Original Article

Tumor Necrosis Factor-Alpha (TNF- α) and Antiviral Activities of Artemisia SPP. Extracts on SARS-COV2

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Abstract

Background

The challenges posed by Coronavirus disease 2019 (COVID-19), including treatment resistance, pandemic threats, and vaccine failure, necessitate the need for locally sourced efficacious therapeutic interventions. We investigated the in-vitro antiviral effects of natural medicinal compounds from Artemisia spp., validated by molecular docking, on TNF-α levels in hospitalized SARS-CoV-2 patients from a designated Lagos COVID-19 isolation centre.

Methods

Bioactive chemicals in ethanol and dichloromethane (DCM) extracts from freshly collected, dried, and powdered A. afra and A. annua leaves were characterised using UHPLC. A cross-sectional study of 253 hospitalised SARS-CoV-2 patients was conducted to evaluate the extracts' antiviral activity through serum TNF-α modulation; and docking techniques to determine compound-TNF-α binding affinities.

Results

Phytochemical screening of A. annua revealed therapeutic constituents ((lactones, monoterpenes, flavonoids, and sesquiterpenes (artemisinin, rutin, and phenolic acids)) confirmed by UHPLC. The dichloromethane extract of A. afra demonstrated greater TNF-α inhibition in critically ill COVID-19 patients unlike ethanol extracts. Molecular docking validated TNF-α binding affinity for most isolated compounds.

Conclusions

Artemisia spp. promises antiviral-proinflammatory cytokine regulation, justifying clinical COVID-19 prophylaxis/therapeutic research, especially against resistant SARS-CoV-2 variants. This could reduce vaccination dependency in impoverished nations while addressing vaccine efficacy and local immunity gaps.

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Keywords: Artemisia annua, Artemisia afra, ethanol, dichloromethane, HPLC, TNF-α

Introduction

In recent years, advancements in healthcare delivery and increased survival rates have become key priorities in modern life. Artemisia annua, known as "qinghao" in traditional Chinese medicine, has a long history of use due to its unidentified chemical compounds, primarily as an antimalarial agent, among other applications.[1] Both Artemisia afra and Artemisia annua have attracted significant academic interest for centuries and have since naturalized in multiple countries, including Argentina, Romania, Spain, and the United States.[2]

The recent Coronavirus disease 2019 (COVID-19) pandemic, caused by the highly transmissible Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has led to widespread devastation, severely impacting global economies and healthcare systems. Originating in Wuhan, Hubei Province, China, this highly contagious viral disease has infected millions and resulted in hundreds of thousands of deaths worldwide.[3] SARS-CoV-2 typically induces a robust immune response that can lead to cytokine storm syndrome,[4] respiratory complications, including acute respiratory distress syndrome (ARDS). These severe outcomes are closely associated with an exaggerated innate immune response and cytokine storm, which can cause multi-organ failure and, ultimately, death.[5]

The most severe impacts of this virus disproportionately affect vulnerable populations, particularly socioeconomically disadvantaged individuals, elderly groups, frontline healthcare workers, and hospitalized patients with comorbidities such as autoimmune disorders, hypertension, cardiovascular disease, and diabetes. These high-risk groups often face significant barriers in accessing costly emergency medical care and treatments.[6] Given the demonstrated antiviral efficacy and low cytotoxicity of A. annua and A. afra against other viral pathogens, their phytochemical constituents may theoretically exhibit inhibitory effects against SARS-CoV-2.

According to Rivas and colleagues,[7] the detrimental lung injury and respiratory failure linked to cytokine-mediated inflammatory events has resulted in deaths among COVID-19 patients. Furthermore, one of the notable challenges posed by SARS-CoV-2 was its ´continual´ evolution, which raises important questions regarding vaccine efficacy, human heterologous immunity, and post-vaccination immunity. Then, the evaluation for unknown ´SARS-CoV-3 strains´ remains unavoidable. One of the early high-level pro-inflammatory cytokines, Tumor necrosis factor-alpha (TNF-α), readily produced by innate immunity to enhance immune cell infiltration responds to Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) infections.

TNF-α is an essential cytokine that orchestrates inflammation and immunological responses, significantly influencing both health and illness. The unregulated production of TNF-α has been linked to numerous inflammatory and autoimmune disorders. TNF-α-mediated inflammation in COVID-19 can induce detrimental tissue damage and progressively facilitates lung fibrosis, leading to pneumonia, pulmonary oedema, and acute respiratory distress syndrome; however, its protective and pathological effects render it a crucial target for therapeutic interventions in diverse inflammatory disorders. Understanding the processes of TNF-α helps enhance the development of effective therapeutics, as TNF-α inhibitors are commonly utilised in clinical practice to treat numerous medical conditions by inhibiting its activity.

This study evaluated the phytochemical composition of ethanol and dichloromethane (DCM) extracts from Artemis afra and Artemisia annua, along with their in-vitro antiviral activity against TNF-α, a key pro-inflammatory cytokine in SARS-CoV-2 infection. The investigation focused on cytokine modulation in both symptomatic and asymptomatic COVID-19 patients at a designated isolation centre in Lagos.

Methodology

Study design and study population

We performed a cross-sectional analysis of 253 hospitalised SARS-CoV-2 patients in one of the main COVID-19 isolation centres in Lagos.

Sample Collection and Identification

Informed consent was obtained from patients following a clear explanation of the study's objectives and benefits, while maintaining strict confidentiality regarding their information throughout the research. The individuals enrolled in this study were classified as either asymptomatic or symptomatic SARS-CoV-2 patients and further stratified according to disease progression (mild, moderate, severe, and fatality). Patient data, including medical records, suspected classical symptoms of SARS-CoV-2, cytokine screening were conducted in accordance with the manufacturer's instructions. All clinical samples were under strict medical supervision and oversight. Individuals with congenital disorders were excluded from the study. The resources available to the study include, but are not limited to, the following: protective gear such as facemasks, latex gloves, and disposable isolation gowns; absorbent paper; sterile nasopharyngeal swab sticks; 5ml EDTA blood sample bottles; a -20°C freezer; 5ml syringes and needles; and disposable pipette tips of 200µl, 500µl,and 1000µl. Refrigerator, incubator set at 37°C, de-ionized or double-distilled water, micropipettes, and Eppendorf tubes of various sizes (200, 500, 1000 µl), among others.

Polymerase chain reaction (PCR) and blood plasma concentration of TNF-α

Nasopharyngeal swabs, throat samples, and blood samples from individual patients were screened to confirm SARS-CoV-2 infection through positive reverse transcriptase polymerase chain reaction (qPCR) testing. A retrospective review of the patients' backgrounds and underlying ailments was also documented.

Serum samples of Covid-19 patients

From each patient, 5 mL of venous blood was collected in pre-labeled EDTA vacutainers using standardized phlebotomy protocols. To determine blood plasma concentration of TNF-alpha, in a Microplate Reader, we used ELISA Stat Fax 2100 (Awareness Technology, USA) according to the detailed protocol in the manufacturer's manual.

Plants selection

Fresh leaves of Artemisia afra and Artemisia annua plants were collected from a local Rwanda community, Huye, Rwanda and identities authenticated at the National Industrial Research and Development Agency (NIRDA) botanical herbarium in Huye, Butare, Rwanda. We further confirmed the Herbarium specifications at the Department of Botany, College of Science and Technology, University of Rwanda, Kigali, Rwanda; and all the extraction procedures carried out at the analytical laboratory of NIRDA.

Figure 1(a):Artemisia annua(AA, left): a 30cm upright, non-hairy, brownish-violet brown stem, annual short-day plant of the Asteraceae family(the cultivated plants can grow up to 200cm tall).**(Source coordinate:2.616124N,29.74611E (b):Artemisia afra(AF, right)**: a ridged, hairy, and leafy stem a perennial woody shrub to two meters height; with a length of 8 cm and width of 4 cm, soft feel and are dark green leaves on adaxial surface and lighter green on the abaxial surface **(Source coordinate:2.61389N,29.750027E)**.... adapted from this study

Extraction of phytoconstituents of Artemisia Annua and Artemisia Afra Plants

Pre-extraction procedures

Fresh leaves of A. afra and A. annua were sterilized with distilled water, chopped, and air-dried at 25°C for two weeks in labeled perforated bags. The desiccated leaves were then separately ground using a RETSCH GM 200 electric grinder (A. afra: 700 rpm; A. annua: 500 rpm) with four 30-second cycles to produce homogenized powder (400 µm particle size), following established protocols prior to solvent extraction. [8] To preserve bioactive compounds, we employed Soxhlet extraction with selective solvents to isolate soluble metabolites, following standardized protocols while removing insoluble cellular debris. The resulting crude extracts - containing alkaloids, glycosides, phenolics, terpenoids, and flavonoids - were vacuum-filtered through Whatman No. 1 paper before subsequent analytical processing.

Ethanol extracts of A. Afra and A. Annua plants

Using aseptic techniques, we separately processed 20 g aliquots of powdered A. afra and A. annua through sequential solvent extraction. Each sample was decanted into 500 mL conical flasks with 200 mL dichloromethane (DCM) at a 1:10 ratio (w/v) for 24-hr maceration.[9] This extraction cycle was repeated five times with fresh solvent to maximize yield. The same protocol was replicated using 96.4% ethanol with subsequent ethanol extracts concentrated via rotary evaporation (60 kHz, 32°C) under vacuum before refrigerated storage.[10] The ethanol extract of A. annua was analyzed using an Agilent 1260 Infinity HPLC system (NIMR ID: p1-a-05) equipped with a Poroshell 120 EC-C18 column (4 µm, 150 × 4.6 mm).Separation was achieved at ambient temperature using a binary mobile phase gradient of 0.1% formic acid in water and 0.2% formic acid in methanol, with detection at 210 nm via a variable wavelength detector (VWD).

Dichloromethane extracts of A. Afra and A. Annua plants

Dichloromethane (DCM) extracts of A. afra and A. annua were prepared separately following established protocols under strict aseptic conditions.[11] The solutions were concentrated using a Heidolph rotary evaporator (Germany) at 60 kHz and 30°C, then sequentially processed through: (i) Whatman No. 1 filtration, (ii) centrifugation (10,000 rpm, 30 min, 2°C), and (iii) lyophilization (-80°C, 7 hr). Purified extracts were stored at -30°C until analysis. Prior to phytochemical analysis, plant extracts were solvent-diluted and filtered for concentration determination (mg/mL). Quantitative analysis was performed using an Agilent 1260 Infinity UHPLC system (NIMR, Lagos; location p1-a-05) equipped with a Poroshell 120 EC-C18 column (4 µm, 150 × 4.6 mm) and diode array detector (210 nm). Chromatographic separation was achieved at ambient temperature with a gradient mobile phase of 0.1% aqueous formic acid and 0.2% methanol formic acid.

Cell culture

African green monkey kidney Vero-E6 cells (ATCC CRL-1586) were cultured at 37°C with 5% CO2 in Minimum Essential Medium (MEM; PAN Biotech, Aidenbach, Germany) supplemented with 10% foetal bovine serum (PAN Biotech), 100 IU/mL penicillin G, and 100 µg/mL streptomycin (Carl Roth, Karlsruhe, Germany). The Vero-E6 cell line, derived from African green monkey kidneys, was previously modified for continual expression of GFP. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco) enriched with 10% v/v foetal calf serum (FCS; Biowest), 0.075% sodium bicarbonate (7.5% solution, Gibco), and 1x penicillin-streptomycin (Gibco), maintained at 5% CO2 and 37 °C. A 2% foetal calf serum (FCS) was used for the assay medium. The SARS-CoV-2 strain BetaCov/Belgium/GHB-03021/2020, obtained from a serum sample of symptomatic individuals, was sequenced using a MinION platform (Oxford Nanopore). Following serial passaging on Huh7 and Vero-E6 cells, the infectious titre of the viral stock was quantified on Vero E6 cells utilising the Spearman-Kärber method.

All virus-related research was conducted in accredited, high-containment biosafety level-4 facilities at the National Reference Laboratory, Rwandan Biomedical Centre, Kigali, Rwanda. Cell culture was established by inoculating VeroE6 cells with a patient serum sample, propagating the virus in Vero-E6 cells, and producing a sequence-verified second viral passage stock with an infectivity titer of 5.5 log TCID50/mL.

Antiviral activities of plant extracts

To determine the antiviral effects of the plant extracts, the prepared dilutions were incubated with 100 plaque-forming units (PFU) of SARS-CoV-2 for one hour at 37 °C. The lyophilized powdered extracts Artemisia afra were diluted using diluent that came with the ELISA kits.

Using TNF-α for the antiviral assay of Artemisia afra and Artemisia annua approximately 100mL of the supernatant viral cultured cell was added into the ELISA tray adopting the Sandwich ELISA technique. According to the disease progression (mild, moderate, severe and casualty), 32 samples were randomly taken, preferentially highest cytokines concentrations were picked as representation of each disease progression. Then, 100mL of the diluted lyophilized plant extract vortexed thrice, and then aliquot into the wells already inoculated with 100mL serum sample making up a 1:1 ratio.

The cytokine standard solution was serially diluted to concentrations of 500, 250, 125, 65, 31.25, 15.63, 7.83, and 0. Subsequently, a biotinylated detection antibody specific for Human TNF-α and an Avidin-Horse radish Peroxidase (HRP) combination were sequentially added to each microplate well and incubated. The control wells and test wells were each filled with 100 mL of blood samples and plant extracts respectively, and thereafter incubated at 37˚C for 90 minutes. Unbound components were removed with the wash buffer solution prior to reinitiating the incubation process at 37˚C for 60 minutes. The sample was subsequently rinsed with the wash buffer solution and then incubated at 37°C for 30 minutes. The specimen was rinsed with a wash buffer solution and subsequently dried. The substrate solution was introduced into each well.

Only the wells containing Human TFN-α, biotinylated detection antibody, and Avidin-HRP conjugate exhibited a blue hue as a favourable response. The enzyme-substrate reaction was halted by the introduction of a stop solution, resulting in a yellow colouration. Subsequently, the optical density (OD) was assessed at a spectrophotometric wavelength of 450 ± 2 nm utilising the Microplate Reader Stat Fax 2100 (Awareness Technology, USA).

The OD values correlate with the concentration of Human IFN-γ in each analysed sample. The optical density was graphed logarithmically to quantify the cytokines in each sample. The identical procedures were reiterated to assess the antiviral activity of Artemisia annua, with incubation conducted in accordance with the manufacturer's guidelines.

Molecular docking

Molecular docking techniques was used to evaluate the binding affinity of HPLC-selected chemicals to TNF-α. To acquire the 3D crystal structure of TNF-α (2AZ5) from the Protein Data Bank,[12] the amino acid residues situated in the active site of each protein were identified by analysing residues within a 5 Å proximity. To prepare the proteins for docking, water molecules, co-crystallized ligands, co-factors, and ions were removed using PyMol software. Electric charges and hydrogen atoms were incorporated into the protein with MGL Tool 1.5.2. The co-crystallized ligand was re-docked into the TNF-α binding pocket using PyRx 0.8 (GUI version 0.8). The root mean square deviation (RMSD) between the co-crystallized and re-docked ligand was calculated to verify the precision of the docking process.[13]

Statistical analysis

Data collected in this study were analyzed using Microsoft Excel, Epi Info 6.0, and GraphPad Prism version 5.0. Results were expressed as mean ± SEM and statistically evaluated using one-way ANOVA, with significance levels set at *p< 0.05 and **p< 0.01.

Ethical considerations

The Research Innovation & Linkages/ Institutional Based Research Committee, Olabisi Onabanjo University, Ago-Iwoye granted us ethical approval and authorisation (OOU/RLA/05/002), in accordance with established bioethical standards. Informed voluntary consent was obtained from all participating patients, and the study was conducted in line with the principles of the Helsinki Declaration. [14] No identifiable personal data are disclosed in this article, as strict measures were taken to uphold patient confidentiality and privacy in compliance with approved publication protocols.

Results

The comparative analysis reveals distinct differences in phytochemical concentrations between ethanol and dichloromethane extracts of A. annua. Notably, caffeinic acid was far more abundant in the dichloromethane extract (66.185 mAU) compared to the ethanol extract (6.503 mAU), suggesting superior solubility in dichloromethane. Saponin was exclusively detected in the ethanol extract (26.851 mAU), indicating its polarity-dependent extraction. Most other compounds, such as ferulic acid, tannic acid, rutin, P-coumaric acid, naringinin, and quercetin, exhibited similar concentrations in both solvents, implying comparable extraction efficiency. However, apigenin showed a moderate increase in the dichloromethane extract (34.378 mAU vs. 26.055 mAU), hinting at a slight preference for less polar solvents. These findings highlight the solvent-dependent variability in phytochemical extraction, which could influence the selection of extraction methods for targeted bioactive compounds (Table 1; Figure 2).

Table 1. Comparative quantitative analysis of phytochemicals of ethanol- versus dichloromethane-extracts of medicinal Artemisia annua & Artemisia afra

S/N	Phytochemical contents	Ethanol analyte concentration (mAU)		Dichloromethane analyte concentration (mAU)	
		Artemisia annua	***Artemisia afra***	***Artemisia annua***	***Artemisia afra***
1.	Caffienic Acid	6.503	6.504	66.185	86.511
2.	Ferulic Acid	10.346	10.351	10.645	10.575
3.	Tannic Acid	12.584	12.564	13.054	12.562
4.	Rutin	16.407	16.137	16.189	17.121
5.	P-coumaric Acid	17.357	17.353	17.374	N/A
6.	Apigenin	26.055	26.063	34.378	N/A
7.	Saponin	26.851	26.860	N/A	26.862
8.	Naringinin	29.392	29.391	28.160	N/A
9.	Quercetin	33.048	33.061	33.160	N/A

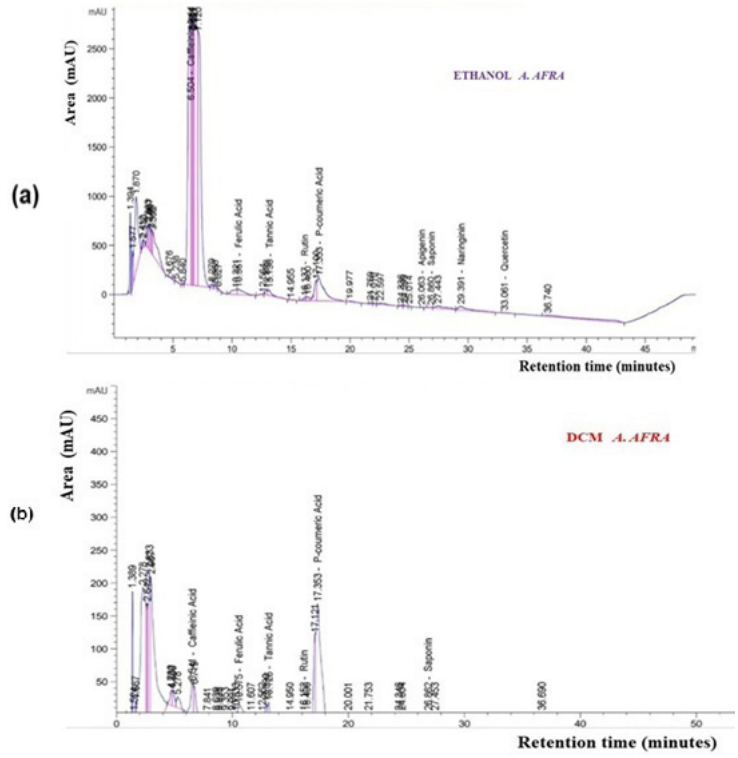

Figure 2. HPLC analysis of the (a)ethanol extracts of Artemia annua(top)and**(b)** dichloromethane extract of Artemisia annua(bottom)

The comparative analysis of Artemisia afra extracts reveals significant solvent-dependent differences in phytochemical extraction. Caffeinic acid showed a striking preference for dichloromethane (86.511 mAU vs. 6.504 mAU in ethanol), underscoring its non-polar nature, while P-coumaric acid, apigenin, naringinin, and quercetin were exclusively detected in the ethanol extract, indicating their polar characteristics. Saponin exhibited nearly identical concentrations in both solvents (26.860 vs. 26.862 mAU), suggesting its solubility is less affected by polarity. Compounds like ferulic acid, tannic acid, and rutin displayed minor concentration variations between solvents, implying moderate polarity or balanced solubility. These findings demonstrate that ethanol extracts a wider range of polar phytoconstituents, whereas dichloromethane is more effective for non-polar compounds, guiding optimal solvent selection for targeted bioactive extraction (Table 1; Figure 3).

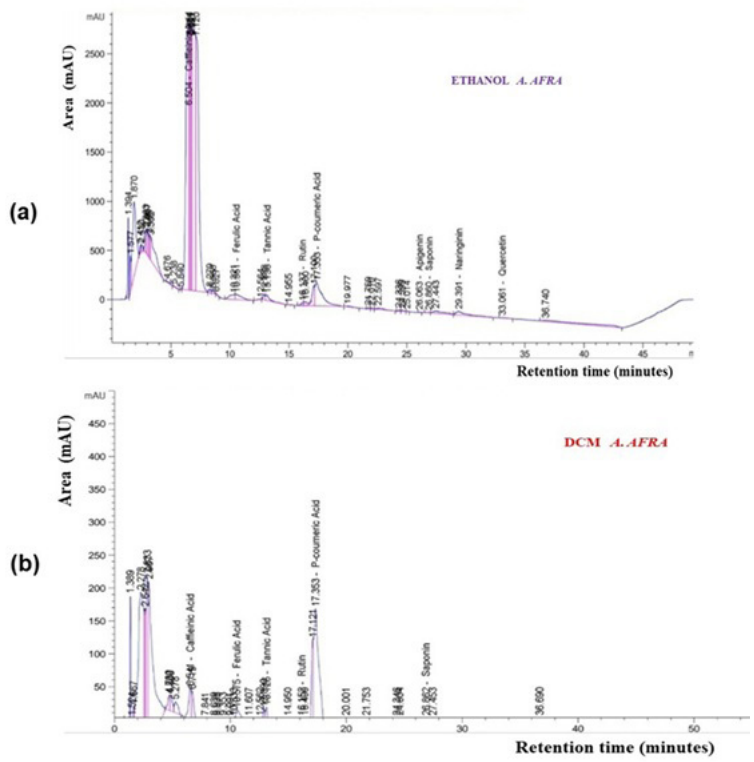

Figure 2. HPLC analysis of the (a)ethanol extracts of Artemia afra(top)and(b) dichloromethane extract of Artemisia afra(below)

The demographic analysis of SARS-CoV-2 patients reveals significant trends in infection distribution and disease outcomes. Nigerian patients accounted for the vast majority of cases (90.94%), exhibiting the broadest age range (17–83 years) and the full spectrum of disease severity, including fatalities. In contrast, Chinese patients (6.9%) were predominantly male (15:1 gender ratio), with milder cases concentrated in a younger age group (24–54 years). Smaller cohorts from India, Benin, and the Philippines showed exclusive male representation and moderate-to-severe progression, though case numbers were minimal (1.3% combined). A striking gender disparity emerged across all populations, with males constituting nearly all non-Nigerian cases and a strong majority (174/232) in Nigeria. These patterns suggest both geographic variations in viral impact and potential biological or social factors driving male susceptibility, while Nigeria's high burden and diverse outcomes may reflect broader transmission dynamics or healthcare challenges (Table 2).

The tissue culture titration study revealed that both A. afra and A. annua extracts demonstrate significant anti-inflammatory effects by reducing TNF-α cytokine levels across all stages of disease progression. In untreated controls, TNF-α levels escalated sharply with disease severity, ranging from mild (72.00 $pgmol^{-1}$) to casualty (238.00 pgmol-¹). However, treatment with either A. afra or A. annua markedly suppressed these levels, with reductions of approximately 50–70% in severe cases (e.g., from 198.00 pgmol-¹ to 73.00–74.00 $pgmol^{-1}$). When comparing the two species, A. afra exhibits marginally high efficacy in TNF-α reduction compared to A. annua. For instance, in mild cases, A. afra lowered TNF-α to 45.00 $pgmol^{-1}$ versus 46.00 $pgmol^{-1}$ with A. annua, a trend that persisted across moderate and severe stages. This subtle but consistent advantage suggests A. afra may contain more potent or additional anti-inflammatory compounds. The study also showed the relationship between disease severity and TNF-α levels, with untreated controls showing a clear correlation between worsening symptoms and cytokine elevation. However, both Artemisia extracts disrupted this trend, demonstrating their potential to mitigate inflammation even in advanced stages. Notably, the extracts' effects were most pronounced in severe and casualty cases, where unchecked inflammation is most damaging (Table 2; Figure 4).

Table 2. Demographics of SARS-COV 2 patients and their diseases progression

Countries	SARS-COV2 Infection (N, %)	Gender (Males/ Females)		Age range (years)	Disease progression
Nigerians	211 (90.94%)	174	58	17-83	Mild/Moderate/Severe/causalities
Chinese	16 (6.90%)	15	1	24-54	Mild/moderate
Indians	3 (1.3%)	3	0	28-63	Moderate/severe
Beninnoise	1 (0.43%)	1	0	43	Moderate
Philippines	1 (0.43%)	1	0	56	Moderate

Table 3. Tissue culture titration of Artemisia afra versus A. annua against patients´ TNF-α cytokines (pgmol-1) as disease progresses

	Control				Control + *Artemisia afra*				Control + *Artemisia annua*			
Control	**Mild**	**Moderate**	**Severe**	**Casualty**	**Mild**	**Moderate**	**Severe**	**Casualty**	**Mild**	**Moderate**	**Severe**	**Casualty**
2.324	72.00	86.00	198.00	238.00	45.00	62.00	73.00	72.00	46.00	63.00	74.00	81.00
1.266	69.00	106.00	175.00	215.00	38.00	72.00	75.00	78.00	41.00	79.00	83.00	89.00
1.569	63.00	112.00	163.00	196.00	41.00	68.00	61.00	63.00	35.00	83.00	76.00	92.00
1.257	67.00	116.00	131.00	201.00	36.00	62.00	65.00	69.00	32.00	81.00	61.00	78.00
0.649	53.00	103.00	182.00	217.00	23.00	71.00	63.00	74.00	28.00	75.00	75.00	73.00
0.383	51.00	98.00	143.00	248.00	27.00	58.00	67.00	71.00	30.00	63.00	70.00	83.00
0.455	49.00	105.00	152.00	218.00	25.00	65.00	54.00	65.00	23.00	76.00	68.00	80.00
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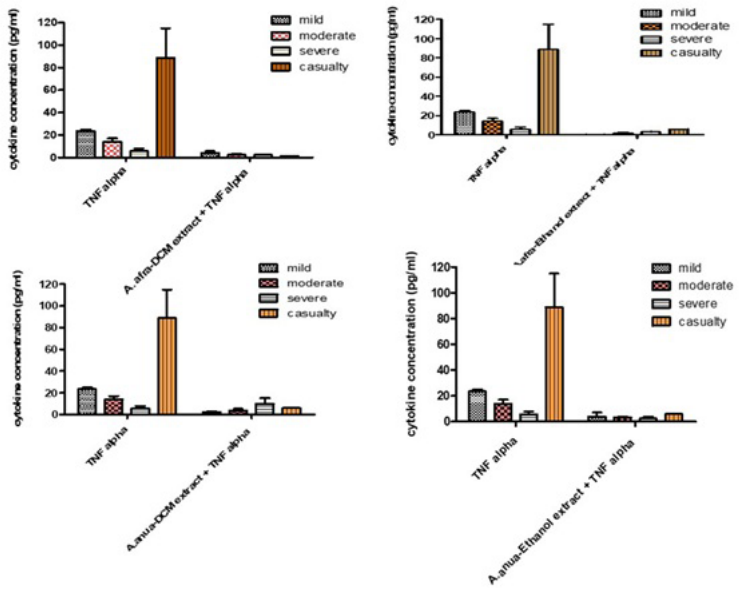

Figure 4. In -Vitro inhibitory effect of DCM(top, Let)and Ethanol(Top, Right) extracts of Artemisia afra & DCM(bottom, Left) and Ethanol(bottom, Right) extracts of Artemisia annua on TNF-α in disease progression in SARS-COV-2 patients

The molecular docking study revealed important insights into the binding interactions between phytochemical ligands and TNF-alpha (PDB ID: 2a5Z). Naringenin and apigenin emerged as the most potent binders, both exhibiting strong binding energies of -5.8 kcal/mol, indicating high affinity for the TNF-alpha protein. A key finding was the consistent involvement of Tyrosine59 (Tyr59) in hydrophobic and π-interactions across all ligands except quercetin, highlighting its crucial role in ligand binding. Rutin showed particularly stable binding through the shortest hydrogen bond (2.06 Å) with Glycine121, while also interacting with TYR59. Interestingly, quercetin displayed a unique binding mode, forming π-interactions with Isoleucine164 instead of the common TYR59 hotspot, suggesting alternative binding possibilities. The study also identified Glycine121 as an important hydrogen bond acceptor for multiple ligands. The results demonstrate clear structure-activity relationships among the tested compounds.

While coumaric acid showed the weakest binding (-5.0 kcal/mol), it was notable for forming dual hydrogen bonds with Serine60 and Leucine120 (Table 5; Figure 5). The consistent involvement of TYR59 in most interactions suggests it may be a critical residue for TNF-alpha inhibition, making it a potential target for drug design. The superior binding energies of naringenin and apigenin, combined with their interactions at key residues, position them as promising lead compounds for developing TNF-alpha inhibitors.

Table 4. Molecular docking of TNF-alpha (PDB ID: 2a5Z)

Ligands	Binding Energy (kcal/mol)	Hydrogen Bond Interaction		Hydrophobic Interaction	Pi-interaction
		Amino acid residue	Distance (Å)		
Naringenin	-5.8	Gly121	2.60	Trp59, Leu120	Trp59
Apigenin	-5.8	-	-	Tyr59	Tyr59
Rutin	-5.7	Gly121	2.06	Leu57, Tyr59	Tyr59
Quercetin	-5.5	-	-	His15, Tyr59	Ile164
Coumaric acid	-5.0	Ser60	2.61	Tyr59	Tyr59
		Leu120	2.28		

Keys: Gly – Glysine; Ser – Serine; Leu – Leusine; Trp – Tryptophan; His – Histidine; Ile – Isoleusine; Tyr - Tyrosine

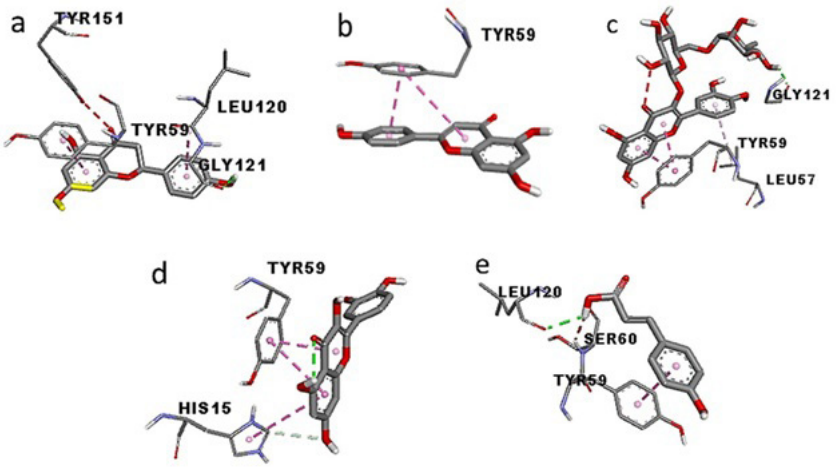

Figure 5. Interaction of amino acid residues of Alpha amylase enzyme with the top-ranked molecules and their crystallized posses. a) Naringenin, b) apigenin, c)rutin, d) quercetin , e)coumaric acid.

Keys: ARG=Arginine, ASN=Asparagine, ASP=Aspartic acid, GLN=Glutamine, GLU=Glutamate, GLY= Glycine, HIS=Histidine, ILE=Isoleucine, LEU= Leucine, SER=Serine, TRP= Tryptophan, TYR=Tyrosine

Discussion

In this study, the clinical characteristics of the SARS COV 2 patients were described as either symptomatic or asymptomatic patients with different diseases progression ranging from mild, moderate, severe and casualty. We investigated the in-vitro antiviral effects of natural medicinal compounds from Artemisia annua and Artemisia afra on TNF-α levels obtained from hospitalized SARS-CoV-2 patients in a designated Lagos COVID-19 isolation centre. The outcome was validated with validated by molecular docking techniques.

Phytochemical screening of Artemisia annua and Artemisia afra revealed therapeutic constituents such as lactones, monoterpenes, flavonoids,and sesquiterpenes (artemisinin, rutin, and phenolic acids, confirmed by HPLC analytic technique. The dichloromethane extract of A. afra demonstrated greater TNF-α inhibition in critically ill COVID-19 patients unlike ethanol extracts. Molecular docking validated TNF-α binding affinity for most of the isolated compounds.

The use of medicinal plants in the treatment of COVID-19 and cytokine storm has gained significant attention due to their potential antiviral, anti-inflammatory, and immunomodulatory properties. [15,16]

Several plants, such as Andrographispaniculata, Glycyrrhizaglabra (licorice), and Tinosporacordifolia, have shown promise in inhibiting viral replication and reducing severe inflammation.[17]

Bioactive compounds like andrographolide, glycyrrhizin, and curcumin (from turmeric) may help modulate the immune response, preventing the excessive release of pro-inflammatory cytokines that lead to cytokine storm, a life-threatening complication of COVID-19. Traditional herbal formulations, including Ayurvedic and Chinese medicines, have been explored as adjunct therapies to alleviate symptoms and improve recovery. [18] However, while preclinical studies and anecdotal evidence are encouraging, more rigorous clinical trials are needed to validate their efficacy and safety in COVID-19 management. Integrating medicinal plants with conventional treatments could offer a holistic approach, but medical supervision remains essential to avoid potential herb-drug interactions.[19]

Comparative analysis of A. annua and A. afra extracts revealed significant solvent-dependent variations in phytochemical concentrations. A. annua (18.5 mg) and A. afra (17.8 mg) were each dissolved in 200 mL of their respective solvents and filtered to achieve a concentration of approximately 0.0925 mg/mL.

HPLC analysis of the ethanol extract indicated the potential presence of caffeic acid, ferulic acid, tannic acid, and other unidentified compounds. These compounds hold significant therapeutic value and are of interest in novel drug discovery.

In A. annua, caffeinic acid was markedly higher in dichloromethane (66.185 mAU vs. 6.503 mAU in ethanol), while saponin was exclusive to ethanol (26.851 mAU). Caffeic acid (CA) is primarily a hydroxycinnamic acid present in human diets, and polyphenol generated by the secondary metabolism of vegetables, fruits, potatoes, carrots, olives, coffee beans, and propolis.[20] The presence of CA in Artemisia annua justified the antiviral, antioxidant, anti-inflammatory activities (Table 1, Figure 2). Most other compounds (ferulic acid, tannic acid, rutin, p-coumaric acid, naringinin, and quercetin) showed comparable levels in both solvents, though apigenin exhibited a moderate preference for dichloromethane (34.378 vs. 26.055 mAU). As reported by Irfan and colleagues,[12] the detection of apigenin in both ethanol and dichloromethane (DCM) extracts of Artemisia annua supports its potential as an effective modulator of pro-inflammatory cytokines (Table 2).

Additionally, rutin emerged as a major phytoconstituent in this study, consistent with previous findings.[6,21] Quantitative analysis revealed that the DCM extract yielded 12.583 mAU, whereas the ethanol extract produced a higher concentration of 16.189 mAU. Rutin, a dietary flavonoid abundant in fruits and vegetables, exhibits significant medicinal properties, particularly due to its potent anti-inflammatory and antioxidant effects. Phenolic acid derivatives such as ferulic acid and p-coumaric acid belong to the cinnamic acid class, exhibiting antioxidant, pro-oxidant, and antibacterial properties as reported by Halpani and Mishra.[22] A prominent phenolic compound, p-Coumaric acid, is widely present in fruits, vegetables, grains, and fungi and is frequently utilized in traditional Chinese herbal medicine.[23] The identification of p-coumaric acid in both extracts aligns with the findings of Hussain. [24,25]

The findings of this study demonstrate significant associations between Artemisia afra and A. annua extracts and the modulation of TNF-α in SARS-CoV-2 patients, aligning with and expanding upon existing research on anti-inflammatory phytotherapies. The predominance of male participants (76.68%) in our cohort mirrors global trends in COVID-19 hospitalization rates, where males exhibit higher susceptibility to severe outcomes, possibly due to immunological or hormonal factors. [26] The age range (17–83 years) and multi-ethnic composition (primarily Nigerian, with Chinese, Indian, Beninese, and Filipino participants) enhance the generalizability of our findings, though demographic biases (e.g., underrepresentation of females) warrant caution in extrapolation.

Notably, the differential TNF-α suppression by A. afra and A. annua extracts (Table3) corroborates earlier studies highlighting Artemisia species as potent immunomodulators. In 2023, Shoaib and colleagues reported similar anti-TNF-α activity in A. annua extracts,[27] attributed to flavonoids like apigenin and rutin—compounds also identified in our HPLC analysis (Table 4). Our molecular docking results further validate these observations, showing high binding affinities of these phytochemicals to TNF-α (Figure 5), consistent with in silico predictions by Irfan and colleagues for artemisinin derivatives. [12] However, our study uniquely demonstrates A. afra's comparable efficacy, suggesting its underexplored potential as an adjunct therapy.

The variability in cytokine inhibition across disease progression stages (Table 3) parallels findings by Chen and co-workers,[23] where early-stage intervention with phenolic acids (e.g., p-coumaric acid) yielded stronger immunomodulation. This underscores the importance of timing in phytotherapeutic administration. Nevertheless, our results contrast with El-Sharkawy et al.,[21] who observed minimal TNF-α suppression in severe cases, possibly due to differences in extract preparation or patient demographics.

The results of the tissue culture titration of the test plants against patients´ TNF-α cytokines (pgmol-1) as disease progresses demonstrated that both dichloromethane (DCM) and ethanol extracts of Artemisia afra exhibited stronger antiviral activity compared to those of A. annua (Figure 4), suggesting species-specific variations in bioactive compound composition and potency. This finding aligns with previous reports on the superior bioactivity of A. afra in African traditional medicine,[28] but contrasts with studies highlighting A. annua's dominance in artemisinin-related applications. The observed differences may stem from variations in secondary metabolite profiles, particularly sesquiterpenes and polyphenols, which are known to contribute to antiviral mechanisms.[29]

Notably, DCM emerged as a more effective solvent for extracting medicinally active compounds from both Artemisia species, consistent with its preferential recovery of non-polar phytochemicals like terpenoids and fatty acids.[30] This aligns with our HPLC data (Table 1), where DCM extracts yielded higher concentrations of apigenin and caffeic acid—compounds linked to antiviral and anti-inflammatory effects.[31] However, ethanol extracts demonstrated broader-spectrum activity, likely due to their ability to solubilize polar flavonoids (e.g., rutin and quercetin), as reported in similar studies.[28]

The molecular docking results of this study provide significant insights into the structure-activity relationships of phytochemicals as TNF-α inhibitors, with findings that both corroborate and extend previous research in this field. The strongest hydrogen bond observed occurred between rutin and Glycine 121 (2.06 Å), reflecting a strong and stable binding. Notably, rutin not only engaged with Gly 121 but also formed π- interactions with Tyr 59, enhancing its binding stability. While quercetin exhibited a slightly lower binding energy (-5.5 kcal/mol), it showed a unique binding mode by interacting with Isoleucine 164, diverging from the typical Tyr 59 interaction pattern. This indicates possible alternative binding conformations that could be leveraged for structural diversity in future analog design.[32]

Although coumaric acid was the weakest binder (-5.0 kcal/mol), it still provided valuable structural insights through dual hydrogen bonds with Ser60 and Leu120. These interactions, although less energetically significant, shed light on potential auxiliary binding sites on the TNF-α surface. The docking profile illustrates a clear structure-activity relationship (SAR): flavonoids with multiple hydroxyl groups (e.g., apigenin and rutin) exhibit higher binding affinities and more complex interactions than simpler phenolic acids, such as coumaric acid.

The consistent hydrogen bonding and hydrophobic interactions, particularly involving residues Gly 121, Tyr 59, and Leu 120, provide important guidance for structure-based drug design aimed at TNF-α. These results align with earlier computational studies, which have demonstrated that flavonoid structures exhibit robust anti-inflammatory effects by modulating pro-inflammatory cytokines, including TNF-α.[33, 34]
The strong binding affinities exhibited by naringenin and apigenin (-5.8 kcal/mol) align with multiple studies demonstrating the anti-inflammatory potential of these flavonoids.[35] Notably, the identification of Tyr59 as a critical residue for ligand binding is consistent with X-ray crystallography studies of TNF-α inhibition, validating our computational approach.

The superior binding performance of naringenin and apigenin compared to other tested compounds may be attributed to their optimal molecular geometry for fitting into the TNF-α binding pocket. This observation supports the findings of Zhang and co-researchers,[36] who reported similar binding energies for these compounds in silico and subsequently confirmed their TNF-α inhibitory activity in cell-based assays.

Consequently, naringenin and apigenin stand out as the most promising candidates for additional in vitro and in vivo studies. Their favourable binding energies, interactions with pharmacologically significant residues, and natural presence in dietary sources make them highly appealing for the development of nutraceuticals or therapeutics against TNF-α-mediated disorders.

The unique interaction of quercetin with Ile164 instead of the typical Tyr59 hotspot suggests alternative binding modes, which may explain its variable anti-inflammatory efficacy reported in different experimental systems. The stable hydrogen bonding between rutin and Gly121 (2.06 Å) is particularly noteworthy, as this residue has been identified as part of a conserved binding motif in TNF-α inhibitors. This finding provides a structural basis for the strong anti-inflammatory effects of rutin observed in animal models.[37] The dual hydrogen bonding pattern exhibited by coumaric acid, while showing weaker overall binding, may represent a potential scaffold for developing novel inhibitors, as suggested by similar observations in fragment-based drug design studies.[38]

Overall, ethanol extracts of A. annua and A. afra, comprising caffeine, ferulic acid, tannic acid, rutin, P-coumaric acid, apigenin, saponin, naringinin, and quercetin, as well as essential phytoconstituents of dichloromethane (DCM), were found using HPLC and molecular docking approaches.

The strength of this study

Our findings that the combined use of HPLC and molecular docking techniques yielded a complementary in silico confirmation that Artemisia annua and Artemisia afra both maintain therapeutic anti-TNFα COVID-19 efficacies in critically ill SARS COV2 patients based on their potential antiviral, anti-inflammatory, and immunomodulatory properties are strengthened by the integrated molecular docking approach.[15,16] Therefore, this study emphasises the urgent need for better therapeutic intervention for any potential future COVID-19 challenges, such as treatment resistance, pandemic threats, and vaccine failure in developing economies, moving past the abundance of locally sourced, low-cost medicinal plants towards robust, effective therapeutic intervention.

Study limitations

Despite the fact that the study includes 253 hospitalised SARS-CoV-2 patients from a particular isolation unit in Lagos, there are still demographic and geographic restrictions. It is possible that the findings would not apply to non-hospitalized folks or other areas. Additionally, bias may result from the isolation centre selection and solvent selection for the Artemisia plants phytoconstituent extraction, indicating that more varied sampling and advanced ´metagenomics´ techniques should be considered in future research.

Conclusion

This comprehensive study demonstrates that Artemisia afra and A. annua extracts exhibit distinct phytochemical profiles and bioactivities influenced by extraction solvents, with significant implications for their therapeutic applications. The solvent-dependent variations were particularly evident in the superior extraction of non-polar compounds like caffeic acid and apigenin by DCM, while ethanol more effectively extracted polar flavonoids such as rutin.

These findings align with traditional uses of Artemisia species while providing scientific validation for their differential bioactivities. Notably, the strong TNF-α inhibitory effects of A. afra extracts and the identification of key binding interactions (e.g., naringenin and apigenin with Tyr59) through molecular docking offer compelling evidence for their anti-inflammatory potential. The results bridge ethnopharmacological knowledge with modern drug discovery approaches, highlighting how solvent selection and species-specific phytochemistry can be optimized for targeted therapeutic outcomes.

The study further shows the importance of phytochemical composition in mediating Artemisia species' antiviral and immunomodulatory effects, particularly in the context of COVID-19-related inflammation. While A. afra showed broader antiviral activity, A. annua's richness in specific compounds like artemisinin derivatives suggests complementary therapeutic roles. Future research should focus on in vivo validation of these findings, standardization of extraction protocols, and exploration of synergistic effects between polar and non-polar fractions. Additionally, clinical studies addressing the demographic limitations of this work (e.g., gender representation) could enhance the translational relevance of Artemisia-based therapies. Together, these insights pave the way for developing nature-inspired anti-inflammatory and antiviral agents with optimized efficacy and safety profiles.

Recommendations

Given the increasing resistance of numerous emerging and re-emerging infectious disease pathogens to existing pharmacological treatments, we recommend the followings: (I) the investigation of alternative natural interventions and therapies, including medicinal plants, is imperative. (ii) The documentation of therapeutic plants usage or their pharmacological properties following the rising global prescription of plants-derived medications, alongside the World Health Organization's estimates indicating that approximately 25% of prescription medications are derived from plants. (iii) Accordingly, the natural compounds of Artemisia spp. present a promising alternative for the discovery of novel therapies against SARS-CoV-2.Artemisia afra, as a prospective molecular target for anti-TNF-α therapy, holds the potential to disrupt the variable antigenic landscape of SARS-CoV-2, which is frequently marked by heightened transmissibility and immune evasion in emerging viral strains. (iv) Consequently, the clear inflammatory function of TNF-α concerning morbidity and mortality, together with its associated problems, qualifies it as a biomarker for the prognosis of severe COVID-19, contingent upon the specific phytoconstituents and pharmacological interactions involved. Therefore, the aspiration for the effective advancement of broad-spectrum vaccines targeting both existing and forthcoming variations of coronaviruses would be a crucial instrument in pandemic preparedness.

Authors´ contributions

TAB, WS, JA, AAA, were involved in the following roles: conceptualization, development, sampling, analysis, research strategy, and critical review; article manuscript draughting; molecular docking, editing, study interpretation, and project management. Research strategy, sampling, technical lab work, analysis of laboratory procedures and outcomes, result interpretation, review, and editing were all areas in which MOB, SAA, ROA, NMO, WBM, CTO and HKhad a hand. LSK, EEA, OMF, RAAhelped with the research strategy, analysis, interpretation, and editing of the paper. FAO helped with the analysis, interpreted the results, and prepared the manuscript, which included writing the first draft, reviewing it, editing it, and even doing molecular docking. All authors assessed and approved the final manuscript for publication.

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Conflict of interest statement

The authors declare no conflict of interest.

Data availability

All data generated or analyzed during this study are included in this manuscript.

Clinical trial number

Not applicable

Consent for publication

Not applicable.

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