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Article

# The analysis of the antimicrobial benefits of *Populus balsamifera*

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## SUMMARY

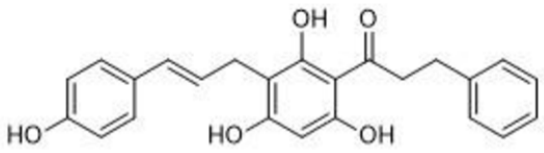
**This study was conducted to explore a new potential treatment for bacterial infections. This study aimed to investigate the antimicrobial properties of the tree species, *Populus balsamifera*. The objective was to determine the effectiveness of extract from the buds of *P. balsamifera* in inhibiting bacterial growth. A Kirby-Bauer assay was used to assess the efficacy of *P. balsamifera* extract against gram-negative and gram-positive bacterial growth. It was observed that the extract of the buds of *P. balsamifera* was highly effective against gram-positive bacteria. This helps to indicate the potential use of *P. balsamifera* in the medical field to eliminate gram-positive bacteria.**

## INTRODUCTION

Today, new sources of medicine are necessary due to the increasing antibiotic resistance of bacterial pathogens. The pathogenic bacterial strains *Staphylococcus aureus* and *Escherichia coli* are the cause of hundreds of thousands of deaths per year (1). Excitingly, natural products from plants are known to be alternative sources of new antimicrobial compounds (2).

One previously unknown source of natural medicine is the tree species, *Populus balsamifera. P. balsamifera*, also known as the Biblical "Balm of Gilead," grows mainly in the Middle East. *Populus balsamifera* is classified as a member of the Salicaceae family (3). It is known to have anti-inflammatory, local anesthetic, and antifungal properties and is found in spices, citrus flavors, soaps, detergents, creams, and lotions (4). *P. balsamifera* is also known as a potent cytotoxic compound (5). Balsacone C, found in *P. balsamifera,* exhibiting potential antimicrobial benefits, was recently discovered (**Figure 1**). Little is known about Balsacone C's effects on *S. aureus* and *E. coli.* Balsacone C may help treat methicillin-resistant *S. aureus* (MRSA) infections and most likely works by altering the structure of bacterial cell membranes (6). Currently, research surrounding Balsacone C and the pathogens it may treat is limited.

The goal of this study was to investigate the efficacy of Balsacone C within *P. balsamifera* against gram-positive and gram-negative strains of bacteria. Whether bacteria are gram-positive or gram-negative is a phenotypic classification that is based on the structure of cell walls. The hypothesis was that the growth of *S. aureus*, a gram-positive bacteria, would be inhibited by the antibiotic, but the growth of *E. coli*, a gram-negative bacteria, would not be. This is because gram-negative bacteria are usually more resistant to antibiotics due to their thick cell walls, which differentiates them from gram-




Balsacone C (16)

**Figure 1: Molecular structure of Balsacone C.**

positive bacteria. Gram-positive bacteria have a thicker wall of peptidoglycan, while gram-negative bacteria have a thinner layer. Gram-positive bacteria are monoderms, meaning that they have no outer lipid membrane, whereas gram-negative bacteria do have an outer lipid membrane. (7). The results showed that *P. balsamifera* is highly effective in eliminating gram-positive, but not gram-negative, bacteria. The results of this study are very important, as staphylococcal infections, including MRSA, spread easily in hospitals from cross-contact and can be deadly if left untreated. Because resistance to MRSA antibiotics continues to grow, it is imperative that alternative treatments be found (8).

## RESULTS

This study was conducted as an analysis of an alternative antimicrobial treatment for *S. Aureus* using a Kirby-Bauer assay. Briefly, two types of bacteria, *S. aureus,* which is gram-positive, and *E. coli,* which is gram-negative, were cultured in Petri dishes. Bacteria were then exposed to filter paper disks containing either *P. balsamifera* extract or nothing as a control. In this assay, a "zone of inhibition" forms during bacterial growth around each filter paper disk, indicating that bacterial growth has been inhibited by the disk and/or what it contains. A zone of inhibition will almost always form, whether or not it contains an antibiotic, and thus the zone must be large to be considered effective.

In the experimental *S. aureus* groups, the zones of inhibition ranged from five to nine millimeters (**Figure 2**), whereas in the experimental *E. coli* groups, the zones of inhibition ranged from one to six millimeters (**Figure 3**). The control group zones of inhibition for *S.aureus* also exhibited a larger range (one to four millimeters) than those of *E. coli* (zero to four millimeters with four being an outlier). It can be concluded that *P. balsamifera* extract may inhibit the growth of *S.aureus* (**Figure 2**, *T*-test, $p$=0.056). However, *P. balsamifera* extract did not significantly influence the growth inhibition of *E. coli* (**Figure 3**, *T*-test, $p$=0.156). Low standard deviations of 1.723 and 1.917 shown in **Figures 2** and **3** indicate consistency among the data collected. These results

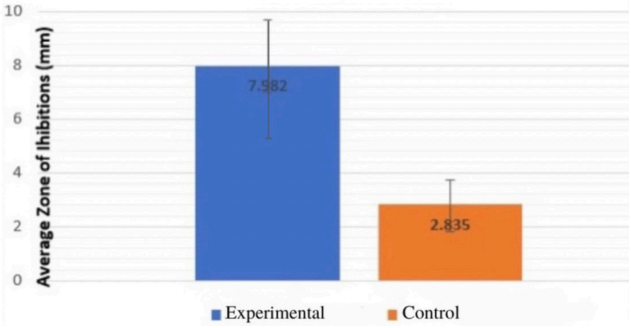



**Figure 2: Average *S. aureus* Zones of Inhibition** Graph representing the difference in zones of inhibition (mm) in *S. aureus* between the experimental group, which was exposed to the *P. balsamifera* extract, and the control group, which was not exposed. The experimental group exhibited a larger average zone of inhibition of 7.982 mm than the control group, which exhibited an average zone of inhibition of 2.835 mm (*T*-test, $p$=0.055958). Data is shown as mean ± SD.

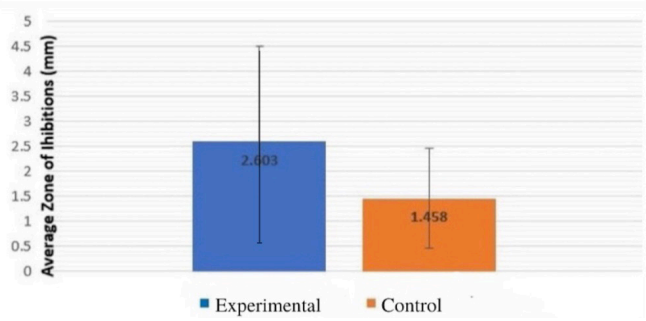



**Figure 3: Average *E. coli* Zones of Inhibition** Graph representing the difference in zones of inhibitions (mm) in *E. coli* between the experimental group, which was exposed to the *P. balsamifera* extract, and the control group, which was not exposed. The experimental group exhibited an average zone of inhibition of 2.603 mm and the control group exhibited an average zone of inhibition of 1.458 mm (*T*-test, $p$=0.155696). Data is shown as mean ± SD.

suggest that *P. balsamifera* may be effective in inhibiting *S. aureus*.

## DISCUSSION

Though $p$-values should be less than 0.05 to be considered statistically significant by custom, the $p$-value for the *S. aureus* experimental group was extremely close (approximately 0.056) and can be deemed as "on the edge of significance" (9). This still may indicate that *P. balsamifera* is indeed effective. This data trends towards significance, indicating that with further examination, *P. balsamifera* may be shown to be an effective antibiotic against gram-positive bacteria. Because *P. balsamifera* extract inhibited the growth of *S. aureus*, *P. balsamifera* may be effective in inhibiting the growth of strains of other gram-positive bacteria.

Sources of error in this study could have been present while measuring the zones of inhibition using ImageJ, which may have impacted the $p$-value and whether the results were statistically significant. Since ImageJ involved using photos and reference measurements, it is possible that the measurements taken were not as accurate as those that would have been acquired manually. Another source of error could have been present while distributing the extract on each filter paper disk. Although I attempted to spread even amounts of the extract, it is possible that some disks may have had slightly more or less extract, which could have led to differences in zones of inhibition. Because the data is considered "on the edge of significance," there are many slight errors that could have impacted the significance of this data. Future research may include the production of ointments or other treatments using *P. balsamifera* extract (10). *P. balsamifera* may potentially provide a useful treatment for *S. Aureus* infections.

Limited research has been conducted on the effects of *Populus balsamifera* on these types of bacteria, so this study neither refutes nor supports other studies. However, it still supports the claim that *P. balsamifera* exhibits antimicrobial properties. More studies should be conducted on *P. balsamifera* because the primary compound it contains, Balsacone C, has been discovered to have many benefits (11). It is possible that there may be many other useful properties of the antibiotic that are unknown, and it could serve many purposes. *P. balsamifera* not only exhibits antimicrobial properties, but it also exhibits antioxidant properties, which could protect against free radicals, preventing diseases like heart disease and cancer (12). Further research on *P. Balsamifera* is strongly encouraged.

## MATERIALS AND METHODS

The Kirby-Bauer assay, also known as the disk diffusion method, was followed. The Kirby-Bauer assay is a fast and effective method used to evaluate the efficacy of antimicrobial compounds against bacterial sensitivity. The assay works by inhibiting bacterial growth using antimicrobial compounds. *S.*

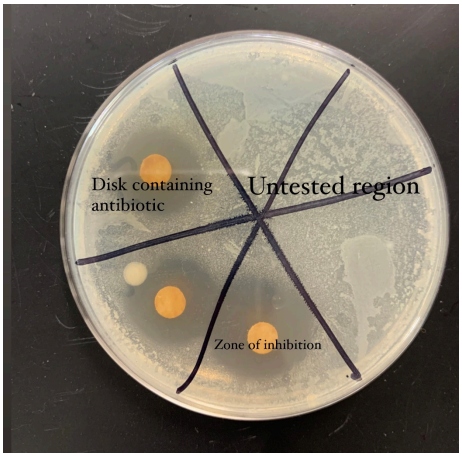



**Figure 4: Zone of inhibition for *S. aureus*.** This image was taken after an eighteen-hour incubation of *S.aureus* cultured on plates. Each disc was inoculated with *P. balsamifera* extract. A zone of inhibition is labeled. Nine disks were tested for each condition, with one Petri dish containing six disks and the other containing three disks. This plate contains three disks.


**Figure 5: Zone of inhibition for *E. coli*.** This image was taken after an eighteen-hour incubation of *E. coli* cultured on plates. Each plate was inoculated with *P. balsamifera* extract-containing disks. A zone of inhibition is labeled. Nine disks were tested for each group, with one Petri dish containing six disks and the other containing three disks. This plate contains three disks, each containing *P. balsamifera*.

*aureus* and *E. coli* were stored at 37°C for 36 hours. Twenty *P. balsamifera* buds were obtained from wildveilperfume.com (13).

The buds were ground into a fine powder using a mortar. The powder was mixed with 90% ethanol until completely submerged and left in an ultralow freezer at -40°C for 24 hours (14). Solid materials were separated using a filter paper with a 20 µm pore size, a funnel, a flask, and a hot plate. A hot plate was used to evaporate the ethanol and concentrate the extract. The mixture was heated until it reached a temperature of 88°C and the extract became free of any excess ethanol (15). After cooling, equal amounts of the solution were poured into eight Petri dishes and were left for 24 hours to solidify. 20 ml were poured onto each plate. Six-millimeter filter paper disks were layered with the extract. The disks were then placed in a sterile hood to dry for 30 minutes. This is to ensure that the extract was fully dried on each disk before being applied to the bacterial plates.

Eight Petri dishes were labeled and grouped into four *S. aureus* and four *E.coli,* with two experimental and two control groups, each labeled A and B. Each Petri dish was divided into six sections. Ten grams of nutrient agar were mixed with 330 ml of water until completely dissolved. Twenty-four hours later, a sterile cotton swab was dipped into the bacterial solutions. For each of the eight plates, *E. coli* and *S. aureus* solutions were swabbed across the Petri dishes to create a uniform bacterial layer. After drying, one filter paper disk, containing either *P. balsamifera* extract or nothing, was placed and secured in each section of each Petri dish. For the experimental groups, nine filter paper disks were distributed among two Petri dishes (six and three). The control groups had seven filter paper disks in total (six and one). The control group, including two Petri dishes for each type of bacteria, used filter paper disks without extract. The plates were incubated for 18 hours at 37°C (16). During incubation, the extract diffused out of the disk, into the media, and was taken up by the growing bacterial cells (17). After the incubation period, the diameter of the zones of inhibition was measured in order to determine whether the bacterial strain was resistant or sensitive to the extract.

The online software, ImageJ, was used to measure the zones of inhibition in millimeters (mm) (18). ImageJ involves using pictures of scientific data through the calibration of objects in person (19). To initiate this, the seven mm filter paper disk was calibrated by entering the information into the software, allowing it to measure the proper values. Multiple measurements were taken and averaged to ensure accuracy. These values are shown in **Figures 2** and **3**. The zones of inhibition of the experimental group and the control group were analyzed using a *T*-test. In addition, the standard deviation of the values was calculated to evaluate the variance of the data. These values are indicated by the error bars in the graph.

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