Scientific Research Publishing

Journal of Biosciences and Medicines, 2021, 9, 42-51
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Morinda citrifolia (Noni) Fruit Juice Inhibits SARS-CoV-2 Spike Protein Binding of Angiotensin-Converting Enzyme 2 (ACE2)

Brett J. West, Shixin Deng

Research and Development, NewAge, Inc., American Fork, Utah, USA
Email: brett_west@newage.com

How to cite this paper: West, B.J. and Deng, S. (2021) *Morinda citrifolia* (Noni) Fruit Juice Inhibits SARS-CoV-2 Spike Protein Binding of Angiotensin-Converting Enzyme 2 (ACE2). *Journal of Biosciences and Medicines*, **9**, 42-51.
https://doi.org/10.4236/jbm.2021.911005

Received: October 11, 2021
Accepted: November 12, 2021
Published: November 15, 2021

Copyright © 2021 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

COVID-19 is a global pandemic that has claimed millions of lives. This disease is caused by a coronavirus, SARS-CoV-2, which requires the binding of its spike protein to angiotensin-converting enzyme 2 (ACE2) for infection of the host cell. *Morinda citrifolia* (noni) fruit juice has antiviral activity that involves enhancement of immune system function. SARS-CoV-2 spike-ACE2 interaction experiments were carried out to further investigate the antiviral properties of noni juice and its major iridoids. Noni juice inhibited binding by approximately 69%. Scandoside was the most active of the three iridoids evaluated, reducing average spike protein-ACE2 interaction by 79.25%. The iridoids worked synergistically towards inhibiting spike protein binding when assayed together, improving activity by more than 22% above the expected level. But the modest activity of the most abundant iridoid, deacetylasperulosidic acid, indicates that other phytochemicals (*i.e.* scopoletin, quercetin, rutin and kaempferol) are also involved. Our results suggest that the presence of several biological active phytochemicals in noni juice enhances resistance to SARS-CoV-2 by interfering with its ability to bind ACE2. This is a new and significant anti-viral mechanism of noni juice that does not directly involve its immunomodulatory properties.

Keywords

Coronavirus, COVID-19, SARS-CoV-2, Spike Protein, *Morinda citrifolia*, Noni, Iridoid

1. Introduction

Coronaviruses are a group of viruses known for the characteristic glycoprotein

projections, or spike proteins, from the viral envelope which give them the crown-like (corona in Latin) appearance from which their name is derived [1]. First isolated in the 1960s, coronaviruses were originally associated with nasopharyngitis, or the common cold [2]. However, these RNA viruses have since been found to be responsible for producing much more severe symptoms in the upper respiratory tract as well as adversely affecting other organs throughout the body, often with lethal outcomes [3]. The recent coronavirus disease 2019 (COVID-19) pandemic was caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), resulting in more than 4.18 million deaths worldwide as of July 2021 [4] [5].

More than 20 years ago, angiotensin-converting enzyme 2 (ACE2) was discovered [6] [7]. This enzyme is attached to cell membranes and converts angiotensin II to angiotensin (1 - 7), thereby influencing blood pressure [8]. This enzyme is distributed widely throughout many organs of the human body. But they are especially abundant in the epithelia of the lungs and small intestine [9]. In 2003, ACE2 was reported to be a functional receptor for SARS-CoV, which preceded the appearance of SARS-CoV-2 by 16 years [10]. Since then, SARS-CoV-2 was also found to have an ACE2 receptor-binding domain on its spike protein [11] [12]. Infection of the cell relies on the binding of ACE2 by the spike protein, which must first undergo proteolytic activation by host transmembrane protease, serine 2 (TMPRSS2) and furin [13] [14]. The role of the SARS-Cov-2 spike protein and ACE2 interaction was confirmed in experiments in which polyclonal antibodies inhibited spike protein-mediated entry of viral RNA into the cell [15].

Plant-derived foods support human health by providing nutrients and phytochemicals, which are biologically active secondary plant metabolites [16]. *Morinda citrifolia*, commonly known as noni, is a tropical tree that produces fruit year-round [17]. It has a well-documented history of traditional use as both food and medicine, has been rigorously evaluated for safety, and has received official approval as safe food by governmental agencies [18]. Some of the perceived health benefits of noni juice, both historical and modern, have been substantiated by human intervention studies. Among these are antioxidant, anti-inflammatory and immunomodulatory properties [19]. For example, increased serum interleukin-2 (IL-2) concentrations and natural killer (NK) cell activity were observed in healthy volunteers who drank noni juice daily for two months, demonstrating enhanced innate and adaptive immune system function [20]. The same effects were observed in immunosuppressed mice that were fed noni fruit juice extract and its major phytochemical constituent, deacetylasperulosidic acid [21]. In another clinical trial, four weeks of daily noni juice ingestion reduced DNA adduct formation in the lymphocytes of cigarette smokers, thereby preserving immune function against the damaging effects of tobacco smoke [22]. These findings demonstrate the potential of noni juice in preventing and mitigating viral infections.

Other *in vivo* and *in vitro* studies further our understanding of how noni juice

influences immune responses to viral exposure. Chickens fed noni juice experienced increased humoral immune response as determined by the hemagglutination assay following infectious bursal disease virus challenge [23]. Neonatal bovine CD4+ and CD8+ T cell activation increased when animals were fed noni fruit puree from French Polynesia for two weeks [24]. A follow-up study revealed that noni puree supplementation for the first three weeks of life also reduced respiratory treatments by 61% [25]. After three weeks of Tahitian Noni® Juice ingestion, peripheral blood CD4+/CD8+ ratio and IL-2 levels increased significantly [26]. Noni fruit extracts also stimulated splenocyte proliferation *in vitro* and increased the humoral immune response of immunosuppressed rats [27]. Finally, 16-days of Tahitian Noni Juice ingestion increased interferon-gamma expression in splenocytes and peritoneal exudate cells [28].

The studies discussed above have centered largely on the ability of noni juice to mount an immune response to infected cells. The purpose of our present investigation was to evaluate additional antiviral properties of noni juice and its major phytochemical constituents (iridoids), with specific interest in SARS-CoV-2. Accordingly, we focused on the mechanism whereby this virus enters host cells, spike protein binding of ACE2.

2. Materials and Methods

2.1. Sample Material and Iridoid Analysis

Noni fruit was harvested by hand in French Polynesia and allowed to fully ripen. The fruit was then processed into a puree by mechanical removal of the seeds and skin with a micro-mesh screen in a commercial fruit pulper. This puree was then pasteurized at 87˚C for 3 seconds at a good manufacturing certified fruit processing facility in Mataiea, Tahiti. This is the same source of noni fruit puree that was approved by the European Commission in 2003 as a safe novel food for use in pasteurized beverages [29]. The puree was then filtered through a 0.45 µm PTFE filter to produce a clarified noni juice for testing.

Previously, we identified iridoids as the major phytochemical constituents of noni fruit. We confirmed the presence of these iridoids (deacetylasperulosidic acid, asperulosidic acid and scandoside) according to our validated method [30]. The filtered puree was diluted with methanol-H_2O (1:1), and chromatographic separation was performed using a C-18 column (4.6 mm × 250 mm; 5 µm, Waters Corporation, Milford, MA, USA) and two mobile phases: A; acetonitrile (MeCN), and B; 0.1% formic acid in H_2O (v/v). A linear gradient of 100% B for 0 - 5 minutes was followed by 70% B and 30% A for 40 minutes at an elution rate of 0.8 mL/min. Eluted compounds were detected by photodiode array (PDA) within 210 - 400 nm. Pure deacetyl asperulosidic acid, asperulosidic acid and scandoside were also obtained for use in the spike protein binding experiments.

2.2. SARS-CoV-2 Spike Protein-ACE2 Binding Inhibition Assay

The ability of noni juice and iridoid samples to inhibit the binding of SARS-CoV-2

spike protein to ACE2 was measured by an enzyme-linked immunosorbent assay kit (Cayman Chemical Company, Ann Arbor, Michigan, USA) involving recombinant rabbit immunoglobulin (Fc)-tagged SARS-CoV-2 spike S1 receptor binding domain (RBD) and His-tagged ACE2. In this assay, a polystyrene 96-microwell plate is precoated with a mouse anti-rabbit monoclonal antibody attached to the rabbit Fc-tagged spike S1 RBD. In the absence of an inhibitor, a recombinant histidine (His)-tagged ACE2 protein binds the spike S1 RBD. The resulting complex is detected with a horseradish peroxidase (HRP)-conjugated anti-His antibody which is quantified by measuring absorbance at 450 nm in a microplate reader (Synergy™ HT, BioTek Instruments, Winooski, Vermont, USA). The absorbance measured without any inhibitor served as the initial activity control, representing 100% spike protein binding. Prior to absorbance measurements, the experiment was performed as follows. A buffer composed of 0.14 M NaCl, 10 mM dibasic sodium phosphate, 0.1% bovine albumin, 1.76 mM monobasic potassium phosphate and 2.68 mM KCl was prepared. 100 μL of this buffer was added to the precoated microwells. Iridoid samples were prepared by dissolving them in the buffer. Next, 50 μL of the dissolved sample solutions, noni juice or buffer (initial activity control) were added to the microwells. Lyophilized His-tagged ACE2 protein (Cayman Chemical Company) was dissolved in the buffer, and 50 μL of this solution was added to each microwell. The Lyophilized SARS-CoV-2 spike RBD (Cayman Chemical Company) was also dissolved in buffer, with the 50 μL of the resulting solution being added to each microwell. The microwell plate was incubated 60 minutes at room temperature on an orbital shaker. Following this initial incubation, the wells were washed three times with 300 μL of a wash buffer composed of 7.61 mM dibasic potassium phosphate and 2.37 mM monobasic potassium phosphate and Polysorbate 20 (500 μL/L). After washing, 150 μL of HRP conjugated anti-His-antibody solution, prepared with a new buffer (0.4 M NaCl, 75.78 mM dibasic potassium phosphate, 23.51 mM monobasic potassium phosphate, 1.1% bovine albumin and 0.038% ETDA tetrasodium salt), was added to each well. The wells were then incubated a second time at room temperature for 30 minutes on an orbital shaker. Next, the wells were emptied and washed three times again followed by the addition of 175 μL of 3,3',5,5'-tetramethylbenzidine (TMB) solution, a substrate of HRP and incubation for 10 to 20 minutes at room temperature. 75 μL of 2.8% sulfuric acid solution was added to each well to stop the enzymatic reaction. Finally, absorbance was read with the microplate reader.

Inhibition percentage was calculated from the difference between control and sample absorbance divided by the absorbance of the control. Replicate samples of the iridoids were prepared and analyzed at 500 μg/mL. A mixture of the three iridoids, at 167 μg/mL each in the final assay, was also evaluated. The expected percent inhibition of the mixture was calculated from the result of each replicate test and the ratio of the concentration of each iridoid in the mixture to its concentration in the individual test. Replicate assays were performed with the noni juice.

3. Results and Discussion

3.1. Iridoid Analysis

Chromatographic analysis confirmed the presence of iridoids in the noni juice samples (Figure 1). Their concentrations were within the ranges that we had observed previously in pasteurized noni fruit puree [31]. The average (± standard deviation) deacetylasperulosidic acid, asperulosidic acid and scandoside contents of the samples were, respectively, 1.40 ± 0.12, 0.41 ± 0.06 and 0.04 ± 0.01 mg/g.

3.2. SARS-CoV-2 Spike Protein-ACE2 Binding Inhibition Assay

The results of the SARS-CoV-2 spike protein binding inhibition experiments are summarized in Table 1. Noni juice exhibited significant anti-spike protein potential by reducing average spike protein-ACE2 binding by 69.14%. Deacetylasperulosidic acid did not exhibit significant activity, only reducing binding by approximately 8%. Asperulosidic acid was somewhat more active, reducing binding by almost 15%. Scandoside, however, displayed a high degree of biological activity. On average, it prevented 79.25% of spike protein-ACE2 binding.

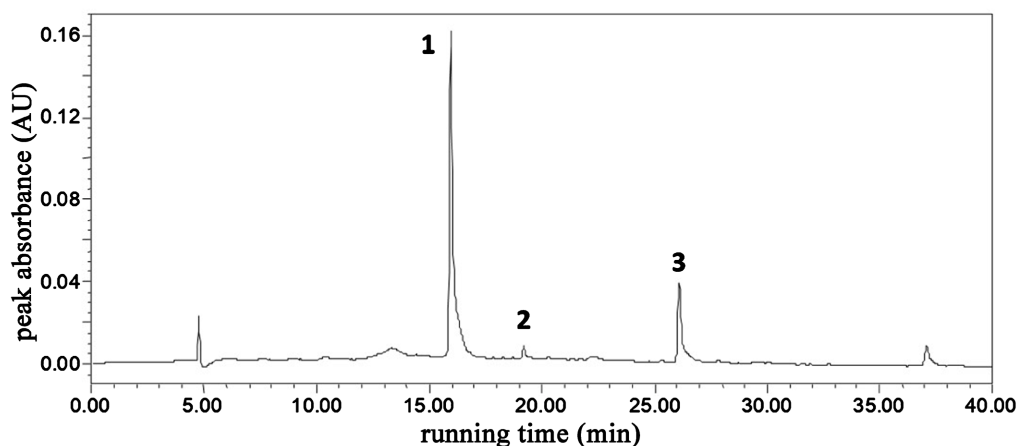

Figure 1. HPLC chromatogram of noni puree juice: 1-deacetylasperulosidic acid, 2-scandoside acid and 3-asperulosidic acid.

Table 1. Percent inhibition (mean ± standard deviation) of SARS-Cov-2 spike protein binding to ACE2.

Sample	% Inhibition
Noni juice	69.14 ± 9.27
Deacetylasperulosidic acid (500 μg/mL)	7.99 ± 0.95
Asperulosidic acid (500 μg/mL)	14.82 ± 2.15
Scandoside (500 μg/mL)	79.25 ± 1.70
Mixture of the iridoids (167 μg/mL each)*	55.48 ± 5.25

*The expected % inhibition of the mixture, determined from individual iridoid results and concentration ratios, is 32.60 ± 1.89.

The mixture of these iridoids, with each at lower concentrations, was expected to reduce spike protein-ACE2 interaction by 32.60%. But the mixture was much more active than expected, preventing binding by an average of 55.48%. Deacetylasperulosidic acid is the major phytochemical constituent of noni juice. It has been found to be responsible, to a large degree, for the antioxidant, immunomodulatory and DNA protective properties of noni juice. However, our results suggest that it has a minor role in the ability of noni juice to prevent SARS-CoV-2 binding of the ACE2 receptor. Scandoside displayed very high activity in preventing SARS-CoV-2 binding. But asperulosidic acid and scandoside are present in smaller concentrations in noni juice than those evaluated in our assay. Although it appears that all three iridoids act synergistically when combined, with an additional 22.88% reduction in spike protein binding beyond the expected levels, they do not appear to be responsible for all the inhibitory activity of noni juice.

Other phytochemicals in noni juice may also be contributing to its anti-spike protein binding activity. Scopoletin, rutin, quercetin and kaempferol are present in noni fruit and juice [32] [33]. In silico studies (computer modeling or simulation) have revealed that scopoletin and related compounds have structural properties that allow it to bind to and inhibit SARS-CoV-2 main protease (Mpro) as well as interact with ACE2, thereby interfering with spike protein-ACE2 recognition [34] [35] [36]. Rutin exhibited potent spike protein binding and anti-Mpro activity in molecular docking studies [37] [38]. Quercetin and kaempferol demonstrated high binding affinity for the spike protein-ACE2 complex, potentially perturbing effective binding [39]. Thus, it is likely that the combination of multiple phytochemicals in noni juice act synergistically to prevent the binding of SARS-CoV-2 to ACE2.

Our current experiment focused solely on binding interactions between SARS-CoV-2 spike S1 RBD and ACE2 and did not evaluate inhibition of cellular proteases. As host cell proteolytic priming of S1 RBD enhances binding affinity, inhibition of furin and serine proteases by noni juice and its phytochemical constituents may further impede the ability of SARS-CoV-2 to infect cells. Scopoletin is reported to be a potent furin inhibitor [40]. Molecular docking studies found that quercetin also inhibits furin [41]. Geniposide, an iridoid with a chemical structure very similar to deacetylasperulosidic acid and scandoside, displayed a high degree of interaction with TMPRSS2 in silico [42]. This suggests that phytochemicals in iridoids in noni juice may also limit spike protein priming.

4. Conclusion

Noni juice reduced the ability of SARS-CoV-2 to bind ACE2 *in vitro*. The iridoids in noni juice are also capable of this. But it appears that other phytochemical constituents also contribute to the level of activity observed in noni juice. Synergistic inhibition of spike protein-ACE2 interaction occurs amongst the iridoids, and further synergies can be expected with scopoletin, quercetin and rutin. These findings provide additional insight into biological activities of noni juice

that are not mediated by the immune system but may still improve resistance to SARS-CoV-2 infection.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Lai, M.M. and Cavanagh, D. (1997) The Molecular Biology of Coronaviruses. *Advances in Virus Research*, **48**, 1-100. https://doi.org/10.1016/S0065-3527(08)60286-9

[2] Tyrrell, D.A. and Bynoe, M.L. (1965) Cultivation of a Novel Type of Common-Cold Virus in Organ Cultures. *British Medical Journal*, **1**, 1467-1470. https://doi.org/10.1136/bmj.1.5448.1467

[3] Chen, B., Tian, E.K., He, B., Tian, L., Han, R., Wang, S., Xiang, Q., Zhang, S., El Arnaout, T. and Cheng, W. (2020) Overview of Lethal Human Coronaviruses. *Signal Transduction and Targeted Therapy*, **5**, 89. https://doi.org/10.1038/s41392-020-0190-2

[4] Mohanty, S.K., Satapathy, A., Naidu, M.M., Mukhopadhyay, S., Sharma, S., Barton, L.M., Stroberg, E., Duval, E.J., Pradhan, D., Tzankov, A. and Parwani, A.V. (2020) Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) and Coronavirus Disease 19 (COVID-19)—Anatomic Pathology Perspective on Current Knowledge. *Diagnostic Pathology*, **15**, 103. https://doi.org/10.1186/s13000-020-01017-8

[5] World Health Organization (2021) Coronavirus (COVID-19) Dashboard. https://COVID19.who.int

[6] Tipnis, S.R., Hooper, N.M., Hyde, R., Karran, E., Christie, G. and Turner, A.J. (2000) A Human Homolog of Angiotensin-Converting Enzyme. Cloning and Functional Expression as a Captopril-Insensitive Carboxypeptidase. *Journal of Biological Chemistry*, **275**, 33238-33243. https://doi.org/10.1074/jbc.M002615200

[7] Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., Donovan, M., Woolf, B., Robison, K., Jeyaseelan, R., Breitbart, R.E. and Acton, S. (2000) A Novel Angiotensin-Converting Enzyme-Related Carboxypeptidase (ACE2) Converts Angiotensin I to Angiotensin 1-9. *Circulation Research*, **87**, E1-E9. https://doi.org/10.1161/01.RES.87.5.e1

[8] Keidar, S., Kaplan, M. and Gamliel-Lazarovich, A. (2007) ACE2 of the Heart: From Angiotensin I to Angiotensin (1-7). *Cardiovascular Research*, **73**, 463-469. https://doi.org/10.1016/j.cardiores.2006.09.006

[9] Hamming, I., Timens, W., Bulthuis, M.L., Lely, A.T., Navis, G. and van Goor, H. (2004) Tissue Distribution of ACE2 Protein, the Functional Receptor for SARS Coronavirus. A First Step in Understanding SARS Pathogenesis. *Journal of Pathology*, **203**, 631-637. https://doi.org/10.1002/path.1570

[10] 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., Choe, H. and Farzan, M. (2003) Angiotensin-Converting Enzyme 2 Is a Functional Receptor for the SARS Coronavirus. *Nature*, **426**, 450-454. https://doi.org/10.1038/nature02145

[11] Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., Bi, Y., Ma, X., Zhan, F., Wang, L., Hu, T., Zhou, H., Hu, Z., Zhou, W., Zhao, L., Chen, J., Meng, Y., Wang, J., Lin, Y., Yuan, J., Xie, Z., Ma, J., Liu, W.J., Wang, D., Xu, W., Holmes, E.C., Gao, G.F., Wu, G., Chen, W., Shi, W. and Tan, W.

(2020) Genomic Characterisation and Epidemiology of 2019 Novel Coronavirus: Implications for Virus Origins and Receptor Binding. *The Lancet*, **395**, 565-574. https://doi.org/10.1016/S0140-6736(20)30251-8

[12] Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y. and Zhou, Q. (2020) Structural Basis for the Recognition of SARS-CoV-2 by Full-Length Human ACE2. *Science*, **367**, 1444-1448. https://doi.org/10.1126/science.abb2762

[13] Benton, D.J., Wrobel, A.G., Xu, P., Roustan, C., Martin, S.R., Rosenthal, P.B., Skehel, J.J. and Gamblin, S.J. (2020) Receptor Binding and Priming of the Spike Protein of SARS-CoV-2 for Membrane Fusion. *Nature*, **588**, 327-330. https://doi.org/10.1038/s41586-020-2772-0

[14] Bestle, D., Heindl, M.R., Limburg, H., Van Lam van T., Pilgram, O., Moulton, H., Stein, D.A., Hardes, K., Eickmann, M., Dolnik, O., Rohde, C., Klenk, H.D., Garten, W., Steinmetzer, T. and Böttcher-Friebertshäuser, E. (2020) TMPRSS2 and Furin Are Both Essential for Proteolytic Activation of SARS-CoV-2 in Human Airway Cells. *Life Science Alliance*, **3**, e202000786. https://doi.org/10.26508/lsa.202000786

[15] Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T. and Veesler, D. (2020) Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*, **181**, 281-292.e6. https://doi.org/10.1016/j.cell.2020.02.058

[16] Efferth T. and Koch, E. (2011) Complex Interactions between Phytochemicals. The Multi-Target Therapeutic Concept of Phytotherapy. *Current Drug Targets*, **12**, 122-132. https://doi.org/10.2174/138945011793591626

[17] Morton, J.F. (1992) The Ocean Going Noni, or Indian Mulberry (*Morinda citrifolia*, Rubiaceae) and Some of Its "Colorful" Relatives. *Economic Botany*, **46**, 241-256. https://doi.org/10.1007/BF02866623

[18] West, B.J., Jensen, C.J., Westendorf, J. and White, L.D. (2006) A Safety Review of Noni Fruit Juice. *Journal of Food Science*, **71**, R100-R106. https://doi.org/10.1111/j.1750-3841.2006.00164.x

[19] West, B.J., Deng, S., Isami, F., Uwaya, A. and Jensen, C.J. (2018) The Potential Health Benefits of Noni Juice: A Review of Human Intervention Studies. *Foods*, **7**, 58. https://doi.org/10.3390/foods7040058

[20] Ma, D.L., Jun, Z. and Jianhua, G. (2008) The Effect of Tahitian Noni Juice on Antioxidation and Immune Function. *Chinese Medical Research and Clinical*, **6**, 8-10.

[21] Murata, K., Abe, Y., Futamura-Masudaa, M., Uwaya, A., Isami, F. and Matsuda, H. (2014) Activation of Cell-Mediated Immunity by *Morinda citrifolia* Fruit Extract and Its Constituents. *Natural Product Communications*, **9**, 445-450. https://doi.org/10.1177/1934578X1400900401

[22] Wang, M.Y., Peng, L., Jensen, C.J., Deng, S. and West, B.J. (2013) Noni Juice Reduces Lipid Peroxidation-Derived DNA Adducts in Heavy Smokers. *Food Science and Nutrition*, **1**, 141-149. https://doi.org/10.1002/fsn3.21

[23] Sunder, J., Yasmeen, R.B.R., Kundu, A. and Jeyakumar, S. (2007) Immunomodulator Effect of *Morinda citrifolia* in Poultry. *Indian Journal of Animal Science*, **77**, 1126-1128.

[24] Brooks, V.J., Schafer, M., Sharp, P., Xu, J., Cai, J., Keuler, N.S., Godbee, R.G., Peek, S.F., Schultz, R.D., Suresh, M. and Darien, B.J. (2009) Effects of *Morinda citrifolia* (Noni) on CD4+ and CD8+ T-Cell Activation in Neonatal Calves. *The Professional Animal Scientist*, **25**, 262-265. https://doi.org/10.15232/S1080-7446(15)30716-6

[25] Brooks, V.J., De Wolfe, T.J., Paulus, T.J., Xu, J., Cai, J., Keuler, N.S., Godbee, R.G., Peek, S.F., McGuirk, S.M. and Darien, B.J. (2013) Ethnoveterinary Application of *Morinda citrifolia* Fruit Puree on a Commercial Heifer Rearing Facility with En-

demic Salmonellosis. *African Journal of Traditional, Complementary, and Alternative Medicines*, **10**, 1-8. https://doi.org/10.4314/ajtcam.v10i1.1

[26] Yang, R.B., Liu, J.X., Zhu, K.R., *et al.* (2011) Effect of Tahitian Noni Juice on Immune Function in Mice. *Journal of Chinese Medical Research*, **11**, 401-403.

[27] Nayak, S. and Mengi, S. (2010) Immunostimulant Activity of Noni (*Morinda citrifolia*) on T and B Lymphocytes. *Pharmaceutical Biology*, **48**, 724-731. https://doi.org/10.3109/13880200903264434

[28] Palu, A.K., Kim, A.H., West, B.J., Deng, S., Jensen, J. and White, L. (2008) The Effects of *Morinda citrifolia* L. (Noni) on the Immune System: Its Molecular Mechanisms of Action. *Journal of Ethnopharmacology*, **115**, 502-506. https://doi.org/10.1016/j.jep.2007.10.023

[29] European Commission (2003) Commission Decision of 5 June 2003 Authorising the Placing on the Market of "Noni Juice" (Juice of the Fruit of *Morinda citrifolia* L.) as a Novel Food Ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. *Official Journal of the European Union*, **L144**, 12.

[30] Deng, S., West, B., Palu, A. and Jensen, J. (2011) Determination and Comparative Analysis of Major Iridoids in Different Parts and Cultivation Sources of *Morinda citrifolia*. *Phytochemical Analysis*, **22**, 26-30. https://doi.org/10.1002/pca.1246

[31] West, B.J., Deng, S. and Jensen, C.J. (2011) Nutrient and Phytochemical Analyses of Processed Noni Puree. *Food Research International*, **44**, 2295-2301. https://doi.org/10.1016/j.foodres.2010.09.038

[32] Deng, S., Palu, A.K., West, B.J., Su, C.X., Zhou, B.N. and Jensen, J.C. (2007) Lipoxygenase Inhibitory Constituents of the Fruits of Noni (*Morinda citrifolia*) Collected in Tahiti. *Journal of Natural Products*, **70**, 859-862. https://doi.org/10.1021/np0605539

[33] Deng, S., West, B.J. and Jensen, C.J. (2010) Simultaneous Characterization and Quantitation of Flavonol Glycosides and Aglycones in Noni Leaves Using a Validated HPLC-UV/MS Method. *Food Chemistry*, **111**, 526-529. https://doi.org/10.1016/j.foodchem.2008.04.021

[34] Abdelmohsen, U.R., Albohy, A., Abdulrazik, B.S., Bayoumi, S.A.L., Malak, L.G., Khallaf, I.S.A., Bringmann, G. and Salwa, F.F. (2021) Natural Coumarins as Potential Anti-SARS-CoV-2 Agents Supported by Docking Analysis. *RSC Advances*, **11**, 16970-16979. https://doi.org/10.1039/D1RA01989A

[35] Ikanovic, T., Sehercehajic, E., Saric, B., Tomic, N. and Hadziselimovic, R. (2021) *In Silico* Analysis of Scopoletin Interaction with Potential SARS-CoV-2 Target. In: Karabegović, I., Ed., *New Technologies, Development and Application IV. NT* 2021, Lecture Notes in Networks and Systems Vol. 233, Springer, Cham, 897-903. https://doi.org/10.1007/978-3-030-75275-0_99

[36] Khan, J., Sakib, S.A., Mahmud, S., Khan, Z., Islam, M.N., Sakib, M.A., Emran, T.B. and Simal-Gandara, J. (2021) Identification of Potential Phytochemicals from *Citrus limon* against Main Protease of SARS-CoV-2: Molecular Docking, Molecular Dynamic Simulations and Quantum Computations. *Journal of Biomolecular Structure and Dynamics*. https://doi.org/10.1080/07391102.2021.1947893

[37] Rahman, F., Tabrez, S., Ali, R., Alqahtani, A.S., Ahmed, M.Z. and Rub, A. (2021) Molecular Docking Analysis of Rutin Reveals Possible Inhibition of SARS-CoV-2 Vital Proteins. *Journal of Traditional and Complementary Medicine*, **11**, 173-179. https://doi.org/10.1016/j.jtcme.2021.01.006

[38] Rizzuti, B., Grande, F., Conforti, F., Jimenez-Alesanco, A., Ceballos-Laita, L., Ortega-Alarcon, D., Vega, S., Reyburn, H.T., Abian, O. and Velazquez-Campoy, A.

(2021) Rutin Is a Low Micromolar Inhibitor of SARS-CoV-2 Main Protease 3CLpro: Implications for Drug Design of Quercetin Analogs. *Biomedicines*, **9**, 375. https://doi.org/10.3390/biomedicines9040375

[39] Pandey, P., Rane, J.S., Chatterjee, A., Kumar, A., Khan, R., Prakash, A. and Ray, S. (2020) Targeting SARS-CoV-2 Spike Protein of COVID-19 with Naturally Occurring Phytochemicals: An *in Silico* Study for Drug Development. *Journal of Biomolecular Structure and Dynamics*, **39**, 6306-6316. https://doi.org/10.1080/07391102.2020.1796811

[40] De, U.C., Mishra, P., Pal, P.R., Dinda, B. and Basak, A. (2012) Non-Peptide Inhibitors of Proprotein Convertase Subtilisin Kexins (PCSKs): An Overall Review of Existing and New Data. *Colloquium Series on Protein Activation and Cancer*, **1**, 1-76. https://doi.org/10.4199/C00066ED1V01Y201209PAC003

[41] Milanovica, Z.B., Marko R. Antonijevic, M.R., Amicc, A.D., Avdovicb, E.H., Dimic, D.S., Dejan A. Milenkovic, D.A. and Markovic, Z.S. (2021) Inhibitory Activity of Quercetin, Its Metabolite, and Standard Antiviral Drugs towards Enzymes Essential for SARS-CoV-2: The Role of Acid-Base Equilibria. *RSC Advances*, **11**, 2838-2847. https://doi.org/10.1039/D0RA09632F

[42] Rahman, N., Basharat, Z., Yousuf, M., Castaldo, G., Rastrelli, L. and Khan, H. (2020) Virtual Screening of Natural Products against Type II Transmembrane Serine Protease (TMPRSS2), the Priming Agent of Coronavirus 2 (SARS-CoV-2). *Molecules*, **25**, 2271. https://doi.org/10.3390/molecules25102271