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Nanoemulsions: The rising star of antiviral therapeutics and nanodelivery system—current status and prospects

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Abstract

Nanoemulsions (NEs) of essential oil (EO) have significant potential to target microorganisms, especially viruses. They act as a vehicle for delivering antiviral drugs and vaccines. Narrowing of drug discovery pipeline and the emergence of new viral diseases, especially, coronavirus disease, have created a niche to use NEs for augmenting currently available therapeutic options. Published literature demonstrated that EOs have an inherent broad spectrum of activity across bacterial, fungal, and viral pathogens. The emulsification process significantly improved the efficacy of the active ingredients in the EOs. This article highlights the research findings and patent developments in the last 2 years especially, in EO antiviral activity, antiviral drug delivery, vaccine delivery, viral resistance development, and repurposing EO compounds against SARS-CoV-2.

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Microemulsion, Nanoemulsion, Antiviral activity, Drug-delivery, Vaccine delivery, Drug resistance, Drug repurposing.

Introduction

The world has persistently witnessed a multitude of viral disease outbreaks and epidemics since ancient times. Although there are several advanced life-saving medical technology available today, emerging new viral infections pose a significant threat to global health. In the past 25 years, several viral disease outbreaks have occurred around the world, in particular, the 1997 avian influenza A (H5N1) viral epidemic in which the virus directly spread from poultry to humans [1], the 1999 Nipah virus outbreak in Malaysia and Singapore caused severe encephalitis in humans [2], and 2002 SARS (severe acute respiratory syndrome) by novel coronavirus SARS-CoV occurred in China and spread to 37 countries with 9.6% mortality rate [3]. Apart from these outbreaks, significantly higher mortality and morbidity cases were reported in the 2009 influenza pandemic by a swine H1N1 influenza A virus [4], the 2012 MERS (Middle East respiratory syndrome) by MERS-CoV [5], the 2010 severe fever with thrombocytopenia syndrome (SFTS) caused by SFTS bunyavirus [6], the 2014 Ebola outbreak caused by Western African Ebola virus [7], the 2015 Zika fever caused by Zika virus [8], and currently ongoing COVID-19 pandemic caused by SARS-CoV-2 [9]. A detailed list of viral disease outbreaks in the past 25 years is available in Table 1.

Although there are several advancements available in antiviral therapy, the newly evolving novel strains and mutation-mediated drug resistance development in viruses certainly decrease the efficacy of many antiviral therapies. For instance, there were multiple levels of antiviral drug resistance recognized in the herpes simplex virus (HSV) [10], hepatitis C virus (HCV) [11,12], and HIV strains [13,14]. Now there is no specific antiviral medication available to cure or manage the COVID-19 [15,16]. Therefore, it is highly essential to develop a broad-spectrum, multitargeting antiviral agent to control these viral infections. Presently, scientists around the globe are working intensively to develop drugs and vaccines against the SARS-CoV-2 virus. Several research groups were involved in repurposing US Food and Drug Administration (FDA)-approved synthetic drugs and well-known antiviral phytochemicals to address the emergency. EOs are concentrated liquid consisting of hydrophobic phytocompounds, traditionally used in several parts of the world to treat ailments. Over the past few decades, EOs acquire immense recognition as a potent reservoir with ample antiviral and

Table 1

Details of viral diseases outbreaks over the past 25 years.

Disease outbreak/epidemic/pandemic	Year	Affected areas	Virus	Reference
Avian influenza epidemic	2013–2019	China	Influenza A virus subtype H7N9	FAO [108]
Chikungunya outbreak	2013–2015	Americas	Chikungunya	Deilgat et al. [109]
COVID-19 pandemic	2019 to present	Worldwide	SARS-CoV-2 virus	Dong et al. [110]
Dengue fever epidemic	2019 to present	Asia–Pacific, Latin America	Dengue fever	Moloo [111], WHO [112,113]
Dengue outbreak	2011	Pakistan	Dengue fever	
Dengue outbreak	2013	Singapore	Dengue fever	
Dengue outbreak	2017	Pakistan	Dengue fever	
Dengue outbreak	2017	Sri Lanka	Dengue fever	
Ebola epidemic	2018–2020	The Democratic Republic of the Congo and Uganda	Ebola	Aceng et al. [114]
Ebola outbreak	2020	The Democratic Republic of the Congo	Ebola	
Hand, foot, and mouth disease epidemic	2011	Vietnam	Hand, foot, and mouth disease	Khanh et al. [115]
Japanese encephalitis outbreak	2017	India	Japanese encephalitis	Kulkarni et al. [116]
Lassa fever epidemic	2019 to present	Nigeria	Lassa fever	Adenola and Ilemobayo [117]
Measles outbreak	2010–2014	The Democratic Republic of the Congo	Measles	Hachiya et al. [118], Kalil et al. [119], Mahase [120], Samaraweera et al. [121]
Measles outbreak	2013–2014	Vietnam	Measles	
Measles outbreak	2019–2020	The Democratic Republic of the Congo	Measles	
Measles outbreak	2019 to present	New Zealand	Measles	
Measles outbreak	2019 to present	Philippines	Measles	
Measles outbreak	2019	Malaysia	Measles	
Measles outbreak	2019 to present	Samoa	Measles	
Middle East respiratory syndrome coronavirus outbreak	2012 to present	Worldwide	Middle East respiratory syndrome/MERS-CoV	Zumla et al. [5]
Nipah virus outbreak	2018	India	Nipah virus infection	Arunkumar et al. [122]
Novel bunyavirus outbreak	2020 to present	China	Severe fever with thrombocytopenia syndrome	Yu et al. [6]
Jaundice outbreak	2014–2015	India	Primarily hepatitis E, but also hepatitis A	Rakesh et al. [123]
Swine flu outbreak	2015	India	Influenza A virus subtype H1N1	Murhekar and Mehendale [124]
Yellow fever epidemic	2020 to present	Nigeria	Yellow fever	Lucey and Gostin [125] and Nwachukwu et al. [126]
Yellow fever outbreak	2012	Sudan	Yellow fever	
Yellow fever outbreak	2016	Angola and DR Congo	Yellow fever	
Zika virus epidemic	2015–2016	Worldwide	Zika virus	Bogoch et al. [8]

other bioactive compounds. These EO bioactive compounds are absorbed in the intestine as micellar structures [17,18].

Nanoemulsion (NE) is a fine, transparent oil-in-water or water-in-oil dispersion system stabilized by an interfacial film of surfactant molecule having a droplet size range of 20–500 nm. NE can be formulated in three types: (1) oil-in-water NE wherein oil is dispersed in the continuous aqueous phase, (2) water-in-oil NE in which water droplets are dispersed in the continuous oil phase, and (3) bicontinuous NE where the microdomains of oil and water are interdispersed within the system [19]. The long-term stability, spontaneous emulsification, extended shelf-life, photostability [20], and high rate of drug solubilization capacity makes the MEs/NEs as a potent antimicrobial agent as well as a drug delivery vehicle [21–24]. This review aims to highlight the past 2 years' research findings and patent developments in antiviral NEs, antiviral EOs repurposing, and EO's role in antiviral drug delivery and vaccine formulation with a special focus on SARS-CoV-2 infection.

Emulsion components delivering antiviral activity

Essential oils

Unlike synthetic drugs, most of the essential oils (EOs) are nontoxic and listed as generally regarded as safe by FDA. Many researchers have begun to explore the effect and efficacy of EOs against various infectious, acute, and chronic diseases [25–28].

Inhibition of virus by EOs and EO substances

The *in vitro* evaluation of camphor comprising EOs of *Chrysanthemum indicum* (36.69%) and *Chrysanthemum morifolium* (14.56%) [25] and the humulene epoxide and caryophyllene oxide constituting EO of Egyptian *Cyperus rotundus* rhizomes (38.43% and 21.03%) [26] displayed dose-dependent inhibitory effect against HSV-1 and hepatitis A virus. In addition, the former EOs have a significant inhibitory effect against vesicular stomatitis virus (VSV), and the Cyperus EO inhibited the Coxsackie B4 virus [25,26]. The EO of tea tree (*Melaleuca alternifolia*) comprising terpinene-4-ol, limonene, γ- and α-terpinene, cineol, and α-terpinolene and the volatile oil (VO) of *Cymbopogon citratus* has strongly inhibited the oral and genital herpes viruses [29,30], and the oregano EO rich with monoterpenic phenol, carvacrol (5-isopropyl-2-methylphenol), and its isomeric analog thymol (2-isopropyl-5-methylphenol) derived from *Origanum vulgare* plant showed a potent protective effect against HIV and simian immunodeficiency viruse [31], and the oxygenated and unoxygenated monoterpenes- and sesquiterpenes-rich herbal *Hornstedtia bella* EO [27], and a monocyclic sesquiterpene, germacrene-rich *Rhizoma curcuma* EOs [28] have inactivated the vaccinia and pseudorabies virus, respectively. Furthermore, the aglycones molecules, especially, quercetin, myricetin, and quercetagetin, and flavonoids, such as apigenin, baicalein, biochanin A, kaempferol, luteolin, and naringenin, have exhibited broad-spectrum antiviral activity toward a wide range of enveloped (hepatitis B virus [HBV], HCV, HIV, African swine fever, influenza A, dengue, respiratory syncytial and Newcastle disease virus [NDV]) and nonenveloped (foot and mouth disease and enterovirus) viruses [32].

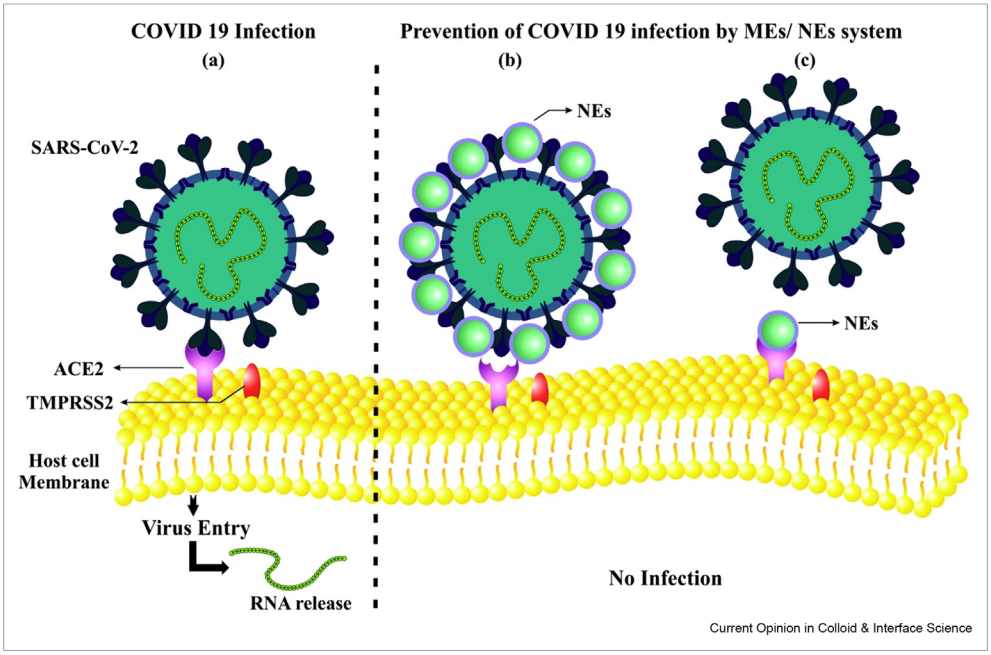
The synergistic antiviral effect between EOs

The synergistic activity of the EO mixture displayed a remarkable activity against numerous virus infections. For example, a mixture of EOs prepared from the dry leaves of thyme (*Thymus capitatus* (L.) Hoffmanns, et Link), sage (*Salvia fruticosa* Mill.), and dittany (*Origanum dictamnus* L) containing carvacrol (53%), eucalyptol (13%), β-Caryophyllene (3%), Borneol (1.68%), p-Cymene (1.32%), γ-Terpinene (1.17%), and α-Terpineol (1.06%) presented substantial inhibition of influenza A and B and human rhinovirus [33]. This EO mixture caused a nucleoprotein trafficking defect in the H1N1 virus, signifying that the nucleoprotein could be the primary target site for EO's antiviral activity [33]. Another synergy essence containing carvacrol, thymol, paracymene, and secondary constituents of thyme oil, oregano oil, and/or cinnamon oil was found to be effective against bovine rotavirus and epizootic hemorrhagic disease virus, equine herpes virus-1, enterovirus 71, H5N1, HBV, HIV, NDV, MS-2 bacteriophage virus, parvovirus, porcine epidemic diarrhea virus, bovine viral diarrhea virus, porcine respiratory, reproductive syndrome virus, and transmissible gastroenteritis virus [34].

Prevention of viral infection by EOs and EO compounds

Virus entry into the host cells is one of the early key steps in viral infections, and hence, inhibition of virus–host interaction could be a successful prevention strategy (Figure 1). Currently, only a few synthetic viral entry inhibitors are available to prevent the viral infection and spread. The EO substance thymohydroquinone dimethyl ether (THQDE) derived from the *Ayapana triplinervis* plant has shown successful prevention of viral infection to human lung epithelial cells in which the THQDE inhibited the internalization process, thereby prevented the Zika virus disease [35]. Similarly, the EOs of *Piper aduncum* L and *Ocotea quixos (Lam.)* Kosterm showed remarkable host cell protection against the West Nile virus, where the EOs might have interfered/masked the virion envelope structure or viral structure to block the virus internalization. Here, the suspected EO constitutes that blocked the virus entry are dillapiole (48.21%) and 1,8-cineole (39.15%) [36]. In addition, the *Hornstedtia bella* EO alone or in combination with mycophenolic acid (antiorthopoxviruses drug) protected the Caco-2 epithelial cell monolayer from vaccinia virus infection [27].

Figure 1



Schematic illustration of the NEs mediated inhibition of SARS-CoV-2 entry and spread into the host. (**a**) COVID 19 infection, (**b**) NEs (20–30 nm) masking SARS-CoV-2, (c) NEs (20–30 nm) blocking the ACE2 receptor.

Mechanism of action of antiviral EOs and EO compounds

Until now, very few studies have demonstrated the mode of action of EOs on viruses. Most of the studies predicted that the EOs could interfere with the virion envelope structure or mask the virus structure, which subsequently blocks the virus adsorption and penetration into host cells [37]. Vimalanathan and Hudson showed the effective inhibition of the membrane proteins (neuraminidase and hemagglutinin) of H1N1 by *Cinnamomum zeylanicum* EO [38]. The vaporized EOs of *C. zeylanicum*, *Pelargonium graveolens*, *Salvia officinalis*, *Thymus vulgaris*, and *Cymbopogon flexuosus* displayed a strong inhibitory effect against the H1N1 hemagglutinin. It suggests that the viral glycoproteins could be the primary target for the EOs action against viruses. The receptor-binding domains of the surface spike glycoprotein of SARS-CoV-2 have pockets of a tube-like shape and a size matching that of the free fatty acid molecules [39], which could facilitate the binding of EO-based NEs (Figure 1).

On the other hand, the EOs components, namely, 1,8-cineole, apigenin, baicalein, biochanin A, ellagic acid, isoquercitrin, kaempferol, luteolin, myricetin, naringenin, quercetagetin, quercetin, sesquiterpenes, and terpenes, have effectively inhibited many viruses (as described previously). Among these substances, the quercetin showed a significant inhibitory effect on the nonstructural protein-3 proteases of HCV [40], protein synthesis of rhinovirus, and replication of dengue virus type 2 [41]. Similarly, the baicalein, myricetin, quercetin, and quercetagetin effectively inhibited the reverse transcriptase of HIV and Rauscher murine leukemia virus, and except baicalein, other flavonoids inhibited the DNA polymerase also. Certain EO flavonoids, namely, apigenin, baicalein, biochanin A, kaempferol, luteolin, and naringenin, inhibited the nucleoprotein production in the H5N1 virus [32]. These observations demonstrate that the primary target of both EOs and EO constitutions is the surface proteins.

Surfactants and cosurfactants

A surfactant denotes the surface-active agent acting as the interface between the hydrophilic part and aqueous phase or aqueous and hydrophilic phase during the emulsification process. Surfactants are amphiphilic substances comprised of the hydrophilic head and lipophilic tail groups. The head groups may be nonionic, anionic, cationic, or zwitterionic and used in the wetting, dispersion, lubricant, and foaming process. Besides emulsification, surfactants also presented a wide range of bioactive properties, specifically, antimicrobial, anticancer, anti-inflammatory, antiaging, and so on. Hence, the selection of surfactants for NEs/MEs formulation remains a critical part. The surfactant that directly affects the virion or inhibits its entry into the host could be an appropriate choice for a new antiviral emulsion or nanodelivery system development. The nonionic polysorbate surfactants, such as Tween 20, 40, 60, and

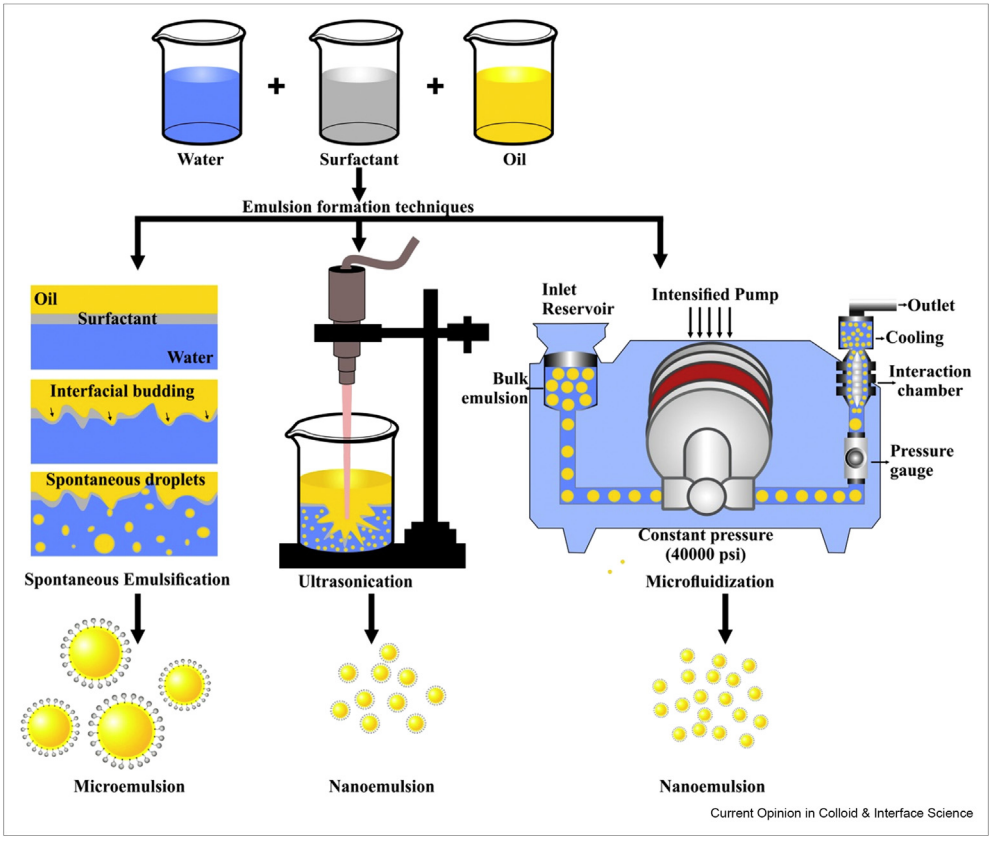
80, have shown moderate to strong viricidal activity against California encephalitis virus, HSV, NDV, poliomyelitis virus type 1, vaccinia virus, and VSV [42]. Another nonionic surfactant, nonoxynol (spermicide), has shown moderate viricidal activity against HIV and other sexually transmitted infections [43]. On the other hand, the cationic surfactant chlorhexidine exhibited an inhibitory effect against HSV-1 and HIV-1 virus [44].

Cosurfactants are the short-chain alcohols, organic acids, and salts used to improve the emulsification of a selected surfactant. They are weak amphiphilic molecules and do not self-aggregate but strongly support and concentrate the primary surfactant aggregates formed on the surfactant layer. The organic acid cosurfactant, short-chain caprylic acid, the key antiviral constituent in the ViroSAL preparation, showed a significant inhibitory effect against a wide range of viral infections, especially, Ebola, Epstein–Barr, HSV, Lassa, measles, orf (parapoxvirus), SARS-CoV-1, pseudoviruses, VSV, and Zika virus [45]. Similarly, the organic sodium salt and sodium cholate displayed strong anti-HIV-1 activity [46]. At present, no guaranteed antiviral agent against the SARS-CoV-2 virus is available that underlines the urgent need for an effective preventive and treatment system. Exploiting the previously mentioned safe, antiviral emulsion components for the EO-based NEs/MEs formulation could result in a multifaceted broad-spectrum agent against the SARS-CoV-2 virus (Figure 1).

Formulation of EO-based NEs/MEs

The NE/ME system with different physical and physicochemical properties was prepared using several techniques. MEs are formulated spontaneously by low-energy emulsification or phase-inversion temperature method exploiting the thermodynamic equilibrium between the oils, water, surfactants, and cosurfactants (optional). Under the low-energy technique, MEs can be prepared by stirring either the oil and surfactant concoction (internal phase) with water or water and surfactant concoction (external phase) with oil or all together (Figure 2). In the second method, the phase-inversion was achieved at a specific temperature, namely, phase-inversion temperature, where water-in-oil emulsion converts into the oil-in-water emulsion, usually in the presence of nonionic surfactant [47]. On the basis of our decade of experience, we recommend stirring the oil and surfactant concoction with water as the most appropriate and suitable EO-based MEs formation technique for antimicrobial studies. For

Figure 2



Schematic illustration of MEs and NEs preparation by spontaneous, ultrasonic, and microfluidization techniques.

instance, a spontaneous ME system was formulated via dropwise addition of internal phase (cinnamon oil and nonionic surfactant—Tween 20) into a constantly stirred water phase [48], which prevented the bacterial sepsis in the Wistar rat model.

On the other hand, the NE systems (20−500 nm) are formed under the thermodynamically nonequilibrium state using an external energy input by ultrasonicator or high-pressure homogenizer (called high-energy method) or without an external energy/device (low-energy method) [49]. In the high-energy method, the NEs are formulated by breaking large coarse emulsions into mini-droplets using high-energy mechanical devices. For example, in the microfluidizer or high-pressure homogenizer, the macro/micro-emulsions are pumped into a narrow valve at constant high pressure that subsequently breaks down the coarse emulsion into NEs. Here, the emulsion size can be controlled by the number of passes and pressure applied (Figure 2). Nabila et al. [50] prepared coarse emulsion (1:8:1 ratio) of castor oil, cremophor RH40 (surfactant), and polyethylene glycol 400 (cosurfactant) by stirring at 200 rpm for 2 h. Then the coarse emulsion was sonicated for 1 h for producing the NEs and diluted in deionized water (Figure 2). In contrast, dissolving the surfactant with an external (water) phase for coarse emulsion preparation was carried out by Franklyne et al. [21]. Initially, the macroemulsion was formulated from basil, turmeric, black seed, clove, and cinnamon oils by stirring with an external phase containing surfactant (cremophor EL or Tween 20) and water. Then the macro emulsions were microfluidized or ultrasonicated to achieve NEs. NEs/MEs formation, components properties, and stability have been extensively reviewed elsewhere [49].

EO-based NEs/MEs antiviral and drug delivery system

The MEs and NEs formulated using EOs have shown potent antibacterial [51−63], antifungal [54,55,64], and antiviral [65] activities. In addition, the EO-based nanodrug delivery system provided solutions to major problems, such as drug−drug interactions, short half-life, drug resistance development, low bioavailability, viral sequestration, viral latency, selectivity, and deficient broad-spectrum activity in treating viral infection [66]. Despite the proven antiviral activity of EOs, only a small number of antiviral NEs/MEs systems have been formulated [32,67].

Antiviral activity of NEs against enveloped viruses

Dengue viruses

Dengue disease is caused by four different serotypes of dengue virus, transmitted through mosquito bites to the human population, and now, half of the world's population at risk. At present, there is no effective antiviral drug available against this virus. Recently, curcumin produced by *Curcuma longa* (L) has shown significant antiviral activity against HCV, HSV-1, HSV-2, VSV, para-influenza 3, and respiratory enteric orphan 1 viruses [50]. Padilla et al. suggested that curcumin inhibits dengue virus replication by inhibiting the ubiquitin−proteasome system [68]. However, the poor aqueous solubility and cell uptake subsequently reduced the curcumin bioavailability and applicability. The curcumin-loaded castor oil NE system with an average droplet size of 40.85 ± 0.919 nm formulated using the self-nano-emulsification technique showed increased stability and delivery of curcumin. Furthermore, this NE system was found to be effective against all the four dengue virus serotypes derived from patients. Particularly, the standard plaque assay showed greater inhibitory profiles against serotypes 1 and 2 [50].

Hepatitis B and C viruses

Because of the extensive absorption in the lymphatic fluid, the lipid based NEs become an excellent delivery system for poorly soluble antiviral agents [69,70]. The *in vivo* murine study revealed 11.5-fold increase in the bioavailability of baicalin (a flavonoid used to treat viral hepatitis) in lymph nodes when delivered with a NE carrier [71]. This nanocarrier system could be used to target HBV that sequesters in the lymphatic system. Similarly, the HBV nucleoside reverse transcriptase inhibitor adefovir dipivoxil−loaded nanostructured lipid carrier was formulated using Capmul MCM (glycerolized fats and oils product), precirol ATO-5 (solid lipid), and cremophor RH40 or Pluronic F68 (surfactant) for the successful delivery of the drug into the Swiss Albino mice liver [72]. Likewise, the improved water solubility and pH-specific intestinal release of silibinin, a potent anti-HCV compound, were achieved when delivered with a polyvinylpyrrolidone NE carrier [73].

Human immunodeficiency virus

The nanoformulations of antiretroviral drugs have a significant advantage over bulk drug formulations. The advantages include bypassing the first-pass metabolism, improving half-life, and improving blood−brain barrier permeability [74,75]. The branched pH-responsive copolymers of oligo (ethyleneglycol) methacrylate (OEGMA), methacrylic acid, and ethylene glycol dimethacrylate polymerized under atom transfer radical polymerization conditions to form NEs with castor oil, peanut oil, and soybean oil. To the NEs, the lopinavir (Lpv) that inhibits the protease-mediated maturation of virions or efavirenz (Efz) that inhibits the viral RNA reverse transcription was loaded, and both the antiretroviral drug-loaded NEs exhibited enhanced permeability and antiviral activity against HIV-1 (IIIB)-infected MT4 cells. The apparent permeability of Lpv

was almost an order of higher magnitude after 2 h compared with the control aqueous-DMSO drug solution [76]. The self-nano emulsifying oil formulations of Efz prepared with caproyl 90 and kolliphor EL showed a low percent dissolution efficiency and a high dissolution half-life less than 9 min for Efz under *in vitro* condition compared with free Efz [77]. Similarly, low-energy water-in-oil NEs of cold-pressed flaxseed oil containing Efz formed using span 20 (28.5%), Tween 80 (28.5%), and ethanol (42.8%) by D-optimized design was found to a stable antiretroviral emulsion [78].

Apart from Lpv and Efz, other antiretroviral drugs were also incorporated in the NE drug delivery systems and used for HIV treatment. For example, Karami et al. formulated lactoferrin-modified NEs containing indinavir (Idv) for brain delivery, wherein the olive oil containing Idv, oleic acid, α-tocopherol, and span 8 were homogenized in a polysorbate 80 solution to form Idv-NE followed by coupling with lactoferrin [79]. The hydrodynamic diameter, polydispersity index, and zeta potential of Idv-NEs were 112 ± 3.5 nm, 0.20 ± 0.02, and 33.2 ± 2.6 mV, respectively. The *in vivo* brain delivery study in the rat model presented 1.6- and 4.1-fold higher concentrations of Idv in the Idv-NEs and lactoferrin-treated Idv-NEs than the drug alone treated animals. The brain uptake clearance of Idv delivered orally via Idv-NEs, and lactoferrin-treated Idv-NEs were 393- and 420-times higher compared with the free drug [79]. Likewise, the oral delivery of Idv-loaded methoxypoly (ethyleneglycol)-poly (e-caprolactone) nanoparticle in the rat showed increased bioavailability of the drug in plasma compared with the Idv solution [80]. We believe that the assembly of multiple retroviral drugs into a single NEs carrier system may empower the effective treatment of HIV infection. In addition, a single NEs system could accommodate the water-soluble drugs such as the nucleoside and nucleotide analogs of the anti-HIV drug (e.g., tenofovir) and highly water-insoluble drugs (e.g. raltegravir or paclitaxel) and exhibit targeted and long-acting characteristics *in vivo* [70].

Herpesviruses

C. citratus VO-loaded nanosuspension was prepared by adding the VO into the poly (D, L-lactide-co-glycolide) dissolved in aqueous saturated ethyl acetate. This organic phase was dropwise added into the ethyl acetate saturated distilled water containing polyvinyl alcohol in an ice-cool bath under continuous homogenization. Prepared VO-NEs showed very high inhibition potency against HSV-1 and HSV-2 [30]. Furthermore, the hydrogel form of the previously mentioned nanoparticles showed significant inhibition at eightfold lesser concentration. The coumestrol (found in soybeans, alfalfa, and red clover) was efficiently combined in positively charged NEs dispersed in hydroxyethyl cellulose gel and tested for its topical delivery to mucosa tissues. After 8 h of permeation, the concentration of coumestrol reserved into cut out intact porcine esophageal mucosa was twofold higher than the formulations containing fluid phospholipid (dioleylphosphocholine) (5.12 ± 0.63 μg/cm^2) when compared with those containing rigid phospholipid (di-stearoyl phosphocholine) (2.39 ± 0.16 μg/cm^2). Similarly, the coumestrol retention into injured mucosa was also twofold higher than formulations containing dioleylphosphocholine (4.72 ± 0.51 μg/cm^2) when compared with those containing di-stearoyl phosphocholine (2.43 ± 0.60 μg/cm^2). Low IC_{50} values demonstrated an increased anti-HSV-1 and -HSV-2 activity when coumestrol incorporated into NEs containing dioleylphosphocholine [81].

Spherical chitosan nanospheres (CNS) containing acyclovir (200 nm) with a zeta potential value of −40.0 mV were prepared by Donalisio et al. The loading capacity of the drug was found to be 8.5%, with 30% release under *in vitro* conditions after 6 h. The *in vitro* skin permeation studies confirmed an improved amount of permeated acyclovir (55%) over the commercial cream (10%) at 24 h. IC_{50} values against HSV-1 at postinfection (48 h) were 0.012 and 0.156 μM for acyclovir-loaded CNS and free acyclovir, whereas IC_{50} values against HSV-2 postinfection (24 h) were 0.100 μM and 1.608 μM for acyclovir-loaded CNS and free acyclovir, respectively [82]. Similarly, the acyclovir containing water-in-oil MEs was formulated with isopropyl myristate, span 20, Tween 20, water, and DMSO, which showed complete inhibition of cutaneous herpetic lesions development in female Balb/c mice with HSV-1-induced infection compared with the commercially marketed cream [83]. Ionic liquid (triethylammonium acetate and diethylammonium acetate) containing microemulsion formed using isopropyl myristate, Tween 80 and span 20 demonstrated very long-term stability (42 days) of acyclovir and methotrexate compared with the commercially available formulations containing 1-ethyl-3-methylimidazolium acetate [84].

Antiviral activity of NEs against nonenveloped viruses

Human papilloma viruses

An interesting NE-based curcumin delivery system for the photodynamic therapy against vulvar intraepithelial neoplasia associated with the human papilloma viruses (HPV) infection has been proposed by Bonfim et al. [85]. The antiviral and antineoplastic compound curcumin was solubilized in the oil phase and then homogenized with a hydrophilic emulsifier for improving its performance in therapies. The curcumin-loaded NEs displayed an efficient internalization and high toxicity to the HPV-16 E6 expressing cells. Furthermore, the expression of apoptosis executioner proteins (caspase 3/7) was high in the cells treated with

the curcumin-loaded NEs. Photoactivation of curcumin loaded in NEs by exposing to light at 430 nm strongly potentiated the effect of curcumin on the HPV-16 E6 expressing cells [85]. As curcumin inactivates a wide range of viruses, such as HCV, HSV-1, HSV-2, VSV, parainfluenza 3, and respiratory enteric orphan 1 viruses [50], similar photodynamic therapy approaches combined with curcumin-loaded NEs has to be established against these viral infections.

Role of EO-MEs and EO-NEs in viral vaccine delivery

Apart from antiviral drug delivery, the NE system was effectively used as an immunization vehicle to deliver/present the inactivated viral proteins to the host immune system. NE was prepared by emulsification of soybean oil, Tween 80, cetylpyridinium chloride, ethanol, and water and used to develop intranasal vaccine containing HSV-2 surface glycoproteins gD2 and gB2 (NE01-gD2/gB2) [86]. The guinea pigs were immunized either intranasally or intramuscularly with NE01-gD2/gB2 at 63, 42, and 21 days before viral treatment. The intranasal delivery of NE01-gD2/gB2 in animals induced increased levels of neutralizing antibody than the monovalent NE01-gD2 vaccine but lesser than the intramuscular Alum/MPL-gD2 vaccine. Following the HSV-2 intravaginal exposure, the animal immunized intranasally with NE01-gD2/gB2 vaccine displayed significantly reduced scores for acute and recurrent disease. Furthermore, only 1 in 12 NE01-gD2/gB2 intranasally vaccinated animals was detected with the latent virus at dorsal root ganglia. In the therapeutic study, a significant reduction in the recurrent lesions in the guinea pigs immunized with NE01-gD2/gB2 (intranasal) was observed [86]. In the same NE01 system, a plant-derived recombinant influenza H5 (rH5) antigen was incorporated to produce a novel intranasal influenza vaccine. The mice and ferrets were immunized intranasally thrice at 4-week intervals using the rH5-NE01 vaccine, which significantly increased the rH5-specific IgA and IgG antibodies in both animals. In addition, the rH5-NE01 intranasal vaccine increased the antigen-specific interferon -γ and interleukin (IL)-17 production in the CD-1 mice model. In ferrets, the vaccination prevented the infection of the intranasally challenged H5N1 virus [87].

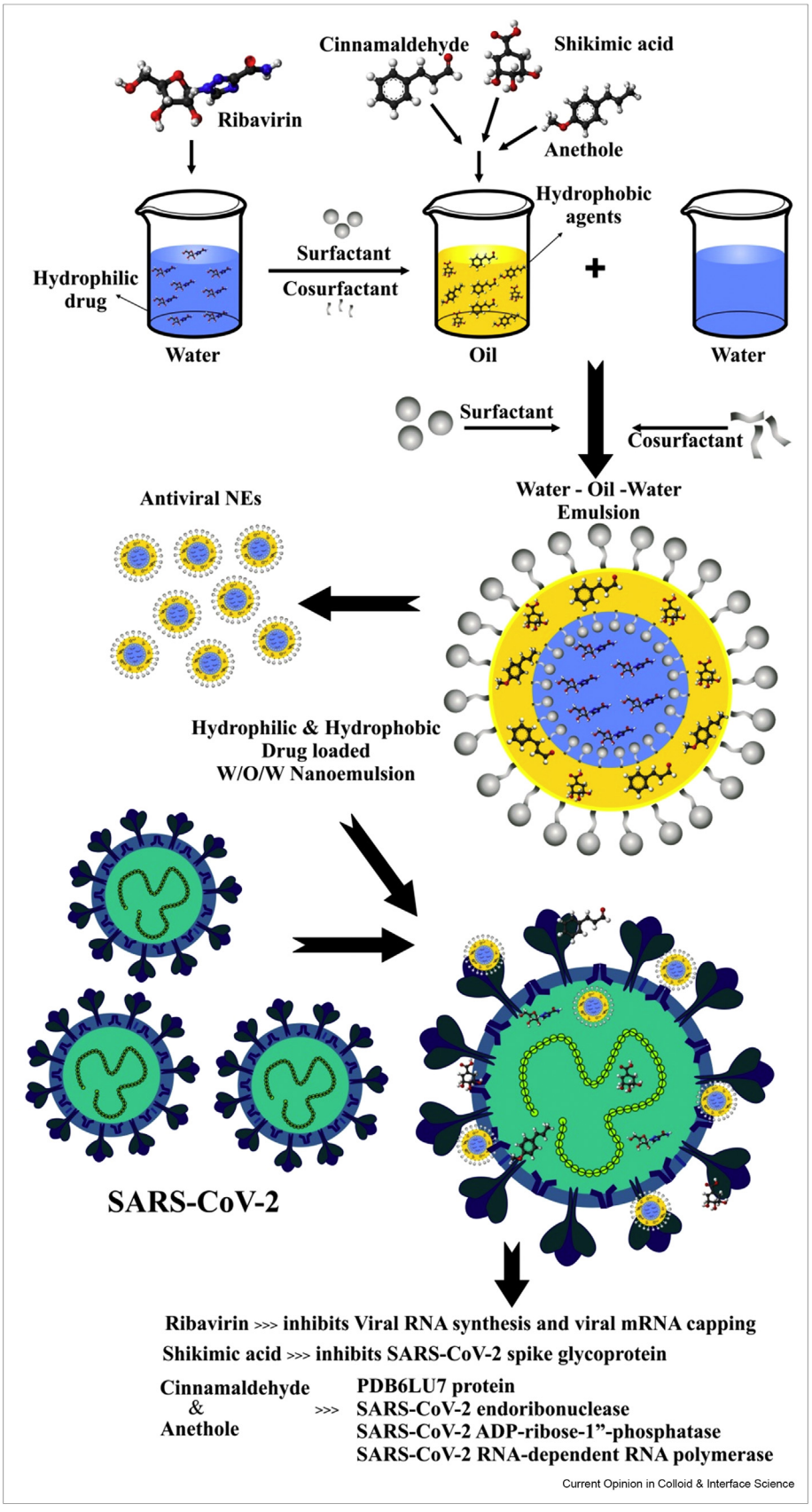
Similarly, intranasal immunization of mice using inactivated A/Puerto Rico/8/1934 (H1N1) (PR/8) adjunct with NE/Sendai viral defective interfering RNAs or NE/influenza viral defective interfering RNAs (3php) showed a synergetic rise of systemic PR/8-specific IgG antibody with significantly high avidity and virus neutralization efficacy than the individual adjuvants. The results revealed that the enhanced immunogenicity of the adjuvant combinations was synergistic and not simply additive [88]. Isopropyl myristate containing NEs was used as an adjuvant for the inactivated influenza H3N2 vaccine. The NEs with 80% or 85.6% water concentration presented higher hemagglutination inhibition titer than aluminum hydroxide or complete Freund's adjuvant and showed higher antigen delivery efficiency in female ICR mice [89]. Minz and Pandey described a recombinant HBV surface antigen–loaded solid fat NE as an adjuvant-carrier system preparation for deep pulmonary vaccination in rats [90]. The vaccine system showed significant (***$P < 0.001$) humoral (sIgA and IgG) and cellular (IL-2 and IF-γ) immune responses compared with naive antigen solution (a recombinant surface antigen without carrier) [90]. The truncated ORF2 proteins (54 kDa and 26 kDa) of the hepatitis E virus were loaded in a chitosan NE system using ultrasonic waves and used as a vaccine candidate. The NE system showed an entrapment efficiency of 70% and 59% for 26 kDa and 54 kDa proteins, respectively. The prepared NE system was nontoxic to HeLa and THP1 cells up to 100 μg/mL concentration. This highly immunogenic ORF2 protein-loaded chitosan NE system can be used as a vaccine candidate against hepatitis E virus [91]. The role of nanotechnology in vaccine development and advanced clinical trials against COVID-19 are reviewed elsewhere [54,92].

On the basis of the studies reviewed herein, above we recommend that the EO-NEs encapsulated with two or more antiviral drugs or with antiviral phytochemicals may synergistically prevent and cure the COVID-19 infection (Figures 1 and 3). On the other hand, the inactivated virus proteins may immunize the susceptible populations against the SARS-CoV-2 virus.

Recent patents on emulsion-based antiviral drugs

Our systematic search showed a limited number of granted patents on the EO emulsion–based antiviral therapy, especially in the past 4 years. For instance, the EO emulsion with a droplet size of <25 microns was prepared from the mixture of thyme oil, oregano oil, and/or cinnamon oil, in combination with natural emulsifiers arabinogalactan and/or tannin compounds. The EO composition was found to be effective against bovine rotavirus and epizootic hemorrhagic disease virus, equine herpesvirus-1, enterovirus 71, H5N1 virus, bovine viral diarrhea virus, HBV, HIV, NDV, MS-2 bacteriophage virus, parvovirus, porcine respiratory and reproductive syndrome virus, porcine epidemic diarrhea virus, and transmissible gastroenteritis virus [34]. A novel submicron oil-in-water dispersion antiviral drug delivery system with 100–1000 nm particle size was prepared in a vegetable oil matrix. In this, ailanthone, an *Ailanthus altissima* (Mill.) plant derivative with significant antiviral activity against adenovirus, astrovirus, enterovirus, HBV, rotavirus, and Norwalk virus, was encapsulated in the oil using phospholipid emulsifier and water

Figure 3



Schematic illustration of multidrug-loaded double emulsion system (~20 nm) targeting multiple sites in SARS-CoV-2 virus, an alternative antiviral agent to treat and cure COVID-19 infection.

phase. The nanoformulation showed a target selectivity, prolonged half-life, stability, significant curation at low drug concentration, reduced adverse reactions, and reduced nontarget tissue delivery compared with positive test drugs [93].

The phytochemical chloroquine has a broad-spectrum anti-HIV activity against HIV-1 A and E and inhibits the glycosylation of the gp120 viral envelope protein leading to reduced viral particle formation with severely reduced infectivity. The chloroquine-loaded water-in-oil emulsion system was developed to prepare chitosan nanosphere gel. Initially, chloroquine chitosan nanospheres (100−800 nm) were prepared by W/O emulsion system, wherein the aqueous phase comprising water-soluble chitosan and chloroquine phosphate was added in corn oil/olive oil matrix using Tween-20 emulsifier. Then the nanosphere was precipitated using sodium hydroxide-n-propanol mixed solution for the final gel formulation for antiviral of external genitalia [94]. Similarly, the water-insoluble anti-HIV drugs Efz, ritonavir (Rtv), and Lpv were dissolved in water-immiscible, oily/organic solvent to procedure an oil phase for NE (200−400 nm) preparation in an aqueous phase containing water-soluble polymer/cellulose and suitable surfactants to treat viral infections [95].

Studies have shown that the efficacy and effectiveness of Lpv/Rtv association and chloroquine and hydroxychloroquine against COVID-19 are poor [96,97]. Hence, the previously mentioned formulations with these drugs can be tested against SARS-CoV-2. Besides, the prepared EO compositions with broad-spectrum antiviral activity can be effectively screened against COVID-19.

In silico screening of EOs components for repurposing against SARS-CoV-2

The SARS-CoV-2 virus has recently emerged as a global pandemic and induced disease (COVID-19) in more than 143 million people and more than 3 million deaths around the globe (according to World Health Organization report as of 6:02pm CEST, April 22, 2021 ([https://covid19.who.int/]). Great efforts being devoted across the globe to find new drugs to control/prevent the disease. Parallelly, the repurposing of known drugs and natural compounds against COVID-19 are also being explored to manage the disease. The garlic EO principal active organosulfur compounds, such as allyl disulfide, allyl methyl trisulfide, allyl(E)-1-propenyl disulfide, allyl (Z)-1-propenyl disulfide, diallyl tetrasulfide, allyl trisulfide, 1,2-dithiole, 2-vinyl-4H-1,3-dithiine, 3-vinyl-1,2-dithiacyclohex-4-ene, carvone, trisulfide, 2-propenyl propyl, methyl allyl disulfide, diacetone alcohol, trisulfide (1E)-1-propenyl 2-propenyl, allyl sulfide, 1-propenyl methyl disulfide, and trisulfide (1Z)-1-propenyl 2-propeny have shown a wide range of therapeutic activity [98]. Molecular docking of these organosulfur compounds against the angiotensin-converting enzyme 2 (ACE2; a host receptor for SARS-CoV-2) and the PDB6LU7 protein (main protease of SARS-CoV-2) exhibited strong synergistic inhibition [98]. ACE2 is the cellular receptor for SARS-CoV-1 and SARS-CoV-2. Blocking this receptor could be the basic choice for preventing the virus infection [39]. Likewise, the S1 unit of SARS-CoV-2 spike glycoprotein responsible for binding to the host ACE2 protein could be putatively inhibited by the anethole (phenylmethyl ether), a major constituent of star anise (*Illicium verum* Hook. f.) EO and fennel (*Foeniculum vulgare*) EO, oseltamivir intermediate shikimic acid of star anise EO, monoterpenoids (thymol and carvacrol) and acyclic monoterpene alcohol (geraniol) of Lamiaceae and Geraniaceae plants EOs, phenylpropanoids (cinnamaldehyde and cinnamyl acetate) of cinnamon EO, L-4-terpineol in tea tree and lavender EOs, and other terpenes, namely, pulegone, camphene, menthol, and ocimene. These components may form a stable complex with SARS-CoV-2 spike glycoprotein because of high affinity and strong hydrophobic interactions, thereby inhibit virus attachment and replication in the host (Figure 3) [99]. Except for shikimic acid, other spike glycoprotein inhibitors have also shown synergistic interaction with other SARS-CoV-2 main proteases, SARS-CoV2 ADP-ribose-1″-phosphatase, SARS-CoV-2 endoribonuclease, SARS-CoV2 RNA-dependent RNA polymerase, and human ACE [100]. Apart from plant oils, the parenteral fish oil lipid emulsion rich in eicosapentaenoic acid and docosahexaenoic acid from Omega-3 polyunsaturated fatty acid (n-3 PUFAs) also been proposed as adjuvant immune pharmacotherapy for hospitalized COVID-19 patients [101]. The molecular docking analysis of ∼200 EO components against SARS-CoV-2 proteins identified that the nano/micro/bulk EO could be used effectively for the prevention and inhibition of SARS-CoV-2 infection [98,100].

Advantages of EO NE as an antiviral agent

The nanoplatforms could be a dedicated antiviral therapy system over the conventional chemotherapeutic agents. Nanoplatforms can be categorized into two groups: organic and inorganic nanoplatforms. The organic nanomaterials are carbon-based materials, especially NE (20−200 nm), liposomes (50−900 nm), polymers (100−900 nm), and dendrimers (3−20 nm). The inorganic nanoplatforms are generally composed of an inorganic core (carbon nanotubes, quantum dots, gold, and silica) and a shell (organic polymers or metals). Each type of these nanosystem has its own unique identity and features suitable for specific and specialized applications. However, the organic nanomaterials such as spongosome lipid nanoparticles and cubosome lipid nanoparticles and NEs, showed a remarkable drug-delivery potency [102,103]. In addition, a single NEs system can solubilize and deliver both hydrophilic and

lipophilic drugs. NEs can protect the drugs from hydrolysis and oxidation and improves the bioavailability of the formulation. As a result of the thermodynamic and kinetic stability, nontoxicity, and nonirritant property, the NEs can be formulated as foams, creams, liquids, and sprays and administered by oral, nasal, pulmonary, enteric, topical, transdermal, and intravenous routes for preventing or treating the COVID-19 infection [39].

Development of virus resistance against oil components

The emergence of drug resistance in the virus has become a matter of great clinical concern over the past few decades. Because of the magnitude and rate of viral replication and extended exposure to antiviral substances, the emergence of drug resistance remains inevitable. Continuous exposure of HIV strain NL4-3 to carvacrol, a major component of oregano oil, resulted in a slow resistance development over 7 months. The NL4-3 strain achieved resistance through mutations in the envelope glycoprotein, particularly a single-point silent mutation in gp120 protein residues, Ala578Thr mutation in the gp41 residue's pocket-forming domain in the N-terminal heptad repeat, and Ala839Thr mutation in the cytoplasmic tail domain of gp41. Depending on the number of mutations in the envelope glycoproteins, the resistance level toward carvacrol increased [31]. Similarly, chicoric acid, a major component present in the *Ocimum basilicum* and *Echinacea purpurea*, turns out to be ineffective against the NL4-3 strain upon 3 months exposure at low concentration. The resistance strains presented serine amino acid instead of glycine amino acid at position 140 of HIV integrase enzyme [104]. Certainly, this resistance development demands an antiviral agent/system with multifaceted target sites to overcome the resistance development in the virus. We strongly recommend that the multidrug–loaded NE/ME system could be an appropriate, alternative, broad-spectrum antiviral agent to treat viral diseases, including SARS-CoV-2 (Figure 3).

Future scope

The poor water solubility of drugs remains a common and enduring problem causing difficulty in the administration and bioavailability. In addition, the solid formulations of drugs are difficult to swallow by patients with chronic diseases, especially advanced stages of AIDS. Therefore, formulating the aqueous insoluble drugs into soluble and bioavailable NEs is of great interest. Although there are a wide variety of antiviral substances reported, there is a huge lacuna in testing these compounds against many viral infections and implementation of EO-NEs as therapeutic options. In addition, many studies have not demonstrated the *in vivo* efficacy of EO substances and nanoformulations. In light of the current COVID-19 pandemic, the plethora of studies on the disease pathogenesis and development of therapeutic and preventive options, there are unexplored areas, such as repurposing and reformulating currently available antivirals, especially as nanoformulations. Many researchers have suggested that curcumin may play a prominent role in COVID-19 therapy [105–107]. A safe NE formulation with antiviral oil, antiviral surfactant/cosurfactant, and antiviral drugs/substances may provide a promising solution for COVID-19 therapy.

Furthermore, a variety of antiviral, anticancer, and anti-inflammatory agents such as ribavirin and interferon-β, a combination of Lpv/Rtv with ribavirin, remdesivir, nelfinavir, arbidol, chloroquine, and renin-angiotensin system inhibitors are also being recognized as potential agents that can target COVID-19. However, many of these drugs are insoluble in water and less bioavailable. The nanoformulations with EOs could improve their delivery, availability, and efficacy and also deliver both water-soluble and water-insoluble drugs that are needed to be administered. In sum, the present review emphasizes that NEs prepared with antiviral components could be a potent therapeutic agent for treating COVID-19 and other diseases and warrants additional research into these areas.

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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