ABSTRACT

The aim of this study was to investigate potential antimicrobial activity of phytochemical extracts of commercially available species of Plantago. Plantago is a medically relevant genus, with several species traditionally used worldwide to treat inflammation and various infections. Chemical compounds (especially terpenoids) extracted from several species were tested against seven different bacterial species (Staphylococcus aureus, Bacillus megaterium, Klebsiella pneumoniae, Mycobacterium smegmatis, Pseudomonas aeruginosa, Serratia marcescens, and Staphylococcus aureus). Minimum inhibitory concentration (MIC) assays were performed using 96-well microtitre plates against seven different bacterial species (Escherichia coli, Bacillus megaterium, Klebsiella pneumoniae, Mycobacterium smegmatis, Pseudomonas aeruginosa, Serratia marcescens, and Staphylococcus aureus). Final results were obtained from only the nonpolar (hexane) and polar (methanol) extracts from P. eriopoda. No tests using either P. eriopoda extract showed bacterial inhibition. However, tests using the methanol extract have yet to be completed for four bacterial species. Further review of the literature using P. major suggests that concentrations used may have been insufficient to exhibit antimicrobial activity. Future studies are planned using extracts from several other Plantago species, starting at higher maximum concentrations.

INTRODUCTION

The genus Plantago contains several medically relevant species that have been used in the treatment of various diseases. Worldwide, several species have been traditionally used (and explored in modern studies for their potential utility) to treat inflamed and infected wounds (1-5), respiratory infections (6-7), urinary tract infections and kidney stones (3-4, 9), and digestive tract infections (1, 5, 7-9). Most of these studies focused on three species: P. major (1-3, 6-8), P. lanceolata (5,10), and P. ovata (9). Several scientific publications also document the antimicrobial activity of several species. However, Plantago is a spesecie genus, containing approximately 265 species, with the potential antimicrobial activity of most species being relatively unexplored. It is hypothesized that the close phylogenetic relationships among congeners is a likely underlying factor that potentially correlates with similar biochemical profiles, which may mark other Plantago species as possible sources of other antimicrobial phytochemicals. The commercial availability of extracts from several Plantago species, coupled with the relatively scarce research on the antimicrobial activity of several of those species, makes this a particularly attractive area of potential phytochemical research.

METHODS

Extraction

Aerial portions of plants were harvested pre-reproductive maturity, dried, and ground in a coffee grinder. Ground plant material was subjected to 72 cycles of Soxhlet extraction for each solvent (hexane and methanol; hexane only for P. subnuda). Solvents were removed using a rotary evaporator, and remaining residue was resuspended in DMSO and autoclaved. The hexane extract of P. subnuda became contaminated and was thus unusable for antimicrobial assays.

Minimum Inhibitory Concentration (MIC) Assays

Each resuspended extract was diluted to 128 μg/mL in sterile nutrient broth (NB), followed by six serial two-fold dilutions, resulting in extract concentrations ranging from 2-128 μg/mL for testing (128, 64, 32, 16, 8, 4, and 2 μg/mL), with a maximum DMSO concentration (in the 128 μg/mL extract) of 3%. Each extract concentration was tested in triplicate with 96-well sterile microtiter plates against seven different bacterial species (E. coli, B. megaterium, K. pneumoniae, M. smegmatis, P. aeruginosa, S. marcescens, and S. aureus). To each well, 250 μL of the appropriate concentration of extract was added, along with 25 μL of the appropriate bacterial culture, and 10 μL of a 0.675% solution of resazurin, an indicator used to visually assess microbial growth by a shift in color from blue/purple to pink. Additionally, positive growth controls were established using appropriate cultures in the absence of extract, and negative controls using non-inoculated sterile dH2O. To account for potential DMSO, an additional column was run using 3% DMSO, inoculum, and resazurin as solvent controls.

RESULTS

The negative data for the MIC assays may be a result of testing the extract at concentrations that were too low. A 2018 paper examining the antimicrobial properties of P. major found a minimum MIC of 500 micrograms/mL for Enterococcus faecalis, Staphylococcus aureus, and Staphylococcus epidermidis, with an even higher MIC of 1000 micrograms/mL for Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, and Shigella flexneri (12). Maximum concentrations used were based on a 2006 paper by Cos et al, advocating for more standardized protocols as a ‘proof of concept’ in studies of anti-infective potential of phytochemical compounds (11). However, in order to use the extract at higher concentrations, the DMSO could potentially inhibit microbial growth. If 10% DMSO does not cause inhibition of bacterial growth, both P. eriopoda extracts will be tested at concentrations starting at _____.

Two other Plantago species (P. coronopus and P. subnuda) will be tested for antimicrobial properties. The first plants grown. Another direction of this study will be to determine any cytotoxicity properties on mammalian cells to determine if the extracted plant material could be beneficial in medicinal use in eukaryotes.

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