Antibacterial effect of aqueous leaf extracts of five spices

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Submitted on 17.04.2024 and accepted for publication on 30.05.2024

ABSTRACT

Introduction: Antimicrobial drug resistance has increased dramatically in the past years. Therefore, the urge to discover alternative natural antimicrobial agents has increased. Plants used in traditional medicine can be identified as potential candidates for the synthesis of novel drug compounds to act against antibiotic-resistant bacteria. This study aims to determine the potential antibacterial effects of aqueous extracts of five Sri Lankan medicinal plants against two human bacterial pathogens.

Methods: *Trachyspermum roxburghianum* L. *(Ajwain), Murraya koenigii* L. *(Curry leaves), Pandanus amaryllifolius* Roxb. *(Screw pine), Cymbopogon citratus* Stapf. *(Lemongrass), and Coriandrum sativum* L. *(Coriander)* were used in the study. Plant extracts were tested against two clinically important bacterial strains: *Staphylococcus aureus (ATCC 25923)* and *Escherichia coli (ATCC 25922*). The antibacterial activity of the plant extracts was evaluated using the agar well diffusion method. Eight concentrations of each extract were tested to determine the minimum inhibitory concentration (MIC) by a 2-fold serial dilution of the original extract.

Results: Aqueous extracts of *T. roxburghianum.* gave the zones of inhibition of 9.67 ± 0.1 mm, MIC values of 400 mg/ml, and MBC values of 800 mg/ml respectively, against *S. aureus.* Aqueous extracts of *C. citratus* gave the maximum zones of inhibition of 10.67 ± 1.1 mm, MIC value 400 mg/ml, and MBC value 800 mg/ml, respectively, against *Escherichia coli.* None of the other plants were effective against either of the microorganisms used for the study.

Conclusions: *T. roxburghianum* and *C. citratus* aqueous extracts exhibit significant invitro antibacterial activity against *S. aureus and E. coli* respectively under the tested conditions, and the active compounds from them can be potential sources for the synthesis of alternative antibacterial drugs.

Keywords: *Ajwain, Antibacterial Activity, Aqueous leaf extracts, Lemongrass, Spices.*

Introduction

For thousands of years, natural and herbal products have been used in traditional medicine worldwide and predate the introduction of antibiotics and modern drugs (1). The efficacy of antimicrobial properties attributed to some plants is beyond belief. It depicted that local communities have used about 10% of all flowering plants to treat various infections, although only 1% have gained recognition by modern scientists. Because of their famous use as remedies for many infectious diseases for centuries, searches for herbal plants containing antimicrobial substances are frequent (2).

Murraya koenigii L. (Curry leaves/කරපිංචා) extracts, which had been used in many traditional cultures namely Indian, Ayurvedic, and Unani has demonstrated antibacterial effects, particularly on *Escherichia coli* (E. *coli), Staphylococcus spp.* including *Staphylococcus aureus (S. aureus), Streptococcus spp.*, and *Proteus spp.* in comparison to antibiotics such as gentamycin and amikacin (3). *Trachyspermum roxburghianum* L (Ajwain/ අසමෝදගම්) contains a higher content of phenols (28.5%), and proteins and carbohydrates are in low amounts (viz 1.3% and 9% respectively) (4). *Coriandrum sativum* L. (coriander/ කොත්තමල්ලි) is a well-known herb widely used as spice, or in folk medicine, and in the pharmacy and food industries. Coriander seed oil is the world's second most relevant essential oil, exhibiting antimicrobial activity against both Gram-positive and Gram-negative bacteria (5). In addition, *Cymbopogon citratus* L. (lemongrass/ ඉඟුරු) essential oils have demonstrated its powerful antimicrobial potential, thus being incorporated into the food products (6). *Pandanus amaryllifolius* Roxb. (screw pine/ රම්පේ), leaf extract contains a substantial phenolic content, which was suggested to be the major contributor to their antioxidant and antimicrobial activities against oral bacteria including its cytotoxicity (7).

Natural chemicals from plants are a source of antimicrobial activities, they have less side effects and are safe for humans and animals (8). As such, they have been identified as alternatives to treat food-borne pathogens and therefore promotes the reduction of antibiotic use (1). When considering the extracts of these plants, water is not considered a proper solvent due to the high sugar content in water extracts; sugars can stimulate microbial growth (9). However, we used water in our experiment because it is cost-effective to use water compared to alcohol in our setting. Further, whatever component available in the water extract (e.g. saponins, hydroxycinnamic acids esters of quinic acid) can be examined for its antimicrobial properties. Moreover, this would support 'green chemistry' and save the environment from 'hard chemicals.'

The study aimed to find out the antimicrobial activity of aqueous extracts of *T. roxburghianum, M.koenigii*, *P. amaryllifolius*, *C. citratus*, and *C. sativum* against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922). *S. aureus* and *E. coli* were selected for this study because they are both commonly associated with foodborne illnesses worldwide. *S. aureus* is the leading cause of foodborne illness around the globe (10). Therefore, the findings of this study will contribute to the role of herbal medicine in treating and controlling *S. aureus* and *E. coli* infections.

Methods

Extraction of plants

The plants were collected from local markets in Sri Lanka and identified at the Bandaranaike Memorial Ayurvedic Research Institute. The selected infection-free healthy plant materials were cleaned thoroughly with distilled water twice and dried in a shade until they achieved a constant mass. A mass of 250 g of the dried plant materials was ground to obtain powder form and was kept soaked in distilled water for two days (9). After two days, plant extracts were filtered through a muslin cloth. The filtered extract was then allowed to evaporate in the air at room temperature for two weeks. Then the weight of the dried extracts was separately measured and resolubilized with 10% dimethyl sulfoxide (DMSO) to make 2 ml of 800mg/ml concentration (concentrated sample) from each of the five plant specimens. Finally, the extracts were stored at 4^0C in air-tight bottles until further studies commenced (11).

Determination of the antibacterial activity using the well diffusion method

Both *S. aureus* and *E. coli* ATCC control strains were sub-cultured on separate Muller Hinton Agar (MHA) plates and incubated at 37°C for 24 hours. Inoculums from 24-hour-old cultures were prepared in sterile saline. The turbidity of the suspensions was adjusted to the 0.5 McFarland turbidity standard. A cork borer was used to cut 5 wells on each 90 mm MHA plate. The bottom of the wells was sealed by adding a drop of sterile molten agar to the wells. A volume of 150 microliters of each concentrated, resuspended using 10% DMSO plant extract (50 mg/ml), (100 mg/ml), (200 mg/ml) and (400 mg/ml), positive control (vancomycin and cefotaxime against *S. aureus* and *E. coli* respectively.) and negative control (10% DMSO) were loaded into wells using

a micropipette. The culture plates were incubated at 37°C for 24 hours and any resulting inhibitory zones were recorded. The diameter of the zones of inhibition was measured in millimetres. All tests were performed in triplicate (12).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

All plant materials were further tested for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A dilution series of plant extracts was prepared using a macro broth dilution series of 2 ml for each tube using 10% DMSO ranging from 800mg/ ml to 6.25 mg/ml. A bacterial suspension of 1 - 2 × 10^8 CFU/mL (0.5 McFarland turbidity) was prepared using sterile normal saline for each isolate. Each tube was inoculated with 100 microliters of bacterial suspension and incubated for 24 hours at 37^0C to detect minimum inhibitory concentration (13). Due to the colour of plant extracts, another series was made without the bacterial suspension to compare with the test series. MBC was determined by subculturing a loopful of plant extract-bacterial suspensions on MHA agar, from the dilution tubes (800 mg/mL – 6.25 mg/mL). Subcultured plates were incubated at 37°C for 24 hours to detect viability. All the experiments were performed in triplicate (14,15). Results were expressed as means along with the standard deviation (SD) of two parallel measurements.

Results

Antibacterial activity of plant extracts

The well diffusion against *S. aureus* which was done after the preparation of the extract, (stored at 4^0 C) showed an average zone of inhibition (ZOI) of 9.67 ± 0.1 mm only for 400 mg/ml concentration, with *T. roxburghianum* aqueous extract compared to the positive control (vancomycin), which exhibited a zone of inhibition of 26.67 ± 3.22 mm, indicating the extract's potential antibacterial activity against Gram-positive bacteria. Representative plates are shown in Figure 1.

The well diffusion against *E. coli* which was done after the preparation of the extract, (stored at 4^0 C) showed an average zone of inhibition (ZOI) of 10.67 ± 1.1 mm only for 400 mg/ml concentration, with *C. citratus* aqueous extract in comparison to the positive control (cefotaxime), which produced a zone of inhibition of 54.00 ± 1.00 mm, highlighting its effectiveness against Gram-negative bacteria under the tested conditions. Representative plates are shown in Figure 1.

The minimum inhibitory concentration and minimum bactericidal concentration were checked for the positive well diffusion results.

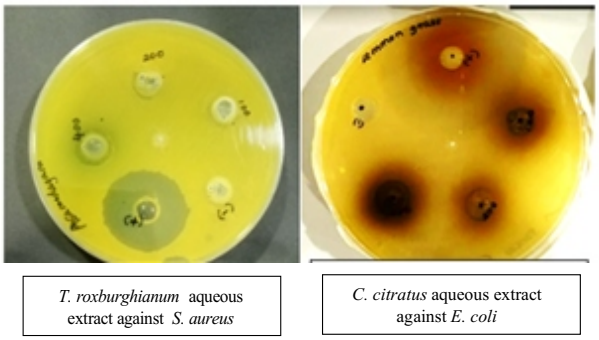
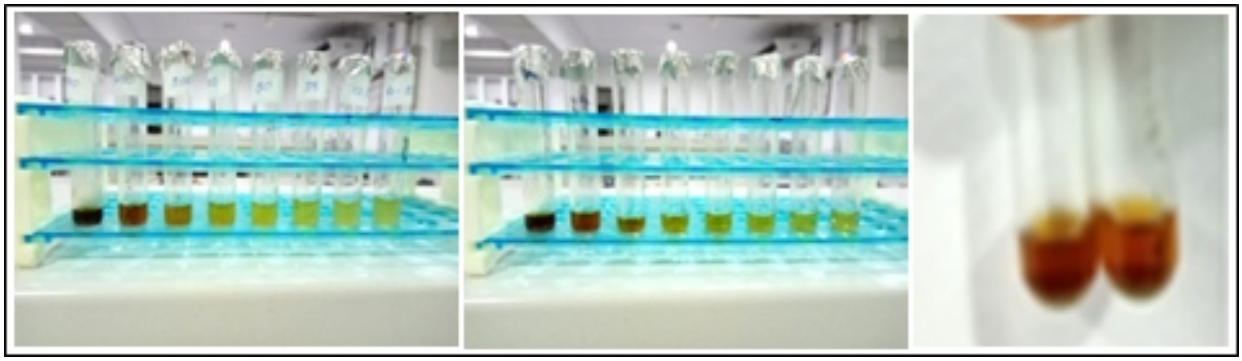


Figure 1: Well-diffusion results of *T. roxburghianum (asamodagam)* and *C. citratus (lemongrass)*

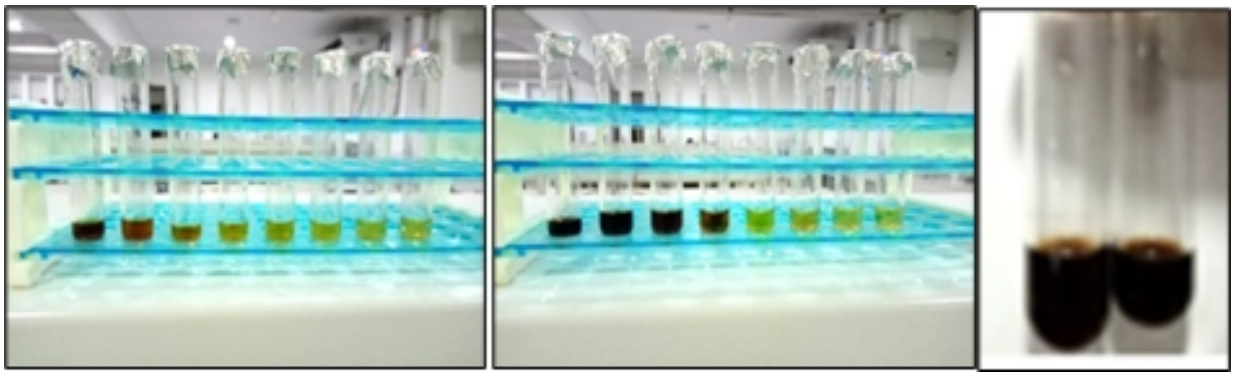
The Figure 1 shows the inhibition zones produced by aqueous extracts of *T. roxburghianum* against *S. aureus* and *C. citratus* (Lemongrass) against *E. coli*. The wells were loaded with 400 mg/ml of plant extract, and the diameter of the inhibition zones was measured in millimeters after 24 hours of incubation at 37°C. Positive controls (vancomycin for *S. aureus* and cefotaxime for *E. coli*) and negative controls (10% DMSO) were also included (Figure 2 & 3). MBC of plant extracts tested against the *S. aureus and E. coli* are shown in the Figure 4. Interestingly both *T. roxburghianum* and *C. citratus* showed an MBC of 800 mg/mL for both *S. aureus* and *E. coli* tested

Figure 4 illustrates the MBC results of aqueous extracts of *T. roxburghianum* and *C. citratus* tested against *S. aureus* and *E. coli*, respectively. The MBC values were determined by subculturing the treated bacterial suspensions on MHA plates after exposure to plant extracts at concentrations ranging from 800 mg/ml to 6.25 mg/ml. The MBC was identified as the lowest concentration that resulted in no visible bacterial growth. Both extracts demonstrated MBC of 800 mg/ml against the tested bacterial strains.



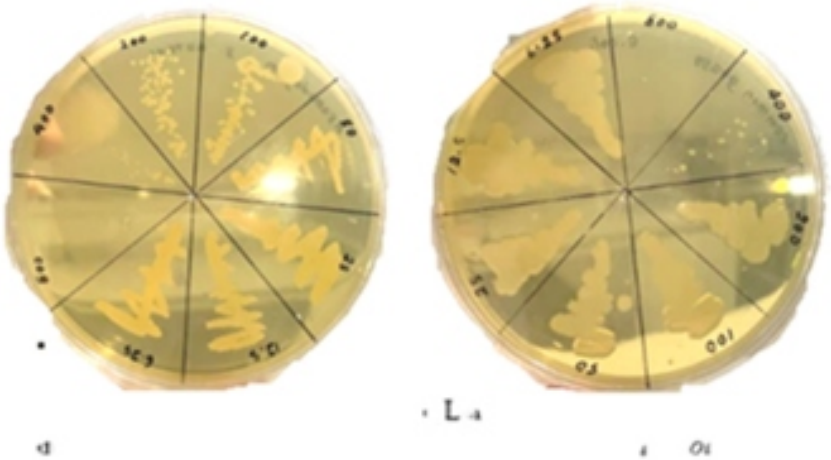
Test Negative Control 400 mg/ml

Figure 2: MIC results of *C. citratus*



Test Negative Control 400 mg/ml

Figure 3: MIC results of *T. roxburghianum*



T. roxburghianum aqueous extract against *S. aureus*

C. citratus aqueous extract against *E. coli*

Figure 4: MBC results of *T. roxburghianum* (Ajwain) and *C. citratus* (Lemmongrass)

Discussion

Increasing resistance to antibacterial agents is indeed a global problem. Further, the toxicity and side effects of currently used antibacterial agents are yet another concern. Therefore, the use of herbs and herbal preparations as alternative agents for antibiotics especially in the control of foodborne infections is considered an interesting alternative.

In this experiment, the selected method of extraction was the aqueous maceration extraction method which was based on the technique carried out by the traditional healers in Ayurvedic practice (4,12). These extraction methods are advantageous when considering factors like less cost, reduced toxicity and simplicity of the technique. When substances are extracted this manner the products are less toxic. For this study, one of the selected organisms was a gram-positive, *S. aureus, a*nd the other was a gram-negative*, E. coli, to* distinguish any difference in the mechanism which aqueous extracts depict against gram-negative and positive bacteria.

The results for the aqueous extract of *T. roxburghianum* against *S. aureus* showed positive inhibition zones proving that it possesses anti-bacterial activity against the organism. However, the same aqueous extract of *T. roxburghianum* didn't depict a zone of inhibition against *E.coli* for any concentrations under the tested conditions. This indicates that the aqueous extract of *T. roxburghianum* possesses an antibacterial effect against gram-positive bacteria than the gram-negative bacteria under the tested conditions. We believe that the antibacterial activity of *T. roxburghianum* observed in this study may be attributed to the high concentration of phenolic compounds present in the plant, as noted in previous literature (4). Phenolics compounds are known for their ability to disrupt bacterial cell walls and inhibit protein synthesis, particularly in Gram-positive bacteria like *S. aureus* (1, 9). The observed zone of inhibition (9.67 ± 0.1 mm) indicates that *T. roxburghianum* could be a promising candidate for further exploration as an antibacterial agent against Gram-positive pathogens.

Aqueous extract of *C. citratus* leaf showed a positive inhibition zone against *E. coli* proving that it possesses antibacterial activity against *E. coli*. However, the same aqueous extract of *C. citratus* did not depict a zone of inhibition against *S. aureus* for any concentrations under the tested conditions. This indicates that the aqueous extract of *C. citratus* possesses an antibacterial effect against Gram-negative bacteria than Gram-positive bacteria under the tested conditions.

Interestingly, the other plant extracts, including *M. koenigii*, *P. amaryllifolius*, and *C. sativum*, did not exhibit significant antibacterial activity against either of the tested bacteria under the tested conditions of this study. This could be due to several factors, such as the absence of certain bioactive compounds in the aqueous extracts or the possibility that these plants are more effective against different strains of bacteria not tested here. For example, studies on *M. koenigii* have shown varying results depending on the extraction method and bacterial strain tested (3, 11). Similarly, research on *P. amaryllifolius* suggests that ethanol-based extracts may be more effective in exhibiting antimicrobial properties (7).

Though this study provides valuable insights into the antibacterial potential of *T. roxburghianum* and *C. citratus*, it is important to acknowledge its limitations. Only two bacterial strains were tested, which limits the generalizability of the results. Future studies should investigate a wider range of bacterial pathogens (12, 15). Furthermore, the study focused on aqueous extracts, but other solvents may yield more potent extracts by isolating different bioactive compounds. A more comprehensive phytochemical analysis of the active compounds in *T. roxburghianum* and *C. citratus* would provide deeper insights into their mechanisms of action (4, 13).

Conclusions

Aqueous extracts T. roxburghianum and *C. citratus* demonstrated significant antibacterial activity against *S. aureus* and *E. coli* under the tested conditions, fulfilling the objective of identifying potential antibacterial agents. Further research is needed to isolate the active compounds responsible for the observed effects in *T. roxburghianum* and *C. citratus* and to explore their potential against other pathogens.

Ethical approval for the study was obtained from Ethics Review Committee at Kaatsu International University, Battaramulla and authors declare no conflicts of interests.

Acknowledgments

The authors would like to thank the Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura for providing the clinical isolates used in this study. This study was funded by Kaatsu International University, Battaramulla.

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