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Review article

Fruit extracts to control pathogenic *Escherichia coli*: A sweet solution☆

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ABSTRACT

Escherichia coli is a major cause of diarrhea and is as well responsible for extraintestinal infections in humans and animals. Many pathotypes have been defined for this ubiquitous microorganism on the basis of the virulence attributes. For the last 70 years, antibiotics have been used to control infections caused by *E. coli*. However, with the resistance observed with many strains these drugs are less recommended. Plant extracts, in particular fruit, represent a source of bioactive compounds that could be beneficial in the control of infectious diseases caused by *E. coli*. These could have bacteriostatic or bactericidal potential or could be used as synergic agents to amplify the activity of antibiotics for which the germs present some level of resistance. Certain studies also revealed that fruit extracts could act directly on virulence characters to attenuate the pathogenic capacity of microorganisms. This review intent to expose the scant but rapidly growing information available that shows that fruit, used as crude extracts or purified molecules, should be considered to manage diverse types of infections caused by *E. coli*.

1. Introduction

Microbial infections are a worldwide cause of mortality and morbidity in humans and animals. Specifically, *Escherichia coli* is a bacterium associated with infections of the gut and extra-intestinal illnesses [1, 2]. We now recognize many pathotypes (a type of microorganism that causes a specific disease in a particular host or range of hosts) that are classified based on the virulence factors they express [3]. For more than half-century, antibiotics have been currently used to treat such infections. However, the efficiency of these drugs has been hampered by the emergence of resistant bacterial strains [4]. Therefore, treatment of infections has become a dreadful challenge and efforts to search for alternative therapies have expended [5].

Recent replacement cures considered are often based on exploitation of natural antimicrobial products as well as purified molecules to either control the microorganisms or target virulence factors responsible for the disease incidence. For example, the potential of such compounds to reduce toxin production was reported [6]. Likewise, the use of bacteriophages is an avenue that is considered and it already shows promising results [7, 8]. Plant-derived compounds represent a source of molecules that could be safe and effective in the treatment of disease-producing microbes [9, 10]. To our knowledge, this review is the first to report trials to control pathogenic *E. coli* infections using fruit extracts.

2. Phytochemicals

Phytochemicals are compounds occurring naturally in plants. They confer colour, aroma, and texture to the various parts of the plant. These chemical compounds are important for the plants as they evolved to outcome the deleterious effects of free radicals and microorganisms (viruses, bacteria, and fungi) on their health. A majority of these chemicals correspond to secondary metabolites produced in response to stimuli from the environment and plant pathogenic microbes [11]. Thus, no direct effect can be ascribed on plant physiology, their roles being solely in defence and plant health. Based on their chemical structures these substances can be classified into several groups. More precisely, alkaloids, sulfur-containing molecules, terpenoids, and polyphenols are produced by distinct plant parts including fruits [12].

In the last decades, many phytochemicals showing antimicrobial activities were described [13]. However, several of the identified compounds were not used individually due to their minimal inhibitory concentration (MIC) that is greater than conventional antibiotics. Thus, combination of two or more phytochemicals with or without an antibiotic have been evaluated as a way to affect microbial growth and thus the infection process. One of the outcomes of these results was that these molecules could be used in combination with antibiotics for which the bacteria were resistant in order to modulate its potency [14]. In this

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fashion, phytochemicals also represent a feasible strategy to develop anti-virulence agents.

3. *E. coli* as a pathogen

A variety of *E. coli* type exists and is found in the environment, food, and intestine of humans and animals [15]. A normal inhabitant of the gastrointestinal tract as part of the microbiote, *E. coli* can also be a dreadful foe. Indeed, intestinal as well as extraintestinal infections in man and animals can be linked to the presence of this pathogen. This bacterium can be categorized on the basis of the virulence attributes they have acquired over evolution. To be successful, pathogenic bacteria have to adhere to epithelial cells, benefit from the presence of nutrients for multiplication and secrete molecules that can adversely affect infected tissues of individuals [16]. Thus, specific and peculiar mechanisms related to such process define distinct *E. coli* pathotypes. The fundamental intestinal pathogenic groups include enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), diffusely adherent (DAEC) and enteroaggregative *E. coli* (EAEC) [3]. Moreover, in 2000, Russo and Johnson suggested the term extra-intestinal pathogenic *E. coli* (ExPEC) as a descriptor for all non-commensal *E. coli* isolates capable of causing extra-intestinal disease [17]. Unlike commensal *E. coli*, ExPEC have the ability to cause disease outside the host gut reservoir due to specific virulence factors. *E. coli* has emerged as major players with multidrug resistance (MDR) characteristics now being relatively common [18, 19, 20]. In fact, *E. coli* has been included in a list of microorganisms of international concern causing the most common infections in different settings; the community, in hospitals or transmitted through the food chain [21]. Worldwide this microorganism is a major cause of diarrheal diseases, peritonitis, colitis, bacteremia, infant mortality, and urinary tract infections that cost billions of dollars to treat. It kills roughly 2 million humans each year [22, 23] and some strains cause cancer [24]. *E. coli* strains are also the cause of intestinal, urinary tract or internal infections and pathologies, in many animal species [25].

Fruit extracts shown to control various *E. coli* pathotypes and their detrimental effects will now be considered. These are grouped according to the effect(s) exhibited in *in vitro* and *in vivo* studies.

4. Antimicrobial activity

Over the hundred thousands of years, *Homo sapiens* have been inhabiting planet earth [26], plants have been used as a remedy for treatment of numerous illnesses with more or less success. Various plant parts, including the reproductive body that we call fruit were components of poultices, elixirs, and other potions. These were used over centuries in an attempt to control some health conditions with more or less success.

After many trials and errors, some of these fruit extracts were found to have reproducible positive effects on disease remediation. However, for centuries even though we could prepare sophisticated preparations the active compounds responsible for the observed conclusive effects remained unidentified. Technologies are now available to isolate and characterize the biologically active chemicals.

One activity easily observable is the antimicrobial potential of fruit preparations against microorganisms [13]. The active principles present in these solutions, decoctions or extracts when added to solid medium or in a liquid culture medium show bactericidal or bacteriostatic effects. Antimicrobial activities based on bacterial targets not presently exploited by antibiotics could represent a major breakthrough in infectious diseases treatment. These compounds could also be relevant for treatment of antibiotic resistant microorganisms. Ideally, these should be active on specific microorganisms (narrow spectrum) as they would lightly alter or not at all the microbiota.

Phytochemicals have shown interesting antimicrobial activities against several human and animal pathogens [12, 13, 27]. Many mechanisms were observed explaining the effect observed including bacterial membrane damage, suppression of virulence factors targeting enzymes and toxins, and inhibition of biofilm formation. *In vitro* antibiotic resistance modifying activities showing synergistic effect with conventional antibiotics were also reported [28, 29]. A combinational approach that permits synergistic interaction between plant extracts and conventional antibiotics is probably the most effective method to combat antibacterial resistance. For example, combination of β-lactams with α-mangostin isolated from mangosteen fruit substantially increase the efficacy of the treatment in β-lactam resistant bacterial strains [30]. In the same way, quercetin and kaempferol from various fruits could clearly act in a synergistic fashion with amoxicillin [31].

Numerous studies have reported on the antimicrobial activities of fruit extracts as already mentioned. In some investigations, specific molecules either kill or alter the growth of bacteria. Further, these compounds tested against antibiotic resistant strains in presence or absence of the antibiotic for which the strain had shown resistance in order to identify new molecules able to affect adversely bacteria and to recognize molecules that could restore the microbicidal capacity to an antibiotic.

5. Antidiarrheal activity

ETEC are associated with nearly 60% of deaths resulting from diarrhea [32]. The enterotoxins produced by these *E. coli* are directly responsible for the intestinal secretion observed [15]. Yao et al. [33], assesses the antidiarrheal activity of a crude extract of Chinese bayberry fruit (Biqi cultivar) used as a folk medicine to cure diarrhea. The bayberry extract was antibacterial against Gram-negative bacteria including *Shigella* (Shigellae are phylogenetically *E. coli* that was later classified as separate species on the basis of biochemical characteristics and clinical relevance) [34]. A positive relationship exists between the antibacterial activity observed and the total polyphenol and flavonoid contents. Separation of the molecules on a polyamide column indicated that the compound with the most activity comprised flavonoids, which included cyanidin-3-O-glucoside, myricetin deoxyhexoside, quercetin-3-O-glucoside, and quercetin deoxyhexoside. These compounds also possessed antidiarrheal activity at 80 mg kg^{-1} as revealed using a mouse model. These findings provided scientific evidence supporting the role of bayberry fruit as an antidiarrheal remedy.

Olive is a fruit that needs to be fermented to be palatable. During industrial fermentation of green olives, exopolysaccharides (EPS) are produced [35]. Analysis of their monosaccharide composition by gas liquid chromatography revealed that the main components are glucose and galactose followed by rhamnose and arabinose. *In vitro* tests demonstrated the ability of EPS at 1% w/v to attach specifically to ETEC K88 fimbriae an important structure for adherence to host cells. On porcine intestinal epithelium, carbohydrates are generally recognized as part of the receptor structure [36]. Competition tests (using the same concentration) did not show the ability to block ETEC K88 adhesion to IPEC-J2 (porcine intestinal epithelium) cells whereas a displacement test indicated that all EPS samples effectively removed the attached pathogen. Obviously, EPS produced during the fermentation of green olives could interfere with the attachment of opportunistic pathogens onto the intestinal epithelial cells.

In vivo experiments using piglets and cranberry extracts conducted in order to evaluate its potential to control neonatal and post-weaning diarrhea (PWD) [37] indicated that a water extract of dried cranberry powder (*Vaccinium macrocarpon* Ait.) inhibited F4- (neonatal and PWD) and F18-positive (PWD and edema disease) *E. coli in vitro* adhesion to pig intestinal epithelium. Moreover, the extract (10 mg or 100 mg) could also abolish *in vivo* binding of both fimbriae to the pig intestinal epithelium in ligated loop experiments. Supplementation of feed (10 g kg^{-1}) and water with cranberry extract reduced excretion and diarrhea occurrence upon oral challenges with F18-positive *E. coli*. No effect was observed in piglets receiving cranberry extract only in feed. The decreased infection was concomitant with a lowered

antibody response to F18-positive *E. coli* indicating a reduced exposure to this pathogen.

The bottom line is that although there are limited studies, some indicate potential to control diarrhea caused by *E. coli* strains. This is decisive as diarrhea is a dominant condition resulting from infection with ETEC. Many other studies will have to be conducted before we can definitively conclude on the potential of the identified molecules and the concentration needed to be effective *in vivo*.

6. Direct impact on toxins

6.1. E. coli toxins

A study by Lescano et al. [38], on the peel extract of *Campomanesia adamantium*, (gabiroba) a medicinal plant found in the Brazilian cerrado, showed that phenolic compounds were present in a 100% methanol extract. In the disk diffusion test, the extract failed to show antibacterial activity against *E. coli* up to 3.12 mg ml^{-1}. T84 (human colon epithelia) cell proliferation was inhibited by the peel extract but no toxic effect was observed. Gallic acid present in the extract tested at 10 μM significantly decreased the levels of cGMP in T84 cells. cGMP is a molecule that accumulates due to the action of STa toxin on its receptor, guanylate cyclase C (GC-C) and leads to diarrhea [39]. It was postulated that a direct interaction between STa and phenolic compounds and between phenolic compounds and residues in the extracellular domain of GC-C could be occurring preventing activation of guanylate cyclase and thus diarrhea.

Fresh and dried fruits of *Chaenomeles speciosa* (Chinese quince) were used for centuries in traditional Chinese medicine and other oriental medicine systems. Chen et al. [40], demonstrated that the fruit extract inhibited *E. coli* heat-labile toxin (LT)-induced diarrhea in mice with a concentration-dependent decrease in the gut to carcass ratio. The ethyl acetate fraction was the most active fraction abolishing the binding of LTB toxin subunits to G_{M1}, its receptor with an IC_{50} of 2.4 ± 0.6 mg ml^{-1}. From the ethyl acetate fraction, oleanolic acid, ursolic acid, and betulinic acid (IC_{50} values of 202.8 ± 47.8, 493.6 ± 100.0, and 480.5 ± 56.9 μM, respectively) blocked the toxin effects resulting in the suppression of LT-induced diarrhea. Therefore, these components were considered as active therapeutic agent able to suppress LT toxicity.

The antidiarrheal activity of *Aegle marmelos* unripe fruit (bael or Bengal quince) used in indigenous systems of Indian medicine was evaluated [41]. A hot aqueous decoction of 1 g of dried fruit was not antibacterial against various *E. coli* pathotypes, including ETEC, EPEC, and EIEC even at a 1/10 dilution. However, bacterial adherence to and invasion of Hep-2 cells was significantly reduced following treatment with the decoction at the same dilution. Most importantly, the extract inhibited the production of cholera toxin (CT, a toxin structurally and mechanistically related to *E. coli* LT with GM1 as its receptor) and the binding of CT and LT to G_{M1}. As there was no killing of the bacteria observed, suppression of CT production suggests that the fruit decoction directly affects the bacterial metabolism. Still, no effect on LT and STa synthesis was noted for this fruit extract showing a targeted action toward CT.

A chestnut extract was also investigated for its efficacy on an experimental ETEC F4 diarrhea infection model in piglets [42]. The ETEC F4 strain used in the study was representative of strains found in post-weaning diarrhea as it harboured the adhesion factor F4ac and two toxins, LT and STb. A 1% chestnut tannins extract (containing 54% of hydrolyzable tannins) was used to supplement a piglet diet. The results of two trials indicated that in the infected animal group, more than 50% developed diarrhea within six days post infection. Feed intake and average daily gain were not affected by the treatment. In the animal receiving the diet supplemented with the chestnut extract, the average fecal score, the number of piglets with diarrhea, and its duration were reduced. However, the supplemented diet did not completely prevent the occurrence of post-weaning diarrhea and did not reduce shedding in the feces. As tannins are able to bind a variety of substrates, binding to fimbrial proteins or toxins could explain the observed outcome of the treatment.

Prosopis alba Griseb and *Ziziphus mistol* Griseb (white carob) fruit extracts were shown to inhibit the toxic action of *E. coli* Shiga toxin [43]. Components of both fruits increased the viability of Vero (African green monkey kidney epithelium) cells treated with Shiga toxin as determined by the neutral red vital staining when the extracts were preincubated with the toxin for 1h. The ethanol extract was more active than the water extract even if it contained fewer dissolved solids. Both extracts were less effective if co-incubated with the toxin. The pro-oxidant action on Shiga toxin favoured the cells and enhanced the protective action of both fruits. On the other hand, in another study, grape seed and pomace extracts (0.5 mg ml^{-1}) showed a reduction in the expression of *E. coli* Shiga toxin 1 and 2 genes using a cell-based *in vitro* assay [44]. In this case, gene transcription was directly affected. The active component of grapes represents most probably a fraction of the extract and thus it must be functional in lower quantities than used in the study.

As demonstrated, toxicity of *E. coli* toxins can be altered using specific fruit extracts. This can be the result of highly specific interactions or due to non-specific binding or aggregation with protein toxins. Direct action on toxin synthesis/regulation illustrates a significant manner to manage these fundamental virulence features.

6.2. Cholera toxin

Grape extracts were evaluated for anti-CT properties [45]. Two commercial grape extracts sold as nutritional supplements inhibited CT and *E. coli* LT activity on cultured CHO–K1 (Chinese hamster ovary epithelium) cells and in pig intestinal loops. CT intoxication was blocked even when the extracts were added 1h after initial exposure to the toxin (10 mg grape seed extract or 1 mg pomace extract). These were able to block toxin binding to the cell surface, prevented unfolding and translocation to the cytosol of the CTA1 active subunit, abolishing its catalytic activity.

Applephenon, a compound obtained from green apples, is composed of chlorogenic acid, catechins, condensed catechins and procyanidins [46]. The latter compound inhibited the binding of CT to Vero cells in a concentration-dependent manner. As well, toxin internalization was suppressed at 200 μg ml^{-1} of applephenon. The mechanism most probably involves the precipitation of CT from solution or perhaps on the cell surface creating large inactive aggregates.

More recently, Cherubin et al. [47], used a cell culture system to identify 12 phenolic compounds from grape extract that inhibited CT. Two of these phytochemicals (epigallocatechin gallate (EGCG) and procyanidin B2 (PB2)) inhibited CT binding to the cell surface and could even dislodge CT from the plasma membrane of target cells (CHO–K1 and Vero cells). Two phenol compounds (kaempferol and quercitrin) at 10 ng ml^{-1} could directly act on the enzymatic activity of CTA1 and four others blocked the activity of CT in the cytosol without affecting the enzymatic activity by disrupting host-toxin interactions needed for the cytosolic activity of CTA1.

7. Uropathogenic *E. coli*

Vollmerhausen et al. [48], determined the effects of seeds of *Citrus reticulata* Blanco (mandarin) on adherence and invasion of the uroepithelium by *E. coli*. The water decoction of mandarin seeds (dilutions of 1/100 and 1/1000) decreased ß1 integrin expression (a common receptor for uropathogens mediating bacterial invasion) and reduced bacterial invasion while adherence to uroepithelial cells was not affected. An effect on biofilm thickness was also excluded. Caveolin-1 (previously reported to reduce bacterial invasion) expression was not influenced and the extract did neither exhibit any direct antimicrobial effects nor did it interfere with type 1 fimbriae binding. The water extract had no effect on cell viability after 48 h, indicating its innocuous nature and the fact that this decoction may help to prevent or treat urinary tract infections.

Liya et al. [49], examined methanol and ethanol extracts (at 0.2 mg μL^{-1} and 0.6 mg μL^{-1}) of many commercial fruits. These included apple (*Malus domestica*, green apple), papaya, lemon, and strawberry. The extracts were tested against *E. coli* responsible for urinary tract infections, using the agar well diffusion method. Lemon and strawberry showed inhibition zones against the *E. coli* strains tested; the ethanol extract being more potent. As apple was previously shown to be active against EAEC, it was concluded that the concentration of the active molecule(s) could vary with the cultivar [50].

As demonstrated with plant parts beside fruits, the cultivar, the ripeness and the time of the year it is harvested can have a dramatic impact on polyphenol content [27].

8. CFTR

Cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel expressed primarily in the apical membranes in the intestinal mucosa [51]. Apical Cl^- secretion is observed in response to many agonists including *E. coli* LT enterotoxin and cholera toxin. Cocoa beans are rich in polyphenols and were historically used as a treatment for diarrhea. Schuier et al. [52], tested the effect of flavonoids compounds present in cocoa or molecules closely related. When tested on forskolin-stimulated CFTR-mediated Cl^- secretion across T84 (a human colon cell line) cells in Ussing chambers it caused a partial inhibition of cyclic AMP production when added on the mucosal side. cAMP is the effector that provokes phosphorylation of CFTR and leads to Cl^- secretion. Epicatechin, catechin, a standardized cocoa preparation (containing approximately 57 mg of mixed flavonols g^{-1} cocoa), and procyanidin B2 where, in that order, the best blockers of LT identified. Effects of test compounds on mucosal redox potential did not correlate with the blocker activity. These data indicate that cocoa flavonols, as epicathechin and catechin, target intestinal CFTR Cl^- transport and could serve as a mild inhibitor of cAMP-stimulated Cl^- secretion in the intestine. As flavonols are poorly absorbed in the human gut it results in high concentrations in the intestinal lumen. For this reason, the authors believed that normal cocoa consumption could supply sufficient concentrations of the active ingredients to affect intestinal CFTR-mediated salt and water concentrations by the small intestine.

9. Anti-biofilm activity

Biofilm formation is an important virulence mechanism by which many microorganisms can resist antibiotic treatment [53]. Today, MDR strains progress and these augment the complexity and severity of the diseases they cause. A methanol pomegranate (*Punica granatum* L.) extract showed anti-biofilm activity against bacteria and fungi including *E. coli* [54]. This extract containing ellagic acid as the major component was able to inhibit the formation of biofilm by *E. coli*. Ellagic acid is a bioactive tannin that could inhibit the bacterial growth at concentrations higher than 75 $\mu g\ ml^{-1}$ and biofilm formation at less than 40 $\mu g\ ml^{-1}$. The extract shown efficacy under both nutrient repleted and nutrient depleted conditions, a clear indication of the pomegranate extract as a bioactive agent. A synergistic activity of pomegranate with antibiotics against pathogens resistant to antibiotics was also observed [51, 55, 56].

Grapefruit juice contains bioactive compounds such as furocoumarins [57]. This molecule showed more than 95% inhibition of autoinducer AI-1 (oligopeptides and N-acylhomoserine lactones) involved in intraspecific communication in Gram-positive and Gram-negative bacteria and of AI-2 (boronated-diester molecules) involved in interspecific communication among both Gram-positive and Gram-negative bacteria as revealed by the *Vibrio harveyi*-based autoinducer bioassay. Grapefruit juice and furocoumarins also inhibited biofilm formation by *E. coli* O157:H7 through inhibition of AI-2 activity. The structural similarity between the autoinducer molecules and the furocoumarins is the furan moiety and this structure could be responsible for competitive binding which resulted in quorum sensing inhibition. Thus, furocoumarins were not only able to interfere with cell-cell signalling but could also inhibit biofilm formation representing a desirable attribute of a treatment. As well, citrus (including grapefruit) flavonoids inhibited biofilm formation by *E. coli* O157:H7 [58,59]. Kaempferol, naringenin, quercetin and apigenin are active flavonoids produced as secondary metabolites in citrus species. From these, naringenin emerged as a potent and possibly a nonspecific inhibitor of AI-2-mediated cell-cell signalling and *E. coli* O157:H7 biofilm.

It was observed that grape seed extract, a by-product of the wine industry, had an effect on the growth, quorum sensing, and virulence factors of six non-O157 Shiga toxin-producing *E. coli* in other studies [60]. A 10% hot ethanol extract (at 0.5 mg ml^{-1}) affected quorum sensing by reducing considerably the production of AI-2 of all *E. coli* strains tested. The inhibitory effect was proportional to the concentration of the extract. The swimming motility was reduced in two isolates complying with the production of flagellar protein FliC and its regulator FliA. In addition, production of Shiga toxin was inhibited at 4 mg ml^{-1} in the same two strains indicating the multiple targets of the active substance(s).

Considering the central role played by biofilm in the resistance of microorganisms to antibiotic treatment, it is promising to realize that some of the tested compounds present in fruit extracts are able to restrain biofilm formation. As this phenomenon is observed at lower concentrations than the one showing antimicrobial activity it also plaid for the specific characters of these molecules and the likelihood of their usage *in vivo*.

10. Immunomodulatory function

In calves fed *Morinda citrifolia* (Noni) puree (30 ml day^{-1}), bactericidal assays performed after 14 days on the animal blood showed significant more *E. coli* killing than the control [61]. The authors speculated that Noni puree either could stimulate the innate immunity resulting in enhanced bactericidal immunity or could potentiate immune function by enhancing colostral-acquired innate immunity. This may be linked most specifically to the polysaccharides found in Noni puree that could function as immunomodulators. This hypothesis will have to be infirm or confirm by thorough investigation.

Cnidium monnieri fruit (Monnier's snowparsley) tested as an immune enhancer in an *E. coli* K18 mouse infection model (300 mg kg^{-1} per day) proved to augment the concentrations of nitric oxide (NO) and tumour necrosis factor alpha (TNF-α) as well as the phagocytosis activity in macrophages [62]. Oral administration of the fruit in mice prolonged the survival rate. The fruit components had no direct bacteriostatic or bactericidal activity *in vitro*. Clearly, *Cnidium monnieri* fruit could stimulate the macrophage function as measured by the level of NO and TNF-α as well as phagocytosis. This study was carried to evaluate the synergistic effects of diverse molecules showing bioactivity using whole fruit instead of purified components. In fact, bioactivity was linked to chromones, coumarins, monoterpenoids, and phenolic glycosides [63, 64, 65, 66, 67, 68, 69].

Once again, the studies bring to light the performance of the complex mixture found in the extracts letting us wonder if using individual molecules could represent an achievable solution in the long term.

11. Conclusions

This succinct review is probably only a foretaste to the multiple biological activities that fruit extracts can exert on the diverse *E. coli* virulence attributes (Table 1). Looking for antibiotic alternatives using fruits is a legitimate choice. Overall, we have to choose the appropriate technique for identifying the component(s) and refer to a pertinent animal model to determine which extract/molecule(s) to use for treating the infection or disease.

The combination or synergistic actions exhibited by the compounds present in a fruit extract could be due to multiple targets on which these

Table 1. Reported fruit extracts active against *E. coli* virulence attributes.

Fruit extract	Extraction/Concentration	Active molecule(s) identified	Effect observed	Reference
Chinese bayberry fruit		Cyanidin-3-O-glucoside, myricetin deoxyhexoside, quercetin-3-O-glucoside, quercitin deoxyhexoside	Antidiarrheal	Yao et al., [33]
Green olive		Exopolysaccharides (glucose, galactose, rhamnose, arabinose)	Antidiarrheal, EPS attach to K88 fimbriae	Zhu et al., [35]
Cranberry	Water extract, 10mg or 100 mg	?	Antidiarrheal, inhibit F4 and F18 fimbriae attachment	Coddens et al., [37]
Campomanesia adamantium	100% methanol extract	Gallic acid	Decrease cGMP accumulation (act on guanylate cyclase)	Lescano et al., [38]
Chaenomeles speciosa	Ethyl acetate fraction	Oleanolic acid, ursolic acid, betulinic acid	Abolish binding of LTB to GM1	Chen et al., [40]
Aegle marmelos	Hot aqueous decoction	?	Inhibit adherence to and invasion of Hep-2 cells, inhibition of CT production	Brijesh et al., [41]
Chesnut	1% hydrolyzable tannin extract	?	Reduce average fecal score, number of piglets with and duration of diarrhea	Girard et al., [42]
Prosopis alba Griseb	Fruit extract	?	Inhibit Shiga toxic effect	Pellarin et al., [43]
Ziziphus mistol Griseb	Fruit extract	?	Inhibit Shiga toxic effect	Pellarin et al., [43]
Grape seed and pomace extract		?	Reduction of Shiga toxin expression (gene transcription level)	Quinones et al., [44]
Grape	Commercial extract	?	Block toxin binding to cell surface, prevent unfolding and translocation into cytosol of CTA1, abolish catalytic activity	Reddy et al., [45]
Applephenon		Procyanidins	Inhibit binding of CT to Vero cells, suppress internalization	Morinaga et al., [46]
Grape	Fruit extract	Epigallocatechin gallate and procyanidin B2,	Inhibit CT binding to cell surface	Cherubin et al., [47]
Idem		Kaempferol and quercitin	Block enzymatic activity of CTA1	Cherubin et al., [47]
Idem		4 other compounds	Block CT activity	Cherubin et al., [47]
Cocoa	Beans extract	Epicatechin, catechin, procyanidin	Inhibit cAMP production (target CFTR)	Schuier et al., [52]
Pomegranate	Methanol extract	Ellagic acid	Antibiofilm activity	Bakkiyaraj et al., [54]
Pomegranate	Methanol extract	Ellagic acid	Synergistic effect with antibiotics	Endo et al., [55]
Grapefruit		Furocoumarins	Inhibit autoinducer AI-1 and AI-2, inhibit O157:H7 biofilm formation	Girennavar et al., [57]
Citrus		(Flavanoids) Kaempferol, naringenin, quercetin, apigenin	Non-specific inhibition of AI-2 in O157:H7	Vikram et al., [58,59]
Grape seed	10% ethanol extract, 0.5 mg ml-1	?	Effect on quorum-sensing and virulence factors of non O157:H7	Sheng et al., [60]
Idem	4mg ml-1	?	Shigatoxin-producing *E. coli*	Sheng et al., [60]
Citrus reticulata Blanco seeds	Water extract	?	Uropathogenic *E. coli* Beta1 integrin expression, reduce bacterial invasion	Vollmerhausen et al., [48]
Lemon, strawberry	Methanol and ethanol extracts	?	Uropathogenic *E. coli*	Liya et al., [49]
Morinda citrifolia (Noni)	Puree	Polysaccharides	Stimulate innate immunity or potentiate immune functions	Schafer et al., [61]
Cnidium monnieri	Hot water extract	Chromones, coumarins, monoterpenoids, phenolic glycosides	Augment NO and TNF-alpha and macrophage phagocytic activity	Malla et al., [62]

constituents can act. Fruit extracts comprise a mixture of active compounds. For this reason, multiple components present in a crude fruit extract acting at different sites contribute to the overall activity of the extract. In fact, the extracts exert their antimicrobial activity not solely by killing the microorganism but also by affecting key events in the pathogenic process. The antibacterial effects involve either damage to the bacterial membrane (with changes in polarization and interruption in efflux activity) as well as alteration of gene regulation associated with certain virulence attributes including adhesion, motility, invasion, and biofilm formation [13]. This is why the development of bacterial resistance to mixture of these active molecules may be much slower than for single chemical compounds as use in conventional antibiotic treatment. Nevertheless, we cannot ignore the extraordinary adaptive bacterial capability and hence the conceivable development of resistance for some of the molecules.

From the studies reviewed, we can conclude that for many fruit extracts tested the content in polyphenols (flavonoids and terpenoids) was identified as active on various *E. coli* virulence traits. Some of the effects were strikingly specific whereas some were due to non-specific mechanisms (i.e aggregation, precipitation, etc…).

As more studies will be performed with fruit extracts and purified components, we will be able to compare the results between these studies. The observed attributes of the compounds can divert specific *E. coli* from their distinct pathogenic mechanism and prevent them from causing disease. As could be realized from the literature reviewed here, various fruit extracts have potent antimicrobial and anti-virulence activities. In some studies molecule(s) responsible for the virulence alteration was purified and identified. This is a major step as these could be concentrated, dosed and tested by themselves or coupled to other molecules to evaluate their possible synergistic actions. Further, it will be possible to compare the concentrations of the active component(s) tested. Thus, although the antimicrobial effect of numerous fruits extracts cannot be ignored, their activities on distinct pathogenic stages widened their potential exploitation as new ways to control bacterial infections and in particular in *E. coli*.

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Additional information

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