promising candidates for antibacterial and antiviral purposes. Previous investigations have demonstrated the ability of AuNPs to hinder multiple stages in the life cycles of various viruses, including Herpes simplex virus type 1, Zika virus, HIV, dengue virus, and foot-and-mouth disease virus [12,13]. Beta-escin (B-escin) is a natural saponin compound extracted from the seeds of the horse chestnut tree (*Aesculus hippocastanum*). Known for its anti-inflammatory, antiviral, vasoprotective, and anti-edematous properties, it has been used in traditional medicine and continues to be explored for its therapeutic potential [14–16]. Investigating its synergistic potential when combined with NPs in binding to the ACE2-spike complex could reveal novel strategies to combat SARS-CoV-2 [17].

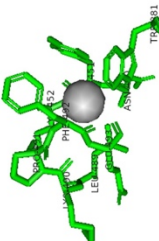
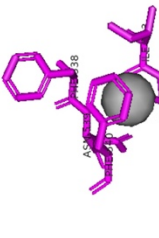
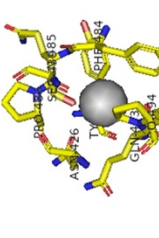
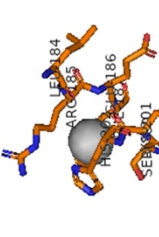
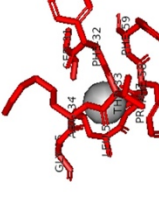
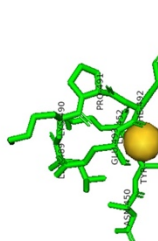
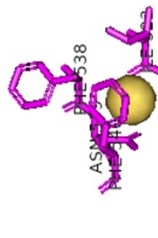
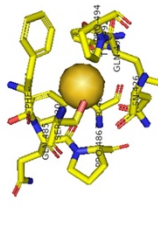
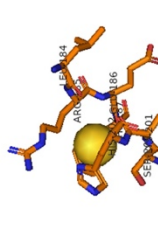
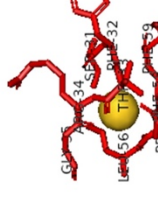
The primary objective of this study is to investigate and elucidate the binding affinities and interaction dynamics among the molecules including the ACE2 receptor and the five SARS-CoV-2 spike protein variants, with the Au and Ag NPs onto these protein-protein complexes, followed by B-escin. And also to explore the potential of AgNPs and AuNPs interactions with key viral targets, namely the Mpro and RdRp, providing insights into their potential therapeutic and diagnostic applications in managing COVID-19 and its variants.

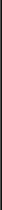
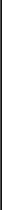
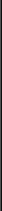
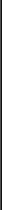
2 Materials and methods

The molecular docking procedure involved utilizing the HDock server to conduct docking simulations for the receptor binding domain (RBD) of five different SARS-CoV-2 spike protein variants: Alpha, Beta, Delta, Omicron, and Gamma (Protein Data Bank [PDB] ID 7R13, 7WEV, 7VHH, 7Q07, and 8DLO, respectively). In this investigation, the amino acid sequence of ACE2 wild type (PDB ID 1R42) served as the ligand, while the SARS-CoV-2 spike protein RBD variants acted as receptors. Subsequently, AuNPs and AgNPs were docked onto the resulting protein-protein complexes. The structural information for AgNPs (CID_23954) and AuNPs (CID_23985) was retrieved from PubChem, and their geometries underwent optimization through Autodock 4.2 [18,19]. Crystal structures' water molecules were removed, polar hydrogen atoms were added, and only the relevant chains were retained. Marsili-Gasteiger partial charges were assigned using a two-phase algorithm. The receptor crystal structures underwent energy minimization via the AutoDockTools

Table 2: Binding energy results of docking AuNPs and AgNPs on ACE2-5 spike receptor variant complexes

Ligand	Score	ACE2-spike Alpha variant	ACE2-spike Beta variant	ACE2-spike Delta variant	ACE2-spike Omicron variant	ACE2-spike Gamma variant
AgNPs	Binding energy (kcal/mol)	−0.21	−0.23	−0.22	−0.18	−0.20
AuNPs	Binding energy (kcal/mol)	−0.25	−0.27	−0.26	−0.20	−0.23

Ligand	ACE2-spike Alpha variant	ACE2-spike Beta variant	ACE2-spike Delta variant	ACE2-spike Omicron variant	ACE2-spike Gamma variant
AgNPs					
AuNPs					

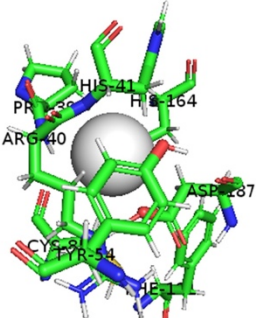
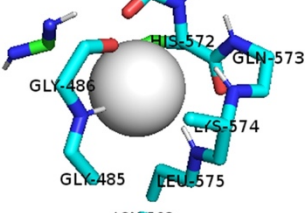
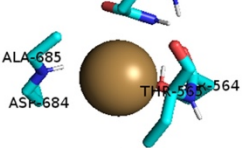
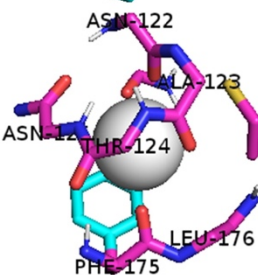
B-escinn-NP complex	ACE2-spike Alpha variant	ACE2-spike Beta variant	ACE2-spike Delta variant
			

(Continued)

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Distinct from the alpha and beta complexes, B-escin adopted two different binding pockets when docked onto Ag and AuNP-ACE2-spike Delta variant complexes. It occupied a terminal pocket in the AgNP-ACE2-spike Delta

Table 6: PDB ID targets and bonded residues' 3D interactions with their receptors and their binding energies

PDB	NP	Binding energy (kcal/mol)	Residues	3D interactions
PDB ID 7CAM (M ^{Pro})	AgNP	-0.16	Pro39, Arg40 His41, Tyr45, Cys85, His164, Phe181, Asp187	
	AuNP	-0.13	Pro39, Arg40, His41, Cys85, Asp187	
PDB ID 6YYT (RdRp)	AgNP	-0.13	Gly485, Gly486, His572, Gln573, Lys574, Leu575	
	AuNP	-0.07	Ser564, Thr565, Asn568, Asp684, Ala685	
PDB ID 6VXX (spike)	AgNP	-0.14	Asn121, Asn122, Ala123, Thr124, Phe175, Leu176	

(Continued)

same as those reported for other reported inhibitors, such as elbasvir (DB11574), daclatasvir (DB09102), and glecaprevir (DB13879) [30].

While this study provides valuable insights into the binding interactions between SARS-CoV-2 spike protein variants, ACE2, NPs, and B-escin, it is important to acknowledge several limitations. First, the results are derived solely from *in silico* molecular docking simulations, which may not fully replicate dynamic biological systems. Experimental validation, including *in vitro* and *in vivo* assays, is essential to confirm these findings. Second, this study does not evaluate the cytotoxicity or biocompatibility of AuNPs and AgNPs, which are critical considerations for therapeutic applications.

NPs, while promising, are known to exhibit dose-dependent toxicity. For example, AgNPs can induce oxidative stress by generating ROS, potentially leading to cellular damage. Furthermore, NP accumulation in vital organs, such as the liver and kidneys, has been observed in pre-clinical studies, raising concerns about their long-term safety. Mitigation strategies, such as NP functionalization with biocompatible materials and rigorous toxicity testing, are imperative before considering clinical applications. These limitations underline the necessity for a cautious approach and further experimental studies to assess the safety and efficacy of these therapeutic agents.

While B-escin has demonstrated promising pharmacological activity, it is not without limitations. Potential side effects include gastrointestinal disturbances such as nausea, diarrhea, and, in rare cases, allergic reactions. Additionally, caution is advised in patients with renal impairment due to potential nephrotoxic effects at higher doses. Regarding the spike protein, it is important to emphasize that its use in this study is limited to *in silico* simulations using structural data from the PDB. In therapeutic applications, proteins or their derivatives undergo extensive modifications to ensure safety and efficacy. Further preclinical studies are required to assess the safety, bioavailability, and therapeutic window of B-escin and NP-based delivery systems before advancing to clinical trials. These steps are crucial to ensure the safety of potential pharmacological interventions.

4 Conclusion

In summary, the docking analysis highlights the robust affinity of AuNPs and AgNPs toward various ACE2–spike receptor variants. Furthermore, the distinct binding pockets and interactions observed underscore the nuanced nature of NP–protein interactions. These findings not only deepen our understanding of NP–receptor interactions but also

underscore their potential as therapeutic agents or diagnostic tools against viral infections. The docking study elucidates the synergistic potential of B-escin with both AuNPs and AgNPs across various ACE2–spike receptor variant complexes. The varying binding affinities and preferred binding pockets of B-escin underscore its dynamic interaction with NP–protein complexes. Despite differences in binding preferences, B-escin demonstrates consistently favorable interactions, suggesting its potential as a therapeutic adjunct in combating viral infections mediated by ACE2–spike receptor variants. Both AgNPs and AuNPs have also shown binding affinities to the Mpro and RDRP, suggesting their potential in antiviral drug development. These insights pave the way for further exploration of B-escin–NP combinations as potential antiviral strategies.

Acknowledgments: We would like to thank Mr. Daniel Clement Agurokpon from Computational and Bio-Simulation Research Group, University of Calabar, Calabar, Nigeria, for editing and reviewing the manuscript.

Funding information: Authors state no funding involved.

Author contributions: All authors accepted the responsibility for the content of the manuscript and consented to its submission, reviewed all the results, and approved the final version of the manuscript. I.Z.: conceptualization, data collection, literature search, data collection, data analysis, manuscript writing, manuscript editing. L.B.: manuscript review, validation, and supervision of the project.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Green-Synthesized Colloidal Metal–B-escin Bioconjugates for Nasal Prophylaxis and Treatment: a Hypothetical Approach Against SARS-CoV-2 Variants

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Zatla, I., & Boublenza, L. (2025). Green-Synthesized Colloidal Metal–B-escin Bioconjugates for Nasal Delivery: A Hypothetical Prophylactic and Therapeutic Product Against SARS-CoV-2 Variants. *Trends in Sciences*, 22(6), 9826.

DOI: 10.48048/tis.2025.9826



○ Objective

In this study, we proposed an innovative prophylactic and therapeutic strategy against SARS-CoV-2 by developing a nasal formulation that combines green-synthesized gold and silver nanoparticles with β -escin, a natural compound known for its antiviral properties. Our objective was to address the ongoing challenges posed by emerging COVID-19 variants and to explore supplementary preventive measures beyond traditional vaccination.

○ Methods

To assess the antiviral potential of the proposed nanoparticle- β -escin conjugates, we employed a comprehensive *in silico* approach. We conducted molecular docking using AutoDock Vina to evaluate the binding affinity of the conjugates to the SARS-CoV-2 S protein. This was followed by molecular dynamics (MD) simulations using GROMACS to analyse the stability of the complexes over time. Finally, quantum-mechanical calculations using density functional theory (DFT) were performed with Gaussian software to assess electronic interactions and binding energies.

○ Results

Docking and simulation results showed that β -escin binds strongly to the SARS-CoV-2 S protein, and that conjugation to AuNPs and AgNPs further enhances binding affinity and complex stability relative to β -escin alone. The nanoparticle- β -escin formulations maintained high binding efficacy across all tested variants, including the highly mutated Omicron strain, and quantum mechanical analysis indicated synergistic effects driven by improved charge redistribution and stronger electrostatic interactions at the conjugation sites. Variant-wide analyses support the proposed nasal formulation as an adaptable prophylactic, with smaller, spherical, thiol- or amine-functionalized nanoparticles displaying superior mucosal penetration, reduced aggregation, and better interaction energies. Given these properties, intranasal delivery of the nanoparticle- β -escin system offers localized action at the respiratory mucosa, facilitating early blockade of viral entry and replication while minimizing systemic side effects.

○ Conclusion

This study provides a solid computational basis for a novel nanobiotherapeutic nasal formulation combining β -escin with AuNPs and AgNPs as a potential antiviral prophylactic

against SARS-CoV-2. The results highlight its molecular stability and strong binding affinity for viral targets, supporting its potential as a non-invasive, accessible preventive strategy.

- **Limitations**

While the computational findings are promising, this study is limited by the lack of experimental validation. Future work should include *in vitro* assays using nasal epithelial cells and *in vivo* testing in animal models to confirm the formulation's safety and antiviral efficacy. It will also be necessary to assess formulation stability, particularly the degradation profile of β -escin within the nasal spray matrix, and to optimize storage conditions. Additionally, challenges related to scaling up the green synthesis process, ensuring batch-to-batch reproducibility, and selecting suitable stabilizers must be addressed before translational development.

- **Perspectives**

The proposed formulation could serve as an affordable and easily deployable nasal prophylactic, especially valuable in low-resource settings. Future research should focus on optimizing dosage, evaluating long-term safety, and expanding its evaluation to different SARS-CoV-2 variants and other respiratory viruses. Successful *in vitro*, *in vivo*, and clinical validation could ultimately lead to large-scale production and integration into global public health strategies.

Green-Synthesized Colloidal Metal–B-Escin Bioconjugates for Nasal Delivery: A Hypothetical Prophylactic and Therapeutic Product Against SARS-CoV-2 Variants

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Received: 21 January 2025, Revised: 4 February 2025, Accepted: 11 February 2025, Published: 10 May 2025

Abstract

The emergence of SARS-CoV-2 variants continues to challenge global healthcare systems, necessitating the exploration of innovative preventive modalities. This study proposes a novel nasal prophylactic approach that combines the antiviral properties of B-escin, a natural compound, with green synthesized gold and silver nanoparticles. B-escin, extracted from horse chestnut seeds, exhibits notable efficacy against coronaviruses, while nanoparticles provide enhanced features for targeted delivery and stability. Through integrated computational analyses, including molecular docking, molecular dynamics simulations, and quantum mechanical calculations, we evaluate the potential of this combined strategy against prevalent COVID-19 variants, such as Alpha, Beta, Gamma, Delta, and Omicron. Specifically designed for nasal administration, our investigation highlights the synergistic interactions between B-escin and nanoparticles, elucidating their collective impact on viral targets specific to each variant within the nasal mucosa. Computational results demonstrate increased antiviral activity and target specificity when combining B-escin with gold and silver nanoparticles, surpassing individual efficacy. Moreover, we explore the influence of nanoparticle characteristics, such as size, morphology, and surface functionalization, on the observed synergistic effects. These findings underscore the potential of a nasal prophylactic utilizing natural compounds and nanotechnology, offering a promising frontline defense against the diverse spectrum of SARS-CoV-2 variants. While these findings underscore the potential of a nasal prophylactic utilizing natural compounds and nanotechnology, this study is entirely computational, and experimental validation is necessary to confirm its real-world applicability.

Keywords: SARS-CoV-2, COVID-19, B-escin, Nanoparticles, Nasal delivery, Antiviral therapy, Computational modeling, Variant-specific targeting

Introduction

The ongoing global pandemic caused by SARS-CoV-2 has presented unprecedented challenges to healthcare systems worldwide. The rapid emergence of multiple variants, including Alpha, Beta, Gamma, Delta, and Omicron, has complicated efforts to control the virus's spread and severity. Although vaccines remain a cornerstone of prevention, the high mutation rate of the virus, combined with vaccine hesitancy and waning immunity, has underscored the need for complementary preventive strategies [1,2].

Among these strategies, nasal prophylactics have garnered significant interest due to their ability to directly target the primary site of viral entry and replication within the upper respiratory tract. Nasal delivery systems provide localized protection, minimizing systemic side effects and enhancing therapeutic efficiency [3].

B-escin, a natural compound derived from horse chestnut seeds, has shown promising antiviral properties against a variety of viruses, including coronaviruses. Its

ability to inhibit viral entry and replication, coupled with its biocompatibility, positions it as a compelling candidate for COVID-19 prophylaxis [4,5]. Additionally, nanotechnology has emerged as a transformative tool in the development of antiviral treatments. Gold and silver nanoparticles (NPs), with their tunable physical and chemical properties, have demonstrated potential for drug delivery, antiviral activity, and stability enhancement [6-15]. However, despite these promising attributes, the potential synergistic effects of combining B-escin with green synthesized nanoparticles (**Figure 1**) for SARS-CoV-2 prophylaxis remain largely unexplored. There is limited understanding of how nanoparticle functionalization influences B-escin's antiviral efficacy, its molecular interactions with viral targets, and its stability in a nasal formulation. This study aims to bridge this gap by employing computational approaches to evaluate the feasibility and effectiveness of a nanoparticle-B-escin combination as a nasal prophylactic against SARS-CoV-2 variants. The interaction between B-escin and

nanoparticles, along with their collective impact on viral targets in the nasal mucosa, is a central focus of this investigation. By targeting the nasal mucosa, the proposed prophylactic could potentially serve as an early-line defense against SARS-CoV-2, complementing existing vaccination efforts and mitigating the spread of new variants. Its localized delivery may offer rapid antiviral action at the primary site of infection, reducing viral load and transmission risk. Furthermore, as a non-invasive, self-administered intervention, a nanoparticle-based nasal prophylactic could provide an accessible and convenient preventive measure, particularly in populations with low vaccine coverage or emerging variant threats. Its ease of use and potential for broad distribution make it a viable adjunct to current preventive strategies. Ultimately, this work provides a proof-of-concept for a nanoparticle-based nasal prophylactic (**Figure 2**), offering a promising frontline defense against the evolving spectrum of SARS-CoV-2 variants.

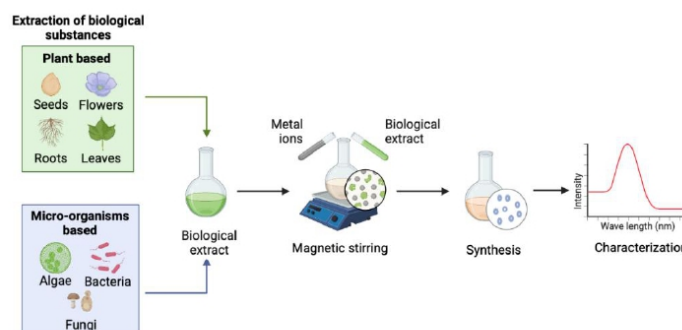


Figure 1 Methodology for the green synthesis of nanoparticles using biological extracts.



Figure 2 Conceptual framework of a nasal formulation incorporating nano-bioconjugates.

Materials and methods

Computational analyses

To evaluate the antiviral potential of the proposed combination treatment, computational approaches were employed to simulate interactions between B-escin, nanoparticles, and SARS-CoV-2 variants spike protein [16-30].

Molecular docking

Molecular docking was conducted to predict the binding affinity and orientation of B-escin within the active sites of SARS-CoV-2 spike protein. The docking was performed using AutoDock Vina, with protein structures obtained from the Protein Data Bank (PDB). The docking results were analyzed for binding energy, hydrogen bonding, and hydrophobic interactions.

Molecular dynamics simulations

MD simulations were performed using GROMACS to evaluate the stability and flexibility of the docked complexes. Systems were solvated in a TIP3P water model, neutralized with ions, and subjected to energy minimization and equilibration under physiological conditions (300 K, 1 atm). Trajectory analysis included root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and interaction energy.

Quantum mechanical calculations

The interaction energy and electronic properties of B-escin with nanoparticles were further analyzed using density functional theory (DFT) in the Gaussian software package. Parameters such as binding energy, charge distribution, and molecular orbitals were assessed to understand the synergistic effects at the molecular level.

Nanoparticle synthesis and modeling

Gold and silver nanoparticles were selected for their antiviral properties, biocompatibility, and ease of functionalization. The following parameters were considered for nanoparticle characterization:

Nanoparticle size and morphology

Particle size (10 - 50 nm) was optimized for nasal delivery to ensure deep penetration into the nasal

mucosa without systemic absorption. Spherical morphology was chosen due to its stability and higher surface area-to-volume ratio.

Surface functionalization

B-escin was conjugated to the nanoparticle surface through thiol or amine linkages to enhance stability and ensure targeted delivery. Surface charge and hydrophilicity were optimized to prevent aggregation and promote mucosal adhesion.

Synergistic interaction evaluation

The combined antiviral activity of B-escin and nanoparticles was assessed *in silico* using interaction energy and docking scores. Simulations included comparative analysis of B-escin-nanoparticle complexes versus standalone treatments, and variant-specific docking simulations against Alpha, Beta, Gamma, Delta, and Omicron with spike proteins.

Hypothetical experimental validation

Though this study focuses on computational approaches, the proposed methodology can be validated through:

In vitro studies

Testing B-escin-nanoparticle formulations against SARS-CoV-2 pseudovirus in nasal epithelial cell cultures.

Ex vivo models

Assessing mucosal adhesion and antiviral activity in human nasal tissue models.

In vivo studies

Evaluating safety and efficacy in animal models infected with SARS-CoV-2 variants.

Results and discussion

Molecular docking revealed strong binding affinities of B-escin to key SARS-CoV-2 spike protein targets and computational simulations demonstrated that the B-escin-nanoparticle treatment-maintained efficacy across multiple SARS-CoV-2 variants (Figures 3 - 7), as detailed in our previously published work [31]. Molecular dynamics (MD) simulations and quantum

mechanical calculations provided valuable insights into the B-escin-nanoparticle complexes, where they confirmed the stability of both the B-escin-protein and B-escin-nanoparticle complexes. Key findings included: Minimal structural fluctuations, as indicated by average root-mean-square deviation (RMSD) values; reduced flexibility in binding site residues, particularly in the spike protein receptor-binding domain (RBD), suggesting a stabilizing effect from the nanoparticle conjugates; and enhanced interaction energies, demonstrating stronger and more stable binding.

Quantum mechanical analysis using density functional theory (DFT) further revealed the synergistic effects of the B-escin-nanoparticle complexes. Both nanoparticles showed stronger binding energies with B-escin, surpassing the standalone binding energy of B-escin to viral proteins. Additionally, the conjugation of B-escin to nanoparticles led to enhanced charge redistribution, optimizing electrostatic interactions with viral targets. Frontier molecular orbital analysis highlighted increased electron density near the conjugation sites, correlating with improved reactivity and antiviral activity.

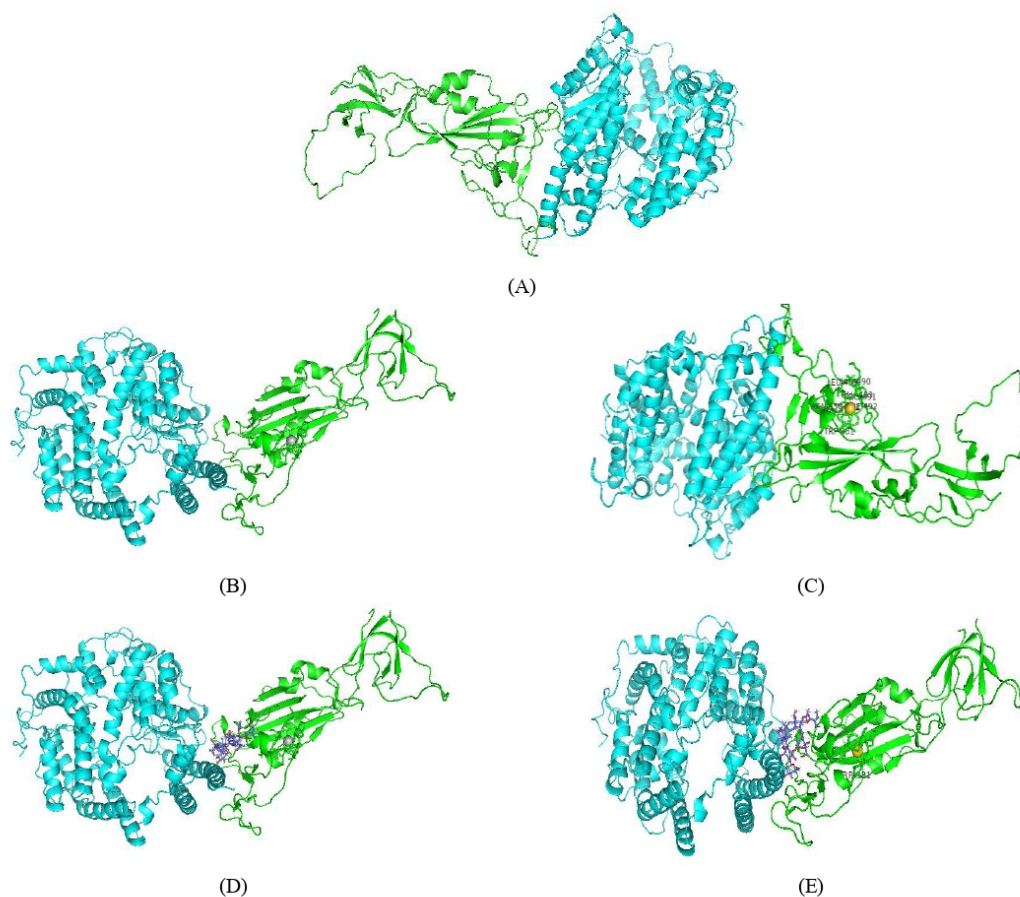


Figure 3 The 3D structure of ACE2-Spike Alpha variant complex docked using HDOCK where ACE2 is shown in cyan and Spike Alpha variant is shown in green (A), 3D structures of silver NP docked in ACE2-Spike Alpha variant (B), 3D structure of gold NP docked in ACE2-Spike Alpha variant (C), 3D structure of ACE2-Spike Alpha variant complex showing the synergetic binding of silver NP and B-escin (D) and gold NPs and B-escin (E).

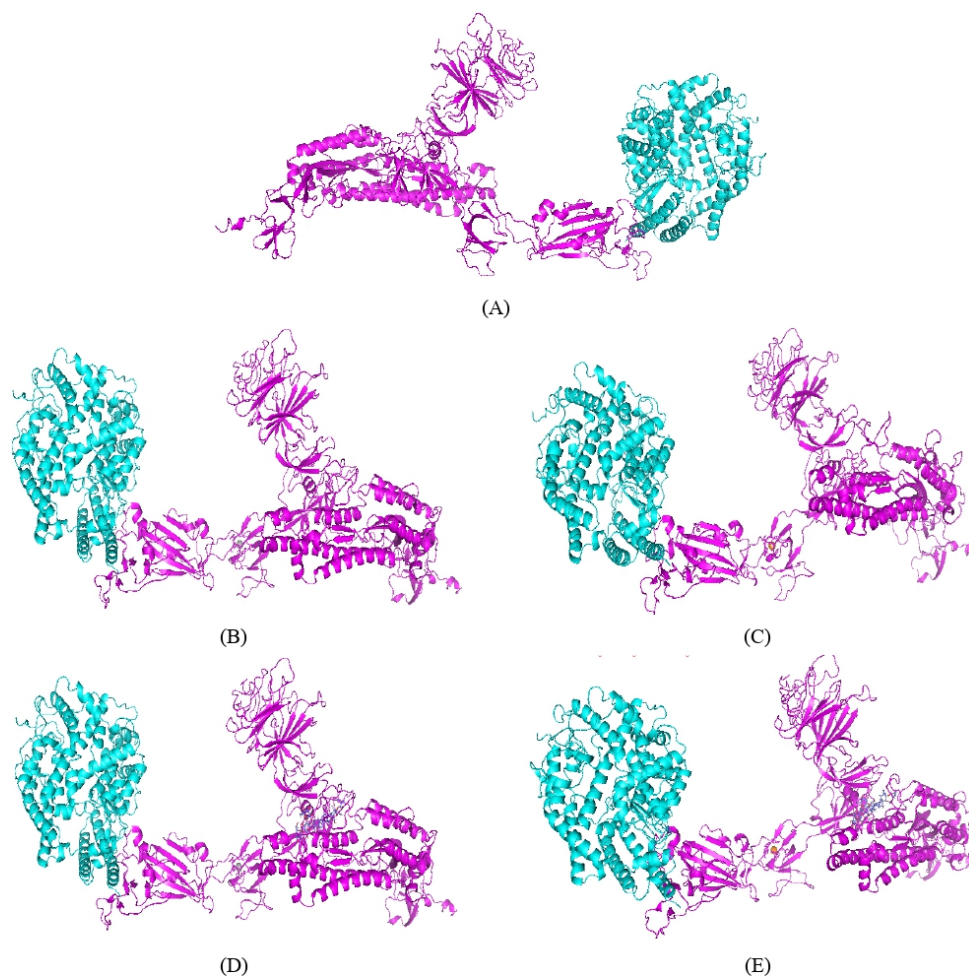


Figure 4 The 3D structure of ACE2-Spike Beta variant complex docked using HDock where ACE2 is shown in cyan and Spike Beta variant is shown in magenta (A), 3D structures of silver NP docked in ACE2-Spike Beta variant (B), 3D structure of gold NP docked in ACE2-Spike Beta variant (C), 3D structure of ACE2-Spike Beta variant complex showing the synergetic binding of silver NP and B-escina (D) and gold NPs and B-escina (E).

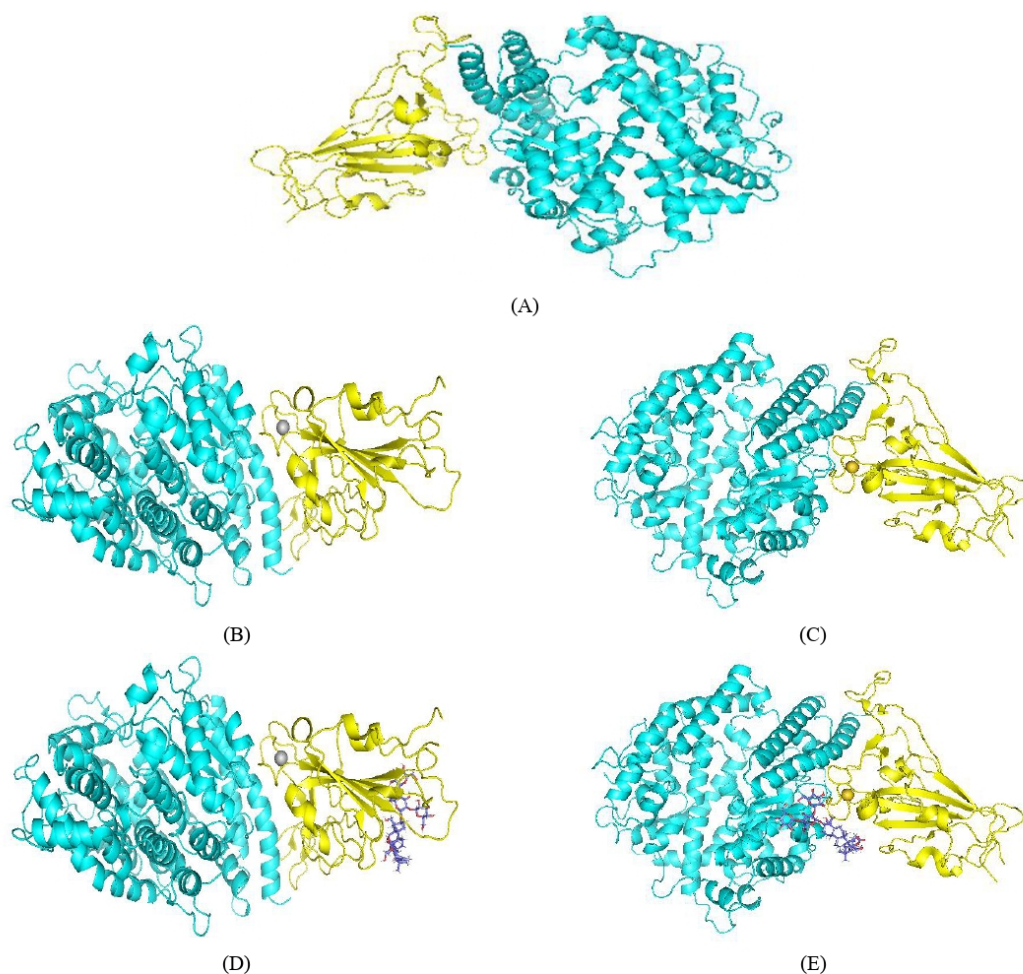


Figure 5 The 3D structure of ACE2-Spike Delta variant complex docked using HDOCK where ACE2 is shown in cyan and Spike Delta variant is shown in yellow (A), 3D structures of silver NP docked in ACE2-Spike Delta variant (B), 3D structure of gold NP docked in ACE2-Spike Delta variant (C), 3D structure of ACE2-Spike Delta variant complex showing the synergetic binding of silver NP and B-escin (D) and gold NPs and B-escin (E).

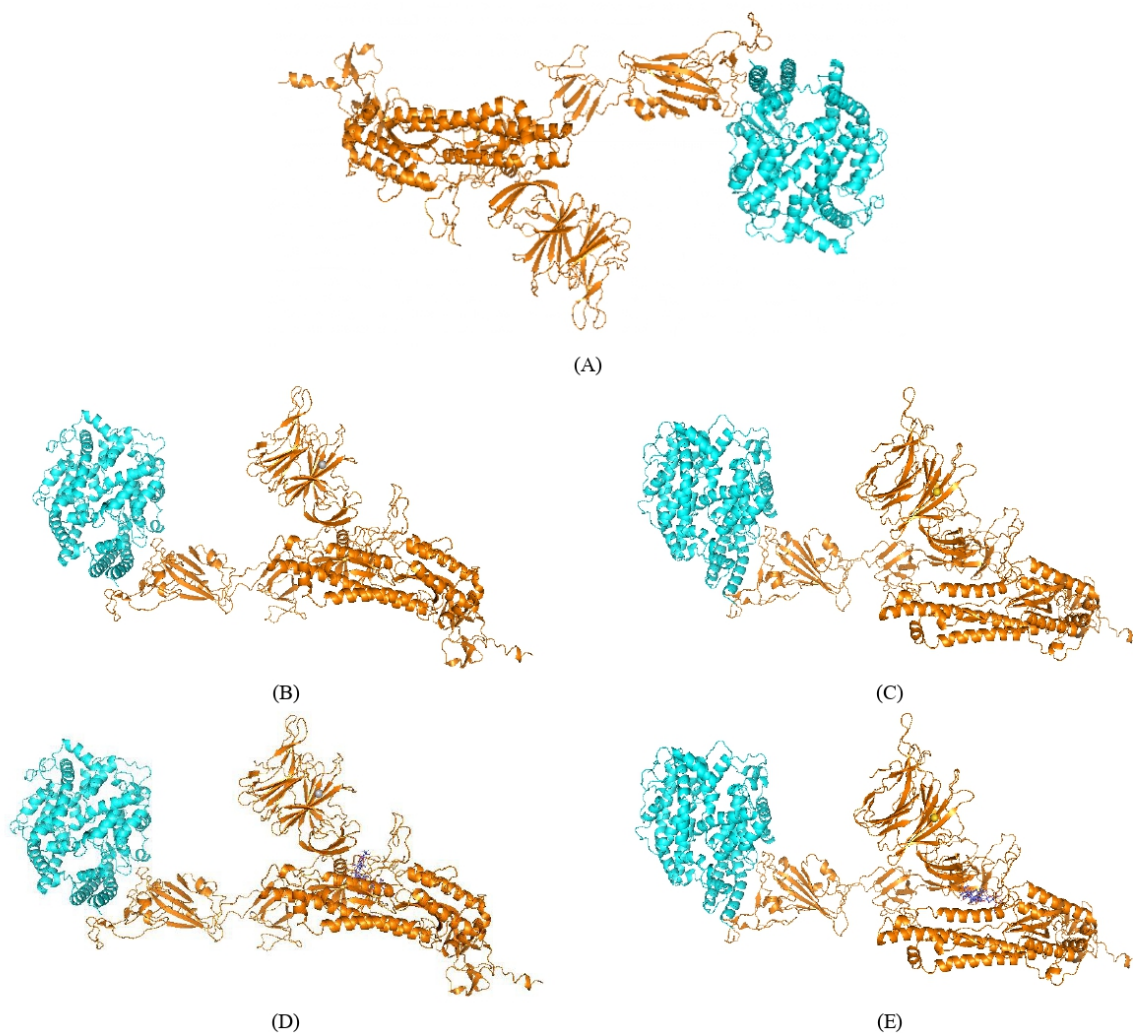


Figure 6 The 3D structure of ACE2-Spike Omicron variant complex docked using HDock where ACE2 is shown in cyan and Spike Omicron variant is shown in orange (A), 3D structures of silver NP docked in ACE2-Spike Omicron variant (B), 3D structure of gold NP docked in ACE2-Spike Omicron variant (C), 3D structure of ACE2-Spike Omicron variant complex showing the synergetic binding of silver NP and B-escin (D) and gold NPs and B-escin (E).

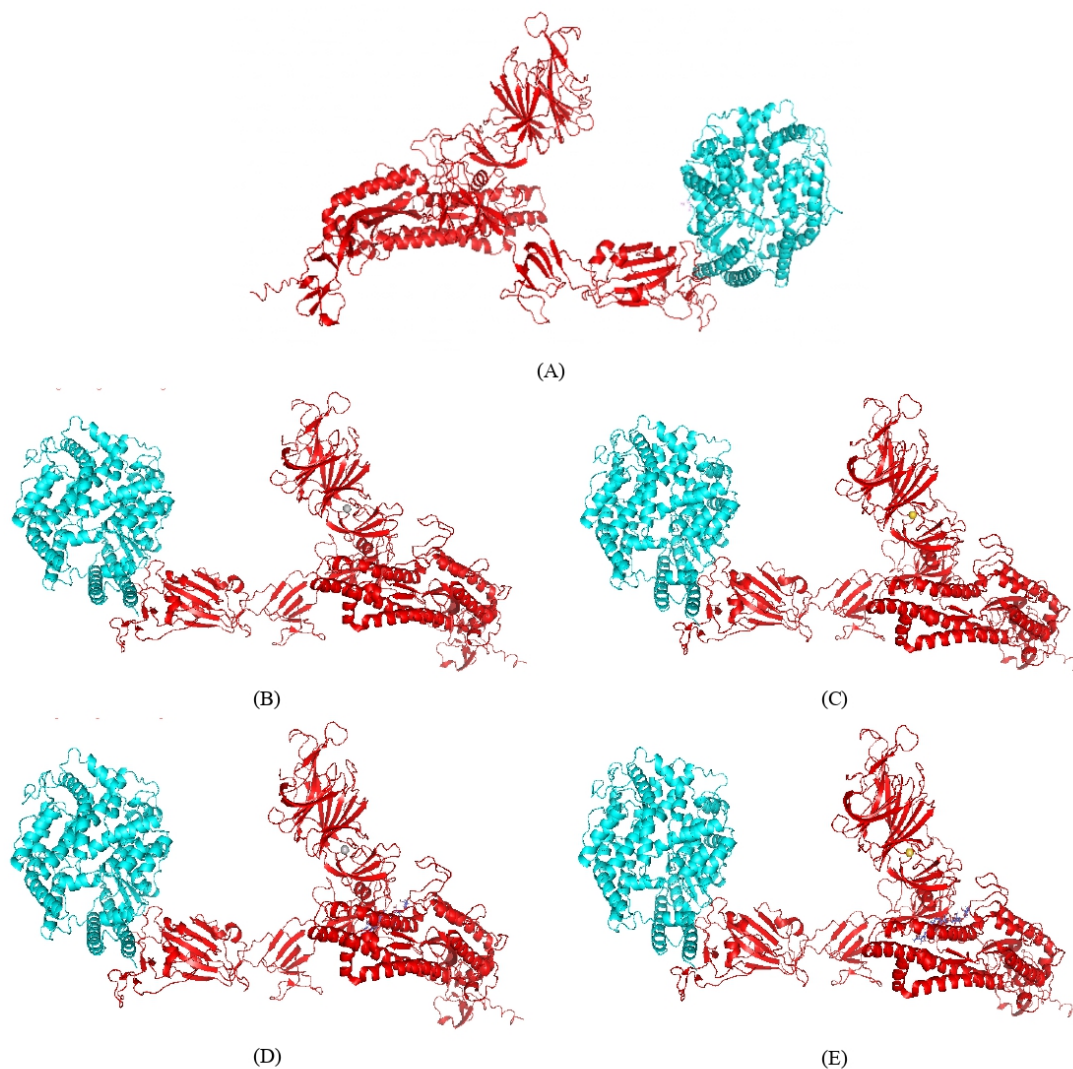


Figure 7 The 3D structure of ACE2-Spike Gamma variant complex docked using HDOCK where ACE2 is shown in cyan and Spike Gamma variant is shown in red (A), 3D structures of silver NP docked in ACE2-Spike Gamma variant (B), 3D structure of gold NP docked in ACE2-Spike Gamma variant (C), 3D structure of ACE2-Spike Gamma variant complex showing the synergetic binding of silver NP and B-escic (D) and gold NPs and B-escic (E).

The findings from this study underscore the potential of a nasal treatment combining B-escic and nanoparticles in combating SARS-CoV-2 variants. The computational analyses demonstrated that this combination exhibits superior binding affinity and stability compared to B-escic alone [32-34]. These

results highlight the advantages of incorporating nanoparticles for targeted delivery and enhanced antiviral activity [35,36].

The molecular docking and molecular dynamics simulations reveal that B-escic-nanoparticle complexes exhibit stronger interactions with SARS-CoV-2 spike

protein [34,37]. The significant improvement in binding energy and stability is attributed to the high surface area and tunable properties of nanoparticles, which enhance the molecular interactions at the viral interface.

The prophylactic effectiveness across multiple SARS-CoV-2 variants, particularly Alpha, Beta, Gamma, Delta, and Omicron, suggests that it could serve as a robust therapeutic option in the face of ongoing viral evolution [37,38]. While a slight increase in binding efficacy was observed with the highly mutated Omicron variant, the combination therapy still outperformed B-escin alone, indicating its adaptability, that could be due to significant mutations in the receptor-binding domain of the Omicron spike protein, which may alter the structure and charge distribution, thereby affecting the interaction with B-escin-NPs.

The size, morphology, and surface functionalization of nanoparticles play critical roles in optimizing the treatment. Smaller nanoparticles demonstrated better mucosal penetration and interaction energies, while spherical shapes provided enhanced stability. Functionalization with thiol or amine groups ensured stronger conjugation with B-escin, preventing aggregation and improving mucosal adhesion [2,5].

The results of this study pave the way for experimental validation and eventual clinical application. Nasal delivery systems are particularly advantageous for treating respiratory infections due to their localized action, ease of administration, and minimal systemic side effects [1]. By targeting the nasal mucosa, this treatment could potentially prevent viral entry and replication at an early stage of infection.

While computational approaches provide valuable insights, experimental validation is essential to confirm these findings. Future studies should focus on developing scalable synthesis protocols for B-escin-functionalized nanoparticles, conducting *in vitro* and *in vivo* studies to evaluate antiviral efficacy and safety, and investigating the long-term stability and storage conditions of the nasal formulation. While also nanoparticles offer promising advantages for antiviral applications, their potential toxicity, particularly when used in nasal formulations, must be carefully considered. Nanoparticles can interact with biological tissues in ways that may cause adverse effects, such as irritation or damage to the nasal mucosa. Factors such as particle size, surface charge, and surface

functionalization play critical roles in determining biocompatibility. To mitigate these risks, the design of nanoparticles should prioritize biocompatibility, with considerations for size optimization to ensure efficient clearance via the mucociliary system and the use of surface coatings that enhance safety. Additionally, comprehensive toxicological assessments are essential to evaluate the long-term impact of nanoparticle exposure on the nasal mucosa and overall health.

In a broader context, this approach has the potential to address significant global health challenges, particularly in regions with limited access to vaccines or other preventive measures. A nanoparticle-biomolecule-based nasal prophylactic could provide an accessible, non-invasive, and cost-effective solution, complementing existing vaccination strategies and reducing transmission in underserved populations. By offering a rapid and localized antiviral action, such a prophylactic could help mitigate the spread of SARS-CoV-2 and its variants, particularly in areas where vaccine distribution is constrained or uptake is low, ultimately contributing to global efforts to control the pandemic.

Conclusions

This study demonstrates the potential of a novel nasal treatment combining B-escin, a natural compound, and nanoparticles to address the challenges posed by SARS-CoV-2 variants. Our computational analyses revealed enhanced antiviral activity and variant-specific efficacy, highlighting the synergistic interactions between B-escin and nanoparticles, which could potentially offer a more targeted approach against the evolving landscape of SARS-CoV-2. These findings provide a strong rationale for the further development and experimental validation of this approach, with the possibility of creating a new prophylactic strategy to complement existing vaccination efforts. Furthermore, while this study is focused on SARS-CoV-2, the approach holds potential for broader applications against other coronaviruses and possibly even a wide range of respiratory viruses. Given the similarities in viral entry mechanisms, the combination of B-escin and nanoparticles may offer a versatile platform for targeting multiple viral pathogens.

The results underscore the promising role of nanotechnology in improving the delivery and

effectiveness of antiviral compounds, particularly in high-risk populations or regions with limited access to vaccines. However, it is important to note that while computational models offer valuable insights, experimental validation is crucial to confirm the findings and evaluate the safety and efficacy of this treatment *in vivo*. Future studies should focus on optimizing nanoparticle design, evaluating long-term safety profiles, and conducting clinical trials to assess the therapeutic potential of this approach. Additionally, expanding the scope to include other viral variants and exploring combinations with other antiviral agents may further enhance the treatment's effectiveness. Ultimately, this work contributes to the growing body of research on nanotechnology-based solutions for COVID-19 and provides a foundation for the development of effective, accessible, and scalable treatments for global use.

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Enhanced Nanobiotechnological Strategy Against SARS-CoV-2: Synergistic Potential of Rutin

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Zatla, I. & Boublenza, L. (2026). Enhanced Nanobiotechnological Strategy Against
SARS-CoV-2: Synergistic Potential of Rutin. *Coronaviruses*.

DOI: 10.2174/0126667975464024260213054706



○ Objective

This follow-up study advances our previous work on β -escin-functionalized gold and silver nanoparticles by introducing Rutin, a flavonoid with well-established antioxidant and antiviral properties, into the formulation, thereby broadening the scope of the nanobiotechnological antiviral strategy against SARS-CoV-2. The rationale for this study arises from the urgent need for complementary antiviral interventions to overcome global challenges in vaccine distribution, accessibility, and hesitancy, particularly in low- and middle-income regions, and from the growing recognition that natural compounds combined with nanotechnology can offer safe, effective, and targeted solutions.

○ Methods

This computational study used molecular docking and molecular dynamics simulations to characterize the interactions of AuNPs and AgNPs, functionalized with β -escin and rutin, with multiple SARS-CoV-2 protein targets. Seven targets were considered, comprising five ACE2-bound spike variants, the Mpro, and the RdRp, all obtained as high-resolution structures from the PDB. β -Escin and rutin ligands were sketched, energy-minimized with ChemOffice and Avogadro, and then docked using AutoDock Vina, where binding poses were ranked by binding affinity and key hydrogen bonds. The most promising complexes were further examined through 100 ns GROMACS simulations using the CHARMM36 force field in TIP3P water under NPT conditions at 300 K and 1 bar. Trajectory analyses, including RMSD, RMSF, and hydrogen-bond occupancy, were performed to evaluate conformational stability and interaction persistence within each nanoparticle–protein assembly.

○ Results

Analyses revealed clear target-dependent binding preferences, with AuNPs showing stronger affinity for spike-associated complexes and AgNPs favoring RdRp, suggesting complementary antiviral roles. Both nanoparticles stably interacted with Mpro, where they consistently occupied the catalytic pocket, as well as with spike and RdRp targets. Among the ligands, β -escin demonstrated the highest binding affinity, particularly against Mpro and RdRp, involving key catalytic residues such as His41. Rutin exhibited slightly lower affinities but contributed to binding diversity by occupying alternative pockets and forming multiple noncovalent interactions. In NP–ACE2–spike assemblies, rutin showed moderate binding across

distinct sites, while β -escin consistently outperformed rutin, with the strongest interactions observed for β -escin–AuNP–Mpro and β -escin–AgNP–RdRp complexes.

○ Conclusion

This study demonstrates that incorporating rutin into a previously established β -escin–functionalized AuNPs and AgNPs system enhances the formulation's binding stability and multi-target inhibitory potential against SARS-CoV-2 proteins. The results highlight the synergistic antiviral effect of β -escin, rutin, and metallic nanoparticles, which together improve molecular stability and bioavailability. Designed for intranasal delivery, this nanobiotechnological formulation offers a promising prophylactic and therapeutic strategy against SARS-CoV-2 and other respiratory viruses, underscoring the potential of nanoparticle-assisted delivery of natural compounds in next-generation antiviral development.

○ Limitations

Despite encouraging *in silico* results, this study remains computational in scope. Experimental validation is required to confirm molecular interactions and biological efficacy. Key limitations include the need for extended molecular dynamics simulations, the lack of quantitative assessment of β -Escin–Rutin synergism in free form, and the absence of pharmacokinetic and toxicity data. Furthermore, evaluating the cytotoxicity and safety profiles of AuNPs and AgNPs through *in vitro* and *in vivo* assays is essential before advancing toward preclinical stages.

○ Perspectives

Future research should focus on experimental validation, formulation optimization, and translational development of this nasal nanotherapeutic. Emphasis should be placed on optimizing dosage, delivery stability, and mucosal compatibility, as well as testing its efficacy across multiple SARS-CoV-2 variants and other respiratory pathogens. With comprehensive safety profiling and clinical evaluation, this formulation could ultimately emerge as a non-invasive, affordable, and scalable antiviral strategy, complementing existing vaccination efforts and improving global pandemic preparedness.

RESEARCH ARTICLE

Enhanced Nanobiotechnological Strategy against SARS-CoV-2: Synergistic Potential of Rutin

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Abstract: Introduction: This study extends a previously developed nanobiotechnological antiviral platform based on β -escin-functionalized gold and silver nanoparticles (AuNPs and AgNPs) by incorporating rutin, a flavonoid with established antioxidant and antiviral activity. The objective was to determine whether dual phytochemical functionalization enhances molecular interactions and inhibitory potential against key SARS-CoV-2 targets.

Methods: Molecular docking and 100-ns molecular dynamics simulations were conducted using AutoDock Vina and GROMACS 2023 to investigate the binding behavior, stability, and affinity of β -escin- and rutin-conjugated AuNPs and AgNPs against seven SARS-CoV-2 proteins. These included five spike protein variants (Alpha, Beta, Gamma, Delta, and Omicron), the main protease (Mpro), and RNA-dependent RNA polymerase (RdRp).

Results: Both nanoparticle systems demonstrated strong and stable multi-target interactions. AuNP conjugates showed higher affinity toward spike-ACE2 complexes (binding free energy ≈ -0.17 kcal/mol), whereas AgNPs preferentially interacted with RdRp (≈ -0.13 kcal/mol). β -Escin exhibited the most pronounced binding across targets, particularly against Mpro (β -escin-AuNP: -9.02 kcal/mol; β -escin-AgNP: -9.04 kcal/mol), engaging key catalytic residues such as His41. Although rutin displayed slightly lower affinities (-7.35 to -8.78 kcal/mol), it complemented β -escin by occupying alternative binding sites and contributing stabilizing hydrogen bonds and π - π interactions. Dual β -escin-rutin functionalization enhanced complex stability, reduced RMSD fluctuations, and expanded antiviral coverage across all tested variants.

Discussion: The observed synergistic effects between β -escin and rutin highlight the advantage of dual phytochemical functionalization, which enables complementary binding mechanisms, improved conformational stability, and broader antiviral activity against diverse SARS-CoV-2 targets.

Conclusion: The β -escin-rutin-functionalized gold and silver nanoparticle system exhibits enhanced stability and multi-target inhibitory potential against SARS-CoV-2, supporting its translational potential as an intranasal nanotherapeutic strategy for COVID-19 prevention and treatment.

Keywords: SARS-CoV-2, COVID-19, antivirals nanoparticles, biomolecules, molecular dynamics.

1. INTRODUCTION

Despite the global rollout of COVID-19 vaccines, disparities in access remain a significant barrier in many low- and middle-income regions. These inequities are compounded by logistical constraints, distribution challenges, and persistent vaccine hesitancy, often fueled by safety concerns and misinformation. Such gaps underscore the need for complementary or alternative preventive and therapeutic strategies that

are affordable, scalable, and culturally acceptable [1-4]. Natural bioactive molecules, owing to their broad pharmacological activities and favorable safety profiles, represent an attractive foundation for such interventions. When coupled with nanobiotechnology-based delivery systems, these compounds can achieve targeted delivery, enhanced bioavailability, and improved stability, thereby maximizing antiviral potential while minimizing systemic side effects. The convergence of phytochemistry and nanotechnology thus offers a promising avenue for the development of accessible antiviral solutions, particularly in populations with limited vaccine uptake [5, 6].

The COVID-19 pandemic, caused by SARS-CoV-2, has also accelerated the search for novel antiviral agents [7, 8].

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the weakest interaction. Confidence scores exceeding 0.91 further confirmed the strong and probable ACE2–spike binding across all variants.

Subsequent docking of AuNPs and AgNPs onto these ACE2–spike complexes revealed consistent, high-affinity interactions, with binding energies ranging from -0.18 to -0.27 kcal/mol. AuNPs generally exhibited slightly stronger affinities than AgNPs, particularly toward the Beta variant. Both nanoparticles consistently occupied the same binding pocket across all complexes, suggesting a conserved interaction mode.

In this follow-up analysis, Rutin was introduced to investigate its binding profile in conjunction with AuNPs and AgNPs. As shown in Table 1, Rutin demonstrated favorable binding affinities across all ten NP-ACE2–spike complexes, ranging from -7.35 to -8.78 kcal/mol, although these values were generally lower than those observed with β -escin. Notably, Rutin achieved its highest binding affinity with the NP-ACE2–spike Alpha variant. Unlike β -escin, which frequently targeted interface regions, Rutin consistently occupied distinct binding pockets, as summarized in Table 2.

For the Alpha variant, β -escin preferentially bound to a pocket at the ACE2–spike interface, forming eight and six hydrogen bonds in the AgNP and AuNP complexes, respectively. Despite high docking scores (-10.46 and -10.21 kcal/mol), a few unfavorable interactions were detected. Rutin also targeted the ACE2–spike interface in these complexes (Figs. 1A and 1B), forming six and seven hydrogen bonds, together with π - π , π -anion, and π -cation interactions.

In Beta variant complexes, β -escin bound to a central pocket in the spike protein, with binding affinities of -9.22 and -9.35 kcal/mol for AgNP and AuNP complexes, respectively. It established three and five hydrogen bonds, with no unfavorable clashes, in contrast to the Alpha variant complexes. Rutin, however, showed a different preference: it bound within the ACE2 subunit, forming eight and seven hydrogen bonds in the AgNP and AuNP complexes, respectively (Figs. 2A and 2B).

In Delta variant complexes, β -escin adopted two distinct binding sites: a terminal pocket in the AgNP-ACE2–spike complex and an interface pocket in the AuNP-ACE2–spike complex. These interactions yielded the lowest binding affinities observed for β -escin (-8.50 and -8.11 kcal/mol, respectively), but still involved six and four hydrogen bonds. In contrast, Rutin consistently occupied a single interface pocket in both AgNP and AuNP complexes (Figs. 3A and 3B), with affinities of -7.71 and -8.01 kcal/mol, each forming six hydrogen bonds.

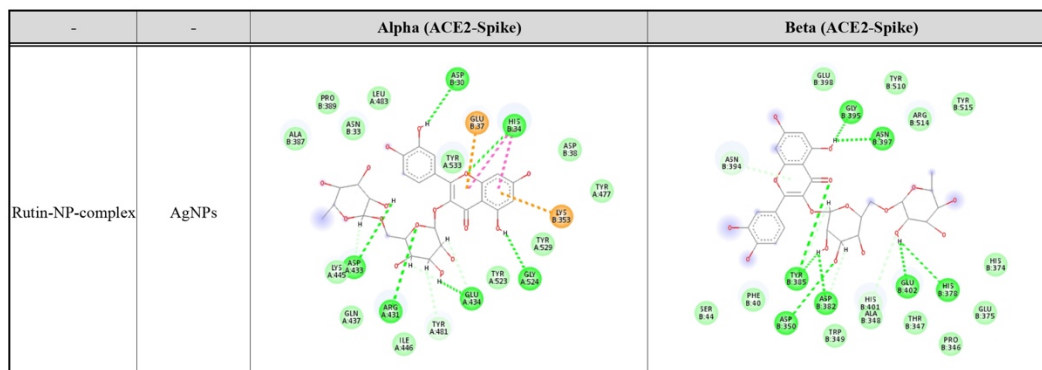
For the Omicron and Gamma variant complexes, β -escin showed comparable binding affinities and consistently targeted central spike protein pockets, similar to its behavior in the Beta variant. Rutin, however, exhibited different binding preferences: in Omicron variant complexes, it favored an internal ACE2 pocket (Figs. 4A and 4B), forming seven and six hydrogen bonds for AgNP and AuNP complexes, respectively. In Gamma variant complexes, Rutin bound at the ACE2–spike interface (Figs. 5A and 5B), but with the lowest binding affinities across all variants (-7.57 and -7.45 kcal/mol for AgNP and AuNP complexes, respectively; Table 1).

Table 1. Docking binding energies of Rutin with AuNP-ACE2–spike and AgNP-ACE2–spike variant complexes.

Ligand	NP-complex	Alpha (ACE2-Spike)	Beta (ACE2-Spike)	Delta (ACE2-Spike)	Omicron (ACE2-Spike)	Gamma (ACE2-Spike)
Rutin	AgNPs	-8.32	-7.71	-7.71	-7.67	-7.57
	AuNPs	-8.78	-7.69	-8.01	-7.59	-7.45
	Without NP	-8.41	-7.83	-7.86	-8.36	-7.44

Note: *Binding energy values shown in this table represent molecular docking scores and are expressed in kcal/mol.

Table 2. Two-dimensional interaction profiles of Rutin with AuNP-ACE2–spike and AgNP-ACE2–spike variant complexes.



(Table 2) Contd...



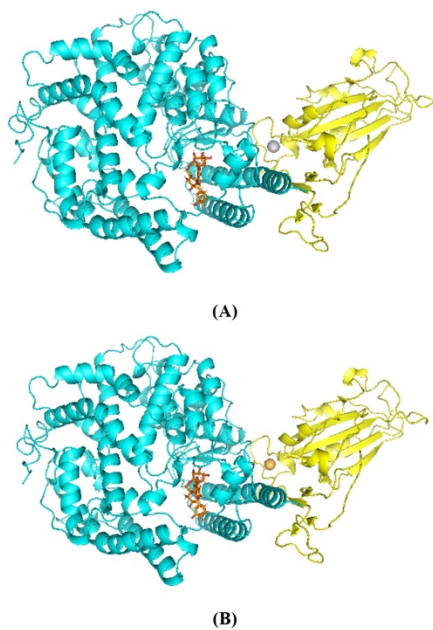


Fig. (3). Three-dimensional structures of ACE2–spike Delta variant complexes showing the synergistic binding of (A) AgNP with Rutin and (B) AuNP with Rutin. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

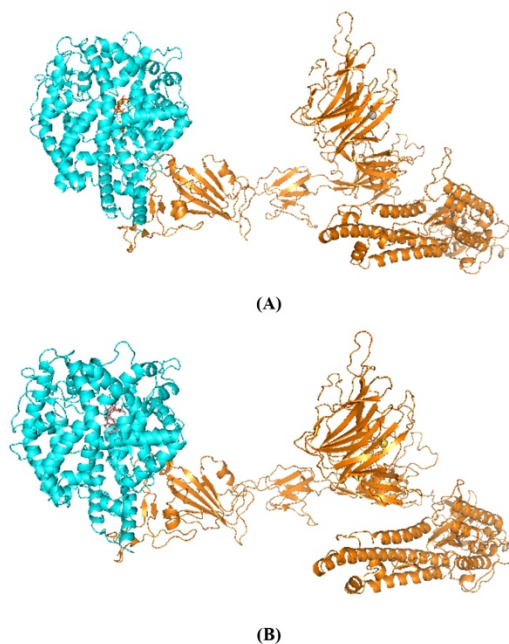


Fig. (4). Three-dimensional structures of ACE2–spike Omicron variant complexes showing the synergistic binding of (A) AgNP with Rutin and (B) AuNP with Rutin. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Docking results for the phytochemicals with AgNP- and AuNP-bound Mpro and RdRp complexes (Table 4) provide further insight into possible synergistic effects between plant-derived compounds and metallic nanoparticles. For Mpro-NP complexes, B-escin consistently demonstrated stronger binding affinities than Rutin across both nanoparticle types. The highest affinity was observed for the B-escin-AuNP complex (-9.02 kcal/mol), followed by the B-escin-AgNP complex (-7.99 kcal/mol). These interactions likely benefited from B-escin's planar polycyclic structure and its capacity for extensive hydrogen bonding and Van der Waals

interactions, further enhanced by the higher surface reactivity of gold.

Rutin, in comparison, showed weaker affinities for Mpro-NP complexes, with binding energies of -7.06 kcal/mol (AgNP) and -6.81 kcal/mol (AuNP). The slightly stronger affinity observed with AgNPs may reflect better geometric or electrostatic complementarity with silver-modified Mpro pockets. However, overall, the results suggest that Rutin is less effective than B-escin in targeting Mpro when combined with nanoparticles.

Table 3. Two-dimensional interaction profiles of B-escin and Rutin with AuNP-Mpro, AgNP-Mpro, AuNP-RdRp, and AgNP-RdRp complexes.

		M ^{pro}	RdRp
B-escin-NP-complex	AgNPs		
	AuNPs		
Rutin-NP-complex	AgNPs		
	AuNPs		

(Table 3) Contd...

multi-target candidate against SARS-CoV-2 proteins [29, 30]. Likewise, the positive results for B-escin are supported by recent experimental findings demonstrating antiviral and virucidal activity of β -escin and *Aesculus hippocastanum* extracts in cell-based assays, including reductions in viral titers and modulation of inflammatory responses [31]. These complementary computational and experimental insights suggest that B-escin may act both by directly disrupting virus-host interactions and through secondary cellular mechanisms.

An increasing body of work highlights the advantages of combining nanoparticles with bioactive phytochemicals for antiviral applications, whether to improve stability, enhance targeted delivery, or exploit synergistic effects. Reviews on metallic nanopatforms for COVID-19 diagnostics and therapeutics report that functionalized Au/Ag surfaces improve ligand presentation and local concentration at target sites [26]. The findings that B-escin and Rutin display enhanced docking interactions in the presence of nanoparticles support these concepts and suggest that nanoparticle-assisted delivery may increase inhibitory potency compared with free compounds.

Building on an earlier work [32], which proposed a green-synthesized Au/Ag-B-escin conjugate as a potential nasal prophylactic against SARS-CoV-2 variants, the present study expands the scope by introducing Rutin and conducting a comparative analysis of both phytochemicals across key viral targets. This progression validates earlier conclusions on the synergistic potential of nanocarriers while revealing that Rutin's unique interaction patterns may complement those of B-escin, strengthening the rationale for multi-phytochemical nanoparticle formulations as broad-spectrum antivirals.

In summary, the docking analyses confirm strong binding affinities of AuNPs and AgNPs toward multiple SARS-CoV-2 targets, including ACE2-spike variants, Mpro, and RdRp. The complementary binding profiles of B-escin and Rutin—each engaging distinct residues—suggest that combining these phytochemicals with nanoparticles may broaden inhibitory potential. Together with current literature, these findings support nanoparticle-assisted delivery as a promising approach to enhance natural compound engagement, positioning B-escin and Rutin as leading candidates for innovative, multi-target antiviral strategies.

Despite the promising findings, this study has several limitations that should be acknowledged. First, while molecular docking provided important insights into binding interactions, only limited molecular dynamics simulations were conducted; extended simulations and free-energy calculations would be necessary to fully validate the stability of the complexes. Second, although the abstract and conceptual framework highlight the dual use of β -escin and Rutin, the present work does not provide a direct quantitative assessment of their synergistic effects in free form, focusing instead on their integration within a nanoparticle-based system. Third, pharmacokinetic aspects such as bioavailability, metabolism, clearance, and *in vivo* biodistribution of the proposed intranasal formulation were not investigated and remain to be explored in future preclinical studies. Fourth, while gold and silver nanoparticles (AuNPs & AgNPs) are

central to the proposed nanopatform, their potential cytotoxicity warrants careful consideration. It should be noted that cytotoxic effects of AgNPs are strongly dependent on particle size, concentration, and surface modification; conjugation with natural bioactives such as β -escin and Rutin may reduce nonspecific toxicity, but systematic *in vitro* and *in vivo* studies will be required to establish their safety profile. Finally, as with any computational study, experimental validation through *in vitro* and *in vivo* assays will be essential to confirm the antiviral efficacy of the proposed nanobiotherapeutic strategy.

CONCLUSION

Building on an earlier work with β -escin-conjugated gold-silver nanoparticles, the incorporation of Rutin has yielded a nanobiotechnological formulation with enhanced binding stability and multi-target inhibitory potential against key SARS-CoV-2 proteins. Molecular docking and interaction analyses revealed synergistic contributions from hydrogen bonding, hydrophobic contacts, and π - π stacking, underscoring the complementary binding mechanisms of the two phytochemicals. These results highlight the innovative potential of integrating natural compounds with distinct yet compatible properties to develop next-generation intranasal nanotherapeutics for the prevention and treatment of COVID-19, as well as other respiratory viral infections. Future studies should prioritize experimental validation, safety profiling, and translational development to advance this formulation toward clinical application.

AUTHORS' CONTRIBUTIONS

The authors confirm their contributions to the paper as follows: I.Z. was responsible for conceptualization, data collection, literature search, data analysis, manuscript writing, and manuscript editing. L.B. supervised the project. All authors accepted responsibility for the content of the manuscript, consented to its submission, reviewed all results, and approved the final version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analysed during this study are included in this published article.

FUNDING

None.

Synergistic Potential of Rutin

Coronaviruses, XXXX, Vol. XX, No. XX

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Chapter III:

Discussion

Discussion

Algeria's response to the COVID-19 pandemic was shaped by substantial structural and human challenges, with multinational analyses highlighting disparities in virological and mortality surveillance across African countries due to fragile health systems, resource constraints, and concurrent epidemics such as malaria and tuberculosis [(Adebisi *et al.*, 2021); (Zatla & Boublenza, 2023a)]. Early containment strategies limited initial transmission peaks (April–July 2020) but imposed a considerable psychological toll on healthcare personnel, with high levels of burnout, depersonalization, and post-traumatic stress disorder (PTSD) linked to prolonged exposure to the coronavirus disease units and personal loss (Uhegwu *et al.*, 2023). Host-related factors, particularly psychological stress and immune dysregulation, further modulate viral illness outcomes, reinforcing the need for interventions that address both viral replication and host resilience [(Shah *et al.*, 2021); (Karimi *et al.*, 2022); (Lemerini *et al.*, 2023)]. This context underscores the urgency of robust mental health support systems and health system reform, as future crises may exacerbate these vulnerabilities [(Lounis, 2021); (Boussouf *et al.*, 2023)].

To better understand Algeria's pandemic trajectory and the factors shaping disease outcomes, diagnostic and epidemiological analyses were conducted across local and global scales. Retrospective analysis in Tlemcen (2021) demonstrated the diagnostic value of integrating RT-PCR with chest CT imaging in resource-constrained environments, revealing strong associations between low Ct values (20–30), high viral loads, and severe radiological findings, and highlighting urban clustering in Tlemcen, Mansourah, and Chetouane where population density acted as a major transmission driver (Zatla *et al.*, 2025a). Rich regional data further illustrated Algeria's heterogeneity: in Oran, the Delta wave (May–September 2021) predominantly affected females and adults aged 31–40 (Belhadj *et al.*, 2024), whereas eastern Algeria (2020–2021) showed older hospitalized cohorts with higher burdens of diabetes and hypertension and markedly elevated mortality, particularly among patients with chronic disease (Abed *et al.*, 2024). Such intra-national disparities argue for tailored, province-specific health strategies and informed subsequent decisions on vaccine allocation and preparedness. Additionally, evidence from Niemi *et al.* (2022) indicates that host genetics influences disease severity. These regional differences

informed vaccine allocation and preparedness strategies, particularly regarding age, comorbidity, and susceptibility.

At the broader North African level, regional epidemiology (Jan 2020–May 2023; ~37.2 million cases, 84k deaths) established 2021 as the pandemic peak. Morocco and Tunisia led in infection rates; Tunisia and Egypt in mortality (**Zatla *et al.*, 2023c**). A comparative analysis of 67 pandemic indicators across continents (January 2020–August 2022), with a specific focus on Europe, revealed that variations in infrastructure and national response strategies generated distinct epidemiological patterns in case incidence and mortality. Determinants identified through Principal Component Analysis included smoking prevalence, cardiovascular comorbidities, and gross domestic product (GDP), factors of particular relevance to the Algerian context (**Zatla *et al.*, 2023d**). Building upon these diagnostic observations, regional and demographic analyses further revealed how Algeria’s pandemic dynamics varied across provinces and populations. Comparative epidemiologic analyses in Europe and the USA identified age, sex, and comorbidities, including trisomy 21, neurological disorders, and obesity in children, as key determinants of severe outcomes [(**García Carretero *et al.*, 2023**); (**Dahu *et al.*, 2024**); (**Doenhardt *et al.*, 2024**)]. These findings mirror Algerian and regional data, emphasising the importance of context-specific surveillance that accounts for demographic risk profiles and social inequities, alongside variant-specific effects on severity. Understanding these demographic disparities underscores the importance of efficient vaccination strategies to protect high-risk groups and ensure equitable immunisation coverage.

The evaluation of Tlemcen’s vaccination campaign revealed that Sinovac doses were widely accepted, Sputnik experienced minimal inventory loss, whereas AstraZeneca wastage was substantial, indicating inefficiencies in inventory management and logistics (**Zatla *et al.*, 2025b**). These local observations reflect common obstacles in many low- and middle-income settings, where infrastructural deficits and weak coordination hamper effective vaccine distribution. **Dagovetz *et al.* (2025)** study shows that supply-chain robustness alone does not resolve fluctuating demand and poor coordination, motivating the use of advanced tools such as dynamic-systems and rolling-horizon inventory models to optimise vaccine allocation [(**Georgiadis & Georgiadis, 2021**); (**Hajibabai *et al.*, 2022**); (**Nahofti Kohne *et al.*, 2022**)]. Tlemcen's recurrent stockouts and delayed deliveries thus exemplify failures in top-down planning, while regional data showing that Morocco distributed nearly half of the region’s vaccines further underscore Algeria’s

comparatively low vaccination rates driven by both logistical and communication challenges (**Zatla et al., 2023c**). The **Atherstone et al. (2023)** paper notes that mass immunisation proved pivotal in reducing peak cases and deaths.

However, logistical efficiency alone proved insufficient, as sociocultural and behavioural dynamics strongly influenced vaccine uptake and risk behaviours. Multinational and local surveys, including our own (n = 756), indicated high adherence to preventive behaviours but suboptimal vaccination and persistent refusal among a sizeable minority, with misinformation, hesitancy, and booster refusal limiting coverage [(**Galende et al., 2022**); (**Lounis et al., 2022**); (**Zatla et al., 2023a**); (**Duggar et al., 2024**)]. Cultural-linguistic barriers among Amazigh communities further constrained distribution (**Minority Rights Group, 2022**), while widespread reliance on natural remedies reflected enduring scepticism toward formal healthcare systems. These real-world constraints highlight the need for adaptive models that bridge theoretical logistics frameworks with context-sensitive implementation and communication strategies.

Beyond acute infection and vaccination, long-term psychological and functional sequelae contributed to the overall burden of COVID-19. International work further links resilience factors (optimism, support) to reduced psychological harm (**O'Connor et al., 2025**), whereas depression, cognitive dysfunction, and impaired quality of life remain common among survivors [(**Mazza et al., 2022**); (**Griggs et al., 2024**)]. Addressing these behavioural and mental-health dimensions requires integrated physical and mental health services and community-based educational approaches to strengthen preventive practices and preparedness

Within this complex landscape, the present work bridges theoretical models and real-world struggles by advocating adaptive, data-driven inventory and distribution frameworks, emphasising decentralised stock monitoring and responsive logistics as critical to equity and minimal waste. Placing Algeria's experience within the broader North African and global context provides comparative insights into shared challenges and policy responses.

At the most immediate level of intervention, system-level preparedness ultimately translates into individual hygiene behaviours that directly shape transmission risk. In this context, interactive hygiene education using Glo Germ in Tlemcen exposed technique gaps despite self-reported high compliance, demonstrating the value of hands-on pedagogical approaches for

improving retention and pandemic preparedness (**Zatla *et al.*, 2024a**). Complementing these behavioural initiatives, continuous genomic and environmental monitoring remains vital for tracking viral evolution and transmission patterns, while post-COVID-19 pandemic awareness surveys that reveal gaps in knowledge about Monkeypox disease (Mpox) emphasize the need for sustained public health vigilance and literacy to prevent future zoonotic crises (**Zatla *et al.*, 2023e, 2023f, 2024b**).

While hygiene interventions mitigate transmission at the individual level, genomic and environmental surveillance are essential for capturing broader transmission dynamics and viral evolution. In this regard, phylogenetic studies identified dominant SARS-CoV-2 clades and their mutation profiles in Algeria (**Triqui *et al.*, 2024a**), while environmental analyses linked UV index and urbanisation to transmission, though climatic effects (temperature, humidity) were secondary to behavioural measures [(**Fadel *et al.*, 2021**); (**Boufekane *et al.*, 2022**); (**Triqui *et al.*, 2024b**)]. The global spread of viral variants (**Erkihun *et al.*, 2024**) underscores the need for ongoing genomic monitoring. Complementing these preparedness efforts, phylogenetic and genomic surveillance played a pivotal role in tracking the infectious agent's evolution and environmental determinants of transmission.

The rapid emergence and succession of the viral pathogen variants have exposed the limitations of conventional therapeutic and vaccine approaches, underscoring the urgency of developing adaptable antiviral strategies [(**Huang *et al.* 2022**); (**Adhikari *et al.*, 2023**); (**Zhang *et al.*, 2024**)]. Insights from genomic surveillance not only enhance preparedness for future emerging pathogens but also guide the design of next-generation therapeutics to address viral evolution. Within this landscape, the present work investigates an innovative nanobiotherapeutic framework that aims to offer sustainable efficacy against continuously mutating viral strains, thereby representing a crucial step toward long-term pandemic resilience.

On this basis, the thesis presents a novel nanobiotechnological approach that combines natural bioactives (β -Escin and Rutin) with gold-silver colloidal nanoparticles (Au-AgNPs) to inhibit SARS-CoV-2. Molecular docking and MD demonstrated that these bio-nanoconjugates form strong, stable interactions with key viral proteins (Spike, Mpro, RdRp), supporting their potential as efficient, stable antivirals for nasal delivery. At the molecular level, computational

simulations provided key evidence for the stability and antiviral activity of the proposed nanobioconjugates.

Compared to earlier computational studies on natural inhibitors such as cannabidiol, capsaicin, rhodotone, and cladosine C (Zatla & Boublenza, 2023b, 2024a, 2024b), the dual-ligand nanocarrier system in this thesis achieved improved binding affinities and stability. Integrating bioactives into nanoscaffolds bridges molecular inhibition with advanced formulation science, thereby presenting a more comprehensive antiviral strategy (Zatla & Boublenza, 2025a, 2025b, 2026).

These observations are further supported and contextualized by regional research in North Africa, which has reinforced the therapeutic value of natural compounds, where recent studies from Morocco, Tunisia, and Algeria have identified potent phytochemicals, such as Taxol, Rutin, Apigenin derivatives, and Isothymol, for their notable inhibition of viral entry and replication [(Abdelli *et al.*, 2020); (Nouadi *et al.*, 2021); (Farhat *et al.*, 2022); (Soudani *et al.*, 2023)]. Ethnobotanical surveys document the use of medicinal plants such as *Aloysia citriodora*, *Thymus vulgaris*, and *Zingiber officinale* in traditional approaches to managing COVID-19 symptoms (Brahmi *et al.*, 2023). This foundation supports the integration of phytotherapeutic wisdom into nanoscale delivery systems, enabling the translation of traditional medicine into advanced nanomedicine solutions. Building on this ethnopharmacological foundation, molecular efficacy studies elucidated the complementary mechanisms of the β -Escin–Rutin–nanoparticle system.

Molecular modelling revealed complementary antiviral interactions of β -escin and rutin against multiple SARS-CoV-2 variants. β -escin showed the strongest binding affinities (−8.11 to −10.46 kcal/mol), stabilising the NP–ACE2–Spike complex through extensive hydrogen bonding, while rutin contributed additional π – π interactions that enhanced complex stability. AuNPs demonstrated preferential binding to the Spike–ACE2 interface, whereas AgNPs effectively targeted RdRp, supporting a dual mechanism involving inhibition of both viral entry and replication. These findings are consistent with previous reports highlighting the broad-spectrum antiviral activity of green-synthesised metallic nanoparticles [(Haggag *et al.*, 2019); (Jeremiah *et al.*, 2020); (Hussain *et al.*, 2022); (Flórez-Álvarez *et al.*, 2023); (Ahmed *et al.*, 2025)].

The observed activity against conserved viral targets, including Spike, Mpro, and RdRp, further supports the ability of metallic nanoparticles to retain antiviral efficacy across viral variants [(Kim *et al.*, 2020); (Raja *et al.*, 2023); (Norouzi *et al.*, 2024)]. Green-synthesised AgNPs and AuNPs derived from biological extracts provide biocompatible and eco-friendly antiviral platforms [(Iravani, 2011); (Al-Sanea *et al.*, 2021); (Daoudi *et al.*, 2024); (Dell'Annunziata *et al.*, 2024); (Omar *et al.*, 2024)]. In this hybrid nano-bio system, AgNPs mainly contribute virucidal activity through disruption of viral membranes and proteins, whereas AuNPs enhance biocompatibility, cellular uptake, and controlled release [(El-Bendary *et al.*, 2020); (Amina & Guo, 2020); (Fan *et al.*, 2020); (Ratan *et al.*, 2021); (Nejati *et al.*, 2021); (Khedr *et al.*, 2024); (Contreras *et al.*, 2025); (Mosidze *et al.*, 2025)]. β -Escin may improve membrane interaction and mucosal penetration, while rutin provides antioxidant and stabilising effects [(Geisler *et al.*, 2019); (Choi *et al.*, 2021)]. Collectively, the β -Escin/Rutin–Ag/Au nanocomplex may act through simultaneous inhibition of viral adhesion and replication, combined with anti-inflammatory and mucosal protective effects.

Furthermore, the proposed intranasal delivery of 10–20 nm nanoparticles offers a rapid, localised, and non-invasive therapeutic strategy with reduced systemic toxicity and improved patient compliance [(Amini, 2019); (Hadjichrysanthou *et al.*, 2022); (Palmieri & Vadalà, 2022)]. To address limitations related to phytomolecule pharmacokinetics, a preliminary pharmacokinetic assessment was included in Appendix 1 as a basis for future *in vitro* and *in vivo* investigations.

In parallel, comparison of the proposed β -escin/rutin–Au–Ag platform with previously reported nanotechnological antivirals highlights its distinctive features, including green synthesis, dual-metal synergy, and intranasal delivery, positioning it as a promising candidate for translational development. These characteristics align with evidence that functionalized nanoparticles, particularly those mimicking the ACE2 interface, can inhibit viral binding [(Mehranfar & Izadyar, 2020); (Ren *et al.*, 2021); (Pagni *et al.*, 2026)]. Notably, the biofunctionalized Au–Ag nanoparticles developed in this study incorporate natural ligands with anti-inflammatory and mucosal protective properties and are optimized for nasal administration. The antiviral potential of eco-friendly silver and gold nanoparticles has also been supported by previous studies (El-Sheekh *et al.*, 2022). However, despite these encouraging computational

findings, translational validation remains essential, including assessment of cytotoxicity, biodistribution, immune response, and safety through in vitro and in vivo studies, potentially using advanced models such as 3D organoids and high-throughput screening platforms.

This thesis advances two major frontiers: (1) the generation of regional insights from Algeria through the integration of behavioural, environmental, and immunological dimensions, emphasizing the importance of strengthened genomic surveillance and evidence-based public health policies; and (2) the development of a nanobiotechnological strategy for SARS-CoV-2 intervention, highlighting the value of interdisciplinary collaboration among nanomedicine, virology, and epidemiology in driving translational progress. Ultimately, bridging laboratory innovation with real-world implementation underscores the translational potential and societal relevance of this work, particularly within resource-limited settings.

Optimising synthesis, characterisation, and mucosal delivery of β -Escin–Rutin–NP conjugates will be critical for clinical deployment. The green nasal spray formulation proposed in this study could complement vaccination campaigns, particularly in low-resource environments, by offering a rapid, targeted, and sustainable antiviral measure. At last, the lessons drawn from both epidemiological and nanotechnological perspectives converge toward a unified vision of sustainable pandemic preparedness.

To safeguard the innovation and therapeutic value of this approach, a formal patent application has been filed for the proposed nasal nanobiotherapeutic system. The patent encompasses the unique formulation of β -Escin and Rutin conjugated with biosynthesised gold and silver nanoparticles, designed for synergistic antiviral action via mucosal delivery. This step not only preserves the formulation's specific composition and preparation method but also ensures the formulation's intended use as a novel intranasal antiviral agent. Securing intellectual property rights paves the way for clinical validation, pharmaceutical development, and eventual commercialisation, thereby maximising the translational potential of this work (**Appendix 2**).

In the final analysis, these findings highlight the interconnectedness of epidemiological behaviour, healthcare systems, and nanobiotechnological innovation in pandemic management. Integrating lessons from both macro- and micro-level analyses is essential to developing adaptable, sustainable health solutions. Effective crisis management must transcend virological containment

and integrate epidemiological, behavioural, educational, and infrastructural measures. The findings advocate scientifically rigorous, culturally sensitive, and psychologically supportive frameworks for future pandemic readiness.

Conclusion

&

Perspectives

Conclusion & Perspectives

SARS-CoV-2 continues to circulate globally, with multiple variants co-existing even during periods of low reported incidence. This persistence underscores that COVID-19 remains a significant public health concern requiring sustained vigilance. Furthermore, the continued activity of other respiratory viruses highlights the broader and enduring complexity of the viral ecosystem. Continuous adherence to preventive measures, along with the integration of molecular insights and clinical experience, is essential for developing adaptive and effective response strategies [(Pilapil *et al.*, 2023); (de Assis Carvalho *et al.*, 2025); (Rizzi & Sainaghi, 2025)].

This thesis provides a comprehensive evaluation of the regional impact of SARS-CoV-2 associated disease in Algeria, emphasizing vaccination dynamics, logistical barriers, clinical surveillance, and the value of data-driven public health interventions. The findings highlight the need to optimize vaccine distribution systems, strengthen healthcare infrastructure, and tailor interventions to ensure equitable and efficient pandemic management. These results also offer a microcosmic perspective of global public health challenges, contributing region-specific insights to the broader pandemic discourse.

Beyond the epidemiological dimension, this work introduces an innovative nanobiotechnological strategy that combines natural biomolecules with metallic nanoparticles to design novel antiviral agents. *In silico* analyses revealed that β -Escin–Rutin–conjugated AuNPs and AgNPs exhibit strong and stable inhibitory interactions with key SARS-CoV-2 proteins. The proposed nasal nanobiotherapeutic formulation offers a localized, non-invasive prophylactic approach that could enhance mucosal immunity while minimizing systemic exposure, presenting a promising adjunct to existing vaccination strategies.

While computational findings provide valuable preliminary insights, experimental validation remains a critical next step. Future investigations should include *in vitro* and *in vivo* studies to assess cytotoxicity, biodistribution, bioavailability, and therapeutic efficacy. Optimizing nanoparticle functionalization and advancing toward translational and clinical trials will be essential for real-world applications.

By integrating epidemiological evidence with nanobiotechnological innovation, this thesis presents a holistic framework for understanding and combating COVID-19. This interdisciplinary

approach, bridging public health surveillance, natural compound discovery, and nanoscale drug design, demonstrates how converging scientific domains can generate resilient, adaptable solutions against emerging viral threats. Ultimately, this work contributes to both regional and global preparedness, paving the way for sustainable strategies to address future pandemics.

“The virus still circulates, and the disease remains among us. We accept this reality, not with resignation, but with resilience. We face what persists, acknowledging what has passed, and choose to focus on solutions rather than problems. In the spirit of this thesis, the true worth of knowledge lies in its sharing; the true worth of discovery lies in its humility; and the true worth of innovation lies in its impact”

Heart of Knowledge – Mind of Science

Knowledge guided by intentionality, fortified with patience and sincerity.

From universals to specifics, a reflection of scientific reasoning.

Each error is a step on a continuum, from the smallest to the greatest.

*Belief in the unseen is the portion of the heart, the formulation of ideas is the portion of the mind,
and from what we possess and what we endure emerges the reality behind our hypotheses.*

Just as faith shapes perception, science shapes understanding, and philosophy shapes purpose.

- Ilyes Zatla

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Appendices

Appendix 1.

Pharmacokinetic Profiling of β -Escin and Rutin

SwissADME and pkCSM analyses indicated that both β -Escin and Rutin deviate from standard drug-likeness criteria and exhibit poor oral bioavailability due to high polarity, low solubility, and limited permeability. However, these pharmacokinetic limitations primarily affect oral formulations and do not preclude their suitability for alternative delivery routes. Intranasal administration, in particular, bypasses first-pass metabolism and depends mainly on mucosal permeability and local tolerability. The use of nanocarrier-based systems, as proposed in this study, could further enhance local retention within the nasal cavity while minimizing systemic exposure. Nevertheless, experimental validation remains essential to assess mucosal safety, cytotoxicity, and *in vivo* tolerability. Overall, although β -Escin and Rutin are unsuitable for oral delivery, intranasal nanobiotechnology-based formulations may offer a viable strategy for their therapeutic application, provided that safety (especially for β -Escin) is thoroughly evaluated.

Table A1. Predicted ADME/Toxicity Properties of β -Escin and Rutin

Rutin	β -Escin	Compound
5280805	4555259	PubChem ID
610.52	1131.26	MW (g/mol)
16	24	NHA
10	13	NHD
-1.51	-0.28	LogP (cLogP)
3	3	Lipinski Violations
-2.89	-2.86	Water Solubility (log mol/L)
-0.94	-0.90	Caco2 Permeability (log Papp, 10 ⁻⁶ cm/s)
23.44	0	HIA (% Absorbed)
-2.73	-2.73	Skin Permeability (log Kp)
1.66	-0.46	VDss (log L/kg)
0.18	0.42	Fraction Unbound (Fu)
-1.89	-3.03	BBB Permeability (log BB)
-5.17	-5.90	CNS Permeability (log PS)
No; No; No; No; No	No; No; No; No; No	CYP Substrate/Inhibitor (2D6, 3A4, 1A2, 2C19, 2C9)
-0.36	0.08	Total Clearance (log ml/min/kg)
No	No	Renal OCT2 Substrate
5000	134	LD50 (mg/kg)
4	3	Toxicity Class
Inactive	Inactive	Hepatotoxicity
Inactive	Active	Carcinogenicity
Active	Active	Immunotoxicity
Inactive	Inactive	Mutagenicity
Inactive	Inactive	Cytotoxicity

Appendix 2.

Patent

Antiviral Formulation Based on *Aesculus hippocastanum* and *Prunus Avium* Against Respiratory Viruses

Ilyes Zatla & Lamia Boublenza

To safeguard the innovation and therapeutic value of this approach, a formal patent application has been filed for the proposed nasal nanobiotherapeutic system. The patent encompasses the unique formulation of β -Escin and Rutin conjugated with biosynthesized gold and silver nanoparticles, designed for synergistic antiviral action via mucosal delivery.

This step not only protects the specific composition and preparation method but also secures the intended use of the formulation as a novel intranasal antiviral agent. Securing intellectual property rights paves the way for clinical validation, pharmaceutical development, and eventual commercialization, thereby maximizing the translational potential of this work.

R2-FO-03 E1DZ

الجمهورية الجزائرية الديمقراطية الشعبية
REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE

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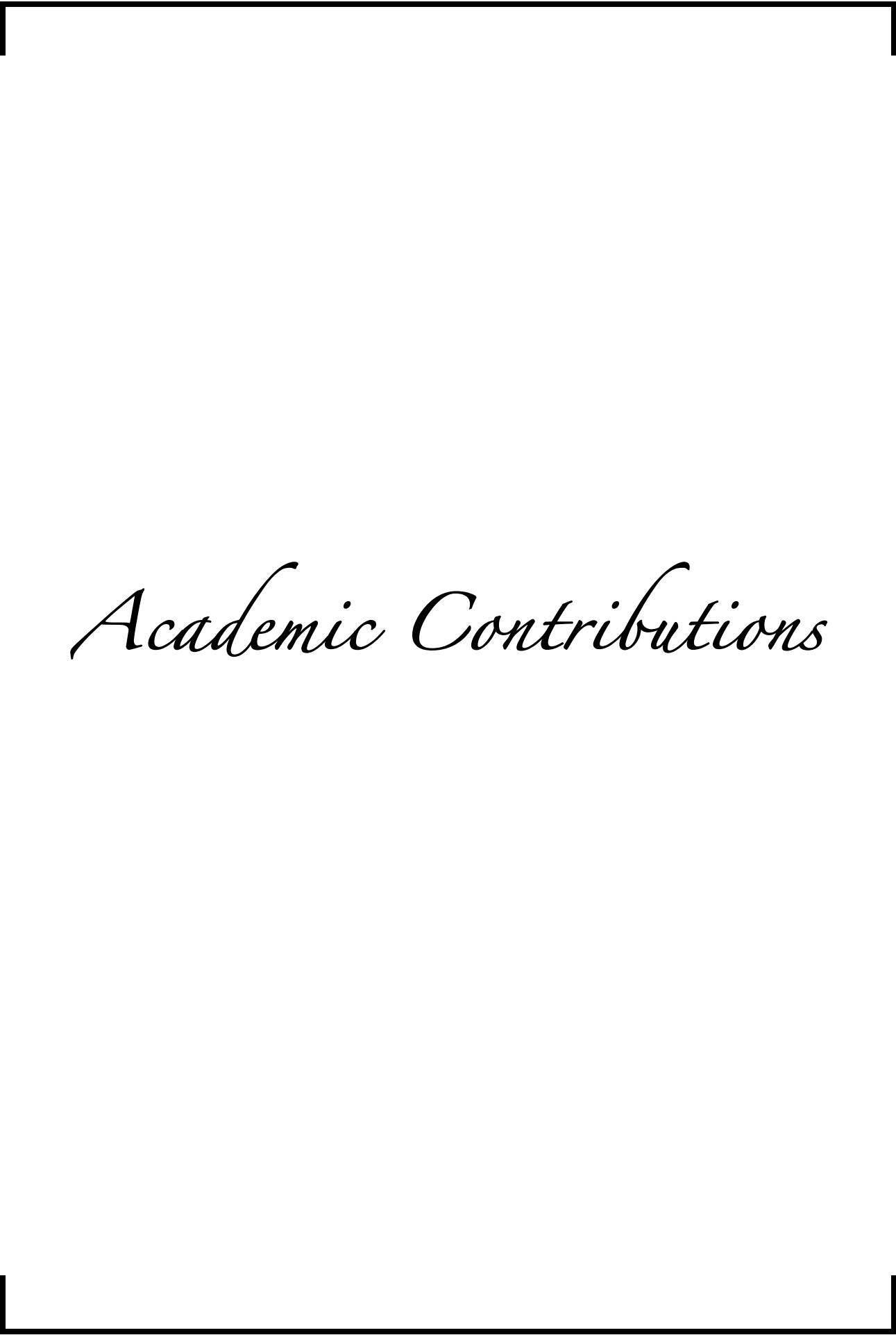
المعهد الوطني الجزائري للملكية الصناعية

REQUETE EN DELIVREANCE D'UN BREVET D'INVENTION

طلب منح براءة الاختراع

1 <u>Nature de la demande de protection</u> طبيعة الطلب Brevet d'invention ☒ Demande divisionnaire ☐ Certificat d'addition ☐ براءة الاختراع ☒ طلب جزئي ☐ شهادة الإضافة ☐ Extension de la demande internationale PCT ☐ الإمتداد غير طلب دولي ☐	6 <u>TITRE DE L'INVENTION</u> عنوان الاختراع 54 Formulation antivirale à base de Aesculus hippocastanum et Prunus avium contre les virus respiratoires.
2 <u>INFORMATION SUR LE DEPOSANT</u> معلومات حول مقدم الطلب 71 Dénomination: Université de Tlemcen إسم الشركة Forme juridique: Etablissement public à caractère scientifique الطبيعة القانونية Secteur d'activité: service قطاع النشاط التجاري Adresse: 22 Rue Abi Ayad F. G. Pasteur BP119 Wilaya: Tlemcen العنوان Tlemcen Commune: Tlemcen الولاية البلدية Téléphone: 0551071258 رقم الهاتف	7 <u>DOMAINE TECHNIQUE DE L'INVENTION</u> المجال التقني للاختراع 51 //////////
3 <u>CODE DU MANDATAIRE</u> رمز الوكيل 74 Nom du mandataire: /////////// إسم الوكيل	8 <u>DONNEES RELATIVES AU DEPOT</u> بيانات الإيداع Date: _____ Heure: _____ تاريخ الوقت Numéro: _____ رقم الإيداع
4 <u>INFORMATIONS SUR L'INVENTEUR</u> معلومات حول المخترع 72 Nom et Prénom: ZATLA الاسم واللقب ILYES Nationalité: DZ, Algeria الجنسية Adresse: Faculté SNV STU, 02-06-2025, 14 cité 20 villas, Hamza العنوان Cherif, Oudjida, Tlemcen Fonction: Etudiant المهنة E-mail: ilyes.zatla@univ-tlemcen.dz البريد الإلكتروني	9 <u>DONNEES RELATIVES A LA DEMANDE INTERNATIONALE</u> بيانات الطلب الدولي Date: _____ (22) تاريخ Numéro: _____ (21) رقم
5 <u>DONNEES RELATIVES A LA PRIORITE</u> بيانات الأولوية 30 Date: _____ Numéro: _____ تاريخ رقم الأولوية Pays d'origine: _____ البلد الأصلي	10 <u>DECHEANCE</u> إبطال La déchéance d'un brevet d'invention intervient en cas de non-acquittement, à la date anniversaire du dépôt, des taxes de maintien en vigueur, يسقط الحق على ملكية براءة الاختراع في حالة عدم تسديد الرسوم السنوية المستحقة
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Academic Contributions

Academic Contributions arising from this Degree

○ *Articles*

• *Reviews*

1. **Zatla, I.**, Boublenza, L., Abid, W. (2023). Re-emergence of monkeypox: a post-COVID-19 challenge. *Current Trends in Immunology*, 24, 21-29.
2. **Zatla, I.**, Abid, W., & Boublenza, L. (2023). Unveiling Monkeypox virus emergence: What unfolded in the aftermath of the COVID-19 threat? *Current Trends in Immunology*, 24, 9-20.
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4. **Zatla, I.**, Boublenza, L., & Hassaine, H. (2022). Infection and viral pathogenesis of SARS-CoV-2: A review. *Research & Reviews: A Journal of Microbiology and Virology*, 12(2), 1–7.
5. **Zatla, I.**, Boublenza, L., & Hassaine, H. (2022). The first days and months of the COVID-19 pandemic. *Research & Reviews: A Journal of Microbiology and Virology*, 12(1), 7–13.
6. **Zatla, I.**, Boublenza, L., & Hassaine, H. (2021). SARS-CoV-2 morphology, genome, life cycle and our bodies' immune response: a review. *Current Topics in Virology*, 18, 15-24.
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8. **Zatla, I.**, Boublenza, L., & Hassaine, H. (2021). Therapeutic and preventive approaches against COVID-19: A review. *Research & Reviews: A Journal of Microbiology and Virology*, 11(3), 26-33.

- **Research Articles**

1. **Zatla, I., & Boublenza, L. (2026).** Enhanced Nanobiotechnological Strategy Against SARS-CoV-2: Synergistic Potential of Rutin. *Coronaviruses*.
2. **Zatla, I., & Boublenza, L. (2025).** Battling COVID-19 leveraging nanobiotechnology: Gold and silver nanoparticle–B-escin conjugates as SARS-CoV-2 inhibitors. *Open Life Sciences*, 20(1), 20221047.
3. **Zatla, I., & Boublenza, L. (2025).** Green-Synthesized Colloidal Metal–B-escin Bioconjugates for Nasal Delivery: A Hypothetical Prophylactic and Therapeutic Product Against SARS-CoV-2 Variants. *Trends in Sciences*, 22(6), 9826-9826.
4. **Zatla, I., Boublenza, L., Lemerini, W., Triqui, C., & Selka, N. (2025).** Profiling COVID-19 Cases in Tlemcen, Algeria: PCR and CT Imaging Insights. *COVID*, 5(9), 149.
5. **Zatla, I., Boublenza, L., Lemerini, W., Behar, D., Dahmani, B., Khouani, A., & Selka, N. (2025).** Assessment of COVID-19 vaccination performance in Tlemcen: Insights into vaccine inventory management. *Microbes and Infectious Diseases*, 6(3), 1036-1043.
6. **Zatla, I., & Boublenza, L. (2024).** Computational Screening of Natural Compounds as Antiviral Candidates Targeting the SARS-CoV-2 Main Protease. *Journal of Integrated OMICS*, 14(3), 1-15.
7. **Zatla, I., & Boublenza, L. (2024).** Molecular simulation of diverse biomolecules inhibiting the SARS-CoV-2 RdRp function. *Current Topics in Peptide and Protein Research*, 25, 13-26.
8. **Zatla, I., Abid, W., & Boublenza, L. (2024).** Assessing the knowledge of smallpox and Monkeypox virus among the University of Tlemcen members in the wake of COVID-19: A 2023 cross-sectional study. *African Journal of Infectious Diseases*, 18(1), 5-10.
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○ ***Proceedings***

1. **Zatla, I.**, & Boublenza, L. (2024). A Computational Study on Gold and Silver Nanoparticles against SARS-CoV-2 Proteins. ***Proceedings***, 103, 23.

○ ***Book Chapter***

1. **Zatla, I.**, & Boublenza, L. (2022). COVID-19 pandemic: A survey study analysis. **In Current Algerian Research Topics in Microbiology (Sarahmed edition)**. Bejaia, Algeria.

○ ***Collaborations***

1. Belhadj, A., Yayaoui, R., **Zatla, I.**, & Sahraoui, T. (2024). Uncovering Clinical Insights: A Comprehensive Analysis of Covid-19 Cases In Oran From May To September 2021. ***Euromediterranean Biomedical Journal***, 19(14), 67-70.

2. Triqui, C., **Zatla, I.**, Chahbar, M., Lemerini, W., Moussi. N., Gaouar, S. B., Mokhtari-Soulmane, N. (2024). Exploring the Interplay of UV Index, Population Density, and SARS-CoV-2 Clades on COVID-19 Propagation: A Comprehensive Analysis of the Pandemic Dynamics. *Infection Epidemiology and Microbiology*, 10(1), 61-70
3. Triqui, C., **Zatla, I.**, Lemerini, W., Benmadani, N., Houari, M. C., Gaouar, S. B., Mokhtari-Soulmane, N. (2024). Navigating the molecular diversity of SARS-CoV-2: early pandemic insights from comparative phylogenetic analysis: SARS-CoV-2 Clades Phylogeny. *Journal of Integrated OMICS*, 14(1), 1-6.
4. Lemerini, W., **Zatla, I.**, & Boublenza, L. (2023). The impact of stress on COVID-19 severity: Exploring antidepressants as a potential intervention. *Current Topics in Pharmacology*, 27, 63-66.

○ **Communications**

• **National Communications**

▪ **Oral Presentations**

1. **Zatla, I.**, Boublenza, L. *Gold and silver nanoparticle complexes of rutin and β -escin as dual inhibitors of SARS-CoV-2 main protease and RdRp*. **1st National Seminar on Biomolecules and Health**, University of Boumerdes, Boumerdes, Algeria, November 12–13, 2025.
2. **Zatla, I.**, Lemerini, W., Boublenza, L. *The use of antidepressants against severe COVID-19 infection*. **World Pharmacists Day & 2nd National Pharmacy Day**, Ain Témouchent, Algeria, September 25, 2023.
3. **Zatla, I.**, Boublenza, L. *Variety of drugs in the time of COVID-19*. **World Pharmacists Day & 1st National Pharmacy Day**, Ain Témouchent, Algeria, February 25, 2022.

▪ **Poster Presentations**

1. **Zatla, I.**, Boublenza, L., Lemerini, W., Triqui, C. *Assessment of COVID-19 clinical features and vaccination campaign in Tlemcen, Algeria: a retrospective analysis*. **22nd**

National Microbiology Days, University of Tlemcen, Tlemcen, Algeria, April 29–30, 2025.

2. **Zatla, I.,** Boublenza, L. *Bio-nanotechnology-enhanced nasal prophylaxis: a computational approach for targeting SARS-CoV-2 variants.* **22nd National Microbiology Days**, University of Tlemcen, Tlemcen, Algeria, April 29–30, 2025.
3. **Zatla, I.,** Boublenza, L. *Nasal treatment development targeting SARS-CoV-2 variants with β -escin and nanoparticles.* **5th National Seminar MGBIR: Between Student and Investor**, University of Tlemcen, Tlemcen, Algeria, May 5, 2024.
4. **Zatla, I.,** Boublenza, L. *In silico evaluation of metallic nanoparticles against SARS-CoV-2 proteins.* **Applications of Natural and Life Sciences & Earth Sciences: Relations with the Socio-Economic Sector**, University of Khemis Miliana, Khemis Miliana, Algeria, April 17–18, 2024.
5. **Zatla, I.,** Boublenza, L. *Molecular simulation of biomolecules against SARS-CoV-2 proteins.* **National Conference on Phytobiotechnology**, University of El-Tarf, El-Tarf, Algeria, December 17–18, 2023.
6. **Zatla, I.,** Boublenza, L., Sebiane, A., Salah, I. *Evolution of COVID-19 in North African countries.* **National Doctoral Days: Applied Sciences and Innovations**, University of Annaba, Annaba, Algeria, November 15–16, 2023.
7. **Zatla, I.,** Boublenza, L., Lemerini, W. *Epidemiological study of COVID-19 in Tlemcen in 2021.* **1st National Doctoral Colloquium of Biological Sciences**, University of Blida, Blida, Algeria, November 8–9, 2023.
8. **Zatla, I.,** Boublenza, L., Boublenza, A. *The beginning of COVID-19 in Europe and its evolution.* **2nd National Hybrid Seminar on Biotechnology and Microbial Biodiversity**, University of Khenchela, Khenchela, Algeria, November 8, 2023.
9. **Zatla, I.,** Boublenza, L., Boublenza, A. *Fluctuation rates of COVID-19 in Europe.* **4th Seminar of Biology and Health**, University of Sidi Bel Abbès, Sidi Bel Abbès, Algeria, October 21, 2023.
10. **Zatla, I.,** Boublenza, L., Sebiane, A., Salah, I. *Tracing COVID-19 in North African countries.* **4th Seminar of Biology and Health**, University of Sidi Bel Abbès, Sidi Bel Abbès, Algeria, October 21, 2023.

11. **Zatla, I.**, Boublenza, L., Zair, S., Diab, N. *University of Tlemcen vaccination campaign for COVID-19: a survey study*. **1st National Seminar on Biodiversity, Health and Environment**, University of Mila, Mila, Algeria, October 3, 2023.

12. **Zatla, I.**, Boublenza, L., Zair, S., Diab, N. *University of Tlemcen vaccination campaign for COVID-19: a survey study*. **3rd National Workshop Between the Student and the Investor**, University of Tlemcen, Tlemcen, Algeria, March 23–24, 2022.

- **International Communications**

- **Oral Presentations**

1. **Zatla, I.**, Boublenza, L. *Innovative nasal therapy for SARS-CoV-2 variants utilizing β -escin and nanoparticle technology*. **UMTEB 16th International Scientific Research Congress**, IKSAD Institute, Turkey, December 6–8, 2024.

2. **Zatla, I.**, Boublenza, L. *In silico investigation of gold and silver nanoparticles with β -escin against COVID-19 variants*. **2nd International Biological & Life Sciences Congress**, Trakya University, Turkey, October 31 – November 2, 2024.

3. **Zatla, I.**, Boublenza, L. *COVID-19 case profiles in Tlemcen: insights from PCR and CT imaging*. **3rd International Conference on Scientific and Innovative Studies**, All Sciences Academy, Turkey, September 11–12, 2024.

4. **Zatla, I.**, Boublenza, L. *Screening of natural antivirals against SARS-CoV-2 main protease*. **3rd International Conference on Innovative Academic Studies**, All Sciences Academy, Turkey, September 26–28, 2023.

5. **Zatla, I.**, Boublenza, L. *Screening of natural antivirals against SARS-CoV-2 RdRp*. **3rd International Conference on Innovative Academic Studies**, All Sciences Academy, Turkey, September 26–28, 2023.

6. **Zatla, I.**, Boublenza, L. *Screening of natural antivirals against the receptor-binding motif of the SARS-CoV-2 spike glycoprotein*. **3rd International Conference on Innovative Academic Studies**, All Sciences Academy, Turkey, September 26–28, 2023.

7. **Zatla, I.**, Boublenza, L. *Survey of vaccination rates against COVID-19 at the University of Tlemcen*. **2nd International Congress on Natural & Medical Sciences**, Turkey, June 12–13, 2023.

8. **Zatla, I.,** Boublenza, L., Boublenza, A. *Europe in the face of COVID-19.* **2nd International Congress on Natural & Medical Sciences**, Turkey, June 12–13, 2023.
9. **Zatla, I.,** Lemerini, W., Boublenza, L. *SARS-CoV-2 and antidepressants: was it all stress?* **2nd International Congress on Natural & Medical Sciences**, Turkey, June 12–13, 2023.
10. **Zatla, I.,** Boublenza, L. *Surface cleaning proof: product or tool?* **1st International Colloquium on Surface Hygiene in the Hospital and Agri-Food (NADAFa)**, University of Tlemcen, Algeria, May 9–10, 2023.
11. **Zatla, I.,** Boublenza, L. *COVID-19 vaccination at the University of Tlemcen.* **10th International GAP Summit Scientific Research Congress**, Gaziantep University & IKSAD Institute, Turkey, March 17–18, 2023.
12. **Zatla, I.,** Boublenza, L., Boublenza, A. *COVID-19 evolution in Europe.* **10th International GAP Summit Scientific Research Congress**, Gaziantep University & IKSAD Institute, Turkey, March 17–18, 2023.
13. **Zatla, I.,** Lemerini, W., Boublenza, L. *COVID-19: what about antidepressants?* **10th International GAP Summit Scientific Research Congress**, Gaziantep University & IKSAD Institute, Turkey, March 17–18, 2023.
14. **Zatla, I.,** Boublenza, L. *Survey analysis of the COVID-19 pandemic.* **International Conference and Exhibition for Science**, King Saud University, Saudi Arabia, February 6–8, 2023. (*Springer indexed*)
15. **Zatla, I.,** Boublenza, L. *Investigation of the COVID-19 pandemic: health, infection, and vaccination.* **1st International Microbiology Seminar**, University of Bejaia, Bejaia, Algeria, June 4, 2022.
16. **Zatla, I.,** Boublenza, L. *Investigation of the COVID-19 pandemic: health, infection, and vaccination.* **6th International Symposium on Pharmaceutical and Biomedical Sciences**, Gaziantep University, Turkey, May 26–28, 2022.

▪ **Poster Presentations**

1. **Zatla, I.,** Boublenza, L. *Bio-nanotechnology-enhanced nasal prophylaxis: a computational approach for targeting SARS-CoV-2 variants.* **5th International Online Conference on Nanomaterials**, MDPI, Switzerland, September 22–24, 2025.

2. **Zatla, I.**, Boublenza, L., Lemerini, W., Triqui, C. *Assessment of COVID-19 clinical features and vaccination campaign in Tlemcen, Algeria*. **1st International Online Conference on Diseases**, MDPI, Switzerland, September 9–11, 2025.
3. **Zatla, I.**, Lemerini, W., Triqui, C. *Hematological alterations in COVID-19 patients: insights from a descriptive study in Tlemcen, Algeria*. **1st International Online Conference on Diseases**, MDPI, Switzerland, September 9–11, 2025.
4. **Zatla, I.**, Boublenza, L. *Beyond vaccines: a nasal nano-formulation and the realities of COVID-19 coverage in Tlemcen*. **2nd International Seminar on Catalysis, Chemical Engineering & Green Chemistry**, University of El-Oued, Algeria, May 21–22, 2025. (Springer indexed)
5. **Zatla, I.**, Boublenza, L. *Design of a nasal prophylactic targeting SARS-CoV-2 variants with β -escin and nanoparticles*. **3rd International Online Conference on Cells**, MDPI, Switzerland, March 25–27, 2025.
6. **Zatla, I.**, Boublenza, L. *In silico evaluation of gold and silver nanoparticles' efficacy against five COVID-19 variants*. **5th International Electronic Conference on Applied Sciences**, MDPI, Switzerland, December 24–26, 2024.
7. **Zatla, I.**, Boublenza, L. *Nasal treatment targeting SARS-CoV-2 variants in the age of vaccines*. **2nd International Biological & Life Sciences Congress**, Trakya University, Turkey, October 31 – November 2, 2024.
8. **Zatla, I.**, Boublenza, L. *Gold and silver nanoparticles with β -escin against COVID-19 variants: an in silico approach*. **3rd International Conference on Clinical and Applied Microbiology**, BioLeagues, Indonesia, July 26–27, 2024.
9. **Zatla, I.**, Boublenza, L. *Gold and silver nanoparticles against SARS-CoV-2 proteins: a computational study*. **1st Pan-Arab Symposium on Parasitology and Infectious Diseases**, University of Relizane, Relizane, Algeria, May 18–19, 2024.
10. **Zatla, I.**, Boublenza, L. *Biomolecules against SARS-CoV-2 main protease, RdRp, and the receptor-binding motif of its spike glycoprotein: an in silico approach*. **1st Pan-Arab Symposium on Parasitology and Infectious Diseases**, University of Relizane, Relizane, Algeria, May 18–19, 2024.

11. **Zatla, I.**, Boublenza, L. *Biomolecules against SARS-CoV-2 main protease, RdRp, and the receptor-binding motif of its spike glycoprotein: an in silico approach*. **3rd International Electronic Conference on Biomolecules**, MDPI, Switzerland, April 23–25, 2024.
12. **Zatla, I.**, Boublenza, L. *A computational study of gold and silver nanoparticles against SARS-CoV-2 proteins*. **3rd International Electronic Conference on Biomolecules**, MDPI, Switzerland, April 23–25, 2024.
13. **Zatla, I.**, Boublenza, L., Lemerini, W., Triqui, C., Selka, N. *Exploring vaccine variants: a study of COVID-19 vaccination in Tlemcen (2021)*. **9th International Seminar MGIBR “Biodiversity: Food Security & Health”**, University of Tlemcen, Tlemcen, Algeria, April 20–22, 2024.
14. **Zatla, I.**, Triqui, C., Lemerini, W. *COVID-19 propagation dynamics: a comprehensive analysis of the pandemic dawn*. **9th International Seminar MGIBR “Biodiversity: Food Security & Health”**, University of Tlemcen, Tlemcen, Algeria, April 20–22, 2024.
15. **Zatla, I.**, Boublenza, L., Sebiane, A., Salah, I. *COVID-19 progression in North Africa*. **6th International Eurasian Conference on Biological and Chemical Sciences**, Turkey, October 11–13, 2023.
16. **Zatla, I.**, Boublenza, L. *Microbial and plant biomolecule evaluation against SARS-CoV-2 main protease*. **1st International Seminar of Microbiology: Functional Micro-ecology in the Service of Living Systems**, University of Tiaret, Tiaret, Algeria, September 20–21, 2023.
17. **Zatla, I.**, Boublenza, L. *GLOGERM: an educational tool against COVID-19 spread*. **1st International Colloquium on Surface Hygiene in the Hospital and Agri-Food (NADAFa)**, University of Tlemcen, Tlemcen, Algeria, May 9–10, 2023.
18. **Zatla, I.**, Lemerini, W., Boublenza, L. *Antidepressants against severe COVID-19*. **International Conference on Clinical and Applied Microbiology**, BioLeagues, India, April 27–28, 2023.
19. **Zatla, I.**, Boublenza, L. *The illustrative virology of SARS-CoV-2*. **International Conference and Exhibition for Science**, King Saud University, Saudi Arabia, February 6–8, 2023. (*Springer indexed*)
20. **Zatla, I.**, Boublenza, L., Zair, S., Diab, N. *Investigation of COVID-19 and vaccination at the University of Tlemcen (Algeria)*. **43rd EORTC-PAMM**, Italy, December 15–17, 2022.

21. **Zatla, I.,** Boublenza, L. *Investigation of the COVID-19 pandemic: health, infection, and vaccination. International Union of Microbiological Societies*, Netherlands, July 20–22, 2022.
22. **Zatla, I.,** Boublenza, L. *COVID-19 pandemic: a survey analysis. 24th Annual Conference of the European Society for Clinical Virology*, United Kingdom, September 7–10, 2022.
23. **Zatla, I.,** Boublenza, L. *Investigation of COVID-19 and vaccination at the University of Tlemcen (Algeria). TeebPedia – COVID Impact: What Changed*, Algiers University 1, Algiers, Algeria, May 27–28, 2022.
24. **Zatla, I.,** Boublenza, L. *Enquête sur la pandémie de COVID-19 : santé, infection et vaccination. BIOMEDJ Congress*, France, May 19–20, 2022.

○ **Student Mentorship**

- **University of Tlemcen Academic Year 2023-2024**

Academic advising and skills tutoring to three students, in general biology support, university orientation, credit-system guidance, exam preparation, and digital educational tools.

○ **Awards**

Award for " **COVID-19: Prevention and Vaccination Awareness Award**" organized by University of Maghnia, Tlemcen, 27-March-2022.

○ **Innovative Projects 1275**

- FSNV-STU-057.

▪ **Patent**

Patent application (pending): "*Antiviral formulation based on Aesculus hippocastanum and Prunus Avium against respiratory viruses*", Inventors: **Ilyes Zatla** and Lamia Boublenza. Application No.: 4295. Filed: 02-Juin-2025. Status: Pending.

Reflection of Life

We are all visitors to this time, this place.

We are just passing through.

Our purpose here is to observe, to learn, to grow, to love...

and then we return home.

- Queen Elizabeth II

(Australian Proverb)

Abstract

The global COVID-19 pandemic has highlighted the urgent need to develop both innovative antiviral therapies and effective public health strategies. This thesis adopts a dual approach to address this challenge: (I) an epidemiological evaluation of the COVID-19 vaccination campaign conducted in Tlemcen, Algeria, between 2020 and 2022; and (II) an *in silico* investigation of novel antiviral formulations based on natural compounds, supported by nanobiotechnological tools.

The first part relies on a retrospective analysis of regional vaccination data, focusing on five major vaccines: *Sputnik V*, *AstraZeneca*, *Sinovac*, *Sinopharm*, and *Janssen*. Descriptive statistical methods were applied to assess vaccination coverage, demographic trends, and logistical management. The second part consists of an *in silico* study using molecular modelling techniques, including molecular docking and molecular dynamics simulations, to evaluate the interactions of gold and silver nanoparticles conjugated to β -escin and Rutin with key SARS-CoV-2 target proteins.

The epidemiological analysis revealed marked disparities in vaccination coverage, particularly among younger populations, as well as challenges related to vaccine hesitancy, logistics, and stock management. The *in silico* findings demonstrated strong and stable interactions between the nanobiological conjugates and viral proteins, suggesting promising antiviral activity and potential immunomodulatory effects.

These findings underscore the need to enhance communication and Organisation in vaccination campaigns while highlighting the potential of nanobiotechnological approaches combining natural compounds and colloidal metals to develop innovative antiviral formulations, particularly for nasal prophylactic applications. This integrated framework contributes to both pandemic preparedness and future therapeutic development.

Keywords: SARS-CoV-2, COVID-19, Nanoparticles, Phytomolecules, *In Silico* Analysis, Public Health.

Résumé

La pandémie mondiale de COVID-19 a mis en évidence la nécessité urgente de développer des thérapies antivirales innovantes et d'optimiser les stratégies de santé publique. Cette thèse adopte une approche double pour répondre à cette problématique : (I) une évaluation épidémiologique de la campagne de vaccination contre la COVID-19 dans la wilaya de Tlemcen (Algérie) entre 2020 et 2022, et (II) une étude *in silico* explorant de nouvelles formulations antivirales à base de composés naturels et de nanotechnologies.

La première partie repose sur une analyse rétrospective des données régionales de vaccination, portant sur cinq vaccins majeurs : *Sputnik V*, *AstraZeneca*, *Sinovac*, *Sinopharm* et *Janssen*. Des analyses statistiques descriptives ont permis d'évaluer la couverture vaccinale, les tendances démographiques et la gestion logistique des campagnes. La seconde partie est une investigation *in silico* utilisant la modélisation moléculaire (Docking moléculaire et Dynamique moléculaire) pour évaluer l'interaction entre des nanoparticules d'or et d'argent, conjuguées à la β -escine et à la rutine, et les protéines cibles du SARS-CoV-2.

Les résultats de l'étude épidémiologique révèlent des disparités marquées dans la couverture vaccinale, notamment chez les jeunes, ainsi que des difficultés liées à l'hésitation vaccinale et à la logistique. Les résultats *in silico* montrent des interactions fortes et stables entre les complexes nanobiologiques et les protéines virales, suggérant une activité antivirale prometteuse et un potentiel d'effet immunomodulateur.

Ces travaux mettent en lumière, d'une part, la nécessité de renforcer la communication et la planification des campagnes de vaccination, et d'autre part, le potentiel des approches nano-biotechnologiques combinant des composés naturels et des métaux colloïdaux pour le développement de formulations antivirales innovantes, notamment à usage prophylactique nasal. Cette approche intégrée apporte une contribution complémentaire à la préparation face aux futures pandémies.

Mots-clés : SARS-CoV-2, COVID-19, Nanoparticules, Phytomolécules, Analyse *In Silico*, Santé Publique.

المخلص

قد سلطت جائحة كوفيد-19 العالمية الضوء على الحاجة الملحة لتطوير علاجات مضادة للفيروسات مبتكرة واستراتيجيات فعالة للصحة العامة. تتبنى هذه الأطروحة نهجاً مزدوجاً لمواجهة هذا التحدي: (I) تقييم وبائي لحملة التطعيم ضد كوفيد-19 التي أجريت في تلمسان، الجزائر، بين عامي 2020 و2022، و (II) دراسة حاسوبية لتراكيب جديدة مضادة للفيروسات مبنية على مركبات طبيعية مدعومة بأدوات التكنولوجيا الحيوية النانوية.

يعتمد الجزء الأول على تحليل رجعي لبيانات التطعيم الإقليمية، مع التركيز على خمس لقاحات رئيسية: سبوتنيك V، أسترازينيكا، سينوفاك، سينوفارم، وجانسين. طبقت أساليب إحصائية وصفية لتقييم تغطية التطعيم، والاتجاهات الديموغرافية، والإدارة اللوجستية. يتكون الجزء الثاني من دراسة حاسوبية باستخدام تقنيات النمذجة الجزيئية، بما في ذلك الالتحام الجزيئي ومحاكاة الديناميكيات الجزيئية، لتقييم تفاعل الجسيمات النانوية الذهبية والفضية والمقترنة بـ β -escin و Rutin ضد بروتينات هدف SARS-CoV-2 الرئيسية.

كشف التحليل الوبائي عن تفاوتات ملحوظة في تغطية التطعيم، لا سيما بين الفئات السكانية الأصغر سناً، بالإضافة إلى تحديات تتعلق بالتردد في تلقي اللقاح، واللوجستيات، وإدارة المخزون. أظهرت النتائج الحاسوبية تفاعلات قوية ومستقرة بين المقترنات النانوية الحيوية والبروتينات الفيروسية، مما يشير إلى نشاط مضاد للفيروسات واعد وتأثيرات مفعلة للمناعة.

تؤكد هذه النتائج على الحاجة إلى تعزيز التواصل والتنظيم في حملات التطعيم، مع تسليط الضوء على إمكانات مناهج التكنولوجيا الحيوية النانوية التي تجمع بين المركبات الطبيعية والمعادن الغروية لتطوير تراكيب مبتكرة مضادة للفيروسات، لا سيما لتطبيقات الوقاية الأنفية. يُسهم هذا الإطار المتكامل في كل من التآهب للجائحة وتطوير العلاجات المستقبلية.

الكلمات المفتاحية: SARS-CoV-2، كوفيد-19، الجسيمات النانوية، الجزيئات النباتية، تحليل حاسوبي، الصحة العامة.