Review Article

Grapefruit Seed Extracts' Antibacterial and Antiviral Activity: Anti-Severe Acute Respiratory Syndrome Coronavirus 2 Impact

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Abstract

The Corona pandemic has affected the entire world. It caused a fatal respiratory illness and threatened public safety and human health. Scientists scrambled to detect an operative drug to counter the SARS-CoV-2 (severe acute respiratory syndrome Coronavirus 2). The hunt for a nutritional supplement to treat the Coronavirus has occupied a large area of scientific inquiry. Grapefruit (GF) (*Rutaceae* family) is a subtropical fruit, it has potent bioactive properties. Natural grapefruit seed extract (GFSE) has been revealed to exhibit beneficial therapeutic effects. The purpose of this review was to compile published research studies demonstrating antibacterial, antifungal, and antiviral activities of GFSE and its bioactive components, besides their ability to inhibit SARS-CoV-2 activity. The GFSE has a high growth inhibition against gram-positive bacteria (*Enterococcus* spp. and *Staphylococcus* spp) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Besides, it showed an inhibition zone against Multidrug-resistant (MDR) bacteria. The antiviral activity of GFSE nasal spray against the new Coronavirus has been recognized. The anti-Coronavirus capabilities of GFSE's main components, such as naringenin, resveratrol, limonoids, and hesperidin, have also been verified. In conclusion, additional *in vivo* and clinical research studies are warranted to endorse the impact of GFSE (spray or syrup) against SARS-CoV-2.

Keywords: Grapefruit seed extract, SARS-CoV-2, Antibacterial, Antiviral

Introduction

Coronaviruses are a diverse group of viruses that cause mild to severe respiratory infections in humans; they have caused fatal respiratory illnesses and threatened public safety [1]. The SARS-CoV-2 has impacted global health systems that have never been witnessed before [2]. Finding effective treatments and vaccines for SARS-CoV-2 is a significant challenge for humankind. Numerous studies are underway to ascertain potential treatments for SARS-CoV-2 to diminish the pandemic [3]. Natural remedies can provide a safe and low-cost foundation to produce new effective medicines to aid in treating SARS-CoV-2 while reducing negative consequences [4, 5].

Grapefruit (GF) *Rutaceae* family (*Citrus paradisi*) is a subtropical fruit [6]. It is the second most important member of the *Citrus* genus worldwide [7]. In several countries, GF has been used in traditional therapy as an antifungal, antibacterial, antimicrobial, antiviral, and anti-inflammatory agent. The effects of GF for cancer prevention, cleansing, detoxification, and cellular regeneration were proven [8]. Moreover, grapefruit seed extract (GFSE) has a potential antiviral substance [9, 10]. Citrus seed extract or GFSE is a liquid obtained from the pulp, seeds, and white film leftover after grapefruit juice is pressed. This fruit is packed with valuable nutrients such as phytochemicals and fiber pectin. It has a pink and red color, which indicates a high content of antioxidants such as lycopene [11]. GFSE is rich in ascorbic acid, tocopherols, flavonoids, limonoids, citric acid, sterols, and many biochemical compounds [9]. It is mainly flavonoids and limonoids, such as narirutin, limonin, naringin or naringenin, hesperidin, and resveratrol, that are found in grapefruit seed [10, 12]. These substances contribute to the antioxidant power of GFSE which has been linked greatly to the prevention of chronic diseases such as diabetes, heart disease, and malignancies [13]. Consumption of GF had been also proven to aid weight loss and enhance lipid metabolism [14]. In folk medicine, GFSE is used to cure urinary tract infections, gastrointestinal disorders, and ulcers [15].

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Traditionally, grapefruit residues were given to cattle and pigs, and these animals were found to be less infected [16]. GFSE was developed as a non-toxic antimicrobial spray for fruits and vegetables [17]. In addition, GFSE has an antibacterial effect against a wide range of foodborne infections [18]. Following its approval as a safe supplement, GFSE was promoted as a dietary treatment for alleviating human bacterial, viral, and fungal infections [18, 19].

This review aimed to demonstrate evidence of the probable antiviral, antifungal, and antimicrobial effects of GFSE and any of its active constituents; in addition to compiling studies on their anti-SARS-CoV-2 activity.

Antimicrobial Effect of GFSE against Bacteria and Fungi

Multidrug-resistant (MDR) bacteria are bacteria that are considered resistant to antibiotics such as penicillin class, cephalosporin class, and methicillin-resistant bacteria. Generally, if patients have MDR bacteria-infected, their health tends to deteriorate with high mortality rate than those infected by sensitive bacteria [20]. The search for new naturally occurring substances that can target MDR bacteria is of increasing importance. The antibacterial effects of GFSE have been proven to show an inhibition zone against MDR bacteria, which was confirmed by a disk diffusion test *via* its large amount of the flavonoids naringin, also the acidic and pH properties of GFSE [12].

An early investigation published in 1990 determined that commercially available GFSE was effective against 794 bacteria types and 93 fungus strains. It examined the antibacterial and antifungal activity of over-the-counter GFSEs (free of triclosan, benzethonium chloride, and benzalkonium chloride). This article raised two open-label trials in people having gastrointestinal issues and severe atopic eczema. In the first experiment, 10 patients took GFSE (200 ml of 0.05% aqueous solution) two times a day for one month. The study revealed no significant alterations in the fecal microflora test; besides, only two out of the 10 patients saw improvement in the gastrointestinal tract symptoms. In the second trial, 15 patients received 50 mg GFSE capsules three times per day for four weeks. The data showed that GSFE exerted high antibiotic activity against three pathogenic gut microbiota (*Candida*, *Geotrichum*, and *hemolytic E. coli*); and little activity against three others (*Lactobacillus sp*., *Staph aureus*, and *aerobic spore formers*). It was found that GSFE did not exhibit action against *Bifidobacteria* and *Klebsiella* species. Subjectively, all patients stated that their gastrointestinal problems improved without side effects [21].

In 2001, research was conducted to measure the effect of GFSE on the yeast-like strain *Candida albicans* growth. The study included 200 *Candida albicans* strains; Five were isolated from patients having various candidiasis symptoms of ontogenesis, and 12 were isolated from patients with dermatophytes and molds. Candida susceptibility was tested using a serial dilution method. GFSE solution of 33% appeared to have significant antifungal effectiveness against yeast-like strains but little efficacy against dermatophytes and fungi [22]. In 2002, GFSE was proved to be efficacious against 67 biotypes of gram-negative and gram-positive bacteria [12].

Cvetnić and Vladimir-Knezevic [9] examined alcoholic GFSE against 20 strains of bacteria and 10 yeast using agar assay and broth dilution test. In the broth dilution test, the extract was effective against all microorganisms. However, it was efficacious only against yeast and gram-positive bacteria on agar plates test at concentrations ranging from 4.13% to 16.5% (m/V).

In another study, under electron microscopy, GFSE was found to damage bacterial cell membranes. This is considered the bactericidal technique of quaternary ammonium compounds in GFSE [23]. Moreover, GFSE has been established *in vitro* to be effective against the *Borrelia burgdorferi sensu lato* bacteria that cause Lyme borreliosis disease. The anti-bacterial effect of GFSE was shown to be due to its active compounds hesperidin, naringenin, and other citrus flavones [24].

The antibacterial efficacy of aqueous GFSE against *Proteus vulgaris*, *Staphylococcus aureus*, *Candida albicans*, and Klebsiella *pneumoniae* was high, whereas the ethanolic GFSE had low antimicrobial action [25]. In another study, 70% ethanol was used to extract grapefruit seeds and then used to produce a 33% (w/v) extract for microbiological testing. The extract was verified against 20 different bacteria and yeasts *via* the agar diffusion method. It displayed the highest antibacterial activity against *Salmonella enteritidis* [15].

Choi *et al*. [26] assessed the antibacterial effect of GFSE against diversified sets of foodborne pathogens such as *Pseudomonas aeruginosa*, *Candida albicans*, and *E. coli*. The results revealed that the concentrations of 0.1 and 0.2 % of GFSE showed capability against these microorganisms. The antimicrobial properties of GFSE are explained through its effects on prohibiting the active transport and glycolysis, which induced microbial growth inactivation, and disruption of bacterial cell membranes [27].

The inhibitory activity of GFSE spray-on *Salmonella infantis* and *E. coli* were also examined. GFSEx1000 inactivated *Salmonella infantis* and *E. coli* within five seconds. The study suggested GFSE as a prospective novel disinfectant against bacteria [19].

In a disc diffusion test, GFSE demonstrated antibacterial activity against multidrug resistance bacteria (vancomycin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*). In the micro diluted minimum

inhibitory concentration (MIC) study, GFSE showed antibacterial activity even at the lowest dose [28].

In their study, Ignacio and Thai [29] investigated the antifungal activity of natural inhibitors of GFSE, tea tree oil, garlic, and probiotic supernatant compared to miconazole nitrate salt for *Candida albicans*. GFSE showed the most anti-Candida effectiveness among the natural inhibitors tested at a concentration of 100-120 µg mL^{-1}, decreasing the growth rate to below 0.02 hr^{-1}.

Recently, the antifungal properties of GFSE treatment were investigated on the cell structures of fungi spores using a transmission electron microscope. The GFSE presented antifungal activity by damaging the cell membrane and thick cell of the spore and losing the spore contents [18].

In vitro and *in vivo*, researchers investigated the anti-helicobacter pylori properties of GFSE alone and combined with the novel *Lactobacillus plantarum* (pH 3A) and monolaurin *in vitro*. According to their results, monolaurin and GFSE inhibited the growth of *H. pylori* at a minimum inhibitory concentration (MIC) of 62.5 ppm. *In vitro*, *L. plantalum* pH 3A reduced *Helicobacter pylori* infection, demonstrating a synergistic effect with monolaurin and GFSE, but *in vivo*, Only *L. plantalum* pH 3A inhibited *helicobacter pylori* infection [30].

Antiviral Activity of GFSE

Chinsembu *et al*. [31] reported that 6,7-dihydroxy-bergamottin of GF extract enhances the bioavailability of HIV protease inhibitor through the P450 iso-enzyme 3A4 in the gut and liver. Avian influenza virus (AIV) is a highly pathogenic virus. Chlorine-based, alkaline, or cationic disinfectants are the recommended antiseptics against AIV. However, these agents may not be suitable and can easily harm human and animals skin and mucous membranes [32]. GFSE is a potential novel safe virus disinfectant. In a previous study, GFSE showed virucidal activity against Newcastle disease virus (NDV) and AIV [33].

The GFSE's active constituents against viruses are yet unidentified; however, aglicons such as flavonoid glycoside, limonoids, quercetin, naringin, hesperidin, apigenin, kaempferol, and unsaturated or saturated fatty acids are probable constituents of GFSE that have antiviral activities [12, 17].

In another study, the results revealed that GFSE contains great quantities of antiviral compounds such as polyphenolic, deacetylnomilin, bioflavonoids, obacunone, deacetylnomilinic acid, nomilin, nomilinic acid, and 17-beta-D-glucopyranosides of limonin [34].

The virucidal efficacy of disinfectant spray containing 0.25% GFSE was assessed against AIV. The spray was diluted several times then added to AIV suspension (2.5 mL). After 30 minutes, inactivated fetal bovine serum (2.5 ml of 10%) was added to neutralize the solutions. An amount of 0.2 ml/ of each diluent was allantoic cavity injected in five chickens (10 days old). The AIV viability of the embryos was evaluated after five days of incubation through a hemagglutination titer. In their studies, they found that the AIV titer of the pathogen control was over 6.1 log10 EID50/mL, without causing embryonic damage. These results support the idea that the disinfectant spray containing GFSE has operative virucidal activity against AIV [35].

Komura *et al*. [19] examined the inhibitory activity of GFSE spray-on infectious AIV, bursal disease virus, and Newcastle disease virus. Diluted GFSE (100, 500, and 1000 in distilled water) proved to be effective. GFSEx100 reduced both bird flu and Newcastle disease virus titers. The bursal disease virus is highly resistant to GFSE. The study suggested GFSE as a prospect novel antiseptic against viruses, proven by its short contact time, effectiveness in contaminated tissue, and spray form. Compared to other disinfectants, GFSE has several advantages. GFSE is neither volatile nor flammable compared to alcoholic disinfectants. GFSE is also non-toxic to human and animal skin and mucous membranes, as opposed to aldehyde, alkali, and phenol-dependent disinfectants. Additionally, GFSE is of low odor, non-corrosive, and will not stain clothing.

Antiviral Effect of GFSE against SARS-CoV-2

The human enteric viruses such as hepatitis A virus (strain HM175) and virus surrogates (murine norovirus, feline calicivirus, and bacteriophage MS2) were significantly reduced when treated at room temperature with GFSE [36]. A recently published study found that six bioactive compounds in GFSE (naringenin, narirutin, naringin, citric acid, ascorbic acid, and limonin) inhibit SARS-CoV-2's main protease Mpro compared to acetoside, remdesivir, and gallic acid [10].

Scientists have found that the nose may be the main gateway and the primary site of replication for SARS-CoV-2; in addition, the droplet methods of transportation have also been found to be effective. Researchers hypothesized that a commercially ready-to-use nasal spray containing GFSE and xylitol, namely Xlear Nasal Spray, may be utilized as an adjuvant remedy for Covid-19. In a newly published study, three symptomatic Covid-19 patients at mild to moderate risk were received intranasal combination therapy as a supplement to current medications. These three patients were given two nasal sprays every six hours. When repeated intranasal swab tests using PCR, they all showed rapid clinical recovery and a shorter time to negative. No safety issues were identified during the treatment course. These results support the idea that the ingredients have a major antiviral activity [2].

The viral damaging effect of Xlear nasal drops on SARS-CoV-2 was also investigated *in vitro*. Two experiments were implemented to test the effectiveness of Xlear (experiment

one) and replicable drug (experiment two) for inactivating SARS-CoV-2. When tested against SARS-CoV-2, Xlear containing GFSE 0.2% was the only compound that reduced the infectious virus to imperceptible levels [37].

Magurano *et al*. [38] investigated the potential effect of GFSE and their primary components belonging to the limonoids class to combat SARS-CoV-2 infection using two dual approaches virucidal-antioxidant activity. GCSEs exhibited substantial antioxidant, virucidal, and cytoprotective effects. Nomilin, obacunone, and limenin functioned successfully against SARS-CoV-2. The IC50 ranged between 15 and 31 µg/mL. Limonoids in GFSE could directly target the SARS-CoV-2 and protect the hosting cell from oxidative damage.

Antiviral Effect of Some GFSE Active Constituents against SARS-Cov-2

The antiviral effects of the grapefruit flavonoid naringenin have been examined against several viruses, including dengue, Zika, hepatitis C, Semliki Forest, chikungunya, yellow fever, herpes simplex 1 and 2, and human immunodeficiency virus. *In vitro* studies have demonstrated naringenin's effectiveness as an antiviral pre- and post-infection treatment [39-41]. As with many natural chemicals, naringenin has been widely studied *in vitro*, but its use as a viral infection model has been limited *in vivo* [41-43]. Once SARS-CoV-2 infects the host cell, proteolytic processing releases polypeptides from polyproteins. In the proteolytic process, 3-chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro) are involved. Several nonstructural proteins that are required for viral replicating are released by the 3CLpro when the polyprotein is bound [44]. A potential treatment for Covid-19 may include 3CLpro inhibitors because it is so critical in the viral cycle of coronaviruses. A research study showed that flavonoids have been evident to suppress the 3CL protease in SARS [45]. Although naringenin was not one of the flavonoids investigated, an in silico analysis found that it can inhibit SARS-CoV-2 3CLpro [46].

According to another study, SARS-CoV-1 and SARS-CoV-2 exhibit 99.02% genetic similarity to 3CL, with only 12 punctual alterations [44], implying that naringenin and many other flavonoids may inhibit 3CL [47]. Another possible method is to inhibit two ion channels (TPC1 and TPC2) [48]. TPC1 and TPC2 inhibition reduce intracellular traffic, MERS-CoV infection, and viral multiplication [49]. Naringenin is a hydrophilic molecule that has a greater affinity for the cytoplasmic membrane, causing naringenin to accumulate within the cell [50]. As a result, this affinity is anticipated to increase intracellular signaling as well as regulation of TPC1 and TPC2 [51].

One of the many active constituents of GFSE is resveratrol. It can also be found among other fruits such as blueberries, grapes, and cranberries [52]. Resveratrol exerts significant antiviral properties on RNA viruses including rhinovirus, influenza, Zika virus, rotavirus, MERS-CoV, and some DNA viruses such as poxvirus and polyomavirus [53-56]. Pasquereau *et al*. [57] observed in their study that resveratrol demonstrated an antiviral response against SARS- CoV-2 and HCoV229E (a coronavirus family member) with reduced cytotoxicity.

Limonoids are tetracyclic triterpenoids widely found in the citrus genus. The seeds of grapefruit contain the greatest amounts of limonoids, with limonin and nomilin being the two most potent constituents [58]. Another study revealed the probable antiviral efficacy of limonoids based on molecular docking and silico ADMET research to inhibit five types of SARS-CoV-2 protein [59]. In a recent study, 14 triterpenoids compounds were tested for their capacity to inhibit SARS-CoV-2 target proteins. Limonoids were found to suppress the reproduction and expansion of the SARS-CoV-2 primary protease (Mpro) [60].

The main functional flavanone identified in flavonoids is hesperidin (3,5,7-trihydroflavanone 7-rhamnoglucoside). It is extracted from citrus fruits such as grapefruit and lemons [61]. Hesperidin was determined to bind to two cellular proteins, angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), which are both required for SARS-CoV-2 to enter the cell. Hesperidin prevented SARS-CoV-2 infection by blocking the S-protein from binding to the cell surface receptor ACE2 and reducing the expression of ACE2 and TMPRSS2 [62].

Conclusion

GFSE's antibacterial, antifungal, and antiviral properties have been established in several early and current research studies. The role of GFSE nasal drops in the prompt elimination of SARS-CoV-2, which resulted in a fast negative PCR test, has also been evident in recent clinical research. Naringenin, resveratrol, and hesperidin have also been demonstrated to have anti-coronavirus properties. In light of GFSE's antiviral activity, further investigation is warranted to provide stronger evidence on the capability of GFSE to serve as a supportive therapy against Covid-19.

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