



Antioxidant and antimicrobial properties of cranberry juice and lemon essential oil

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Abstract— The growing demand for non synthetic preservatives has increased research interest in natural substances with bioactivity. Among recent natural substances investigated for their bio properties are cranberry juice and lemon essential oil. This review discussed the antioxidant and antimicrobial properties of cranberry juice and lemon essential oil.

Keywords— Cranberry juice; Lemon essential oil; Antioxidant property; Antimicrobial property; Preservatives.

I. INTRODUCTION

For decades, food industries have been using synthetic food preservatives to prevent oxidation and microbial contamination. However, due to the adverse effects of synthetic compounds on health and environment and the growing problem of emergence of multi-drug resistant strains, the food industries are shifting their focus towards the use of natural antioxidant and antimicrobial compounds extracted from plants as preservatives [1]. Therefore, efforts to replace synthetic preservatives with natural compounds with antioxidant and antimicrobial activity have been becoming an important research direction [2]. Natural food preservatives are safe, ecofriendly, cost-effective and have broad spectrum in contrast to synthetic compounds [3]. Natural antioxidants are produced in living cells to protect them from the damage due to free radicals produced in chain reactions. In this sense, some fruits and vegetables and their derivatives are good sources of antioxidants [4]. Moreover, natural extracts can also contain compounds with antimicrobial properties [5].

Essential oils extracted from *Citrus* and juice from cranberry (*Vaccinium* spp.) contain various bioactive compounds. Specifically, lemon (*Citrus limon*) essential oil, which is generally recognized as safe (GRAS), is reported of comprising a complex mixture of volatile components including limonene, β -pinene, geranial, and linalool, etc. that exhibit antioxidant and antimicrobial activity [6,7]. Similarly, cranberry juice is reportedly a rich source of valuable flavonols, terpenes, flavanones, and other phenolic acids, and previous studies have demonstrated its antioxidant and antimicrobial properties [8–10]. Thus, both lemon essential oil and cranberry juice possess antioxidant and antimicrobial properties that can be useful in the food industry.

To the best of our knowledge the current state of researches regarding the antioxidant and antimicrobial properties of lemon essential oil and cranberry is not yet reported. Hence, this review aimed mainly at discussing the antioxidant and antimicrobial properties of lemon essential oil and cranberry juice. Prior, a brief description about their source, extraction and composition is provided.

II. CRANBERRY JUICE

2.1. Source, extraction and composition

Cranberry belongs to the *Ericaceae* or *heath* family, to which plants in the genera *Rhododendron* and *Kalmia* (laurels) also belong [11]. Members of this family prefer acidic soils (pH 4-5) that are moist, well drained and high in organic matter (3-15%). Cranberry is usually placed in the genus, *Vaccinium*, which has 22 species [12]. Some botanists also place cranberry in the genus, *Oxycoccus*, leading to some confusion in the literature with regards to nomenclature.

Cranberry fruits are mainly found in Northern American and some countries in Europe and Asia [13]. The North American cranberry (*Vaccinium macrocarpon*) is recognized by the US Department of Agriculture, USDA, as the standard for fresh cranberries and cranberry juice cocktail. The European variety, grown in parts of central Europe, Finland, and Germany known as *Vaccinium oxycoccus* is a smaller fruit with anthocyanins and acid profiles slightly different to that of the North American variety [14].

Cranberry has become the subject of interest of the food industry in the last two decades due to the increased awareness of consumers about functional food and its preventive and positive effects on human health [15]. Cranberry is a rich source of valuable phytochemicals, including vitamins and phenolic compounds [16]. The profile of bioactive compounds in cranberry differs from other types of berries, as it is rich in proanthocyanidins type A, contrary to the majority of fruits in which proanthocyanidins type B are predominate. Cranberries are mostly consumed in processed form (juices, jams, syrups, or dried) since the sour taste of fresh cranberries is widely unacceptable for consumers [15]. Figure 1 show the tree, fruits and juice of cranberry.



Fig.1. Cranberry (*Vaccinium macrocarpon*) tree and fruit and juice. Source: Adapted from [17]

Freeze-thaw treatments are widely used in the cranberry processing industry to increase anthocyanin yield to ~ 50% (vs 7% in untreated fruit) as well as total juice yield [18]. Cell wall deterioration due to ice crystal formation releases phytochemicals from normal cellular

compartmentalization, resulting in higher extraction efficiency of anthocyanin pigments. It is likely that freeze-thaw treatments similarly release other classes of phytochemicals, such as the other flavonoids, but no experimental verification of this is available [19].

There are three main juice extractions methods used in the cranberry juice industry [20]. The first one uses a mechanical press to extract the juice where no heat is needed, preventing deterioration. The second one is mash depectinization, which consists in the addition of enzymes with the aim to reduce the fruit into a mash and then pressed. The last one is a countercurrent extraction of the sliced fruit and water, involving the use of a large screw. These processes yields are 75%, 100% and 90%, respectively [14]. Pectinase is obtained when fermenting *Asperillus niger* with carbon sources, such as glucose, sucrose and galacturonic acid [21].

Processing variables such as heat, increased pH, light, dissolved oxygen, and ascorbic acid, and the presence of certain enzymes (polyphenol oxidases and glycosidases) markedly destabilize cranberry juice color and anthocyanin content, while pigment co-factors (including cinnamic acid derivatives and flavonoids) as well as certain metals (copper, iron, and tin) improve cranberry anthocyanin and color retention during storage [19]. Phenolic condensation via several mechanisms seems likely to be a major mode of phytochemical loss during processing and storage in cranberry products.

Cranberry juice, the most common form in which cranberries are consumed is a food product with attractive red color and high content of acids. The bright red color in cranberries originates from the anthocyanins cyanidin-3-monogalactoside, cyanidin-3-monoarabinoside, peonidin-3-monogalactoside and peonidin-3-monoarabinoside [22]. Historically, cranberry juice has been consumed to prevent urinary tract infections. These health benefits, including reduced risks of cancer and cardiovascular disease associated with the consumption of cranberry juice, are believed to be due to the presence of various polyphenolic compounds, including anthocyanins, flavonols, and procyanidins, and the synergistic effects among them. According to Brown et al. [23] there are approximately 8000–10,000 phytochemicals detected in *V. macrocarpon*, and *V. oxycoccus*. The phenolic compounds are important for plants for their normal growth and defense against biological and environmental stresses, infection, and injury [24]. Generally, cranberries have a diverse phytochemical profile with phenolic acids such as hydroxycinnamic acid, three classes of flavonoids (i.e., flavonols, anthocyanins, and proanthocyanidins), catechins, and triterpenoids [25]. Česonienė et al. [26] compared the amount of biologically active compounds among 40 genotypes (13 certified

cultivars and 27 wild clones) of *V. oxycoccos* fruit of different origins (Estonian, Russian, and Lithuanian), grown under uniform ecological conditions in Lithuania. They found great variation in anthocyanin content, organic acids, and sugar content in fruits of cultivated types and wild clones, therefore the content of presented compounds differs depending on the cultivars. Analogously to the berries of *V. macrocarpon*, *V. oxycoccos* berries also contain citric acid (10.8 to 54.3 g/kg), malic (14.1 to 43.3 g/kg), and quinic (3.81 to 13.3 g/kg) acids as the main organic acids. Figure 2 provides a detailed bioactive compounds description in berry and cranberry.

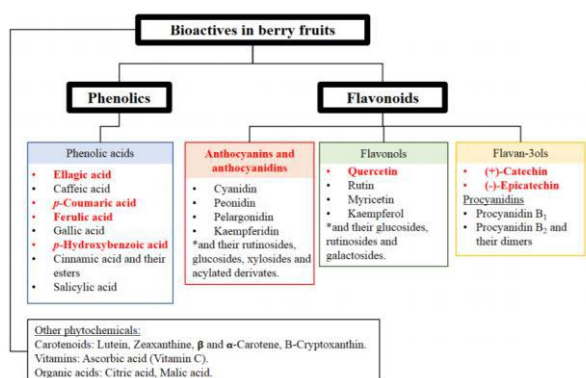


Fig.2. Bioactive compounds in berry. (Bioactive compounds peculiar to cranberry are in red). Source: [17]

2.2. Antioxidant properties

In biology, compounds that can retard or prevent the effects of oxidation have been broadly considered as antioxidants, including compounds that either inhibit specific oxidizing enzymes or react with oxidants before they damage critical biological molecules [27]. Effective antioxidants are radical scavengers that break down radical chain reactions caused by reactive oxygen/nitrogen species or that can inhibit the reactive oxidants from being formed in the first place [28]. These reactions are based on a compound's ability to donate hydrogen as well as to stabilize the resulting antioxidant radical by electron delocalization (resonance) and/or intramolecular hydrogen bonding or by further oxidation. The loss of hydrogen may take place by donation of an electron followed by deprotonation [29,30].

The antioxidant activity of cranberry juice derives from cranberry phenolics and considerable in vitro evidence exists, showing that cranberry phenolics are powerful antioxidants. However, the great diversity of methods applied to studying both the radical-scavenging activity and the antioxidant activity has resulted in great differences in the outcome [31]. Still, cranberry phenolics appear to have free radical-scavenging properties against

superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($-OH$), and singlet oxygen (1O_2), and can also inhibit lipid peroxidation, as well as protein and lipid oxidation in liposomes [32–34]. This could explain why they prevent the oxidation of bulk lipids. The formation of methyl linoleate hydroperoxides for example, was inhibited over 90% by cranberry phenolics at concentrations as low as 500 $\mu g/mL$ [31]. The effect of cranberry juice on oxidative changes occurring in meat products was determined also by Tyburcy et al. [35]. Cranberry juice, in the amount of 5% of the meat weight, was added to the thermally processed pork burgers, which were stored for seven days at 3 to 7°C, and juice was added also to a raw beef stuffing. A 5% addition of the cranberry juice caused decreasing of TBARS values, which are formed as a byproduct of lipid peroxidation of burgers, to twice or by three times the value of the control sample. As mentioned by the authors, cranberry juice was a good color stabilizer of the raw beef stuffing. Another study evaluated the degree of antiradical activity against DPPH in incubated and non-incubated juice and reported an increase in antiradical activity against DPPH• with the increase of the concentration of the juice. The incubated samples showed more expressed antioxidant activity due to the longer exposure to radicals. The determined EC_{50} value of the incubated juice is 5.94 mg/L [15]. These results clearly demonstrated that constituents in cranberry juice exhibit antioxidant activity. In literature however, conflicting reports about the main constituents responsible of antioxidant activity among hydroxycinnamates, flavonols, and proanthocyanidins, etc are found. Indeed, Zheng et al. [36] have reported that the anthocyanins contributed 54.2% of the antioxidant activity of cranberries, whereas the flavonols contributed 34.6% of the observed antioxidant activity. According to Yan et al. [37], the cranberry extract composed primarily of flavonol glycosides showed the best inhibition of oxidative processes measured by DPPH assay (EC_{50} at 30–40 $\mu g/mL$) compared to anthocyanin-rich cranberry extract or to crude phenolic cranberry extract. From the compounds isolated from cranberry extract by He et al. [38], quercetin, 3,5,7,3',4'-pentahydroxyflavonol-3-O- β -D-glucopyranoside, 3,5,7,3',4'-pentahydroxyflavonol-3-O- β -D-galactopyranoside, and 3,5,7,3',4'-pentahydroxyflavonol-3-O- α -L-arabinofuranoside, showed potent antioxidant activities, with lower median effective concentration, EC_{50} , values of approximately 10 μM . Polyphenol and volatile extracts from cranberry were also reportedly effective in reducing nitric oxide production [39].

2.3. Antimicrobial properties

In the past decade, cranberry juice antimicrobial activity was demonstrated toward various groups of bacteria and fungi. Although the antimicrobial activity of cranberry is generally attributed to the presence of phenolic compounds which activity is accredited to the acidic nature of hydroxyl groups, other polymeric tannins and in particular, the proanthocyanidins consisting primarily of epicatechin tetramers and pentamers with at least one A-type linkage, seem to be the protecting element against pathogenic bacteria [31,34,40]. Several mechanisms could explain the growth inhibition of pathogens in presence of cranberry juice—the destabilization of cytoplasmic membrane, the permeabilization of cell membrane, the inhibition of extracellular microbial enzymes, the direct actions on microbial metabolism, and the deprivation of the substrates required for microbial growth [31]. In an experiment by Ermis et al. [10], there was shown a possibility to inhibit the growth of visible colonies of several fungi with cranberry juice concentrate in fruit spreads (raspberry–aloe vera; strawberry–lime) with reduced sugar, which is a main reason for a growth of microorganisms in low-calorie jams. The antifungal activities of cranberry concentrate were studied in vitro against selected fungi *Absidia glauca*, *Penicillium brevicompactum*, *Saccharomyces cerevisiae*, and *Zygosaccharomyces bailii*. The concentrate was able to inhibit growth of visible colonies of most xerophilic and non-xerophilic fungi. For both fruit spreads with cranberry concentrate *A. glauca* was not able to grow, the growth of *P. brevicompactum* on the spread was inhibited at 3% cranberry concentrate, and *S. cerevisiae* could not grow at a concentration of 18%. *Z. bailii* was the most resistant fungus, the highest concentration (24%) was able to inhibit its growth by 29.8% only for raspberry–Aloe vera spread. The antibacterial effects of American cranberry (*Vaccinium macrocarpon*) juice concentrate on foodborne pathogens, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella Typhimurium*, and *Staphylococcus aureus* in vitro were also investigated. BHI data indicated that the 100 µl/ml treatment reduced the four pathogens by 3–8 log compared with the control on day 5 at 21 and 4 °C. TEM revealed damage to the bacterial cell walls and membranes. Cranberry concentrate has antibacterial effects on the four foodborne pathogens. Based on potential health benefits and proven antimicrobial effects, the authors suggested that American cranberry concentrate may have dual applications as a food preservative [41]. Ilić et al. [15] investigated the antibacterial activity of cranberry juice against seven bacteria. Among all tested bacteria, *S. aureus* was the most sensitive. In addition, the juice was more efficient against *E. faecalis* than ampicillin, as well as more efficient

against *S. aureus*, *S. enteritidis* and *K. pneumoniae* than cefalexin. Moreover, the juice was more efficient against *E. coli* than both tested conventional antibiotics. The authors ascribed the antibacterial activity to the presence of organic acids and phenols in cranberries that cause cell lysis and facilitate leakage of cell content, therefore assuring inactivation [15]. In another study the antibacterial activity of cranberry juice concentrate has been evaluated in vitro and in situ against three foodborne pathogenic bacteria. Results showed a high antimicrobial effect with a noticeable inhibition capacity against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium*. In situ studies showed 2.5, 1.8 and 5 log reduction of *E. coli*, *L. monocytogenes* and *S. typhimurium*, respectively in presence of cranberry juice concentrate, on pre-cut red peppers after 7 days of storage at 4 °C. A total inhibition of *L. monocytogenes* on fresh cranberry fruits in primary day of storage, was observed. Cranberries treated with cranberry juice concentrate also showed a 3 log reduction of *S. typhimurium* after 4 days of storage at 4 °C. The results suggest that cranberry juice concentrate can be an effective preservative, source of natural antibacterial, to protect ready to eat foods from foodborne pathogens contamination without effecting on sensorial properties of treated samples and allow to maintain the freshness, sensory and the nutritional quality [42].

III. LEMON ESSENTIAL OIL

3.1. Source, extraction and composition

Citrus are the most important crops in the world in terms of production according to the Food and Agricultural Organisation (FAO), with 13 735 357 million tons produced in 2020 [43]. The genus *Citrus* belongs to the *Rutaceae* family that comprises of about 140 genera and 1300 species [44]. One of the species *Citrus limon* is scattered in tropical and sub-tropical regions of South east Asia and the top producers are India, China, Mexico, Argentina, Brazil, Spain, United States, Iran, Turkey, Spain, Greece and some parts of Italy [45]. This is a small thorny tree bearing oval juicy fruit which is acidic and aromatic in nature. It grows up to a height of about 10-20 feet. Leaves of this tree are elliptical and shiny in nature and the flowers possess a strong fragrance. As compared to the other varieties of lemon, *C. limon* is quite larger in size (Figure 3). Furthermore, this species contains many important bioactive compounds such as essential oil, which are complex mixtures of volatile organic substances produced as secondary metabolites [46]. EOs are highly soluble in volatile compounds such as alcohol, ether, and fixed oils but insoluble in water [47]. Also, they can easily

degrade due to environmental factors such as light and heat and quickly oxidize.



Fig.3. Lemon (*Citrus limon*) tree, fruits and essential oils

Essentials oils from *Citrus* are extracted by methods such as steam distillation, solvent extraction, cold pressing and supercritical fluid extraction and subcritical water [48]. High proportion (93%) of citrus is extracted commercially by traditional methods including hydro-distillation and steam distillation and remaining (7%) by other methods [49]. The yield is a watery emulsion, which is then centrifuged to recover the EOs [50]. CO₂ is the most popular solvent of the supercritical fluid extraction because it is a non-toxic, cheap, readily available, and generally recognized as safe (GRAS) [51]. However, some compounds cannot be fully extracted by using CO₂ only [52]. Therefore, the extraction can be improved by increasing pressure or adding a polar modifier, such as ethanol. The subcritical water or pressurized hot water has been introduced as an extractant under dynamic conditions (pressure high enough to maintain water under liquid state and temperature in the range of 100 to 374 °C). This method is quicker, provides a more valuable essential oil (with higher amounts of oxygenated compounds and no significant presence of terpenes), and allows substantial savings of costs, in terms of both energy and plant material [53]. New extraction method such as solvent-free microwave extraction (SFME) has received increasing attention over the years due to higher yields of EOs, shorter extraction time and less solvent consumption. SFME is a combination of microwave heating and dry distillation, performed at atmospheric pressure without any solvent or water. Isolation and concentration of volatile compounds are performed by a single stage [54]. A novel design for shortening the extraction time to around 20–60 min was documented in a recent study [55]. They reported that solvent-free microwave extraction was an effective method for EO extraction.

Essential oil of *Citrus limon* consist of various compounds such as aliphatic sesquiterpenes, terpenes, oxygenated derivatives and also aromatic hydrocarbons [6,7,56]. Specifically, limonene, α -pinene, geranial, neral, myrcene, linalool, and terpinene are considered to be the major constituents (Figure 4) of the essential oil of *Citrus limon*

[6,7,56]. However, great variability may occur in the composition due to several factors, among others the geographical location, season and environmental factors, such as soil type and climate, genetic factors processing and extraction method and the part of the plant used to extract the oil [57].

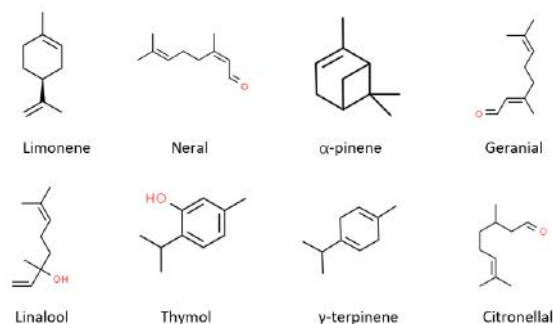


Fig.4. Chemical structures of some popular constituents of lemon essential oil

For instance, in a recent study, it is identified a total of forty-three compounds in *Citrus limon* essential oil, of which limonene (55.40 %) and neral (10.39 %) were found as major compounds followed by trans-verbenol (6.43 %) and decanal (3.25 %) [58]. In another study, the chemical composition analysis by Gas chromatography-mass spectrometry (GC-MS) of *Citrus limon* essential oil from China revealed d-limonene (61.72%), Carene (13.97%), α -pinene (13.67%), Citral (1.88%), geranial (1.29%) as major compounds while the major constituents of essential oil of *C. limon* from Irak were limonene (29.52 %), β -pinene (23.89 %), citronellal (11.53 %) and thymol (9.79 %) [6,46]. Furthermore, limonene (71.81%, 70.36% and 72.48%) with γ -terpinene (8.72%, 8.91% and 8.88%), β -pinene (6.61%, 6.72% and 6.60%), were identified as major monoterpene hydrocarbons in *Citrus limon* extracted by supercritical CO₂ extraction (SFE), cold pressing (CP) and hydrodistillation (HD) methods whereas β -bisabolene (1.42%, 1.41% and 1.22%) and neryl acetate (1.25%, 1.28% and 1.21%) were identified as principal sesquiterpene hydrocarbon and oxygenated compounds respectively in SFE, CP and HD [59]. Extracted essential oil from *C. limon* possesses many medicinal properties. It can be used in different cosmetic industries such as hair oil, beauty soaps, deodorants, and it is most widely used in making soft drinks and in pharmaceutical industries [60,61]. It possesses different anti-microbial, anti-fungal, rich in anti-oxidants and contains anti-cancer properties [7,62]. It also has the great potential for the treatment against cancer [61,63].

3.2. Antioxidant properties

Citrus limon essential oil is a source of natural antioxidants that help in the prevention of oxidative stress and related diseases. It is a good substitute for chemical antioxidants in the food processing industry. The mechanisms by which EOs demonstrate their antioxidant activities depend on the content and composition of active constituents present in them. Due to the large variety of compounds, its antioxidant activity cannot be attributed only to a single mechanism of action [64]. Mainly, their activities are related to the presence of phenolic compounds that have significant redox properties and play important roles in neutralizing free radicals and in peroxide decomposition [65]. The other components such as certain alcohols, ethers, ketones, aldehydes, and monoterpenes: linalool, 1,8-cineole, geranial/neral, citronellal, isomenthone, menthone, and some monoterpenes also play a key role in the antioxidant properties of EOs [66]. Different mechanisms (direct or indirect) to slow down the oxidation reactions including prevention of chain initiation and free-radical scavenging activity are reported [48,67]. Also, continued hydrogen abstraction and terminators, quenchers of singlet oxygen formation, and binding of transition metal ion catalysts are between their modes of actions [53]. EOs activity as antioxidants occurs in three phases: initiation, propagation, and termination as shown in Figure 5.

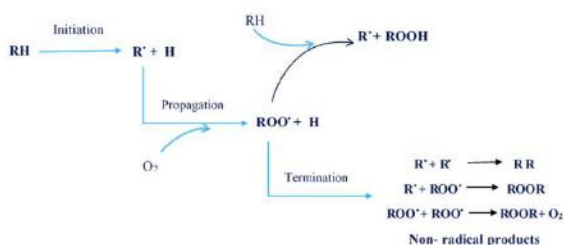


Fig.5. Mechanism of action of essential oil against lipid oxidation. Source : [68]

In vitro DPPH and ABTS assays, conducted on the *Citrus limon* EO, demonstrate its ability to act as an antioxidant [69] while Ben Hsouna et al. [7] reported excellent DPPH scavenging ability of *Citrus limon* EO with IC_{50} of 15.056 $\mu\text{g/ml}$ and a strong β -carotene bleaching inhibition after 120 min of incubation with IC_{50} of 40.147 $\mu\text{g/ml}$. Ben Miri et al. [70] reported IC_{50} and β -carotene/linoleic acid inhibition percentage of *Citrus limon* essential oils were 1570.10 and 752.26 $\mu\text{g/mL}$, while the total phenolic were 16.90 and 10.53 $\mu\text{g/mg}$. The authors concluded that LEO could be good alternative to protect food. Antioxidant activity of EO of *Citrus limon* and other *Citrus* cultivated in China was tested by DPPH and ABTS assays [6]. The

study reported that the essential oil exhibited good antioxidant activity and have a potential to be used as a natural food preservative to prevent oxidation. The antioxidant and antiradical scavenging properties of the six different oils including LEO were tested by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. All examined oils exhibited a free radical scavenging activity, ranging 20–70% of DPPH inhibition. Lemon oil showed the most antioxidant capacity, with DPPH inhibition rate of 70% [71]. Antioxidant assays based on the consumption of stable free radicals (ABTS and DPPH) and assays based in the capacity of antioxidants to reduce ions (FRAP and CUPRAC), were carried out to evaluate the antioxidant capacity of essential oils from *Citrus* species including *Citrus limon* from Argentina and the United States [72]. All essential oils including lemon essential oil showed consistently strong antioxidant activity. Yang et al. [73] compared the antioxidant activities of six popular and commercially available herb essential oils, including lavender (*Lavendular angustifolia*), peppermint (*Mentha piperita*), rosemary (*Rosmarius officinalis*), lemon (*Citrus limon*), grapefruit (*Citrus paradise*), and frankincense (*Boswellia carteri*). *Citrus limon* essential oil showed one of the highest DPPH radical-scavenging activity with RC_{50} values of $2.1 \pm 0.04\%$. Spadaro et al. [74] compared the volatile fraction composition and biological activity of *Citrus limon* essential oils extracted from conventionally grown and biological fruit. Results revealed differences in both oil composition, especially in the content of oxygenated compounds and biological activity with *Citrus limon* from biological production demonstrating greater antiproliferative effect. The authors suggested the antiproliferative effect of the lemon oils could be related to monoterpene hydrocarbons. Dawidowicz et al. [75] reported good antioxidant activity with different essential oils including *Citrus limon* using 2,2'-Diphenyl-1-picrylhydrazyl, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and β -carotene bleaching assays. Nevertheless, the obtained data show that the antioxidant properties of essential oils do not always depend on the antioxidant activity of its main component, and that they can be modulated by their other components. Therefore, when comparing the antioxidant properties of essential oils and their main components, the concepts of synergism, antagonism and additivity are very relevant. Also, the conclusions concerning the interaction of essential oil components may depend on the type of method applied for assessing the antioxidant activity.

3.3. Antimicrobial properties

Investigations have proved that EO of *Citrus limon* has potential application as antimicrobial agents or food additives in the food industry due to their antimicrobial

potential against common food-borne and spoilage microorganisms [6]. The antimicrobial mechanism of essential oils depends mainly on the type and concentration of the chemicals it contains. Different chemical components can act through different mechanisms. For example, the main function of phenolic compounds is to disrupt the structure and permeability of cell membranes and the hydroxyl groups carried in phenolic compounds can impair the activity of enzymes in microorganisms [76]. Also the lipophilic or hydrophobic properties of the components of EOs allow them to interact with the lipids of the microbial cell membrane and mitochondria, thereby making cell structures less organized and thus more permeable. This increased permeability allows the outflow of ions and other cell contents. Although a certain amount of outflow from microbial cells may be tolerated with no loss of viability, substantial loss of cell contents or the loss of vital ions and molecules will lead to cell death [77]. In addition, the EOs activity as an antimicrobial is also varying due to the difference in the cellular structure of the bacterial cell, such as Gram (+) and Gram (-) bacteria, which differ in the structure of the cell membrane [68]. Thus, the action route of essential oil antimicrobial mechanism is no single, but two or more routes exist at the same time [76]. The possible action mechanisms of EO are shown in Figure 6, which illustrates them from four aspects of cell wall, cell membrane, DNA, respiration and energy metabolism, respectively.

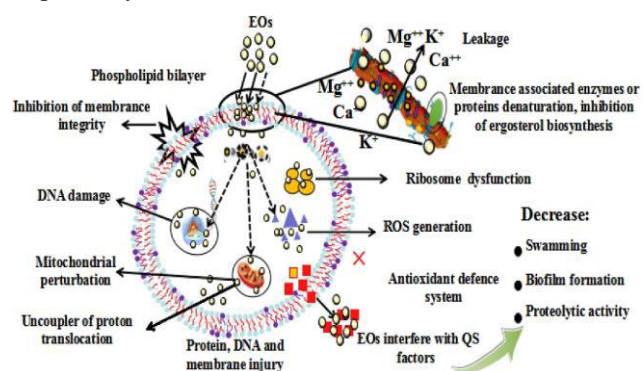


Fig.6. The possible action mechanisms of essential oil.

Source: [76]

Frassinetti et al. [71] investigated the antimicrobial activity of four *Citrus* species essential oils including *Citrus limon* against ten strains of gram-negative and gram-positive bacteria and reported all oils showed good antibacterial activity against both gram-negative and gram-positive bacteria. The minimum inhibitory concentration (MIC) for *Citrus limon* oils ranged 20–200 µg/ml depending on the microorganisms. Ben Hsouna et al. [7] assessed the

antimicrobial activities of *C. limon* essential oil along with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. The MICs varied from 0.039 to 1.25 mg/ml for gram positive bacteria and from 0.25 to 2.5 mg/ml for Gram-negative bacteria. Additionally, *C. limon* essential oil successfully inhibited the development of *L. monocytogenes* in minced beef meat. Antibacterial activity of lemon essential oil was determined against six different fish pathogens (*Yersinia ruckeri*, *Aeromonas hydrophila*, *Listonella anguillarum*, *Edwardsiella tarda*, *Citrobacter freundii* and *Lactococcus garvieae*). The disc diffusion results indicated that essential oil of lemon significantly inhibited the growth of *Y. ruckeri*, *A. hydrophila*, *L. anguillarum* and *C. freundii* [78]. The antimicrobial activity of LEO against 12 bacteria and 4 yeasts was tested by a microdilution broth test [69]. Results showed a low inhibitory activity against food grade and *Lactobacillus* strains, whereas *Listeria monocytogenes* and *Staphylococcus aureus* were inhibited at low EO concentration. Also, *Citrus limon* essential oil demonstrated an antimicrobial activity against yeast, with *Saccharomyces cerevisiae* being the most sensitive strain. Akarca et al. [79] investigated the biological activities of LEO on foodborne pathogenic bacteria and food-borne saprophytic yeasts and molds and reported higher antibacterial effect on gram-positive bacteria used in the study compared to gram-negative bacteria. The highest antibacterial activity was detected on *Staphylococcus aureus* with 22.55 mm. The highest anti-fungal effects were determined on *Candida tropicalis* (23.61 mm) and *Rhizopus nigricans* (14.15 mm). Greater antibacterial activity of EO of *Citrus limon* against gram-positive bacteria compared to gram-negative bacteria were also reported in a recent study [6]. The yeasts were also more susceptible than bacteria to the essential oils. Brahmi et al. [80] reported strongest antifungal power with values of 35 mm for lemon essential among four essential tested. However, no antibacterial activity was detected for the four types of EOs. The authors concluded these EOs would be of interest in both agriculture and the food industry by acting as bio-fungicide but also as additives for compounds of medical and cosmetic interest. Antibacterial activities of essential oils from unripe and ripened lemon were carried out by Mehmood et al. [81]. Results showed that both the isolated LEO exhibited considerable antibacterial activity against *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and *Staphylococcus aureus* with no cytotoxic effects. Nevertheless, a significant variation in biological activities of LEO were observed that can be linked to lemon fruit ripening stages. Yazgan et al. [82] compared the antimicrobial activities of lemon oil based nanoemulsion and two different concentrations of lemon

essential oil (100% and 10%) on food-borne pathogens. According to value of MIC, both nanoemulsion and 100% essential oil inhibited bacterial growth of all of the pathogen bacteria tested whereas they were less effective on inhibition of fish spoilage bacteria. However, 10% essential oil was more effective on spoilage bacteria than pathogens. The minimum bactericidal concentration (MBC) showed that nanoemulsion and 100% lemon essential oil presented a noticeable bactericidal activity against *S. paratyphi* whereas 10% lemon essential oil was found as ≥ 25 mg/mL against pathogens and spoilage bacteria. The authors concluded that the use of nanoemulsion based on lemon essential oil can have potential as a natural antimicrobial agent against food-borne pathogen and spoilage bacteria for fish processing industry.

IV. CONCLUSION

This review focused on antioxidant and antimicrobial properties of lemon essential and cranberry. Our results indicated that the antioxidant and antimicrobial properties of lemon essential oil and cranberry juice depends on the composition and concentration of active compounds. While major components can play a leading role, the antioxidant and antimicrobial effect do not always depend on the antioxidant and antimicrobial activity of their main components, but can be modulated by their other components. Therefore, when comparing the antioxidant and antimicrobial properties of both lemon essential oil and cranberry juice and their main components, the concepts of synergism, antagonism and additivity are very relevant. Compared to the gram-negative bacteria, the gram-positive bacterial strains are more sensitive to their bioactive compounds. Fungal strains on the other hand seem more susceptible than the bacteria. Overall, this review confirmed that the lemon essential oil and cranberry juice could be used not only as source of natural antioxidants and antimicrobials, but also as possible food natural supplements to extend food shelf life.

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ABBREVIATIONS

ABTS	Radical scavenging assay using 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
BHI	Brain Heart Infusion
CP	Cold pressing
CUPRAC	CUPric reducing antioxidant capacity
DNA	Deoxyribonucleic Acid
DPPH	Radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl
EO	Essential oil
EC50	Half maximal effective concentration
FAO	Food and Agricultural Organisation
FRAP	Ferric reducing ability of plasma
GC-MS	Gas chromatography/mass spectrometry
GRAS	Generally recognized as safe
HD	Hydrodistillation
IC50	Half maximal inhibitory concentration
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
RC50	Concentration of antioxidant required to achieve absorbance equal to 50% that of control containing no antioxidant
SEM	Scanning Electron Microscopy
SFE	Supercritical fluid extraction
SFME	Solvent-free microwave extraction
TBARS	Thiobarbituric acid reactive substances
TEM	Transmission electron microscopy