Essential Oil Prevents COVID-19

Subjects: Pharmacology & Pharmacy | Virology
Contributor: Senthil Kumar

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as coronavirus disease-2019 (COVID-19), is a pandemic disease that has been declared as modern history's gravest health emergency worldwide. Until now, no precise treatment modality has been developed. The angiotensin-converting enzyme 2 (ACE2) receptor, a host cell receptor, has been found to play a crucial role in virus cell entry; therefore, ACE2 blockers can be a potential target for anti-viral intervention. In this study, we evaluated the ACE2 inhibitory effects of 10 essential oils. Among them, geranium and lemon oils displayed significant ACE2 inhibitory effects in epithelial cells. In addition, immunoblotting and qPCR analysis also confirmed that geranium and lemon oils possess potent ACE2 inhibitory effects. Furthermore, the gas chromatography-mass spectrometry (GC–MS) analysis displayed 22 compounds in geranium oil and 9 compounds in lemon oil. Citronellol, geraniol, and neryl acetate were the major compounds of geranium oil and limonene that represented major compound of lemon oil. Next, we found that treatment with citronellol and limonene significantly downregulated ACE2 expression in epithelial cells. The results suggest that geranium and lemon essential oils and their derivative compounds are valuable natural anti-viral agents that may contribute to the prevention of the invasion of SARS-CoV-2/COVID-19 into the human body.

Keywords: geranium essential oil ; lemon essential oil ; citronellol ; limonene ; ACE2 ; TMPRSS2 ; SARS-CoV-2 ; COVID-19

1. Introduction

Coronaviridae or coronaviruses are a large family of viruses that can cause disease in mammals and birds. In humans, the viruses cause illness, ranging from common cold to severe respiratory diseases. In 2002, severe acute respiratory syndrome (SARS) emerged, and in 2012, Middle East respiratory syndrome (MERS) emerged—both are betacoronoviruses transmitted from animal to human that result in severe respiratory diseases in affected individuals [1]. In December 2019, a novel SARS-coronavirus (CoV)-2, also known as 2019 novel corona virus (2019-nCoV) or coronavirus disease-2019 (COVID-19), caused a pneumonia outbreak in China and subsequently expanded worldwide, leading to a pandemic. Recently, the first genomic sequence of COVID-19 was released, and through comparing to the genomes of SARS-CoV and MERS-CoV, researchers found that COVID-19 has better genomic sequence homology with SARS-CoV than that of MERS-CoV [2][3].

During the first SARS-CoV outbreak, Li et al. [4] identified angiotensin-converting enzyme 2 (ACE2) as the human host factor or cell entry receptor for SARS-CoV. Overexpression of ACE2 and injection of SARS-CoV spike protein developed severe acute lung failure in mice, which can be attenuated by blocking the renin-angiotensin pathway [5]. Recent studies have revealed that COVID-19 spike protein has strong affinity with ACE2 on host cells, which is significantly higher than that of SARS-CoV [1][5]. These studies also pointed out that treatment with transmembrane protease serine 2 (TMPRSS2) inhibitor significantly blocked SARS-CoV cell entry; therefore, either ACE2 or TMPRSS2 blockers can be a potential targets for anti-viral intervention. Another study reported that the COVID-19 receptor binding domain was capable of entering cells expressing human ACE2, while other receptors are ineffective, confirming that human ACE2 is the prime receptor for COVID-19 [6]. Since the host cell receptor plays a crucial role in virus entry, targeting the precise receptor ACE2 is a promising preventive strategy for COVID-19 infection. Recent studies have demonstrated that ACE2 overexpression was frequently observed in gastrointestinal tissues and colon cell lines [1], which is comparatively higher than that of other tissues, including lung tissues. Therefore, in this study, HT-29, a colon adenocarcinoma cell line, was employed to investigate the ACE2 inhibitory effect of test samples in vitro.

At present, there is no definite treatment or vaccine developed for the coronavirus that causes COVID-19. Nevertheless, many possible treatments for COVID-19 have been thrust into the spotlight by scientists and health industries. For example, the anti-malarial drug combination of chloroquine and hydroxychloroquine as well as anti-HIV drugs ritonavir and lopinavir have been recommended [7][8]. Since the drugs directly target the pathogen, the effectiveness of these drugs are largely anecdotal. Additionally, the development of new drugs for targeting ACE2 and treating COVID-19 could be time-

consuming. Hence, the safety efficacy of the new drugs are a prime concern, which requires a long time for testing, while the infection is growing fast. The traditional medicine systems from many geographical areas use herbs as the primary treatment of viral infections, including those caused by SARS-CoV. For example, leaf extracts of *Toona sinensis* inhibit SARS-CoV replication [9]. Licorice has been suggested as a promising treatment for SARS-CoV [10]. In addition, natural products including diterpenoids, sesquiterpenoids, triterpenoids, lignoids, curcumin, and ginsenosside-Rb1 have been shown to inhibit SARS-CoV [11][12]. A recent in silico study showed that baicalin, scutellarin, hesperetin, nicotianamine, and glycyrrhizin were capable of inhibiting ACE2 [3].

There are a wide-range of essential oils, and their components have been clinically proven to possess antiviral properties [13][14]. A study by Jackwood et al. [15] found that treatment with a mixture of oleoresins and essential oils from botanicals decreased the severity of clinical signs and lesions in chickens that carried the avian infectious bronchitis virus (IBV-CoV). However, the effects of plant essential oils on human coronaviruses are yet to be explored. In particular, their effects on host cell receptors have barely been investigated. Geranium essential oil is derived from the leaves of *Pelargonium graveolens,* which is widely utilized in the cosmetic industry, perfumery, and aromatherapy [16]. Traditionally, geranium essential oil has been used in both physiological and psychological complications, including anxiety, insomnia, high blood pressure, worry, anger, frustration, restlessness, nervousness, weight loss, hypercholesterolemia, gastrointestinal disorders, and respiratory tract infection [17][18]. Geranium oil represents a potent immune modulator, and stimulates and cleans lymphatic system [19]. The oil is clinically used for treating diarrhea, jaundice, diabetes, hepatitis, ulcerative colitis, cholecystitis, and renal stone [20].

2. Discussion

Essential oils have been used in folk medicine throughout history as principal ingredients in aromatherapy and psychotherapy. However, modern preclinical studies have revealed that essential oils possess a broad spectrum of pharmacological activities, including antiseptic, diuretic, choleretic, spasmolytic, hyperemic, expectorant, anti-anxiety, antioxidant, anti-inflammatory, and anticancer activities [21]. The pharmacological properties of essential oils are attributed by their unique major constituents, such as monoterpenoids, sesquiterpenoids, and phenylpropanoids. In addition, there is considerable evidence emerging from in vitro studies and controlled trails of the potential for essential oils as antiviral agents for the treatment of human viral infections, including SARS coronaviruses [22]. These antiviral essential oils were mostly tested against enveloped RNA or DNA viruses such as herpes simplex virus type 1, Junin virus, influenza virus, dengue virus type 2, and SARS coronaviruses, as well as non-enveloped viruses such as coxsackie virus B1, poliovirus, and adenovirus type 3 [21]. Most of these clinically useful antiviral agents are substances that act on specific steps of the viral biosynthesis, inhibiting viral replication in particular. On the other hand, virucidal agents denature the structure or glycoproteins of the virus, thus reducing or completely blocking the infectivity of virus particles [23].

Recently, ACE2 was identified as a functional SARS coronavirus receptor in epithelial cells. In particular, the SARS-CoV spike protein engages ACE2 as the cell entry receptor and employs the cellular serine protease TMPRSS2 for spike protein priming [1]. Thus, either inhibition of ACE2 or TMPRSS2 receptors can be potential targets for SARS-CoV prevention, as ACE2 displays distinct modes of enzyme action and tissue distribution. Abundant endogenous expression of ACE2 has been observed in cardiovascular and colon-specific cell lines, while fainted expression has been noted in kidney cells [24]. Recent studies have also confirmed that the endogenous expression of ACE2 was higher in gastrointestinal tissues [25] and colon cell lines [1], with these reports pointing out that ACE2 expression is comparatively higher in intestinal tissues than in lung tissues. Therefore, in this study, we employed HT-29, a colon adenocarcinoma cell line, which endogenously expresses ACE2, in order to investigate the ACE2 inhibitory effect of essential oils. Initially, we examined the cytotoxic effects of essential oils in HT-29 cells. Assessment of cytotoxicity is clearly an important aspect of the evaluation of a potent antiviral agent, as a potential agent should be selective for virus- or cell-specific processes with no or limited effects on cellular metabolism [26]. Our results indicated that each essential oil exhibits differential cytotoxicity, some of them are strongly cytotoxic and others of them do not exhibit cytotoxicity up to a concentration of 200 µg/mL.

Recent in silico studies reported that organosulfur compounds in garlic essential oil and other natural products, such as baicalin, scutellarin, hesperetin, nicotianamine, glycyrrhizin, (*E*,*E*)-α-farnesene, (*E*)-β-farnesene, and (*E*,*E*)−farnesol, have the potential to bind the human ACE2 receptor, thereby possibly blocking SARS-CoV-2 cell entry [3]. One study evaluated the in vitro antiviral effects against SARS-CoV of seven essential oils from Lebanese species that included *Laurus nobilis* T., *Juniperus oxycedrus* spp. *oxycedrus*, *Thuja orientalis*, *Cupressus sempervirens* spp. *pyramidalis*, *Pistacia palaestina*, *Salvia officinalis*, and *Satureja thymbra*. Among them, the fruit essential oils of *L. nobilis*, *T. orientalis*, and *J. oxycedrus* spp. *oxycedrus* displayed inhibition against SARS-CoV-induced cytopathogenic effect with IC_{50} values of 120 µg/mL, 130 µg/mL, and 270 µg/mL, respectively [26]. However, there are no studies directly exhibiting the ACE2 inhibitory effects of essential oils or their major compounds in vitro or in vivo. In this study, we screened the ACE2 inhibitory effect of 10

essential oils; among them, 8 essential oils exhibited significant ACE2 inhibition. Interestingly, geranium and lemon essential oils strongly inhibited ACE2 activity without displaying cytotoxicity. To the best of our knowledge, this is the first report indicating that geranium and lemon essential oils and their major components citronellol, geraniol, limonene, linalool, and neryl acetate downregulate ACE2 receptor activity in virus–host epithelial cells.

3. Conclusions

The recent COVID-19/SARS-CoV-2 pneumonia pandemic constitutes the largest global public health crisis and has created international anxiety due to its relatively high infectious, rapid progression, as well as its relatively high death rate. Drug development for treating COVID-19 is currently important due to its rapid progression. The conventional antiviral vaccine development is time-consuming and its safety needs to be verified. The fact is that there is no conventional medicine developed for the treatment of COVID-19. However, there is evidence that suggests that essential oils and their major components have displayed potent antiviral activity to other coronaviruses, such as SARS-CoV-1, although the mechanism of action of these oils and their components were found to be mainly through inhibition of viral replication [26]. Recently, ACE2, a receptor in host cell favoring virus cell entry, was recognized as one of the prime targets to minimize the infection. In this study, we presented the first piece of evidence that geranium and lemon essential oils and their major compounds, citronellol, geraniol, limonene, linalool, and neryl acetate, could downregulate ACE2 expression in epithelial cells, thereby blocking virus entry into host cells, and eventually preventing viral infection. However, further studies are highly warranted to unveil the underlying molecular mechanisms of this inhibitory effect.

References

1. Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Kruger, N.; Herrler, T.; Erichsen, S.; Schiergens, T.S.; Herrler, G.; Wu, N.H.; Nitsche, A.; et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020, 181, 271–280e.
2. Lu, R.; Zhao, X.; Li, J.; Niu, P.; Yang, B.; Wu, H.; Wang, W.; Song, H.; Huang, B.; Zhu, N.; et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. Lancet 2020, 395, 565–574.
3. Chen, H.; Du, Q. Potential natural compounds for preventing 2019-nCoV infection. Preprints 2020, 2020010358.
4. Li, W.; Moore, M.J.; Vasilieva, N.; Sui, J.; Wong, S.K.; Berne, M.A.; Somasundaran, M.; Sullivan, J.L.; Luzuriaga, K.; Greenough, T.C.; et al. Angiotensin-converting enzyme 2 is a functional receptor for the sars coronavirus. Nature 2003, 426, 450–454.
5. Ziegler, C.G.K.; Allon, S.J.; Nyquist, S.K.; Mbano, I.M.; Miao, V.N.; Tzouanas, C.N.; Cao, Y.; Yousif, A.S.; Bals, J.; Hauser, B.M.; et al. SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. Cell 2020, 181, 1016–1035.e19.
6. Letko, M.; Munster, V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Microbiol. 2020, 5, 562–569.
7. Pastick, K.A.; Okafor, E.C.; Wang, F.; Lofgren, S.M.; Skipper, C.P.; Nicol, M.R.; Pullen, M.F.; Rajasingham, R.; McDonald, E.G.; Lee, T.C.; et al. Review: Hydroxychloroquine and chloroquine for treatment of sars-cov-2 (covid-19). Open Forum Infect. Dis. 2020, 7, ofaa130.
8. Sanders, J.M.; Monogue, M.L.; Jodlowski, T.Z.; Cutrell, J.B. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): A review. JAMA 2020, 323, 1824–1836.
9. Chen, C.-J.; Michaelis, M.; Hsu, H.-K.; Tsai, C.-C.; Yang, K.D.; Wu, Y.-C.; Cinatl, J.; Doerr, H.W. Toona sinensis roem tender leaf extract inhibits sars coronavirus replication. Ethnopharmacol. 2008, 120, 108–111.
10. Pilcher, H. Liquorice may tackle SARS. Nature 2003, doi:10.1038/news030609-16.
11. Wen, C.C.; Kuo, Y.H.; Jan, J.T.; Liang, P.H.; Wang, S.Y.; Liu, H.G.; Lee, C.K.; Chang, S.T.; Kuo, C.J.; Lee, S.S.; et al. Specific plant terpenoids and lignoids possess potent antiviral activities against severe acute respiratory syndrome coronavirus. Med. Chem. 2007, 50, 4087–4095.
12. Wu, C.Y.; Jan, J.T.; Ma, S.H.; Kuo, C.J.; Juan, H.F.; Cheng, Y.S.; Hsu, H.H.; Huang, H.C.; Wu, D.; Brik, A.; et al. Small molecules targeting severe acute respiratory syndrome human coronavirus. Natl. Acad. Sci. USA 2004, 101, 10012–10017.
13. Astani, A.; Reichling, J.; Schnitzler, P. Comparative study on the antiviral activity of selected monoterpenes derived from essential oils. Res. 2010, 24, 673–679.

14. Swamy, M.K.; Akhtar, M.S.; Sinniah, U.R. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. Based Complement. Alternat. Med. 2016, 2016, 3012462–3012462.
15. Jackwood, M.W.; Rosenbloom, R.; Petteruti, M.; Hilt, D.A.; McCall, A.W.; Williams, S.M. Avian coronavirus infectious bronchitis virus susceptibility to botanical oleoresins and essential oils in vitro and in vivo. Virus Res. 2010, 149, 86–94.
16. Fayed, S.A. Antioxidant and anticancer activities of citrus reticulate (petitgrain mandarin) and pelargonium graveolens (geranium) essential oils. J. Agric. Biol. Sci. 2009, 5, 740–747.
17. Lis-Balchin, M. A chemotaxonomic study of the pelargonium (geraniaceae) species and their modern cultivars. Hort. Sci. 1997, 72, 791–795.
18. Asgarpanah, J.; Ramezanloo, F. An overview on phytopharmacology of pelargonium graveolens l. J. Trad. Knowl. 2015, 14, 558–563.
19. Standen, M.D.; Connellan, P.A.; Leach, D.N. Natural killer cell activity and lymphocyte activation: Investigating the effects of a selection of essential oils and components in vitro. J. Aromather. 2006, 16, 133–139.
20. Peterson, A.; Machmudah, S.; Roy, B.C.; Goto, M.; Sasaki, M.; Hirose, T. Extraction of essential oil from geranium (Pelargonium graveolens) with supercritical carbon dioxide. Chem. Technol. Biotechnol. 2006, 81, 167–172.
21. Reichling, J.; Schnitzler, P.; Suschke, U.; Saller, R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties—An overview. Med. Res. 2009, 16, 79–90.
22. Nadjib, B.M. Effective antiviral activity of essential oils and their characteristic terpenes against coronaviruses: An update. Pharmacol. Clin. Toxicol. 2020, 8, 1138.
23. Zhu, J.D.; Meng, W.; Wang, X.J.; Wang, H.C.R. Broad-spectrum antiviral agents. Microbiol. 2015, 6, 517–517.
24. Warner, F.J.; Lew, R.A.; Smith, A.I.; Lambert, D.W.; Hooper, N.M.; Turner, A.J. Angiotensin-converting enzyme 2 (ACE2), but not ace, is preferentially localized to the apical surface of polarized kidney cells. Biol. Chem. 2005, 280, 39353–39362.
25. Xu, H.; Zhong, L.; Deng, J.; Peng, J.; Dan, H.; Zeng, X.; Li, T.; Chen, Q. High expression of ace2 receptor of 2019-ncov on the epithelial cells of oral mucosa. J. Oral Sci. 2020, 12, 8.
26. Loizzo, M.R.; Saab, A.M.; Tundis, R.; Statti, G.A.; Menichini, F.; Lampronti, I.; Gambari, R.; Cinatl, J.; Doerr, H.W. Phytochemical analysis and in vitro antiviral activities of the essential oils of seven lebanon species. Biodivers. 2008, 5, 461–470.