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Antimicrobial Efficacy of the Phenolic Compounds Ellagic Acid and Epigallocatechin Gallate

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ABSTRACT

Plant phenolic compounds are secondary metabolites that play the essential role of defense mechanism against microorganisms. The antimicrobial activity of ellagic acid and epigallocatechin gallate were determined by using a disc diffusion method that measured the zone of inhibition against *Klebsiella pneuomiae, Staphylococcus epidermis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans*. Of the two phenolics, epigallocatechin gallate exhibited a dose dependent inhibition of antimicrobial activity against *Staphylococcus epidermis* and *Candida albicans.*

Keywords: *Antimicrobial activity, Ellagic acid, Epigallocatechin gallate.*

INTRODUCTION

Phenolic compounds are widely found in a variety of different plant types. They are found in fruits, vegetables, wood, leaves, and plant byproduct (Oliveira et al. 2008). They are a group of diverse secondary metabolites that serve as the plants defense mechanism against microorganisms and other threats, as well as being responsible for pigmentation, growth, and other functions (Cowan, 1999; Lattanzio et al. 2006).

The bioactivities of phenolic compounds are related to the structure of the molecules. Phenolic compounds, by definition, are substances which possess an aromatic ring containing one or more hydroxyl substituents (Nietama et al. 2012). Due to their structure and functionality, phenolic compounds have been found to possess antimicrobial, antioxidant, and anthelmintic effects (Oliveira et al. 2008; Kahkonen et al. 1999; Williams et al. 2015).

Phenolic compounds are present in an array of different classes ranging from simple phenolic acids and tannins to stilbenes and flavonoids (Oliver Chen and Blumberg, 2008). Ellagic acid (EA) is a dimeric derivative of gallic acid, which is a phenolic acid, and has the basic carbon skeleton of C_6-C_1 (Landete 2011; Lattanzio et al. 2006). In many plant sources, ellagic acid is found as ellagitannins and are hydrolyzed in the presence of an acid or base in order to generate ellagic acid (Landete 2011). Epigallocatechin Gallate (EGCG) falls under the class of a flavonoid and follows the basic skeleton structure of C_6-C_3-C_6. EGCG is specifically a galloylated catechin with eight hydroxyl groups on its aromatic rings [18].

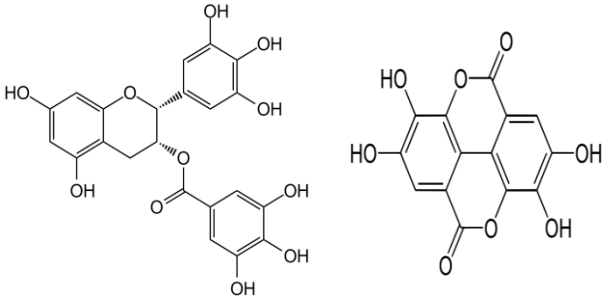

Figure 1. The structure of epigallocatechin gallate and ellagic acid

The hydroxyl groups of phenolic compounds enable phenolic compounds to form complexes with soluble and extracellular proteins (Savoia, 2012). The order of binding to proteins increases as the number of hydroxyl groups increases on that of the phenolic compound (Ozdal et al. 2013). Phenolic compounds, being able to form these complexes, have shown antimicrobial, anti-carcinogenic, and anti-parasitic properties (Rauha et al. 2000; Maddox et al. 2010; Barch et al. 1996; Du et al. 2012).

The importance of identifying the antimicrobial properties of secondary class metabolites, and possibly using them as potential antimicrobial substitutes, is due to the increase in drug resistant pathogens (Savoia, 2012).

In this study, we tested the effectiveness of the two phenolic compounds, ellagic acid and epigallocatechin gallate, against the following microbes: Staphylococcus aureus, Staphylococcus *epidermis*, *Escherichia coli*, Klebsiella *pnuemoniae,* and *Candida albicans.* Previous research has implicated different phenolic compounds being effective against these and other microbes.

The focus of this study will be to evaluate the antimicrobial effectiveness of ellagic acid and epigallocatechin gallate using the disc diffusion method.

MATERIALS AND METHODS

Microorganisms and Growth

The Microorganisms *Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Klebsiella*

Table 1. The zones of inhibitons of the phenolic compounds ellagic acid and epigallocatechin gallate at a concentration of 5mg/ml. Values are means ±SD (n = 2) and in units of mm.

Compounds	*K.pneumoniae*	*S.epidermidis*	*S.aureus*	*C. albicans*	*E.coli*	*P.aeruginosa*
Ciprofloxacin	13.0	11.9 ±0.1	13.0	10.9 ±0.4	13.9 ±0.1	13.0 ±0.5
Ellagic Acid	0	0	0	0	0	0
Epigallocatechin Gallate	0	6.4 ±0.2	0	5.4 ±0.4	0	0

Table 2. The zones of inhibition with an increasing Epigallocatechin Gallate concentration ranging from 2mg/ml to 15 mg/ml. Values are means ±SD (n=2) and in units of mm.

Species	Ciprofloxacin	2mg/ml	5mg/ml	8mg/ml	10 mg/ml	15mg/ml
S. epidermidis	11.5 ±0.5	5.1 ±0.1	6.4 ±0.1	6.8 ±0.3	6.9 ±0.4	7.2 ±0.2
C. albicans	11.8 ±0.3	6.0 ±0.6	7.0 ±0.1	7.1 ±0.3	7.1 ±0.1	8.0

pnuemoniae, Pseudomonas aeruginosa and *Candida albicans* were obtained as live cultures from Carolina Biological Company. The separate bacterial strains were picked with a wire loop from the original culture plates and inoculated in tests tubes with four milliliters of sterile tryptic soy broth using sterile transfer techniques. The test tubes were left overnight in an incubator at 37° C at 150 rpm to ensure that the microorganisms were allowed the same time to grow and to equalize to the same micro-organismal count. The test tubes with equivalent absorbance values between 0.3-0.35, measured with a spectrometer at 600nm, were used for disc diffusion assays.

Phenolic Compounds

The phenolic compounds ellagic acid (EA) and epigallocatechin gallate (EGCG) were purchased from Sigma Aldrich. The compounds were first dissolved in deionized water at a concentration of 5mg/ml. As needed, the concentrations of EA and EGCG were diluted to 2mg/ml, 5mg/ml, 8mg/ml, 10mg/ml, and 15mg/ml in order to determine the response to a change in the concentration of the phenolic compounds.

Disc Diffusion method

Mueller Hinton Agar was prepared and autoclaved for 45 minutes at 121°C. The agar was allowed to cool to room temperature and then stored in an incubator at 65°C until further use. Sterilized cotton swabs were used to spread the cultured bacteria on to the Mueller Hinton plates. ¼ inch discs were immersed in the phenolic compounds for 45-60 seconds and placed on to the plate. Ciprofloxacin was used as a positive control and sterilized tryptic soy broth was used as a negative control. Plates were placed, each with four discs: the negative and positive control, the EA disc and EGCG disc, in an incubator at 37°C and allowed to grow over night. Plates with the varying concentration of EA or EGCG were spotted with ¼ inch discs immersed in their respective solutions for 45-60 seconds. The plates were placed in the incubator at 37°C and allowed to grow over night.

Zone of Inhibition

After overnight Incubation the zone of inhibition was measured with a ruler by measuring the radius of each effective disc and calculating the diameter.

RESULTS

The results of inhibition on the tested microbes by the phenolic compounds, EA and EGCG, can be seen in table 1. EGCG was effective in inhibiting growth of select microbes whereas EA proved to be completely ineffective at 5mg/ml. Increasing the concentration of EGCG showed an increase in inhibition of growth.

EGCG was active against *Staphylococcus epidermis* and *Candida albicans* and proved to be inactive against *Klebsiella pneumonia*, *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aerogenes*. Increasing the concentration of EGCG from 2mg/ml to 15mg/ml showed an increase of inhibition as shown in table 2. EA displayed no antimicrobial effectiveness against any of the tested microbes.

DISCUSSION

This study reports EA as being ineffective against the tested microbial organisms. Previous studies have shown EA to be an effective antimicrobial compound. For instance, EA was shown to be effective against the gram negative bacteria *Helicobacter pylori*. This study also illustrated a dose dependent inhibitory effect against different other bacterial isolates, including those that were highly resistant to antibiotics (Landete 2011). Other phenolic acids such as, caffeic, ferulic, and gallic acid have shown antibacterial activity against *Staphylococcus aureus*, *Escherichia*

coli, and *Pseudomonas aeruginosa* (Daglia M. 2012). Ellagic acid differs from these phenolic acids as it is a dimer derivative of gallic acid linked together by ester bonds and varies in the number of hydroxyl side groups with a one to two difference with respects to the other phenolic acids. The ellagic acid compound used in this study again, showed no antibacterial activity, but does not restrict ellagic acid to possess antipathogenic effects. One study in particular illustrated ellagic acid derivatives being effective in inhibiting biofilm formation of *Staphylococcus aureus* (Quave et al. 2011).

Epigallocatechin gallate showed selective antibacterial activity against only two of the six microbes tested (Table 2). One study showed EGCG as well as ellagic acid to be effective against other *Staphylococcus aureus* at concentrations of 250mg/L and 8000mg/L respectively (Akiyama et al. 2001). This current study conflicts with previous findings as EGCG nor ellagic acid showed effectiveness against *Staphylococcus aureus* at concentrations of 5mg/ml. Increasing the concentrations of both EGCG and ellagic acid could induce an antimicrobial response as shown in Akiyama's study.

Many polyphenolic compounds, particularly flavonoids and tannins, have been shown to suppress the virulence of bacterial strains. They do so by targeting and complexing with surface adhesins, extracellular and membrane bound proteins and enzymes, and cell wall polypeptides (Cowan M, 1999). The hydroxyl groups of phenolic compounds have been thought to be responsible for their antimicrobial activity. Concerning the degree of hydroxylation of the two compounds EGCG contains eight hydroxyl groups whereas ellagic acid contains four. The greater antimicrobial effect between the two compounds is possibly due to the difference in the abundance of hydroxyl groups as it has been found that there is an increase in antimicrobial effect with an increase in the degree of hydroxylation.

Knowledge on antimicrobial activity of phenolic compounds will continue to be of importance as the situation surrounding drug resistant pathogens continues to grow more negative. Understanding which phenolic compounds are effective could lead to possible utilization with current ineffective antibiotics, having a synergistic effect, to combat the drug resistant problem.

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