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ANTIBIOFILM AND ANTIBACTERIAL ACTIVITIES OF ETHANOLIC EXTRACT OF *Swietenia macrophylla* King AGAINST FOODBORNE PATHOGENS

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ABSTRACT

Food poisoning and foodborne illnesses caused by microbial deterioration during preparation and storage are becoming more common across the world. Synthetic additives and antimicrobial agents are used in food processing to alleviate the development of bacteria, minimizing contamination and extending food shelf life. Unfortunately, synthetic chemical additives have harmful effects and might lead to respiratory or other health issues. Due to increasing attention to that, there is a greater need for natural inhibitors as alternatives to synthetic antimicrobial agent. Efficacy of *Swietenia macrophylla* has been investigated in many studies, however its antibiofilm and antibacterial against foodborne pathogens remains not well investigated. The present work was performed to examine the phytochemicals constituents, antibacterial and antibiofilm activities of *S. macrophylla* leaves ethanolic extract against *S. typhimurium* ATCC 14028, *S. sonnei* ATCC 33862, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 10145. Antibiofilm activities of *S. macrophylla* was studied using the crystal violet assay, time killing assay and biofilm inhibitory concentration. Antibacterial activities were done by the determination of minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). Gas Chromatography-Mass Spectrometry (GC-MS) was employed to analyze the phytochemical compounds of the plant. GC-MS of *S. macrophylla* plant leaves extract, revealed the existence of Germanicol (11.50%), 3,4-Altrosan (1.44%), 2,4-Di-tert-butylphenol (0.49%) and n-Hexadecanoic (0.23%). The ethanolic extract of *S. macrophylla* had the lowest MIC values against *P. aeruginosa* (31.25 µg/mL) and *E. coli* (31.25 µg/mL). *S. macrophylla* ethanolic extract significantly ($p<0.05$) reduced biofilm biomass at the concentrations ranging from 31.25 to 1000 µg/mL. For biofilm time-killing assay, *S. typhimurium* and *E. coli* were killed at the concentration of 250 µg/mL after 12 hours of incubation while *S. sonnei* and *P. aeruginosa* were killed after 6 hours at the concentration of 125 µg/mL and 62.5 µg/mL respectively. Biofilm inhibitory concentration (BIC_{50}) for *S. typhimurium*, *S. sonnei,E. coli* and *P. aeruginosa* were 23.19, 9.19, 42.47 and 5.19 µg/mL respectively. It is concluded that *S. macrophylla* contains promising natural antimicrobial and antibiofilm agent.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

The prevalence of diseases caused by foodborne bacteria has increased in recent years, resulting in a significant and serious worldwide health concern (Zhao et al., 2017). Foodborne pathogens are the leading cause of food poisoning and foodborne infections, posing a serious threat to food safety (Adnan *et al*., 2020). Food contaminating microbes have received a lot of attention since they have become the major cause of mortality and morbidity, with a rate of 420,000 fatalities each year (World Health Organization, 2018).

According to the Centre for Disease Control and Prevention (CDC), pathogens that caused food poisoning includes *Escherichia coli*, *Staphylococcus aureus, Shigella flexneri, Listeria* spp., *Clostridium perfringens*, *Campylobacter* spp., and *Salmonella* spp. (Adnan *et al*., 2020). These bacteria can contaminate foodstuffs at any phase during production, distribution, and storage. In food processing, the pathogens may attach to the food, surface of processing devices or non-processing section such as drainpipes and walls further forming biofilm that cause contamination of food and corrosion of equipment (Lin *et al*., 2017). Hence, controlling the growth and development of food pathogens is critical, though removal of these organisms is difficult due to their ability to form biofilm on a wide range of planes (Adnan *et al*., 2020).

A biofilm is a complex matrix of microbial communities built of polysaccharides, proteins, and other organic elements in which cells adhere to produce strong adhesion to biotic or abiotic surfaces. Biofilm that adheres to a surface can survive in the presence of adverse circumstances such as natural host defenses and antimicrobial agents. In addition, biofilm have the ability to rapidly permeate a broad range of external environments, including the human body (Famuyide *et al*., 2019).

According to a recent report by National Institutes of Health (NIH), bacterial biofilms contribute approximately 80% of human soft- and hard-tissue illnesses (Song *et al.*, 2018). Biofilms are believed to be associated in over 60% of microbial illnesses, and two-thirds of all human bacterial infections (Famuyide *et al*., 2019). Biofilm