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Organic Fresh literature review

A literature research document has been composed evaluating the antimicrobial properties and mode of action of the citrus bioflavonoid extract with additional focus on ascorbic acid, rutin, clove oil and *Yucca schidigera* to highlight their antimicrobial activity and potential in applications on the skin and sanitization.

Glycerine, water, ethanol, octanoic acid, ethyl lactate (can be broken down to ethanol and acetic acid) are compounds typically used in formulation and preservation and will not be discussed in detail.

Ascorbic acid (Vitamin C)

Ascorbic acid (Vit C) is an essential vitamin that is not synthesised in the human body. Vit C is essential to membrane structures as it is involved in collagen biosynthesis and repair, deficiencies subsequently result in the periodontal disease observed in scurvy. It has been shown to have a broad role in the human body ranging from an immunomodulatory role, antioxidant capabilities, production of adrenal steroids and catecholamines, metabolism of amino acids and cholesterol, and iron absorption (Johnston et al., 2006, 2007). It also plays a role to combat pathogens through its innate antimicrobial potential towards viruses, fungi as well as bacteria where in the latter it also serves as an adjunct treatment option for the combat of human infections (Mousavi et al., 2019). Part of the mode of antimicrobial activity may be attributed to acidification of the environment, more specific actions related to disruption of the cellular membrane and enzymes (Panda and Arul, 2018), which arise from higher levels of aerobic metabolism of Vit C cause increased oxidative stress (Kallio et al., 2012).

Vit C has been known as early as the 1930's for its antimicrobial properties where it was shown to inhibit *Mycobacterium tuberculosis,* the pathogen causing tuberculosis (Boissevain, C.H. Spillane Jr, 1937; McConkey and Smith, 1933; Myrvik and Volk, 1954; Sirsi, 1952), together with other know antibacterial activities towards the likes of group A haemolytic streptococci (Slade and Knox, 1950), *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Golonka et al., 2017; Kallio et al., 2012) and methicillin-resistant strains of the *S. aureus* (MRSA) (Ali Mirani et al., 2018).

Antimicrobial activity toward other bacterial species is not as clear cut with variability observed depending on the organism and the strain that was targeted. Vit C is able to inhibit *Escherichia coli* O157:H7 strain at 0.2-0.4% as well as synergistically with 0.2% lactic acid *in vitro* in Brain Heart Infusion (BHI) broth and in carrot juice as a food model. This has led to the suggestion that these compounds may have potential as preservatives to inhibit the growth of *E. coli* in food (Tajkarimi and Ibrahim, 2011; Verghese et al., 2017). However, Vit C only had a marginal effect on the growth of *E. coli* ATTC 11775 strain (Kallio et al., 2012). Similar ambiguity has been observed towards *Enterococcus faecalis* where low concentrations of 0.15mg/mL Vit C showed growth inhibition (Golonka et al., 2017) while higher concentrations of 0.22 mg/mL had no effect toward *E. faecalis* in another study(Mehmeti et al., 2013).

In the context of the present document it is noteworthy that Vit C had a synergistic effect on the antimicrobial activity of the flavonoid quercetin (black and green tea, apples, red onions and capers are rich sources) towards *E. coli* ATTC 11775 while this combination has no influence on the probiotic organism *Lactobacillus plantarum* (Kallio et al., 2012). Vit C has a stabilizing effect on the flavonoid by preventing oxidation (Nishinaga et al., 1979; Rajananda and Brown, 1981; Vrijsen et al., 1988), thereby enhance the antimicrobial activity of quercetin (Kallio et al., 2012). Moreover, Vit C together with sucrose and citric acid enhances the absorption and bioavailability of the flavonoids, particularly the catechins (Ferruzzi et al., 2009; Peters et al., 2010). Synergistic antibacterial activity of Vit C combined with linalool and copper towards *Campylobacter jejuni*, *Salmonella enterica* and *Vibrio fluvialis* resulting in severe membrane damage in these pathogens which cause spoilage in the poultry industry (Ghosh et al., 2019). The synergistic interaction of Vit C with other agents has been observed including combinations with: the plant polyphenol epigallocatechin gallate directed even against multidrug-resistant bacterial species MRSA (Hatano et al., 2008), pomegranate rind extracts (McCarrell et al., 2008) and white tea (Holloway et al., 2011) against *S. aureus*; deferoxamine against Gram-positive cocci *S. aureus* and *S. epidermidis*, as well as against Gram-negative *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* (van Asbeck et al., 1983).

Toward the Gram-negative human bacterial pathogens Vit C inhibits *Helicobacter pylori* growth under microaerobic conditions, however, when grown in an aerobic environment the opposite is observed and Vit C enhanced *H. pylori* survival. This conflicting activity can be attributed to antioxidant properties of Vit C, protecting microaerophilic bacteria against toxic effects of reactive oxygen species in an aerobic environment (Tabak et al., 2003). The hypothesis is supported by positive effects observed in humans clinical trials which arose from a low oxygen environment in the gastric intestinal tracts together with Vit C application (Mousavi et al., 2019). Similarly, Vit C application in the microaerobic environment of a broiler-digestive model including the crop compartment, the proventriculus, and the intestine resulted in activity towards *S. enterica* in the crop compartment, while combination with curcumin and boric acid were needed to inhibit the pathogen in the rest of the digestive tract (Hernandez-Patlan et al., 2018).

Vit C has shown antiviral activity towards a range of viruses inhibiting replication of herpes simplex virus type 1 (HSV-1), poliovirus type 1 (Furuya et al., 2008), influenza virus type A (Furuya et al., 2008; Kim et al., 2016) and also inactivated the rabies virus *in vitro* (Madhusudana et al., 2004). Neither Vit C or quercetin showed antiviral activity on their own, however, a combination of Vit C and the flavones quercetin, luteolin and 3-methylquercetin synergistically inhibit the poliovirus, where these combinations have been shown to inhibit viral replication and reduce plaque formation (Vrijsen et al., 1988). Moreover, a combination of red ginseng and Vit C increased the response from immune cells and concomitantly decrease inflammation, while also supressing the progress of viral lytic cycle (Kim et al., 2016).

Citrus extracts

Citrus extracts contain flavonoid compounds which have a range of reported health promoting properties that include antioxidant, protective effect against cardio disease and cancer as well as anti-inflammatory properties (Barreca et al., 2017; Ikawati et al., 2019). The citrus bioflavonoids have proven antimicrobial activity towards *S. aureus*, *P. aeruginosa* and *E. coli* killing 99.9% of these pathogens at dilutions of up to 300x after five minutes contact time when tested in accordance with Section 5.2 of SANS 1615-2011 (SABS Commercial SOC Ltd test report 2425/15-0988/J453835). Valencia orange oil has shown noteworthy activity towards MRSA (Muthaiyan et al., 2012). These orange oil extracts contain polyphenols which are a broad range of plant secondary metabolites, including flavan-3-ols, flavonols, tannins and phenolic acids, display broad antimicrobial activity towards bacteria, fungi and viruses (Barreca

et al., 2017; Daglia, 2012). Antibacterial activity of flavonoids, particularly the catechins (flavonols), has been shown to have greater activity towards Gram-positive than Gram negative bacteria (Cushnie and Lamb, 2005; Cushnie et al., 2008). However, notable activity towards Gram-negative bacteria by citrus bioflavonoids has also been reported (Mandalari et al., 2007). Over and above the antimicrobial activity of the catechins, found in citrus as well as a range of other plant extracts (Tripoli et al., 2007) (tea, apples peels, apricots, cherries, peaches, blackberries, black grapes, strawberries, blueberries and raspberries) (Cushnie et al., 2008); noteworthy antimicrobial activity has been attributed to the citrus flavonoids hesperetin and naringenin. The naringenin derivative 7-O-butylnaringenin has shown an increased antimicrobial effect relative to the latter parent compound (Moon et al., 2013). Similarly, hesperidin and hesperetin-7-O-glucoside exhibit a higher inhibitory effect than hesperetin (Lee et al., 2012). It is noteworthy that the latter flavonol derivatives with increased antimicrobial activity were obtained by enzymatic bioconversion, similarly, the antimicrobial potency of citrus flavonoids increased after enzymatic deglycosylation (Mandalari et al., 2007). These approaches could be considered in future preparations.

The potent activity of citrus flavonoids is found in their multiple modes of action, as summarised in Fig. 1 (Górniak et al., 2019). The flavonoids cause disruption of the bacterial membrane which is responsible for osmoregulation, respiration and transport processes, biosynthesis and cross-linking of peptidoglycan, as well as biosynthesis of lipids. The bacterial membrane disruption by flavonoids also has the added benefit of reducing the ability of bacteria to secrete toxins (Lee et al., 2011; Shah et al., 2008), thereby decreasing their toxicity and prevent food poisoning (Sugita-Konishi et al., 1999). Flavonoids can further inhibit cell envelope synthesis through inhibiting fatty acid synthase (FAS) and the synthesis of Ala–Ala dipeptide needed for peptidoglycan synthesis. Other intracellular targets include inhibition of nucleic acid synthesis through helicase and gyrase/topoisomerase inhibition, and inhibition of ATP synthase which will impact respiration. Flavonoids can also reverse antimicrobial resistance obtained through efflux pumps by inhibiting the activity of the pumps. The virulence of bacteria can further be lessened by flavonoids (particularly catechins) neutralizing toxins released by the bacteria (Ahmed et al., 2016; Choi et al., 2007; Delehanty et al., 2007), causing bacterial aggregation (Cushnie et al., 2007) and by disrupting quorum sensing (Vikram et al., 2010) which impairs biofilm formation (Awolola et al., 2014). The latter attributes of flavonoids are of significant value in the sterilization of food packing and handling facilities where bacterial toxicity and biofilm formation are a significant threat.

Flavonoid containing formulations have been shown to have a beneficial effect toward a broad array of skin conditions due to their antioxidant, antimicrobial and immunomodulatory effects (Lanzendorfer et al., 1999, 2002). Valencia orange oil displayed no toxicity when tested on keratinocyte (skin) cell lines (Muthaiyan et al., 2012). This result is surprising to some in literature (Suroowan and Mahomoodally, 2017) as flavonoids such as fisetin and quercetin have a high to moderate potential to have sensitizing properties (to cause an allergic reaction based on chemical structure), whereas luteolin and rutin have been found to be nonallergenic even though they are expected to be sensitizers (Schmalle et al., 1986). Naringenin, a flavonoid in citrus fruits, has been reported to exhibit anti-inflammatory activities in macrophages *in vitro* by down-regulating the formation of various cytokines such as IL-2, TNF-α, and to have inhibitory effects on the activation and proliferation of T cells and thus being able to alleviate symptoms of contact hypersensitivity (Fang et al., 2010). A similar effect has been demonstrated for the widely distributed flavone luteolin (Kempuraj et al., 2008). Flavonoid compounds can therefore be used as treatment of allergic inflammation, autoimmune diseases and skin cancer, although this still has to be demonstrated in extensive clinical trials. Recent studies have demonstrated that certain flavonoids, in particular quercetin, kaempferol, and myricetin (all flavonoids found in citrus fruits), are able to inhibit collagenases, which are

highly induced in inflamed and photoaged skin. The collagenases break down the dermal matrix proteins such as collagen and elastin; this possibly leads to the prolonged skin damage and wrinkle formation. The citrus flavonoid neohesperidin has been shown to improve microcirculation in the skin and purported to combat long-term ageing (Hu, 2014; Hu and Lan, 2019; Potin et al., 2014) including positive effects observed in clinical trials (Miastkowska and Ikora, 2018). Thus, it appears that topically applied flavonoids may protect against collagen degradation by collagenase inhibition and/or down-regulation of collagenase induction as well as increase skin elasticity and microcirculation (Miastkowska and Ikora, 2018). These activities of flavonoids may contribute, at least in part, to reduced destruction of the dermal tissue and reduced damage of inflamed or photo-aged skin (Potin et al., 2014; Sin and Kim, 2005).

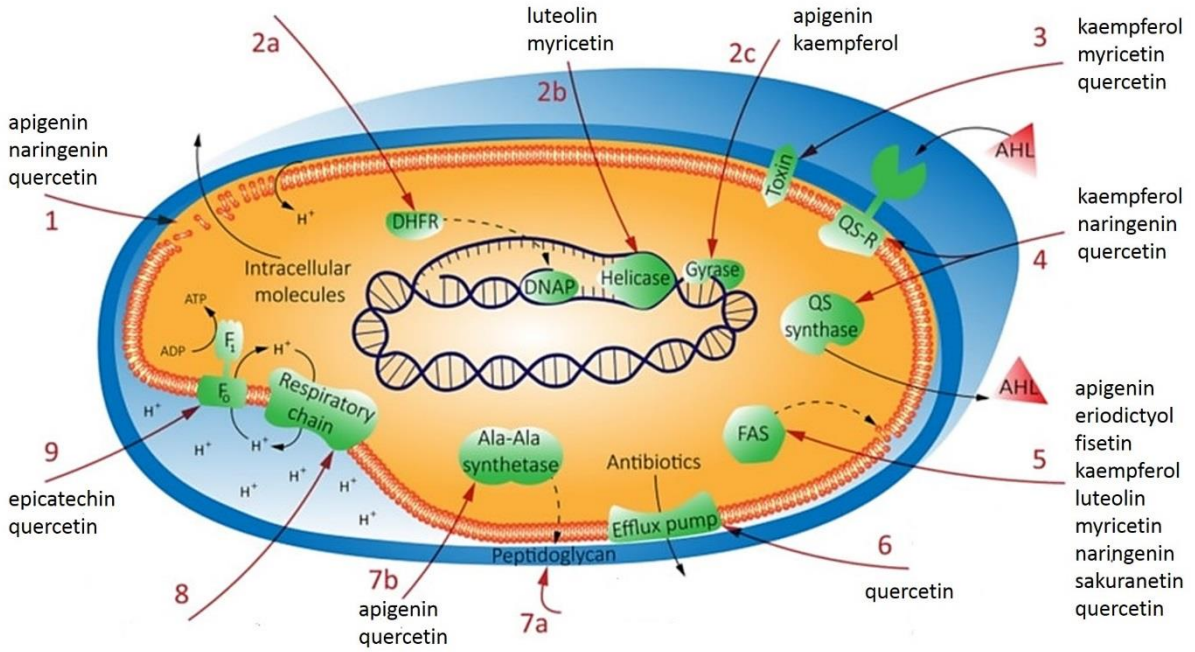

Figure 1: Summary of the modes of action of citrus flavonoids: (1) membrane disruption, inhibit nucleic acid synthesis through (2b) helicase inhibition and (2c) gyrase/topoisomerase, affects virulence of bacteria e.g. (3) toxins and (4) disrupting quorum sensing which impairs biofilm formation, inhibit cell envelope synthesis through (5) inhibiting fatty acid synthase (FAS) and (7b) inhibition of Ala–Ala dipeptide synthesis needed for peptidoglycan synthesis. (6) Flavonoids can inhibit efflux pumps which can lead to reversing antimicrobial resistance and (9) inhibit ATP synthase which will impact respiration. This figure is an adaptation from Górniak et. al. (2019).

Polyphenolic compounds which include flavinoids have been shown to possess antiviral activity toward a range of viruses including RNA viruses (norovirus surrogates, rotavirus, enteroviruses, influenza A and B viruses, respiratory syncytial virus, human immunodeficiency virus-1, hepatitis Cvirus, Japanese encephalitis virus, dengue virus, West Nile virus, and Zika virus) and DNA viruses (hepatitis B virus, herpes simplex virus, and varicella-zoster virus) as stated in the cited review (Seo and Choi, 2018). More specifically related to the current COVID-19 pandemic, the hesperidin flavinoid found in citrus extracts has in computer generated models shown potential as an inhibitor to the development of the SARS-CoV-2 by binding to the viral receptors (Utomo et al., 2020). While the 3CL protease, one of the key proteases in coronaviruses (CoV), which includes the SARS-CoV, is inhibited by a range of flavonoids (Jo et al., 2019, 2020; Ryu et al., 2010) particularly the citrus flavonoids hesperetin (Lin et al., 2005), apigenin, luteolin, and quercetin showed noteworthy antiviral activity through 3CL protease inhibition (Ryu et al., 2010). Citrus flavonoids, particularly naringin, show an immunoregulatory role to regulate the immune response and the so called cytokine storm

(Cheng et al., 2020) associated with the overreaction of the body's immune system which increases the severity as well as complications and high fatalities of COVID-19 (Mehta et al., 2020).

Rutin

Recent reviews on the extraction, bioactivity, formulation and possible application of rutin has been published and will only be discussed in brief (Chua, 2013; Ganeshpurkar and Saluja, 2017; Gullón et al., 2017; Sharma et al., 2013). The compound is a flavonol glycoside commonly found in more than 70 different plant species (Chua, 2013; Gullón et al., 2017) from sources such as fruits, fruit rinds, especially citrus fruits and plant derived beverages such as wine and tea (Ganeshpurkar and Saluja, 2017; Sharma et al., 2013). The main commercial sources of rutin being from buckwheat (*Fagopyrum esculentum* Moench), *Ruta graveolens* L. (Rutaceae), *Sophora japonica* L. (Fabaceae) and *Eucalyptus* spp. (Myrtaceae) (Chua, 2013). It has been found to have multiple benefits including antioxidant, cytoprotective, vasoprotective, antiallergic, anticarcinogenic, neuroprotective and cardioprotective activities (Ganeshpurkar and Saluja, 2017; Sharma et al., 2013). Due to its low solubility in aqueous solutions it has a low bioavailability, especially when administered orally (Miyake et al., 2000). It has been reported that little to no rutin and related flavonoids are absorbed when taken orally due to degradation by gut microflora into various small molecules including quercetin, isoquercetin and other phenol derivatives (Chua, 2013; Griffiths and Barrow, 1972; Winter et al., 1989). Quercetin is a flavonol that has been shown to improve endothelial functioning in a randomized, controlled trial by modulating circulating concentrations of vasoactive nitric oxide products and endothelin-1(Loke et al., 2008), key regulators of vascular homeostasis. The application of rutin, however, is better suited for topical applications, which is ideal as it has also been indicated that hydrogels containing rutin shortened healing time of skin wounds (Almeida et al., 2012). Rutin has been reported to possess activity against Gram-positive and Gram-negative bacteria such as *P. aeruginosa* (Orhan et al., 2010; Singh et al., 2008), *Klebsiella pneumoniae* (Singh et al., 2008), *Acinetobacter baumannii* (Orhan et al., 2010) and *S. aureus* (Orhan et al., 2010). Antifungal activity against multiple *Candida spp*. has been reported (Johann et al., 2011; Orhan et al., 2010). Furthermore, the presence of rutin has been observed to increase the activity of other flavonoids (quercetin, morin, kaempherol, myricetin and fisetin) against *Bacillus cereus* (Arima et al., 2002). An in-silico study suggested that rutin's antibacterial activity was by preventing folic acid synthesis (Ragunathan and Ravi, 2015) whereas an *in vitro* study showed decreased bilayer thickness and disruption of the lipid monolayer structure (Sanver et al., 2016). While the exact mode of action of rutin is not yet clear, its mode of action, together with other flavonoids, is attributed to disruption of the bacterial membrane which has enabled it to act in a synergistic or additive manner with various antibiotics against the multidrug-resistant MRSA (Amin et al., 2015). This activity is noteworthy as *Staphylococcus* species, including MRSA, have been shown to be the primary cause of bacterial dermatological infections in clinical practice (Riain, 2013; Suroowan and Mahomoodally, 2017).

Efforts to increase the bioavalibilty and solubility of rutin and other related flavonoids has primarily entailed increasing aqueous solubility, however, lipid phase solubility is also increased in many of these approaches (Singh et al., 2012). See Fig 2 exemplifying the various modifications (Gullón et al., 2017). Increased solubility can be achieved by a number of means including crystallisation to form water soluble nanoparticles (Mauludin et al., 2009a, 2009b), nanoemulsions (Almeida et al., 2010), introduction of nanocarrier molecules (Babazadeh et al., 2016; Kamel and Basha, 2013; Kumar and Bhopal, 2012; Yang et al., 2016), enzymatic bioconversion (Araújo et al., 2013) and acetylation (Chebil et al., 2006, 2007), encapsulation in cyclodextrins (Sri et al., 2007) or chemical conversion via addition of carboxylate or sulfate groups (Alluis et al., 2000; Pedriali et al., 2008) as well as hydroxyethylation of rutin to ethers of O-3-hydroxyethyl-rutin (Courbat, 1970; Favre, 1961) or hydroxyalkyl ether derivatives of

rutin poly(H)sulfates (Nair et al., 1983). This latter chemical conversion of rutin has been modified and improved to form mono-hydroxyethyl rutin (Zyma, 1975) the tri-hydroxyethyl ether (also known as troxerutin) of high purity with improved wettability (Estanove and Pruvost, 2005). Enzymatic oligomerization using laccase increases the water solubility of rutin 4200 times (Anthoni et al., 2008; Kurisawa et al., 2003). While increased lipid solubility has been achieved by complexation with gelatin nanocapsules (de Oliveira et al., 2016) or phospholipids (Alexander et al., 2016) enabling transdermal application (Das and Kalita, 2014).

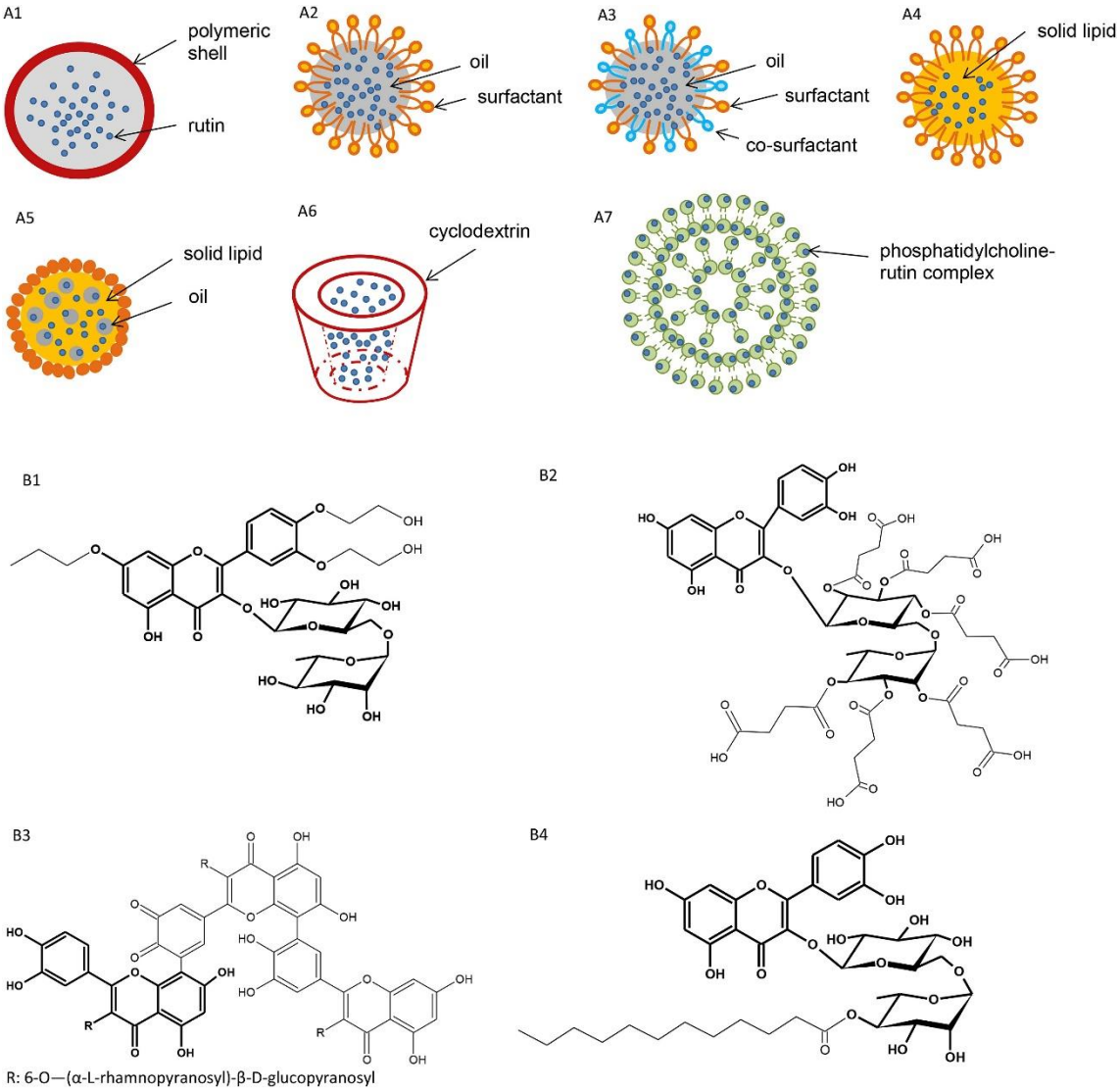

Figure 2: Mechanisms to increase rutin bioavailability. A) Schematic representation of the different kinds of colloidal delivery system: A1: nanocapsules; A2: nanoemulsion; A3: self-emulsifying drug delivery system (SEDDS); A4: solid lipid nanoparticle; A5: nanostructured lipid carrier (NLS); A6: inclusion complex; A7: phytosome; B) Rutin derivatives with enhanced aqueous or lipid solubility: B1: hydroxyethyl derivative known as troxerutin; B2: carboxylated derivative (Pedriali et al., 2008). B3: rutin oligomer (Uzan et al., 2011); B4: rutin ester synthetized (Lue et al., 2010). Original rutin molecule is highlighted in bold. This figure as obtained from (Gullón et al., 2017).

Clove oil

Eugenol is the main non-nutrient phenolic compound found in clove oil that can also be found in cinnamon, bay leaves and basil (Benencia and Courreges, 2000; Lane et al., 2019). It is widely used as an antiseptic, analgesic, and anti-bacterial agent as part of traditional medicine in Asian countries and can also be applied as a pain reliever in dentistry due to its anaesthetic properties (Pramod et al., 2010). Furthermore, eugenol has wide pharmaceutical applications

as a broad-spectrum antimicrobial, antiviral, antioxidant and anti-inflammatory agent (Benencia and Courreges, 2000; Lane et al., 2019; Ogata et al., 2000; Sanla-Ead et al., 2012; Waldman et al., 2009). It was shown to have both activity against human herpes simplex virus HSV-1 and HSV-2 by inhibiting replication (Benencia and Courreges, 2000; Siddiqui et al., 1996). Eugenol was found to have higher viricidal activity than clinical control Acyclovir alone, and combinations synergistically inhibited herpesvirus replication *in vitro* (Benencia and Courrges, 2000; Tragoolpua and Jatisatienr, 2007). It also has activity against the Ebola virus *in vitro* (Lane et al., 2019). Eugenol but not its isomer, isoeugenol (2-methoxy-4-propenylphenol), was found to be a potent inhibitor of melanoma cell proliferation (Ghosh et al., 2005) and act as a tumour suppressor in lung cancer cell proliferation *in vitro* (Fangjun and Zhijia, 2018). It has antifungal activity against wood fungi (white-rot fungi *Lenzites betulina* and brown-rot fungi *Laetiporus sulphurous*) (Cheng et al., 2008), plant pathogens (*B. cinerea* mycelia) (Wang et al., 2010), and human pathogens (*Eugenia cariophyllata*) (Gayoso et al., 2005). Essential oils establish a membrane potential across the cell wall and disrupt ATP assembly, leading to cell wall damage (Tariq et al., 2019). It has also been found to be active against a wide range of pathogenic Gram positive and Gram-negative bacteria such as *Bacillus subtilis*, *E. coli*, *Salmonella typhimurium*, *S. aureus*, *Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *B. cereus*, *E. coli*, *Listeria monocytogenes*, *Micrococcus luteus*, *P. aeruginosa*, *S. enteritidis*, *Enterococcus faecalis* including fungi *Candida albicans Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* (Karapinar and Aktuğ, 1987; Rhayour et al., 2003; Sanla-Ead et al., 2012).

The primary antibacterial mode of action of eugenol appears to be cell membrane permeability (Cui et al., 2018; Devi et al., 2010), but also extends to multiple intracellular targets such as ATPase, amylase histidinecarboxylase, protease (Devi et al., 2010; Thoroski et al., 1989) and causing structural changes of the DNA (Cui et al., 2018). The addition of linalool to *Syzygium aromaticum* (clove) oil (main constituent eugenol) in a synergistic manner enhanced its antimicrobial efficacy against *P. aeruginosa* and *Aspergillus brasiliensis* (Herman et al., 2016). Essential oils (EOs) and their isolated constituents, particularly eugenol and menthol, have shown noteworthy antibacterial activity toward a range of pathogens, particularly *in vitro* studies assessing the effects of EOs on caries-related streptococci (mainly *Streptococcus* mutants) and lactobacilli, and in a limited number of clinical trials, together with a range of cariogenic bacteria which shows the potential of these compounds in oral applications (Freires et al., 2015). A study done by Sanla-Ead *et. al.* (2012) showed that eugenol maintained activity against 10 microbial species in a methylcellulose films suggesting further application in active 'green' biodegradable packaging.

Yucca schidigera

Yucca schidigera has been used as part of traditional medicine practices to treat inflammatory disorders such as headaches, gonorrhoea, arthritis, and rheumatism (Cheeke, 1998). It has GRAS status (Generally Regarded As Safe) which allows for use as foaming agent in soft drinks (root beer), pharmaceutical, cosmetic, food, and feeding-stuffs industries. The main application of yucca products is in animal nutrition, in particular as a feed additive to reduce ammonia and faecal odours in animal excreta (Cheeke, 2000). Dietary supplementation with yucca products is reported to produce positive effects on the growth rates (Anthony et al., 1994; Mader and Brumm, 1987), feed efficiency (Mader and Brumm, 1987) and health of livestock (Anthony et al., 1994; Balog et al., 1994). Steroidal saponins are considered to be the main active compound in *Yucca schidigera* products (Kowalczyk et al., 2011; Wang et al., 2000). However, it also contains phenolic compounds that contributes to its observed benefits: resveratrol, *trans*-3,3',5,5'-tetrahydroxy-4'-methoxystilbene, the spirobiflavonoid larixinol along with novel phenolic derivatives with very unusual spirostructures, named yuccaols A-E and yuccaone A (Oleszek et al., 2001; Piacente et al., 2002, 2004).

Yucca saponins have antibacterial properties (Katsunuma et al., 2000; Wang et al., 2000), although *Lactobacillus spp.* and *E. coli* may be tolerant of yucca extracts and yucca saponins (Katsunuma et al., 2000). It also has growth-inhibitory activity against certain food-deteriorating yeasts (*Candida albicans*), film-forming yeasts (*Debaryomyces hansenii*, *Pichia nakazawae*, *Zygosaccharomyces rouxii*), dermatophytic yeasts (*Candida famata*, *Hansenula anomala*, *Pichia carsonii*), and against brewer's yeast (*Saccharomyces cerevisiae*) (Miyakoshi et al., 2000). Protozoal diseases in which part of the life cycle occurs in the gastrointestinal tract respond to the anti-protozoal activity of saponins. For example, yucca saponins are as effective as the drug metronidazole in killing tropozoites of Giardia lamblia in the intestine (McAllister et al., 2001). Saponins are membrane active, presumably by forming pores (Hassan et al., 2010) which correlates with their character of being natural detergents that form stable foams (Cheeke, 2006). Interactions of saponins with cholesterol and other sterols may also account for many of their biological effects, particularly those involving membrane activity, as it was demonstrated that dietary saponin reduces blood cholesterol levels (Griminger and Fisher, 1958; Newman et al., 1958). Saponin also displays carcinostatic and mutagenesis-inhibitory effects (Man et al., 2009) and it could inhibit the growth of KB human oral epidermoid carcinoma cells (Kaminobe et al., 2002) by inducing cell cycle arrest and apoptosis (Man et al., 2010).

The phenolic extract overall has antioxidant activity, however, *trans*-3,3',5,5'-tetrahydroxy-4'-methoxystilbene has been found to be more active than resveratrol (Piacente et al., 2005). The antioxidant properties of resveratrol is believed to be responsible for the reduced risk of cardiovascular disease associated with a moderate consumption of red wine (Pendurthi et al., 1999; Siemann and Creasy, 1992). Furthermore, it has been shown to be antimutagenic (Uenobe et al., 1997) and cancer preventing (Jang et al., 1997; Surh et al., 1999) by inducing apoptosis in tumour cells. The proposed mode of flavonoid inhibitory action in tumour cells is the binding at the polyphenol binding pocket of ATP synthase and the blockage of clockwise or anticlockwise rotation of the c-subunit (Gledhill et al., 2007). Resveratrol has been shown to modulate the host response during salmonellosis by protecting the host cells from the toxic effects of bacterial infection (*Salmonella typhimurium)* and also by decreasing programmed cell death (Paolillo et al., 2011). It has antiviral activity against a range of targets such as the influenza virus, Epstein-Barr Virus, HSV-1 and HSV-2 infections (Abba et al., 2015). Yucca phenolics have free-radical scavenging effects (Oleszek et al., 2001) and have inhibitory activity against platelet aggregation (Olas et al., 2002, 2003, 2005) which is characteristic of inflammation. The anti-inflammatory activity is also achieved by the inhibition of NF-$\kappa\beta$ activation which controls the expression of inducible nitric oxide synthase (iNOS) which ultimately lowers the production of the inflammatory agent, nitric oxide (Marzocco et al., 2004).

Conclusions

It is apparent from the literature search, as well as the antimicrobial data obtained by The BioConsulting that the components of the Organic Fresh formulation show noteworthy potential to serve as an antimicrobial sanitizing agent. There is evidence in literature to support possible synergistic interactions between some of the constituents of the formulation.

It would be of significant value to the product to further explore the interactions between some of the constituents in antimicrobial activity assays together with analytical techniques, as well as the possible modification of the components using evidence from literature to not only increase antimicrobial activity but also the efficiency of the current production process.

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