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Meta Analysis

Antibacterial and antioxidant activity of catechin, gallic acid, and epigallocatechin-3-gallate: focus on nanoformulations

Mehran Alavi[1,2,*], Michael R. Hamblin[3,4], Ermia Aghaie[5], Seyed Ali Reza Mousavi[6], Mohammad Hajimolaali[7]

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ABSTRACT

Up to one million secondary metabolites are found in plant species, some of which may have desirable therapeutic activities. Among these secondary metabolites, catechin, gallic acid, and epigallocatechin-3-gallate are natural phenolic compounds with promising antioxidant and antibacterial activity. However, these compounds have disadvantages of poor solubility, low bioavailability in physiological conditions, and side effects in patients. Therefore new strategies could rely on formulations with other synthetic and natural materials. Nanoformulations of secondary metabolites could be new efficient strategies to treat many chronic bacterial infections. Combinations and conjugates of catechin, gallic acid, and epigallocatechin-3-gallate with various antibiotics could reduce the dose of these compounds, increase their antibacterial activity, and decrease cytotoxicity against healthy cells. For instance, a smart combination of two or more secondary metabolites may improve therapeutic applications in physiological conditions. In this regard, the growth of antibiotic-resistant bacteria, specifically multidrug-resistant bacteria with overexpression of efflux pumps and expression of the penicillinase enzyme, has been inhibited significantly. According to recent investigations, this review will discuss the advances and challenges of new micro and nanoformulations of these natural products.

1. Introduction

There are between 200000 up to 1 million primary and secondary metabolites found in plants, some of which may have useful therapeutic activity (Figure 1) [1-7]. Nanoformulations (or microformulations) of these metabolites could be new effective strategies for treating many diseases, especially chronic infections [8-12]. Among these secondary metabolites, catechin is a secondary metabolite found in various plant species, such as *Senegalia catechu* (cutch tree), with pronounced antioxidant and antimicrobial properties [13]. Epigallocatechin-3-gallate or epigallocatechin gallate (EGCG) is a polyphenol with the chemical structure of an ester between epigallocatechin and gallic acid [14]. EGCG can be extracted abundantly from the tea plant *Camellia sinensi,* (Figures 2a-c) [15]. Different types of tea, including black, white, and green tea have shown 936, 4245, and 7380 mg per 100 g of dried leaves [16]. Moreover, pecans, hazelnuts, onions, plums, and apples all contain varying amounts of

[1]*Department of biological science, Faculty of Science, Kurdistan University, Sanandaj, Kurdistan, Iran*
[2]*Nanobiotechnology Department, Faculty of Innovative Science and Technology, Razi University, Kermanshah, Iran*
[3]*Wellman Centre for Photomedicine, Massachusetts General Hospital, Boston, MA 02114, USA*
[4]*Department of Dermatology, Harvard Medical School, Boston, MA 02115, USA*
[5]*High Performance Powertrain Materials Laboratory, School of Engineering, University of British Columbia – Okanagan, Kelowna, V1V 1V7, Canada*
[6]*Department of Neuroscience, Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA 92037, San Diego, California, USA*
[7]*Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
***Corresponding Author**: Mehran Alavi (mehranbio83@gmail.com)*

EGCG. Synergistic antimicrobial effects can be found by combining EGCG and antibiotics. For instance, EGCG plus carbapenem showed significant inhibition of carbapenem-resistant *Acinetobacter baumannii* with the ability to produce β-lactamase, with minimum inhibition concentrations (MICs) of ≤ 1.0 μg/mL [17]. EGCG can increase susceptibility to tetracycline antibiotics and chloramphenicol, particularly in the case of *Pseudomonas. aeruginosa* strains with efflux pump expression [18]. Therefore the objectives of this review are to compare the antibacterial activity of these three plant metabolites against sensitive and antibiotic-resistant bacteria and to discuss the promise of new micro and nanoformulations in future investigations.

2. Catechin

Multidrug-resistant bacteria are becoming a major problem in treating bacterial infections [19-21]. Among these bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) can lead to severe infections, particularly in the case of patients with immunodeficiency disorders. Moreover, conventional antibiotics are mostly ineffective against this species [22]. Catechin isolated from *Anacardium occidentale* nutshells showed a minimum inhibitory concentration (MIC) of 78.1 μg/mL and an inhibition zone diameter (IZD) of 19.5 mm against MRSA. In contrast to methicillin, treatment with catechin at the MIC value increased reactive oxygen species (ROS) generation (H_2O_2) by 1.87-fold compared to the control sample. Moreover, the level of superoxide dismutase enzyme in the *S. aureus* cell lysate was also reduced [23].

Derivatives of catechin have attracted attention because of their unique therapeutic effects. For instance, ester compounds between the novel thiol-containing nucleophile tiopronin, (−)-epicatechin-4β-S-tiopronin methyl ester (ECT) and (+)-catechin-4β-S-tiopronin methyl ester (CT) showed higher antibacterial activity towards *S. aureus* and *Escherichia coli* in comparison with epicatechin and catechin alone [24]. These compounds could be loaded into organic or inorganic nanocarriers as a new approach to increase antibacterial activity. The antibacterial mechanism of catechin against *Aggregatibacter actinomycetemcomitans,* an oral bacterium, was suggested to involve blocking the activity of leukotoxin by inhibiting the binding of the toxin to the cholesterol of the cellular membrane [25].

2.1. Nanoformulations of catechin

Nanoformulations with sizes in the nanoscale range of 1-100 nm have been used to improve the solubility and bioavailability of many bioactive materials, with the advantages of biocompatibility and biodegradability [26-28]. For instance, using an ionic gelation reaction, quercetin and catechin were loaded onto chitosan nanoparticles, producing particles with a zeta potential of 31.79 mV, a mean diameter of 180.4 nm, and an ellipsoidal shape. This nano-formulation showed a loading efficiency of 52.23% and 76.35% for quercetin and catechin, respectively. The MIC values for this formulation against *E. coli*, *S. aureus*, and *Bacillus subtilis* were < 4.88 μg/mL, 9.76-4.88 μg/mL, and 9.76-4.88 μg/mL, respectively. In contrast, blank chitosan nanoparticles showed 625 - 312.50 μg/mL, 1250-625 μg/mL, and no antibacterial activity against *E.coli*, *S.aureus*, and *B. subtilis*, respectively [29]. A green tea extract containing catechin was also loaded onto polylactic acid/gelatin microfibers to inhibit bacterial growth [30]. β-Cyclodextrin-metal-organic frameworks were employed as nanocarriers to load catechin and were then incorporated into zein (corn protein) film. In addition to antioxidant activity, the nanocomposite film showed sustained release of catechin with an antibacterial effect against *S. aureus* and *E. coli* [31]. In another investigation, catechin-functionalized ZnO nanoclusters with a mean size of 24.1 nm showed MBC values of 59.5 μg/mL against *E. coli,* compared with catechin and penicillin with values of 1838 μg/mL and 25 μg/mL, respectively [32]. In another study, NPs prepared from catechin and rhenium (III) were coated onto a polyamide membrane to suppress planktonic and biofilm growth of *P. aeruginosa* with an inhibition rate up to >90% [33].

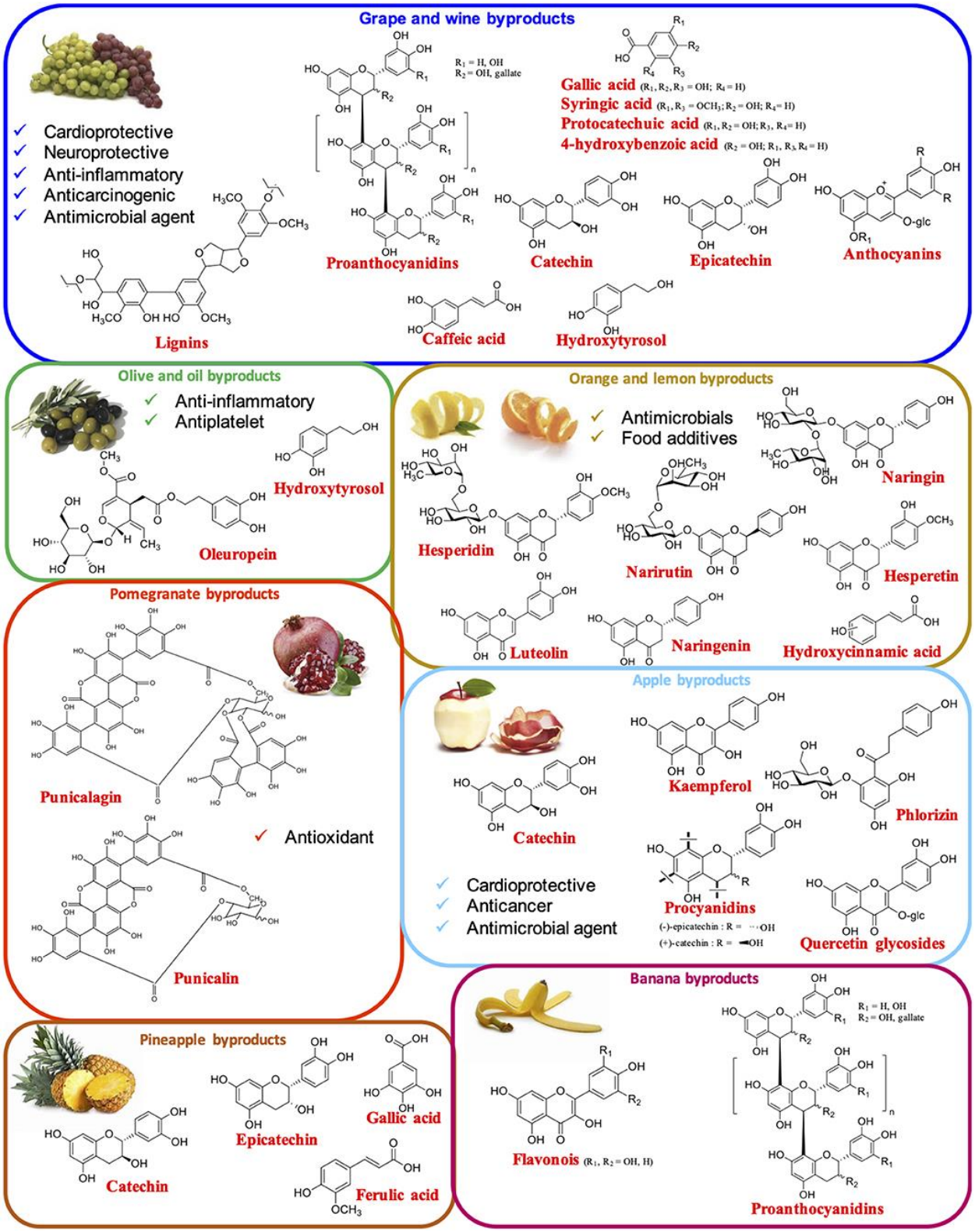
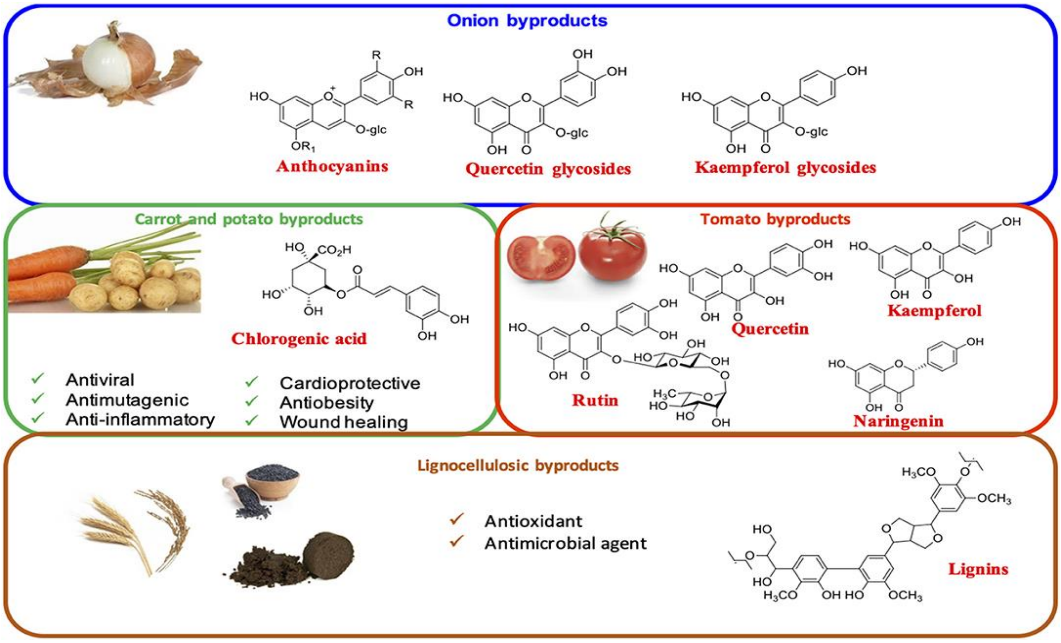
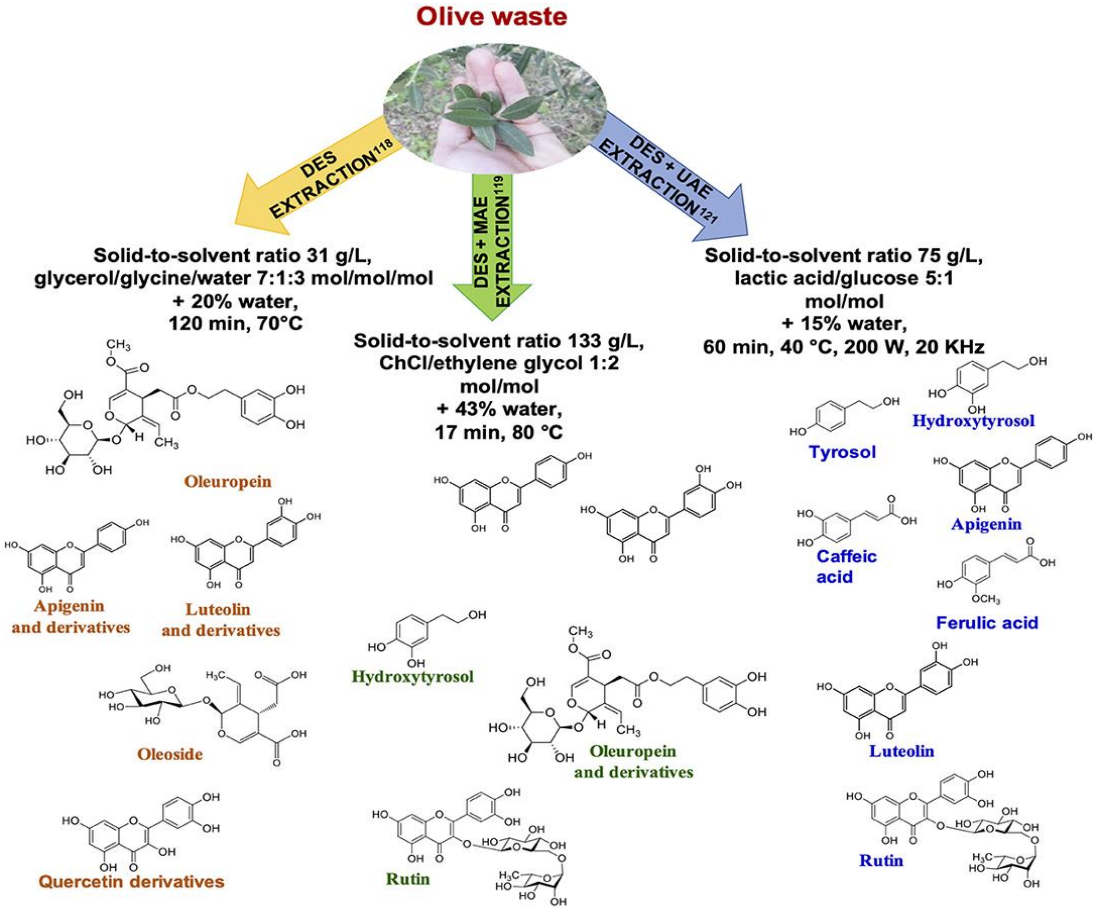


Fig. 1. The main secondary metabolites of phenols and polyphenols with therapeutic activities (copyright under the terms of the Creative Commons Attribution License (CC BY)) [3].





Continuation of **Fig. 1.** [3].

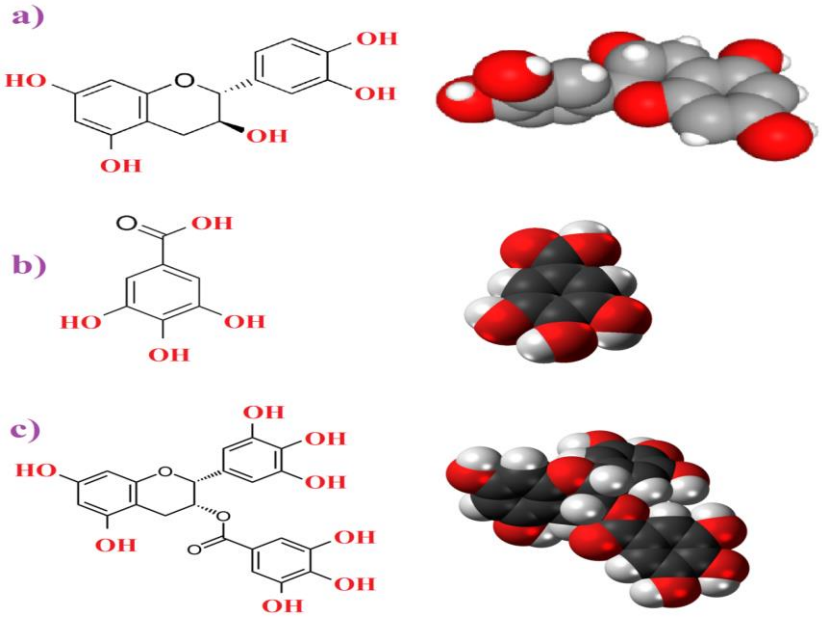


Fig. 2. Chemical structures and space-filling models of (a) catechin; (b) gallic acid; (c) epigallocatechin gallate (EGCG) (https://pubchem.ncbi.nlm.nih.gov).

3. Gallic acid

The smart combination of two or more plant metabolites may improve therapeutic antibacterial effects in physiological conditions. For instance, the grafting of gallic acid onto the acidic polysaccharide pectin was accomplished by using an enzymatic method to attach the 4-OH group of gallic acid to the carboxyl groups (-OH) of pectin. The inhibition rates for *S. aureus* and *E. coli* were 47.87% and 31.56%, respectively, compared to pectin alone, with 8.92% and 2.93%. The expression of the mdoH gene coding for OpgH protein involved in biofilm formation was down-regulated in a gallic acid dose-dependent manner. Additionally, based on the DPPH assay, the antioxidant activity of this formulation was 76.98% relative to pectin, with a value of only 2.68% [34].

Magnetic iron oxide NPs (IONP) were functionalized by gallic acid (Figure 3) with a size range of 5-11 nm, a stable aggregation state, and hydrophilic properties. They showed both antioxidant and antibacterial activity. Percentage inhibition values for IONP-gallic acid at a concentration of 100 mg/mL against *E. coli* and *S. aureus* were 225%, while *B. subtilis* was inhibited by 250% [35].

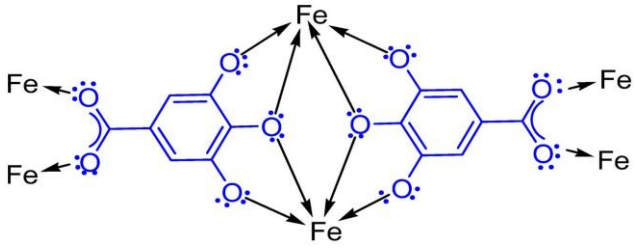


Fig. 3. Probabale chemical structure of iron gallate in NPs [35].

3.1. Nanoformulations of gallic acid

Nanocarriers such as metal or metal oxide nanoparticles, liposomes, lipid nanoparticles, polymeric nanoparticles, and quantum dots have all been investigated, depending on the objectives of the therapy [36]. Phytoliposomes were synthesized via a thin-layer dispersion method based on bonding between the polar head of phospholipids in the liposomes and the OH- groups of gallic acid. In a comparative study, these phytoliposomes (GA-LIP) were compared with phyoliposomes decorated by lactoferrin (a multifunctional iron glycoprotein) to produce LF-GA-LIP. Interestingly, the sizes of these formulations in the simulated oral, stomach, and intestine conditions were 218.3, 243.5, and 486.4 nm, respectively. There was an electrostatic

interaction between lactoferrin and the phospholipid bilayer, while hydrogen bonding connected the hydroxyl groups of gallic acid and the polar phospholipid head group in the liposomes. Both liposomes showed a spherical structure by transmission electron microscopy and atomic force microscopy. LF-GA-LIP displayed a delayed-release effect compared with GA-LIP in simulated digestion. LF-GA-LIP showed MIC values of 0.25 μg/mL against *E. coli* and *S. aureus,* while GA-LIP was less effective, and pure gallic acid had a MIC of 1 μg/mL [37].

In comparison to silver NPs, copper NPs show lower cytotoxicity along with the suitable antibacterial activity. Copper NPs were coated with chitosan and gallic acid by a microwave irradiation method. The chitosan-copper-gallic acid nanocomposites (NCs) showed peroxidase-like and oxidase-like activity. The NCs exhibited strong growth inhibition against *E. coli* and *S. aurues* with values of 91% and 99.9% at 20 μg/mL, by a mechanism involving leakage of the bacterial contents followed by the death of the bacteria. In contrast, chitosan-copper-tannic acid at a similar concentration demonstrated no bactericidal effects against *E. coli* and only 0.9% inhibition against *S. aureus* [38].

4. Epigallocatechin-3-gallate (EGCG)

Oral rehydration treatment is the primary therapy for diarrheal diseases and cholera caused by *Vibrio cholerae*, although treatment with antibiotics is recommended for severely ill patients. However, infection with *V. cholerae* multidrug-resistant (MDR) strains can lead to worse outcomes for patients [39]. As shown in Figure 4, MDR *V. cholerae* underwent membrane disruption after treatment with purified EGCG (98%) extracted from *Camellia sinensis* L., at a concentration of 0.5 mg/mL and 37 °C for 2 h. Moreover, a synergistic effect was found for the combination of EGCG and tetracycline with MIC values of 0.061, 0.008, and 0.004 μg/mL against MDR strains of *V. cholerae* P48 (O1), 22136 (O139), and N16961, respectively [40].

EGCG was conjugated to chitooligosaccharides extracted from the squid pen (*Loligo formosana*) via a free radical grafting reaction, and the antibacterial activity was evaluated against *E. coli* and *Listeria monocytogenes*. The MBC values of this conjugate were measured as 1 mg/mL and 0.05 mg/mL compared to unmodified chitooligosaccharides with MBCs of 2 mg/mL and 0.1 mg/mL against *L. monocytogenes* and *E. coli,* respectively [41].

4.1. Nanoformulations of EGCG

The major disadvantage of using EGCG in clinical applications is its poor solubility and low bioavailability. However, EGCG can be employed as an active natural filler to formulate polymeric films, hydrogels, and nanoparticles on the micro or nanoscale, to improve the antioxidant and antibacterial activity of a packaging film [42, 43]. In this regard, nanocomposites were prepared with dopamine hydrochloride and EGCG, which showed antioxidant activity with values of 70.93% and 56.68% for the DPPH assay and ABTS scavenging assay, respectively. The MIC values of this formulation against *E. coli* and *S. aureus* were 1.6 and 0.4 mg/mL, respectively. The antibacterial mechanism for this nano-formulation may be different for Gram-negative and Gram-positive bacteria. In the case of Gram-positive bacteria, direct binding of the NCs to the naked peptidoglycan layer and for Gram-negative bacteria, oxidative stress by generating H_2O_2 could result in bacterial inhibition and death [44].

Bare or functionalized metal or metal oxide nanoparticles, with higher activity in vitro and in vivo compared to bulk materials, can be utilized to formulate EGCG [22, 45-47]. The growth of *E. coli*, *P. aeruginosa* (Gram-negative), *Enterococcus faecalis* and *S. aureus* (Gram-positive) were inhibited, with MICs in the range of 1-120 μg/mL after treatment with gold (Au) nanoparticles loaded with EGCG. These showed low cytotoxicity against human keratinocytes (HaCaT) and murine fibroblast cells (L929) [48].

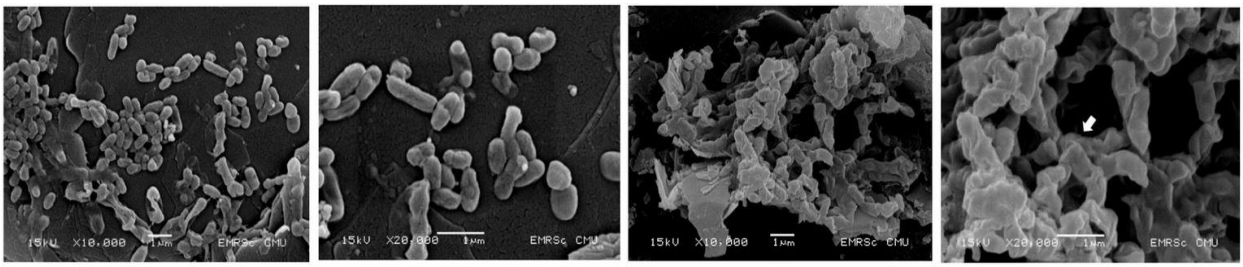


Fig. 4. Disruption of the *V. cholerae* bacterial membrane after treatment with EGCG; a) and b) are control samples, c) and d) are bacteria treated with EGCG at 0.5 mg/mL and 37 °C for 2 h; scale bar is 1 µm with ×10,000 and ×20,000 magnifications [40].

5. Conclusions

Various reports have demonstrated the bacteriostatic and bactericidal effects of EGCG against drug-resistant bacteria. However, EGCG has the disadvantages of low bioavailability and, when taken orally in patients, has adverse side effects of nausea, liver toxicity, and heartburn. Therefore, new formulations should be designed to overcome these limitations. One option may be the combination of EGCG with conventional antibiotics to reduce the EGCG dose and enhance the antibacterial activity of antibiotics. In this regard, antibiotic-resistant bacteria that show overexpression of efflux pumps and express penicillinase have both been significantly inhibited. Future studies should focus on optimizing these formulations with other antibacterial agents to hinder the development of antibiotic resistance, specifically in MDR bacteria. In the case of catechins, the primary antibacterial mechanism may be blocking the action of leukotoxin by inhibiting toxin binding to the cholesterol of the cellular membrane. Low bioavailability is the main disadvantage for the medical application of EGCG, catechin, and gallic acid. However, this limitation may be overcome by new formulations on the micro and nanoscale or as active natural fillers in polymeric nanoparticles, packaging films, and hydrogels.

Abbreviations

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
CT: (+)-catechin-4β-S-tiopronin methyl ester
DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate
ECT: (−)-epicatechin-4β-S-tiopronin methyl ester
EGCG: Epigallocatechin gallate
GA-LIP: Gallic acid-liposome
IONP: Iron oxide NPs
IZD: Inhibition zone diameter
LF-GA-LIP: Lactoferrin- gallic acid-liposome
MBC: Minimum bactericidal concentration
MDR: Multidrug-resistant
MIC: Minimum inhibition concentration
MRSA: Methicillin-resistant Staphylococcus aureus
NCs: Nanocomposites
NPs: Nanoparticles
ROS: Reactive oxygen species

Conflict of Interest

All authors state that they are free of any conflicts of interest related to this paper.

Author's contributions

All authors confirm that they have read and approved this manuscript.

Consent for publications

All authors have read and approved the final manuscript for publication.

Availability of data and material

The authors have embedded all data in the manuscript.

Ethics approval and consent to participate

The authors did not use human or animals in the research.

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