SARS-CoV-2 omicron variants succumb in vitro to Artemisia annua hot water extracts

Nair*, M.S., Huang*, Y., Weathersb*, P.J.

*aAaron Diamond AIDS Research Center, Columbia University Irving Medical Center, New York, NY, USA.
bDepartment of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609, USA.

* Corresponding author:
Pamela Weathers
Department of Biology and Biotechnology
Worcester Polytechnic Institute
100 Institute Rd
Worcester, MA 01609 USA
Email: weathers@wpi.edu
Phone: 508-831-5196
FAX: 508-831-6362

Author email addresses:
Manoj Nair, mn2947@cumc.columbia.edu;
Yaoxing Huang, yh3253@cumc.columbia.edu
Pamela Weathers, weathers@wpi.edu

Abstract:
The SARS-CoV-2 (COVID-19) global pandemic continues to infect and kill millions while rapidly evolving new variants that are more transmissible and evading vaccine-elicited antibodies. Artemisia annua L. extracts have shown potency against all previously tested variants. Here we further queried extract efficacy against omicron and its recent subvariants. Using Vero E6 cells, we measured the in vitro efficacy (IC50) of stored (frozen) dried-leaf hot-water A. annua L. extracts of four cultivars (A3, BUR, MED, and SAM) against SARS-CoV-2 variants: original WA1 (WT), BA.1.1.529+R346K (omicron), BA.2, BA.2.12.1, and BA.4. IC50 values normalized to the extract artemisinin (ART) content ranged from 0.5-16.5 µM ART. When normalized to dry mass of the extracted A. annua leaves, values ranged from 20-106 µg. Although IC50 values for these new variants are slightly higher than those reported for previously tested variants, they were within limits of assay variation. There was no measurable loss of cell viability at leaf dry weights ≤50 µg of any cultivar extract. Results continue to indicate that oral consumption of A. annua hot-water extracts (tea infusions) could potentially provide a cost-effective approach to help stave off this pandemic virus and its rapidly evolving variants.

Key Words:
Artemisia annua; tea infusions; Omicron; COVID-19; SARS-CoV-2; WA1; BA.1.1.529+R346K; BA.2; BA.2.12.1; BA.4

1. Introduction:
The novel coronavirus, SARS-CoV-2, with its rapidly evolving variants continues to plague the global population with nearly 600 million cases and >6 million deaths (https://coronavirus.jhu.edu/map.html, accessed June 26, 2022). The past year the omicron (B.1.1.529) variant of concern (VOC) emerged along with a number of subvariants, especially BA.4 and BA.5 [1]. These are highly transmissible (Omicron RO ≥ 10, Delta RO = 7 [2]) infecting even vaccinated individuals, albeit with less severe outcomes (https://www.healthdata.org/covid/COVID-19-vaccine-efficacy-summary, accessed June 26, 2022).
Omicron (B.1.1.529) and its B.1.1.529+R346K isolate have shown resistance to neutralization by antibodies in patients who have had COVID-19 or been vaccinated and even boosted with one of the widely used vaccines [3-5]. Recently, variants, BA.2.12.1 and BA.4/5 were shown to be 1.8 and 4.2 times, respectively, more resistant to sera from individuals who were vaccinated and boosted [6]. Additionally, recent clinical case studies showed that vaccinated and boosted individuals who took a course of Paxlovid™ have shown relapse and post relapse can accidently infect family members [7]. This presents an even more pressing need for an expanded diversification of therapeutics, which may also serve as prophylactics in a population setting.

Although a number of different drugs have been trialed [8] there are few approved small molecule drugs available to treat COVID-19. The antiviral drug Paxlovid™ was recently approved as a per os combination drug of nirmatrelvir (or PF-07321332) with ritonavir, developed by Pfizer with good anti-SARS-CoV-2 efficacy and relatively few adverse drug reactions [9,10]. Nirmatrelvir acts as a main viral protease inhibitor [9] with ritonavir enhancing its pharmacokinetics by inhibiting hepatic CYP3A4 [11]. Despite this success, access may be limited (https://www.nature.com/articles/d41586-022-00919-5, accessed June 26, 2022). While generic production is estimated at ≤25 USD, it also may be unaffordable to many, especially in low and middle income countries (https://www.reuters.com/business/healthcare-pharmaceuticals/generic-drugmakers-sell-pfizers-paxlovid-25-or-less-low-income-countries-2022-05-12/, accessed June 26, 2022). Thus, there remains a need for more cost-effective therapeutics to treat the global population.

Previously we and others showed that extracts of dried leaves of many cultivars of the medicinal plants, Artemisia annua L., which produces the antimalarial sesquiterpene lactone artemisinin (ART; Figure 1), and A. afra Jacq. ex Willd., a related perennial species lacking artemisinin, prevented SARS-CoV-2 replication in vitro [12-15]. Although ART has some anti-SARS-CoV-2 activity, we showed that antiviral efficacy was inversely correlated to ART content [12]. Here we report in vitro efficacy for four of the seven originally studied A. annua L. cultivars against omicron (B.1.1.529) and three of its subvariants: BA.2, BA.2.12.1, and BA.4.

![Figure 1. Artemisia annua L. and artemisinin.](image)

2. Results and Discussion:
Hot-water extracts of four cultivars of A. annua inhibited the recently evolved omicron and its three tested subvariants of SARS-CoV-2 with IC50 values calculated and normalized to the ART content of each
tested tea infusion ranging from 0.5-16.5 µM ART (Figure 2; the lower the IC50, the more potent the drug/extract). When the IC50 values were instead normalized to the dry mass of the extracted A. annua

![Graphs showing inhibition percentages for different cultivars of A. annua](image-url)

| CV | WA1 | BA.1.1.529 + R346K | BA.2 | BA.2.12.1 | BA.4 |
|----|-----|-------------------|------|-----------|------|
| SAM| 4.9 | 8.2               | 7.6  | 5.3       | 16.5 |
| A3 | 1.2 | 1.8               | 1.0  | 1.5       | 2.6  |
| MED| 4.2 | 4.7               | 2.1  | 3.9       | 6.4  |
| BUR| 0.7 | 1.2               | 0.6  | 0.5       | 1.1  |

**Figure 2.** SARS-CoV-2 variant inhibition by four cultivars of A. annua L. hot water extracts normalized to their artemisinin content and compared to WT. WT, USA/WA1; variants: BA.1.1.529+R346K, omicron; omicron subvariants: BA.2; BA.2.12.1, and BA.4 at a multiplicity of infection (MOI) of 0.1 in Vero E6 cells. Data are plotted from an average of three replicates from each of two experiments ± SE.
leaves, values ranged from 20-106 µg (Figure 3). Although the values for these new variants are for the most part slightly higher than the IC₅₀ values reported for variants previously reported [12,13] and all are summarized in Table 1, they fell within limits of assay variation. As already reported for extracts used in this study, there was no measurable loss of cell viability at a dry weight of ≤50 µg for any cultivar extract [12]. Others have reported in vitro efficacy of A. annua [14,15] and A. afra [14] extracts against earlier variants of SARS-CoV-2; however, to our knowledge there are no reports showing efficacy against omicron or its variants.

Although ART IC₅₀ values are shown in this study, we previously reported that potency was inversely related to ART concentration [12] and others showed that A. afra, a species lacking ART, was also highly effective in vitro against SARS-CoV-2 [14]. Nevertheless, ART has some anti-SARS-CoV-2 activity as we and others showed [12,14-19]. Although there have been some clinical studies using ART, it was used as a combination therapy. For example, in a small non-randomized controlled trial where patients were treated with ART-piperaquine (ART-PPQ) or placebo the mean time for recovery where there was no longer PCR-detectable virus was 10.6 d for ART-PPQ treated patients vs. 19.3 d for those receiving placebo [20]. All patients treated with ART-PPQ were virus-free after 21 d compared to 36 d for placebo. In another small trial patients had faster recovery vs. placebo in those who used ArtemiC, an oral spray containing ART, curcumin, frankincense, and vitamin C [21]. To our knowledge, however, there are no reports of clinical trials using A. annua or its extracts.

Because we and others [12,14] showed that ART is not the most likely anti-SARS-CoV-2 therapeutic phytochemical in A. annua extracts, questions remain regarding the identity of these non-ART phytochemicals. To resolve that question, several groups have used in silico approaches [22,23]. Tang et al. screened the Traditional Chinese Medicines for systems Pharmacology Databased and Analysis Platform to identify all phytochemicals reportedly in A. annua then ranked them according to oral bioavailability and drug likeness (OB and DL, respectively). That list was narrowed to 19 compounds within their OB and DL limits of ≥30% and 0.18, respectively. They concluded that many on the list of 19 compounds had anti-inflammatory, immune regulatory, and therapeutic properties. Among the top therapeutic candidates were luteolin and isorhamnetin. Using a ZINC library the Efferth lab also screened an in silico library of >39,000 natural product compounds including some from plants with known antiviral activity and narrowed their hits to 33 likely compounds [22]. Of the top 12, three, isorhamnetin, luteolin, and rosmarinic acid, are present in A. annua and when tested in vitro had IC₅₀ values of 8.42, 11.81, and 9.43 µM, respectively, against the main protease in SARS-CoV-2, 3CLₚₜ, a chymotrypsin-like protease involved in viral replication. Along with reports of anti-SARS-CoV-2 activity of other A. annua phytochemicals, e.g., quercetin and myricetin against NTPase/helicase [24,25], many other small molecules, especially flavonoids, are showing antiviral potential and likely work in combination (synergistically) in these extracts to achieve the therapeutic response. Future studies are needed to identify and validate the activity of these anti-SARS-CoV-2 therapeutic compounds.
Figure 3. SARS-CoV-2 variant inhibition by four cultivars of *A. annua* L. hot water extracts normalized to their *A. annua* leaf dry mass (DW) and compared to WT. WT, USA/WA1; variants: BA.1.1.529+R346K, omicron; omicron subvariants: BA.2, BA.2.12.1, and BA.4 at a multiplicity of infection (MOI) of 0.1 in Vero E6 cells. Data are plotted from an average of three replicates from each of two experiments ± SE.
Table 1. Comparative IC$_{50}$ values of *A. annua* L. hot-water extracts (10 g/L) against all tested strains of SARS-CoV-2 based on either artemisinin content or leaf dry weight (DW).

| Cultivar | Potency normalized to artemisinin content | IC$_{50}$ µM artemisinin | Potency normalized to dry mass of leaves used in tea infusion | IC$_{50}$ µg leaf DW |
|----------|------------------------------------------|---------------------------|-------------------------------------------------------------|---------------------|
| | WA1* (WT) | B.1.1.7* (alpha) | B.1.351* (beta) | P.1** (gamma) | B.1.617.1** (kappa) | B.1.617.2** (delta) | WA1 (WT) | BA.1.1.529+R346K (omicron) | BA.2 | BA.2.12.1 | BA.4 |
| SAM | 3.4 | 4.9 | 8.4 | 7.9 | 7.0 | 7.0 | 4.9 | 8.2 | 7.6 | 5.3 | 16.5 |
| A3 | 0.8 | 1.1 | 2.0 | 1.9 | 1.9 | 2.1 | 1.2 | 1.8 | 1.0 | 1.5 | 2.6 |
| BUR | 0.4 | 0.3 | 0.8 | 1.2 | 1.1 | 1.2 | 0.7 | 1.2 | 0.6 | 0.5 | 1.1 |
| MED | 2.9 | 2.0 | 3.6 | 2.9 | 2.5 | 4.8 | 4.2 | 4.7 | 2.1 | 3.9 | 6.4 |
| SAM | 21.5 | 31.3 | 53.7 | 50.7 | 45.0 | 45.1 | 31.4 | 52.5 | 48.9 | 34.1 | 106.0 |
| A3 | 15.7 | 22.1 | 39.6 | 38.2 | 37.0 | 42.4 | 23.9 | 36.7 | 20.0 | 30.7 | 50.9 |
| BUR | 15.1 | 11.0 | 32.5 | 50.1 | 44.7 | 49.8 | 27.5 | 48.9 | 23.6 | 22.7 | 45.2 |
| MED | 41.7 | 28.2 | 51.5 | 41.0 | 37.0 | 67.7 | 59.7 | 67.0 | 30.0 | 56.0 | 90.6 |

* Values taken from Nair et al. [12]; ** values taken from Nair et al. [13].
IC$_{50}$s are values where virus is 50% inhibited. Data are an average of three replicates.
3. Materials and methods

3.1 Plant material, extract preparations, and artemisinin analyses:
Hot-water extracts (tea infusions) were previously prepared from dried leaves of *Artemisia annua* L. (SAM, MASS 00317314; BUR, LG0019527; A3, Anamed; MED, KL/015/6407) In brief: 10 g dried leaves/L were boiled in water for 10 min, solids removed via sieving, then 0.22 µm filter-sterilized and stored at -80°C for this study and as detailed in Nair et al. [12]. ART analyses of tea infusions were by gas chromatography-mass spectrometry and detailed in Martini et al. [26] with ART contents detailed in [12]: ART in µg/mL were: 42.5 for A3; 20.1 for BUR; 59.4 for MED; and 149.4 for SAM.

3.2 Viral culture and infection
Cultivation of Vero E6 cells (ATCC CRL-1586) and viral infection are detailed in [12]. SARS-CoV-2 isolates (Table 2) were sourced from BEI Resources ([www.beiresources.org](http://www.beiresources.org)). To determine their tissue culture infectious dose (TCID), viruses were titrated after propagation in Vero E6 cells, aliquoted, and frozen at -80°C until later use. Multiplicity of infection (MOI) was 0.1 as used in other studies [27].

Table 2. SARS-CoV-2 isolates used in this study.

| SARS-CoV-2 isolate | BEI Resource Catalogue number |
|--------------------|-------------------------------|
| USA/WA12020        | NR-52281 SARS-Related Coronavirus 2, Isolate USA-WA1/2020 |
| Omicron B.1.1.529 + 346K | NR-56475 SARS-Related Coronavirus 2, Isolate hCoV-19/USA/HI-CDC-4359259-001/2021 |
| Omicron BA.2       | NR-56520 SARS-Related Coronavirus 2, Isolate hCoV-19/USA/CO-CDPHE-2102544747/2021 |
| Omicron BA.2.12.1  | NR-56781 SARS-Related Coronavirus 2, Isolate hCoV-19/USA/NY-MSHSPSP-PV56475/2022 |
| Omicron BA.4       | NR-56806 SARS-Related Coronavirus-2, Isolate hCoV-19/USA/MD-HP30386/2022 |

3.3 Drug inhibition assays of SARS-CoV-2 and cell viability
Dilutions of extracts were incubated for 1 h in 96-well tissue culture plates having a monolayer of Vero E6 cells seeded the prior day at 20,000 cells/well. SARS-CoV-2 virus was added to each well one h after extract addition to a final MOI of 0.1. Cells were cultured for 3 days in 5% CO₂ at 37°C and then scored for cytopathic effects as previously detailed [27] and values converted into percent of control. Drug concentrations were log transformed and the concentration of drug(s) that inhibited virus by 50% (*i.e.*, IC₅₀), and the concentration of drug(s) that killed 50% of cells (*i.e.*, CC₅₀; viability), were log transformed and determined via nonlinear logistic regressions of log(inhibitor) versus response-variable dose-response functions (four parameters) constrained to a zero-bottom asymptote by statistical analysis. We already reported viability of Vero E6 cells post extract treatment in Nair et al. [12] for the same extracts. To normalize the IC₅₀ values for the new variants tested or the WT and variants tested previously, dry mass of leaves and total ART contents measured in the *Artemisia* extracts were used as reported in Nair et al. [12].

3.4 Chemicals and reagents
Reagents were procured from Sigma-Aldrich (St. Louis, MO). Renilla-Glo was from Promega (E2720). EMEM (Cat # 30-2003) and XTT reagent (Cat # 30-1011k) were from ATCC.

3.5 Statistical analyses
The anti-SARS-CoV-2 analyses were done at least in triplicate. Plant hot water extracts had n≥6 independent assays as documented in Nair et al. [12]. IC₅₀ values were calculated using GraphPad Prism V9.3.

4. Conclusions
Hot-water (tea infusion) extracts of *A. annua* continue to show activity against SARS-CoV-2 and the newest VOCs including omicron and three of its highly transmissible subvariants. Although the specific phytochemicals have not yet been identified, there are a number of possible candidates emerging in the literature. Validation of *A. annua* extracts against SARS-CoV-2 VOCs in a rodent model are needed as a next step towards human trials. Nevertheless, this plant is safe to use, and we urge testing in clinical trials sooner rather than later. WHO announced in 2021 that through its COVID-19 Solidarity Therapeutics Plus Trial that it has included intravenous artesunate as one of three repurposed drugs to treat COVID-19 [28]. Results are not anticipated until 2023, (last accessed July 11, 2022, https://www.isrctn.com/ISRCTN18066414). Regardless of outcome, and based on the continuing efficacy of *A. annua* extracts against all tested variants (10 to date), we again urge the WHO to consider including encapsulated dried leaf *A. annua* as a separate arm in their trial. Use of *A. annua* does not induce ART resistance [29], but the plant could be crucial in helping many in the world where access to vaccines and standard therapeutics is logistically challenging.

**Author Contributions:** Conceptualization, M.S.N., P.J.W.; methodology, M.S.N., Y.H.; formal analysis, M.S.N., Y.H.; writing—original draft preparation, P.J.W.; writing—review and editing, M.S.N. and P.J.W.; supervision, M.S.N. All authors have read and agreed to the published version of the manuscript.

**Funding:** Award Number NIH-2R15AT008277-02 to PJW from the National Center for Complementary and Integrative Health funded phytochemical analyses of the plant material used in this study. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Complementary and Integrative Health or the National Institutes of Health.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data are contained within the article.

**Acknowledgments:** We thank Prof. David Ho of the Aaron Diamond AIDS Research Center at Columbia University for supporting the live virus work in his lab. Prof. David Fidock, Columbia University, and Dr. Melissa Towler, Worcester Polytechnic Institute, provided critical review of the manuscript.

**Conflicts of Interest:** The authors declare that there are no conflicts of interest.

**References:**
1. Tegally, H.; Moir, M.; Everatt, J.; Giovanetti, M.; Scheepers, C.; Wilkinson, E.; Subramoney, K.; Moyo, S.; Amoako, D.G.; Baxter, C.; et al. Continued Emergence and Evolution of Omicron in South Africa: New BA.4 and BA.5 lineages. *medRxiv* **2022**, 2022.05.2001.22274406, doi:10.1101/2022.05.01.22274406.
2. Burki, T.K. Omicron variant and booster COVID-19 vaccines. *The Lancet Respiratory Medicine* **2022**, 10, e17.
3. Cao, Y.; Yisimayi, A.; Jian, F.; Song, W.; Xiao, T.; Wang, L.; Du, S.; Wang, J.; Li, Q.; Chen, X. BA. 2.12. 1, BA. 4 and BA. 5 escape antibodies elicited by Omicron infection. *Nature* **2022**, 1-3.
4. Iketani, S.; Liu, L.; Guo, Y.; Liu, L.; Chan, J.F.-W.; Huang, Y.; Wang, M.; Luo, Y.; Yu, J.; Chu, H. Antibody evasion properties of SARS-CoV-2 Omicron sublineages. *Nature* **2022**, 604, 553-556.
5. Liu, L.; Iketani, S.; Guo, Y.; Chan, J.F.-W.; Wang, M.; Liu, L.; Luo, Y.; Chu, H.; Huang, Y.; Nair, M.S. Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *Nature* 2022, 602, 676-681.

6. Wang, Q.; Guo, Y.; Iketani, S.; Nair, M.S.; Li, Z.; Mohri, H.; Wang, M.; Yu, J.; Bowen, A.D.; Chang, J.Y. Antibody evasion by SARS-CoV-2 Omicron subvariants BA. 12. 1, BA. 4, & BA. 5. *Nature* 2022, 1-3.

7. Charness, M.; Gupta, K.; Stack, G.; Strymish, J.; Adams, E.; Lindy, D.; Mohri, H.; Ho, D. Rapid Relapse of Symptomatic Omicron SARS-CoV-2 Infection Following Early Suppression with Nirmatrelvir/Ritonavir. *Research Square Preprint* 2022, doi:https://doi.org/10.21203/rs.3.rs-1588371/v3.

8. Sakamuru, S.; Huang, R.; Xia, M. Use of Tox21 Screening Data to Evaluate the COVID-19 Drug Candidates for Their Potential Toxic Effects and Related Pathways. *Frontiers in Pharmacology* 2022, 13, doi:10.3389/fphar.2022.935399.

9. Hung, Y.-P.; Lee, J.-C.; Chiu, C.-W.; Lee, C.-C.; Tsai, P.-J.; Hsu, I.-L.; Ko, W.-C. Oral Nirmatrelvir/Ritonavir Therapy for COVID-19: The Dawn in the Dark? *Antibiotics* 2022, 11, 220.

10. Lamb, Y.N. Nirmatrelvir plus Ritonavir: first approval. *Drugs* 2022, 1-7.

11. Owen, D.R.; Allerton, C.M.; Anderson, A.S.; Aschenbrenner, L.; Avery, M.; Berritt, S.; Boras, B.; Cardin, R.D.; Carlo, A.; Coffman, K.J. An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19. *Science* 2021, 374, 1586-1593.

12. Nair, M.S.; Huang, Y.; Fidock, D.A.; Polyak, S.J.; Wagoner, J.; Towler, M.J.; Weathers, P.J. *Artemisia annua* L. extracts inhibit the in vitro replication of SARS-CoV-2 and two of its variants. *J. Ethnopharmacol.* 2021, 274, 114016, doi:https://doi.org/10.1016/j.jep.2021.114016.

13. Nair, M.S.; Huang, Y.; Fidock, D.A.; Towler, M.; Weathers, P. *Artemisia annua* L. hot-water extracts show potent activity in vitro against Covid-19 variants including delta. *J. Ethnopharmacol.* 2022, 284, 114797.

14. Nie, C.; Trimpert, J.; Moon, S.; Haag, R.; Gilmore, K.; Kaufer, B.B.; Seeberger, P.H. In vitro efficacy of Artemisia extracts against SARS-CoV-2. *Sci Rep* 2021, 11, 1-14.

15. Zhou, Y.; Gilmore, K.; Ramirez, S.; Settels, E.; Gammeltoft, K.A.; Pham, L.V.; Fahnøe, U.; Feng, S.; Offersgaard, A.; Trimpert, J.; et al. In vitro efficacy of artemisinin-based treatments against SARS-CoV-2. *Virol J* 2021, 18, 182.

16. Hu, Y.; Liu, M.; Qin, H.; Lin, H.; An, X.; Shi, Z.; Song, L.; Yang, X.; Fan, H.; Tong, Y. Artemether, artesunate, arteannuin B, echinatin, licochalcone B and andrographolide effectively inhibit SARS-CoV-2 and related viruses in vitro. *Frontiers in cellular and infection microbiology* 2021, 526.

17. Cao, R.; Hu, H.; Li, Y.; Wang, X.; Xu, M.; Liu, J.; Zhang, H.; Yan, Y.; Zhao, L.; Li, W. Anti-SARS-CoV-2 potential of artemisinins in vitro. *ACS Infect Dis* 2020, 6, 2524-2531.

18. Gendrot, M.; Andreani, J.; Boxberger, M.; Jardot, P.; Fonta, I.; Le Bideau, M.; Duflot, I.; Mosnier, J.; Rolland, C.; Bogreau, H. Antimalarial drugs inhibit the replication of SARS-CoV-2: An in vitro evaluation. *Travel Med Infect Dis* 2020, 37, 101873.

19. Gendrot, M.; Duflot, I.; Boxberger, M.; Delandre, O.; Jardot, P.; Le Bideau, M.; Andreani, J.; Fonta, I.; Mosnier, J.; Rolland, C. Antimalarial artemisinin-based combination therapies (ACT) and COVID-19 in Africa: In vitro inhibition of SARS-CoV-2 replication by mefloquine-artesunate. *Int J Infect Dis* 2020, 99, 437-440.

20. Li, G.; Yuan, M.; Li, H.; Deng, C.; Wang, Q.; Tang, Y.; Zhang, H.; Yu, W.; Xu, Q.; Zou, Y. Safety and efficacy of artemisinin-piperaquine for treatment of COVID-19: an open-label, non-randomised and controlled trial. *Int. J Antimicrob. Agents* 2021, 57, 106216.

21. Hellou, E.; Mohsin, J.; Elemy, A.; Hakim, F.; Mustafa-Hellou, M.; Hamoud, S. Effect of ArtemiC in patients with COVID-19: A Phase II prospective study. *J. Cell. Mol. Med.* 2022, 26, 3281-3289.
22. Shahramzehi, N.; Abdelfatah, S.; Efferth, T. In Silico and In Vitro Identification of Pan-Coronaviral Main Protease Inhibitors from a Large Natural Product Library. *Pharmaceuticals* 2022, 15, 308.

23. Tang, Y.; Li, X.; Yuan, Y.; Zhang, H.; Zou, Y.; Xu, Z.; Xu, Q.; Song, J.; Deng, C.; Wang, Q. Network pharmacology-based predictions of active components and pharmacological mechanisms of *Artemisia annua* L. for the treatment of the novel Corona virus disease 2019 (COVID-19). *BMC Complementary Medicine and Therapies* 2022, 22, 1-16.

24. Russo, M.; Moccia, S.; Spagnuolo, C.; Tedesco, I.; Russo, G.L. Roles of flavonoids against coronavirus infection. *Chem Bio Interact* 2020, 328, 109211.

25. Solnier, J.; Fladerer, J.-P. Flavonoids: A complementary approach to conventional therapy of COVID-19? *Phytochem. Rev.* 2021, 20, 773-795.

26. Martini, M.; Zhang, T.; Williams, J.; Abramovitch, R.; Weathers, P.; Shell, S. *Artemisia annua* and *Artemisia afra* extracts exhibit strong bactericidal activity against *Mycobacterium tuberculosis*. *J. Ethnopharmacol.* 2020, 262, 113191.

27. Liu, L.; Wang, P.; Nair, M.S.; Yu, J.; Rapp, M.; Wang, Q.; Luo, Y.; Chan, J.F.-W.; Sahi, V.; Figueroa, A. Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature* 2020, 584, 450-456.

28. Kupferschmidt, K. WHO relaunches global drug trial with three new candidates. *Science* 2021, 373, 606-607.

29. Elfawal, M.A.; Towler, M.J.; Reich, N.G.; Weathers, P.J.; Rich, S.M. Dried whole-plant *Artemisia annua* slows evolution of malaria drug resistance and overcomes resistance to artemisinin. *Proc Natl Acad Sci* 2015, 112, 821-826.