Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Anti-COVID-19 potential of *Azadirachta indica* (Neem) leaf extract

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ABSTRACT

COVID-19 is caused by infection with the "severe acute respiratory syndrome coronavirus-2" (i.e., SARS-CoV-2). This is an enveloped virus having a positive sense, single-stranded RNA genome; like the two earlier viruses SARS-CoV and the Middle East respiratory syndrome (MERS) virus. COVID-19 is unique in that, in the severe case, it has the propensity to affect multiple organs, leading to multiple organ distress syndrome (MODS), and causing high morbidity and mortality in the extreme case. In addition, comorbidities like age, cardiovascular disease, diabetes and its complications, obesity, are risk factors for severe COVID-19. It turns out that a most plausible, simple, single explanation for this propensity for MODS is the pivotal involvement of the vascular endothelium (VE). This is a consequence of the fact that the VE seamlessly connects all the entire vascular bed in the body, thus linking all the target organs (heart, lungs, kidney, liver, brain) and systems. Infection with SARS-CoV-2 leads to hyper-inflammation yielding uncontrolled production of a mixture of cytokines, chemokines, reactive oxygen species, nitric oxide, oxidative stress, acute phase proteins (e.g., C-reactive protein), and other pro-inflammatory substances. In the extreme case, a cytokine storm is created. Displacement of the virus bound to the VE, and/or inhibition of binding of the virus, would constitute an effective strategy for preventing COVID-19. In this regard, the acetone-water extract of the leaf of the Neem (*Azadirachta indica*) plant has been known to prevent the adherence of malaria parasitized red blood cells (pRBCs) to VE; prevent cytoadherence of cancer cells in metastasis; and prevent HIV from invading target T lymphocytes. We therefore hypothesize that this Neem leaf acetone-water extract will prevent the binding of SARS-CoV-2 to the VE, and therefore be an effective therapeutic formulation against COVID-19. It is therefore advocated herein that this extract be investigated through rigorous clinical trials for this purpose. It has the advantages of being (i) readily available, and renewable in favor of the populations positioned to benefit from it; (ii) simple to prepare; and (iii) devoid of any detectable toxicity.

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Abbreviations

ACE	Angiotensin converting enzyme
CRP	C-Reactive Protein
COVID-19	SARS-CoV-2 disease 2019 [Severe Acute Respiratory Syndrome-Coronavirus-2 disease 2019]
COX	Cyclooxygenase
ECs	Endothelial cells
HIV	Human immunodeficiency virus
IL	Interleukin
MERS	Middle East Respiratory Syndrome
MODS	Multiple organ distress syndrome MODS
NO	Nitric oxide
NOS	Nitric oxide synthase
pRBCs	Parasitized red blood cells
PG	Prostaglandin
ROS	Reactive oxygen species
SARS-CoV-2	Severe Acute Respiratory Syndrome-Coronavirus-2
TNF	Tumour necrosis factor
VE	Vascular endothelium

Introduction

The search for a direct and specific cure for COVID-19 has so far been unsuccessful. Consequently, the current approach to anti-COVID-19 strategies relies on repurposed drugs, e.g., remdesivir [5]; hydroxychloroquine [17]; dexamethasone [20]; and/or resort to traditional medicine (TM) [3,12,22,23,39,40]. These strategies yield some sort of palliative quelling of the symptoms, as the body's innate and acquired immune surveillance mechanisms are deployed to gradually subdue the infection to obscurity in the mild case. As alluded to by Fara et al. [16] regarding resolution of the cytokine storm, "*Initially, the localized response is meant to eliminate the trigger and involves protective mechanisms* ….". The vascular endothelium (VE) plays a pivotal and intricate role in all aspects of COVID-19, as will be reviewed herein.

Functions of the vascular endothelium (VE) derive from its unique structure

The VE is a continuous monolayer of cells (endothelial cells, ECs) physically delineating the blood with its circulating elements in the lumen (of every blood vessel) from the vascular smooth muscle layer of the wall of all blood vessels. Thus, VE seamlessly links them all from the largest arteries and veins, to the capillaries that connect the arterial and venous systems. It is a highly dynamic organ system that engages in various far-reaching physiological homeostatic functions in which it serves as a signal transducer [13,14,19,35,36,60]. The VE is therefore not merely only a simple physical barrier, but should also be visualized as a *bona fide* endocrine organ being the source of various cellular signaling factors [13]. Another important implication here is that this VE single continuous monolayer of cells pervades through the entire body of the individual, connecting the blood, lungs, heart, liver, kidney, brain, and other metabolic niches. Consequently, the VE serves as the link connecting various cardiometabolic, pulmonary, septic, and renal diseases [30]. Indeed, its involvement in neuronal pathology has been implicated as well [18,38,47]. Any perturbation in the system would therefore spell danger, as in the development of activation, yielding reactive oxygen species (ROS), nitric oxide (NO), cytokines, acute phase proteins (e.g., C-reactive protein, CRP), and other oxidative stress determinants, leading to triggering the formation of an atherosclerotic plaque, for instance [13]. The central and intricate involvement of the VE in many physiological and pathophysiological situations has been recently reviewed [47,50,60].

The quiescent, normal, or "healthy" endothelium

The quiescent endothelium is that in which the component ECs remain non-activated. Such is the normal "healthy" physiological state. In this state, the VE is a primary sensor of biomechanical stimuli which are transduced into biological responses. For instance, regular steady smooth laminar flow of blood, consistent with stable shear stress on the wall, i.e., against the endothelium itself, invokes the homeostatic physiological processes. This signals the activation of constitutive enzymes, namely endothelial nitric oxide synthase (eNOS or NOSIII) and cyclooxygenase-1 (COX-1), leading to synthesis of the appropriate low levels of NO by the eNOS (NOSIII), and the prostanoid prostacyclin (prostaglandin I_2; PGI_2) from arachidonic acid by COX-1. Both NO and prostacyclin are powerful vasodilators, and thus relax blood vessels. In addition, ECs also produce endothelin-1 and angiotensin II, which are potent vasoconstrictors, as well as other vasoactive factors like

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the prostanoid thromboxanes [19,27,35]. To ensure vascular tone, homeostasis is maintained by a fine balance of these vasodilators, vasoconstrictors and the other vasoactive factors in the normal quiescent state [19,35,36]. To exert its action, NO diffuses to the vascular smooth muscle cells where it stimulates soluble guanylate cyclase leading to enhanced cyclic guanosine monophosphate (cGMP) synthesis, causing relaxation. It also diffuses into the lumen, affecting the platelet and blood element functions; it prevents thrombosis and renders the blood more fluid by inhibiting platelet adhesion and aggregation [36]. Prostacyclin, like NO, is also antithrombotic, and causes smooth muscle relaxation by activating adenylate cyclase to increase cyclic adenosine monophosphate (cAMP) production [35,36].

Endothelial dysfunction and the pathological state

Conversion of the quiescent state to the activated state of the endothelium creates "endothelial dysfunction", which is a state of inflammation. It has been indeed referred to in various terms, e.g., as belonging to a "diseased vessel" [36]. It is characterized by the usual hallmarks of inflammation, including up-regulation of the inducible nitric oxide synthase (NOSII; iNOS), and inducible cyclooxygenase-2 (COX-2), respectively and forming copious amounts of NO and prostanoids (e.g., prostacyclin, thromboxanes) from arachidonic acid in the vascular smooth muscle cells, associated with the "diseased" endothelium. Some of these prostanoids are pro-inflammatory and drive disease pathogenesis, e.g., prostacyclin, though usually anti-inflammatory, paradoxically acts pro-inflammatory in rheumatoid arthritis [51]. Production of pro-inflammatory cytokines, such as IL-6, TNFα, IL-1β, is increased; and upgrading of other innate and adaptive immune factors and processes, such as acute phase proteins like CRP [21,32,36,50,60] is enhanced as well, exacerbating oxidative stress.

Production of chemokines (e.g., IL-8), cytokines, and adhesion molecules, which recruit leukocytes and platelets, would cause inflammation in specific tissues for clearing intruding foreign particles and pathogens (viruses, bacteria, etc.). In the VE, this transformation from quiescence to diseased state could be triggered by cardiovascular risk factors [13]. In such circumstances, the homeostatic balance that characterizes the healthy quiescent state (arising from the interplay of cytokines, chemokines and the other factors) is breached [60].

Acute and chronic inflammation

In acute and chronic inflammation caused by any of the triggers, e.g., microbial pathogens; Gram-negative lipopolysaccharide (LPS) or Gram-positive lipoteichoic acid (LTA); and dead cell debris, these reactions and products are exacerbated, leading to overwhelming excesses, and causing sepsis and, at a more aggravated state, septic shock [29,37,41,43,60]. The dysregulated oxidative stress in these circumstances responds to antioxidative and anti-inflammatory management approaches. This is the basis of the multiple effects of the antioxidant vitamin C in various pathological situations [2,6].

When COVID-19 is the cause of endothelial dysfunction

The manifestations of COVID-19 range from asymptomatic to mild, to severe ill-health conditions like respiratory failure, sepsis and subsequently to multi-organ dysfunction syndromes [16]. The reason for this can be linked to the key involvement of the vascular endothelium [24]. The viral pathogen SARS-CoV-2 is the trigger as it infects the VE for instance, and sets the entire inflammatory cascade into motion, creating endothelial dysfunction, with the oxidative stress and other manifestations described [60].

From early infection to the cytokine storm and severe COVID-19

Early in the infection, the respiratory tract is affected, yielding the early symptoms of mild COVID-19 [16]. The triggered innate and adaptive immune responses would attempt to resolve the infection [11]. Should this mitigation strategy not succeed, the result is acute respiratory distress syndrome. Then, due to the interconnectedness of the vascular endothelium, the prevailing inflammatory response is cascaded far and wide through the vascular bed, and so, in addition, affects cardiovascular, renal, and other targets as well. The outcome of this is the exacerbated "endothelial dysfunction" consistent with severe COVID-19, caused by the created "Cytokine Storm" with the associated exaggerated thrombotic and other consequences [60]. The cytokine storm is essentially the exaggerated mixture of pro-inflammatory cytokines (TNFα, IL-6, IL-1β) [60], chemokines (IL-8), and CRPs [16,21,44,55,60] .

Compromised health conditions as risk factors for serious COVID-19

A compromised health status involving cardiovascular, respiratory, renal, or other systems would be a determinant for the serious consequences (enhanced morbidity and mortality) of COVID-19 [60]. So far in the pandemic, intervention strategies with chemical and natural product antioxidants, anti-inflammatory agents, and immunomodulators, aimed at ameliorating the cytokine storm or its production have been under investigation [6,40,42,45,52]. These efforts have yielded promising results and some treatments(e.g., with vitamin C) are in clinical trials [6].

It is therefore clear that the VE is a main focus of the unique pathological hallmarks of COVID-19 [24,33,59], which result from a complex blend of vascular dysfunction, dysregulated inflammation and thrombosis [59,60]. There is direct viral

infection of the endothelium in different organs [1,47,59]. Also, pericyte cells, which have an exaggerated concentration of ACE2 (the receptor of SARS-CoV-2) are in proximity to the lung endothelial cells and, therefore, would exacerbate the endothelial cell injury [4,60] Normal function of the pericytes is maintenance of micro-vessel integrity. But on binding SARS-CoV-2, the consequences are grave. This explains why pre-existing conditions that negatively impact vascular endothelial homeostasis cause severe COVID-19 [59]. Therefore, preventing the access of SARS-CoV-2 to the vascular endothelial cells and pericytes and dislodging the bound virus from such sites have become reasonable strategies for avoiding the attendant problems, i.e., dysregulated inflammation.

COVID-19 and other disease severities caused by cytoadherence to vascular endothelium and other sites

Malaria, Gram-negative and Gram-positive infections, LPS (endotoxin) and LTA [9,10,37,55], and COVID-19, cause severe diseases involving the vascular endothelium and hyper-inflammation. The hallmarks of the disease in each case include a combination of various levels of exacerbated dysregulated cytokine production, NO and ROS release, oxidative stress, "cytokine storm", thrombotic events and others. Thus, in the Gram-negative bacterial and LPS triggered events, the outcome is endotoxin stress (sepsis), and septic shock. In the case of COVID-19, the critical severe outcomes have been linked to the cytokine storm, and some of the characteristics resemble sepsis-associated immune dysregulation [28]. In these cases, effective intervention strategies have included the use of antioxidants, anti-inflammatory agents and immunomodulators [6,16,2].

Cytoadherence to vascular endothelium

Udeinya et al. [56,57] and others [31,34] have established that in the case of *Plasmodium falciparum* malaria infection, parasitized red blood cells (pRBCs) containing schizonts and trophozoites are preferentially sequestered by specific binding to the VE of the venules and capillaries through the parasite's knobs. This specific event protects these malaria parasitic stages from the spleen, thereby saving them from immune destruction and clearance by the spleen. The malaria therefore persists, via this evasion strategy.

Cytoadherence also contributes prominently in the pathogenic mechanisms of other diseases like cancer metastasis [7,53], as well as bacterial [48] and viral infections [8]. In the case of HIV, invasion of the target cell occurs when the viral surface envelope spike glycoprotein binds both CD4 and a seven-transmembrane coreceptor of the target lymphocyte. These interactions induce a conformational change in the spike protein resulting in the fusion event internalizing the HIV in the target cell [8].

Neem leaf acetone-water extract as potential mitigation strategy against COVID-19

Udeinya and associates [56] further discovered that the acetone-water extract of *Azadirachta indica* (Neem) dislodged the trapped pRBCs from the VE, which made it possible for these pRBCs to be conveyed to the spleen for immune killing and clearance from the system. The same Neem extract has also been shown to prevent the invasion of lymphocytes by HIV both in vitro and in vivo in humans. Thus, the Neem acetone-water extract displayed a broad-spectrum effect by inhibiting adhesion of malaria-infected pRBCs, adhesion of cancer cells, and invasion of human lymphocytes by HIV. In addition, it was reported that the extract had no observable toxicity among the cohort of individuals who received the experimental treatment at the University of Nigeria Teaching Hospital (tested in the limited clinical trials performed) [56].

On account of these findings, especially the fact that the pRBCs (just like the SARS-CoV-2 virus) bind to the VE, it is reasonable to hypothesize that the same acetone-water neem leaf extract would be effective in dislodging SARS-CoV-2 (the causative agent for COVID-19) from binding the cells. This would be a game changer in the fight against COVID-19 because the VE underpins the various pathologies and multi-organ involvements and disease severities. Part of the advantage is that, if the extract is effective as expected, it could be possible to administer it via a simple route; for instance, as a food or nutritional additive or an adjuvant to another drug, say remdesivir.

Indeed nature has provided the remedies for ailments and diseases for the benefit of humankind since ancient times. *Azadirachta indica* is one of the accredited and trusted sources of herbal therapy against a plethora of diseases and ill-health conditions since antiquity [15,25,26,61]. This acetone-water extract, considered in isolation, qualifies to be one of the numerous ways in which Neem phytochemicals (either as single applications, or in combination as cocktails with other components) safeguard humans against the onslaught of different pathogens.

Potential anti-covid-19 phytochemicals in *Azadirachta indica* (Neem) and other efficacious herbal resources

The desperate and worrisome absence of drugs for direct attack on the pathogenic agent SARS-CoV-2 has inspired and encouraged the exploitation of the properties of natural products against COVID-19 and its pathogenesis, symptoms and sequelae. *Azadirachta indica* (Neem), a prehistoric source for remedies against numerous ill-health conditions for various indigenous populations in Africa and other parts of the world presents, for advantage in this context, these age-old phytochemical agents endowed within its various parts. Excellent reviews on this topic regarding *A. indica*, and the other herbal and other resources abound [25,49,54,58,61].

Rigorous clinical trials and phytochemical studies advocated

For this immediate moment therefore, it is recommended that appropriate rigorous clinical trials be done on the acetone-water extract, as currently prepared [63], administered to a large enough population of COVID-19 patients and non-infected controls. This is to unequivocally establish the appropriate dosage, and its efficacy, and empower its use as anti-COVID-19 remedy for people in Africa, India, and other zones endowed with abundant and renewable supplies of the Neem plant in their environments around the globe.

Thereafter, it would become necessary to further study the said acetone-water extract to isolate, identify and characterize the specific bioactive phytochemical components, as well as their levels present therein. This later phase of the work would inform the next step: formulating anti-COVID-19 remedies from the pure compounds.

Prerequisite in vitro confirmation that the Neem Extract Inhibits SARS-CoV-2 Binding on VECs:

As mentioned earlier (*vide supra*) in this paper, the Neem extract displayed *in vitro* broad-spectrum effects, and it: prevented malaria parasitized red blood cells (pRBCs) from adhesion to endothelial cells; cancer cell cytoadherence to endothelial cells, as well as cancer cell metastasis; human immunodeficiency virus (HIV) from binding to target lymphocytes. Given this broad-spectrum effect, we hypothesized (*vide supra*) that the Neem extract will bind to the vascular endothelial cells (VECs) and prevent access of the spike glycoprotein of the SARS-CoV-2 to its main receptor, the VEC angiotensin converting enzyme 2 (ACE2). Also, as mentioned earlier, the inhibition of HIV is caused by the Neem extract preventing the viral surface spike glycoprotein from engaging the CD4 and a seven-transmembrane coreceptor of the target lymphocyte. We present this hypothesis, considering (the speculation) that this could be one of the unique characteristics that have enabled the Neem to be such an effective remedy against diseases over the millennia of human existence on our planet [15,25,26,61]. However, to make assurance doubly sure, the plan being proposed herein is that the clinical trials will be preceded by *in vitro* confirmatory investigations involving SARS-CoV-2 (or simply SARS-CoV-2 spike glycoprotein) and the Neem leaf extract. The Neem leaf extract will be obtained as per the published procedure [63].

Udeinya and associates [56] had reported the *in vitro* antiretroviral activity of the Neem extract, and concluded that the mechanism of action may involve inhibition of cyto-adhesion. The work on viral interactions with the extract was performed [56] using HIV, and was patented [62]. These authors reported that in the presence of the extract at 10 microgram/ml *in vitro*, 75% of the target lymphocytes were protected from HIV invasion [56]. Adherence to, and replication in VECs have been highlighted as hallmark phenomena associated with various types of viral infections of humans and animals (e.g., SARS-CoV-2, Hantavirus, Influenza A, H5N1, H7N1, Ebola virus, Zikavirus, West Nile virus, Dengue virus, among others [60].

In a recent Case Report [64], it was concluded that circulating soluble ACE2 inhibited COVID-19. This is by binding of the soluble ACE2 to the VEC membrane-bound ACE2 receptors, thereby excluding SARS-CoV-2 from binding. In the said Case Report, the patient produced an overwhelming amount of soluble ACE2 as a defensive mitigation strategy against the SARS-CoV-2 infection and disease progression. This phenomenon has been reported for recombinant ACE2 administered to animal models of SARS-CoV-2 infection, which resolved the infection [64,65]. In addition, intravenously administered recombinant ACE2, ameliorated COVID-19 in seriously sick human patients [60].

Embarking on the proposed clinical trials with the Neem extract would therefore be predicated on the success of the Neem extract to inhibit the binding of SARS-CoV-2 (or simply its spike glycoprotein) to the membrane-bound ACE2 of the VECs.

Vascular-centric endothelial protective therapy

This simple proposed remedy is consistent with the "*vascular-centric endothelial protective therapies*" that have been advocated by Mangalmurti and collaborators [24,33]. However, if effective, the remedy proposed herein is indeed a superior approach given the fact that the Neem leaf extract presents the advantages of (i) being locally available to the target population; (ii) being easy to process; (iii) being inexpensive; and (iv) having no detectable toxicity at the appropriate low doses. These special attributes are in line with, and satisfy the conditions for applying natural products and resources as therapeutics to less privileged, resource-limited parts of the world [15,61].

Conclusion

Azadirachta indica (the Neem plant) is an age-old resource for traditional remedies against numerous diseases. The acetone-water extract of the Neem leaf has been reported to display broad-spectrum effects, including (i) dislodging of parasitized erythrocytes from cytoadhesion to the vascular endothelium; (ii) inhibition of cancer cells from adhesion and metastasis; and (iii) inhibition of HIV from binding to and invading target T lymphocytes.

COVID-19 results from the attack of SARS-CoV-2 virus on the vascular endothelium. This causes extreme stimulation of the innate and adaptive immune system, yielding hyper-inflammation, with attendant combination of levels of exacerbated thrombotic consequences, oxidative stress, the "cytokine storm," and other pro-inflammatory outcomes. The unique feature of this insult is that it has the propensity of pervading the entire vascular bed. Thus, all the organs and niches seamlessly

connected by the VE are potentially involved and compromised. This creates acute respiratory distress syndrome and in the extreme case the possibility of multiple organ distress syndrome with high morbidity and mortality.

Given its broad-spectrum effect, the Neem acetone-water extract is expected to dislodge SARS-CoV-2 from the vascular endothelium. Success with this process prevents the creation of endothelial dysfunction and the implicit uncontrolled inflammation, which may circumvent COVID-19. It is hereby advocated that the acetone-water Neem extract be subjected to rigorous clinical trials to create the appropriate dosage for efficacy. This would serve the needs of populations of the African continent, the Indian sub-continent, and other under-served marginalized peoples in zones endowed with abundant and renewable supplies of the Neem plant. Thereafter, further studies on the extract should establish the bioactive phytochemical principles for the purposes of formulating anti-COVID-19 remedies from pure compounds.

Authors' contributions

Michael Eze: Conceptualization, Investigation, Validation, Writing - original draft, Writing - review & editing. Chibuike Udenigwe: Conceptualization, Investigation, Validation, Writing - review & editing. Iroka Udeinya: Conceptualization, Writing - review & editing. Patrick Ifeonu: Conceptualization, Writing - review & editing. Chukwunonso Ejike: Writing - review & editing. Peter Uzoegwu: Writing - review & editing. All authors have approved the final article.

Declaration of Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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