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Inhibitory and Bactericidal Potential of Some Indigenous Functional Food-Plants Used in the O.R. Tambo District Municipality of South Africa

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Abstract

Antimicrobial resistance is a major problem in the management of infectious diseases. African indigenous functional food-plants such as *Chenopodium album* and *Solanum nigrum* may constitute important sources of phytochemical constituents for the synthesis of antimicrobial compounds against infectious organisms. The objective of this study was to determine the antimicrobial properties of *Chenopodium album* and *Solanum nigrum*-leaves used as functional food-plants in the O.R. Tambo district municipality of South Africa. Organic and aqueous solvent-extracts of *C. album* and *S. nigrum* were tested against *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC127853), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (25922) and *Enterococcus faecalis* (51299) using standard microbiological techniques. Ciprofloxacin was included in all the experimental runs as positive control antibiotic. The aqueous extracts of both plants were the most active with zones of inhibition diameters ranging from 0 mm - 20 mm and minimum inhibitory concentration (MIC_{50}) values ranging from 0.63 mg/mL - 10 mg/mL. The positive control antibiotic was highly active with zones of inhibition diameters ranging from 17 mm - 31 mm and MIC_{50} values from 0.0003 mg/mL - 0.0005mg/mL for all the bacteria tested. Both extracts were bactericidal with minimum bactericidal concentration (MBC) ranges from 2.5mg/mL - 20mg/mL. From the results, it can be concluded that both plants possess compounds with antimicrobial properties, thus validating scientifically their use in traditional medicine. However, more studies to document the respective plant-principles responsible for antimicrobial activity of these plants would shed more light on their functional properties.

Keywords

Antimicrobial Resistance, Sensitivity Tests, Indigenous Leafy Vegetables, Eastern Cape Province, South Africa

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1. Introduction

Bacterial antimicrobial resistance against commonly used antibiotics is distressingly on the rise [1]. Patients infected with resistant organisms are more likely to have longer more expensive hospital stays [2]. The modification of chemotherapeutic agents to limit this problem has been greatly successful. However, many reports also indicate that many of the drugs are being rendered obsolete by microbial drug-resistance [3]. As a result, the treatment of microbial infection is becoming increasingly complicated. Physicians have now resorted to the use of combination therapy, increasing the cost of treatment even more. Reports on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis* infections and antibacterial resistance reveal the need for a constant search of new drugs against these organisms [4] [5]. *E. coli* is a major cause of travellers' diarrhoea, one of the most common forms of diarrhoea worldwide [6] [7]. Both *E. coli* and *P. aeruginosa* are also major causes of urinary tract infections while *S. aureus* and *E. faecalis* are common causes of nosocomial infections [8] [9]. *B. subtilis* infections are not common but few cases have been reported in the literature in patients with oesophageal perforations [10]. The use of medicinal plants in the treatment of human infections is a common practice in many remote areas of Africa with inadequate health care facilities. *Chenopodium album* and *Solanum nigrum* are functional food-plants with wide nutritional and medicinal importance among rural communities in the O.R. Tambo District Municipality of South Africa [11]. They are jointly referred to as *imifino ezikhulelayo* in isiXhosa, meaning indigenous vegetable. *C. album* is locally known as *imbikicane* while *S. nigrum* is known as *umsobo* [12] [13]. Both plants grow wildly in bushes, barren land and roadside paths from where they are harvested either for nutritional or medicinal purposes. In some parts of India, *C. album* is also used in ayurveda for treating anorexia, cough, dysentery, diarrhoea, oedema, piles and worm infestations [14]. Despite their medicinal uses, very little information is available in the literature about their pharmacological potential. This is surprising considering the ever-increasing rate of antimicrobial resistance of human infectious organisms against currently used drugs. The aim of this study therefore was to investigate the antimicrobial properties of these plants in an attempt to identify cheap sources of compounds for the synthesis of new drugs against medically important bacteria.

2. Materials and Methods

2.1. Bacterial Strains

Standard bacterial strains including *Pseudomonas aeruginosa* ATCC 127853, *Escherichia coli* ATCC 25922, Staphylococcus *aureus* ATCC 29213, *Enterococcus faecalis* ATCC 51299 and *Bacillus subtilis* ATCC 6051 (American Type Culture Collection, Rockville, MD) obtained from the stock culture of the National Health Laboratory Services (NHLS), Nelson Mandela Academic Hospital, Mthatha were used in this study. Ethical clearance was obtained from the Eastern Cape Department of Health and the Ethics Committee of the Faculty of Health Sciences, Walter Sisulu University (WSU). The organisms were cultured on nutrient agar (Oxoid Llt., Basingstoke, UK).

2.2. Collection and Preparation of Plant Material

The leaves of *C. album* and *S. nigrum* were harvested from home gardens and along bush paths in the vicinity of WSU main campus in Mthatha in October 2012. The plants were identified by Dr. Kathleen Immelman of the Department of Botany at WSU and voucher specimens were prepared and deposited in the Kei herbarium (CN01 and CN02). The plant leaves were washed with tap water to remove dirt and soil particles. The plant leaves were placed on cardboards and dried at 50 ˚C for 24 hours in a hot air oven (Heraeus, Schutzart). The plant material was powdered (ATO Mix, Cambridge) and stored in airtight containers at 5 ˚C for further analysis.

2.3. Preparation of Plant Extracts

Approximately 400 g of dried powdered plant material was exhaustively extracted in different solvents. The plant material was separately soaked in 700 mL of concentrated hexane, acetone, ethanol, methanol and water in 2L volumetric flasks (Schott, Durban). The flasks were placed in an orbital shaker incubator (labcon, Maraisburg) for 48 h [15]. The plant material was centrifuged at 1006.2 x g for 5 minutes and filtered through a fritted

filter funnel of pore size 60 Å. The procedure was repeated twice and the three extracts combined and concentrated to dryness under vacuum (Büchi, Switzerland). The dried crude extract was collected in porcelain evaporating dish (Haldenwanger, Berlin) and left open in a biosafety class 2 cabinet (Durban, South Africa) for complete evaporation of residual solvents. The aqueous extracts were lyophilized [16]. A 2-g sample of each extract was used for the preliminary bioassay, and where possible, another 2 g or more was put in universal bottles and kept in the extract bank. Stock solutions were prepared by dissolving the extracts in 80% acetone (a concentration we found to be non inhibitory to any of the bacterial strains tested).

2.4. Screening of Crude Extracts for Antibacterial Activity

The agar-well diffusion method was used for this analysis [17]. Briefly, each bacterial suspension prepared in 0.9% saline (McFarland turbidity standard 0.5) was inoculated by spreading on Mueller Hinton agar (Oxoid Llt., Basingstoke, UK) plates and allowed to dry for 15 minutes. Wells (6mm in diameter) were punched into the agar using a sterile stainless steel borer and filled with 70 µL of the extract at 100 mg/mL. Seventy microliters of 0.005 mg/mL ciprofloxacin and 80% acetone were included in all experiments as positive and negative controls, respectively. The plates were incubated at 37˚C for 24 hours, after which the diameters of zones of inhibition were measured in millimetres. The experiment was repeated twice, and means for zones were recorded.

2.5. Determination of Minimum Inhibitory Concentration (50% Susceptibility)

Based on their good antimicrobial activity in the screening, the aqueous extracts were selected for determination of minimum inhibitory concentration (MIC_{50}) using the micro broth dilution technique performed in 96-well plates [18]. Two-fold dilutions of the extract and control antibiotic (Ciprofloxacin) were prepared in the wells containing Mueller Hinton broth. The final extract concentration ranged from 20 - 0.31 mg/mL while that of the control antibiotic ranged from 0.005 - 0.00015 mg/mL. Exactly 20 µL of an 18-hour old broth culture (McFarland turbidity standard 0.5) of the bacteria was inoculated into 180 µL of extract-containing culture medium. Negative control wells were prepared with culture medium only and bacteria suspension and broth only respectively. An automatic ELISA micro plate reader (Tokyo, Japan) adjusted to 590 nm was used to measure the absorbance of the plates before and after 24-hour incubation. The absorbencies were compared to detect an increase or decrease in bacterial growth and the values plotted against concentration. The lowest concentration of the test extract resulting in inhibition of 50% of bacterial growth was recorded as the MIC.

2.6. Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined following well established procedures [19]. Briefly, the entire content of the MIC well (≈200 µL) was serially tenfold diluted in 0.9% saline. A loop-full was taken from each tube and inoculated into Mueller Hinton agar plates and incubated for 24 h at 37˚C. The MBC was recorded as the lowest concentration of the extract or antibiotic that gave complete inhibition of colony formation of the test bacteria at the later cultivation.

2.7. Statistical Analysis

The statistical package used for analysis was SPSS v18.0 (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) was used to compare the mean difference in inhibitory activities of extracts and control antibiotic, followed by Turkey's post-hoc test. Differences were considered significant at $P < 0.05$.

3. Results

The zones of inhibition diameters of active plant extracts ranged from 0 mm - 20 mm while those for the control antibiotic ranged from 17 mm - 31 mm. Hexane extracts of *C. album* and methanol extracts of both plants were inactive (**Table 1**).

3.1. Minimum Inhibitory Concentration of Active Crude Extracts and Control Antibiotic

Based on agar-well results, the most active extracts (aqueous) were selected for MIC and MBC determination alongside the positive control antibiotic. The activity of the aqueous extracts was confirmed with MIC_{50} values

Table 1. Antimicrobial activity of crude extracts of *S. nigrum* and *C. album* as revealed by the agar-well diffusion technique.

	S. nigrum					*C. album*					
	H	**A**	**E**	**M**	**W**	**H**	**A**	**E**	**M**	**W**	**CIP**
Bact											
Bs	15	0	10	0	17	0	13	0	0	14	22
	11	10	10	0	20	0	21	0	0	17	29
	10	0	12	0	15	0	11	0	0	17	31
Sa	0	0	10	0	10	0	0	0	0	11	19
	7	0	10	0	12	0	0	0	0	10	19
	9	0	9	0	14	0	10	9	0	10	17
Ef	0	0	11	0	0	0	0	0	0	17	21
	0	0	15	0	0	0	0	0	0	14	19
	0	0	11	0	0	0	11	0	0	14	21
Ec	9	0	9	0	11	0	10	9	0	9	21
	0	0	10	0	10	0	0	0	0	10	18
	0	11	13	0	10	0	10	0	0	9	18
Pa	0	0	10	0	13	0	0	0	0	15	19
	0	0	13	0	12	0	0	10	0	17	27
	0	0	9	0	10	0	0	0	0	16	23
Mean ± SD	**4.1 ± 5.4**	**1.4 ± 3.6**	**10.8 ± 1.7**	**0**	**10.3 ± 6.0**	**0**	**5.7 ± 6.8**	**1.8 ± 3.8**	**0**	**13.3 ± 3.1**	**21.6 ± 4.2**

Last row data are Mean ± SD of 15 determinations for each plant crude extract; H: hexane; A: acetone; E: ethanol; M: methanol; W: water; Bact: bacteria; Bs: *Bacillus subtilis*; Sa: *Staphylococcus aureus*; Ef: *Enterococcus faecalis*; Ec: *Escherichia coli*; Pa: *Pseudomonas aeruginosa*.

of 0.63 mg/mL - 10 mg/mL and 0.63 mg/mL - 7.5 mg/mL for *S. nigrum* and *C. album* respectively (**Figure 1**).

3.2. Minimum Bactericidal Concentration of Active Crude Extracts and Control Antibiotic

Aqueous crude extracts of both plants were also bactericidal against the tested bacteria with MBC values ranging from 2.5 mg/mL and 5.0 mg/mL - 20 mg/mL for *C. album* and *S. nigrum* respectively (**Figure 2**). MIC and MBC values of 0.0003 mg/mL and 0.001 mg/mL respectively were recorded for the control antibiotic and were the least values in the entire study (**Figure 1** & **Figure 2**).

4. Discussion

Medicinal plants may constitute an important source of therapeutic compounds against human infectious organisms. Many plants have been reported to contain flavonoids, alkaloids, tannins, phenols, saponins or other secondary metabolites which serve as defence mechanisms against micro organisms, insects and animals [20]. These compounds are known to act in different ways to exert antimicrobial action. The results of this study indicate that crude extracts of *C. album* and *S. nigrum* have the potential for further evaluation in the search for antibacterial compounds. Gram-positive organisms; *S. aureus*, *E. faecalis* and *B. subtilis* were the most susceptible in the entire study while Gram-negatives; *E. coli* and *P. aeruginosa* were less susceptible (**Table 1**, **Figure 1** & **Figure 2**). The difference in susceptibility between Gram-negative and Gram-positive bacteria to antimicrobial agents has been reported by other researchers [20] [21] and may be attributed to structural differences in the cell wall of both organisms. Gram-negative bacteria have a lipid protective sheath around their cell walls which seems to shield them from the effects of antimicrobial agents [22]. All the bacteria tested were highly

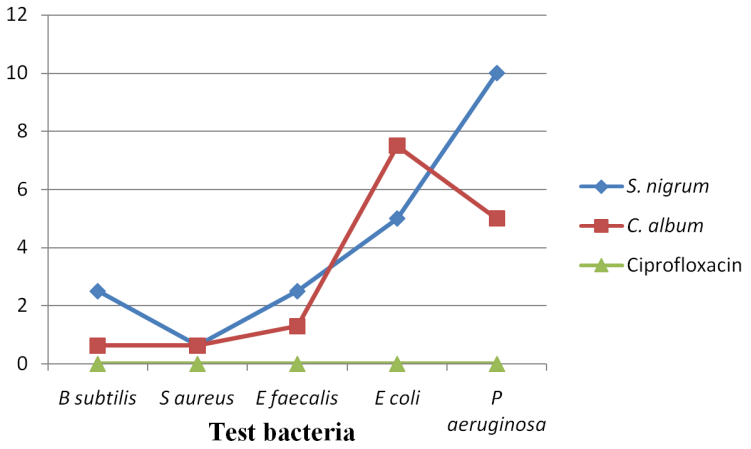

Figure 1. Minimum Inhibitory Concentration (mg/mL) values of plant crude extracts and control antibiotic tested against some bacteria of medical importance.

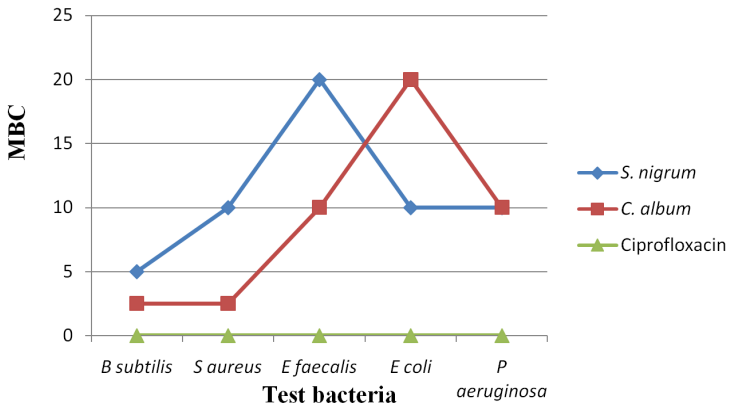

Figure 2. Minimum Bactericidal Concentration (mg/mL) values of plant crude extracts and control antibiotic tested against some bacteria of medical importance.

susceptible to Ciprofloxacin, the control antibiotic (**Table 1**, **Figure 1** & **Figure 2**). The plant crude extracts were relatively less active when compared to Ciprofloxacin ($P < 0.05$). This was expected as the control antibiotic is a purified compound with excipients to facilitate activity. The crude extracts on the other hand are made of numerous compounds; some of which may have antagonistic properties against each other. Equally important is the fact that the quantity of the active ingredient in the crude extracts may be in minute quantities, not enough to exhibit the type of activity demonstrated by the control antibiotic. Of all the bacteria tested, *B. subtilis* and *S. aureus* were most susceptible to aqueous extracts of the plants, producing large zones of inhibition diameters (**Table 1**), low MIC and MBC values (**Figure 1** & **Figure 2**). However, there were no significant differences in antibacterial activity between the aqueous extracts of *C. album* and *S. nigrum* ($P > 0.05$).

5. Conclusion

The current study illustrates the antibacterial properties of crude extracts of *C. album* and *S. nigrum* against some selected bacteria of medical importance. The study shows that aqueous extracts of both plants are inhibitory and bactericidal to *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli* and *E. faecalis*. These findings are consistent with their folkloric use in the treatment of stomach-related morbidities in the O.R. Tambo District Municipality of South Africa. However, more studies to document the plants active ingredients will shed more light on their pharmacological relevance as antibacterial agents.

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