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Research Article

Antimicrobial, Antioxidant and Cytotoxicity Studies of Medicinal Plants Used in the Treatment of Sexually Transmitted Diseases

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ABSTRACT

Context*:* The World Health Organization reported that more than 1 million sexually transmitted infections (STIs) are acquired daily, with an estimated 357 million new infections caused by either chlamydia, gonorrhoea, syphilis or trichomoniasis. Aim: The present study was aimed to evaluate antimicrobial, antioxidant and cytotoxicity activities of ethanol extracts of ten ethnobotanical selected plant species used to treat STD's and related symptoms. Methods: The determination of antimicrobial susceptibility of plant extracts was done using the broth micro-dilution assay against five microorganisms. The free radical scavenging activity was determined by using DPPH (2, 2-diphenyly-1-picrylhydrazyl). Cytotoxicity activity of the plant extracts was done on Vero African monkey cells lines with 2, 3-bis [2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) reagents. Results: Our results suggest that extracts of *Acacia karroo* and *Rhoicissus tridentata subsp. cuneifolia* are potential candidates with a good antimicrobial, antioxidant and low cytotoxicity activities. This results may support the anecdotal claims for the use of the selected plant species to treat venereal diseases.

Keywords: Antimicrobial, Antioxidant, cytotoxicity, sexually transmitted diseases.

INTRODUCTION

Sexually transmitted diseases are caused by microorganisms that can survive in warm, dark places including the anus, the genital areas of both males and females, and the mouth. According to the World Health Organization, more than 1 million sexually transmitted infections (STIs) are acquired every day worldwide[1]. The report further stated that an estimated 357 million new infections with 1 of 4 STIs is caused by chlamydia, gonorrhoea, syphilis or trichomoniasis annually. STIs such as gonorrhoea and chlamydia are the major causes of pelvic inflammatory disease (PID) and infertility in women. In South Africa, about 11 million cases are reported annually and young women are particularly vulnerable to STIs being one of the major contributors to the human immunodeficiency virus (HIV) epidemic[2,3]. *Neisseria gonorrhoeae* is a Gram-negative pathogenic bacterium responsible for a range of diseases ranging from urethritis to disseminated gonococcal infections. It causes asymptomatic infections resulting in severe complications of PID[4]. *N. gonorrhoeae* is a common sexually transmitted pathogen that significantly impacts female fertility, neonatal health, and transmission of HIV worldwide[5]. Sexually transmitted and urinary tract infections (UTI's) are believed to be the most frequent bacterial infection in women[6-8]. About three quarters of the world population depend on traditional medicines for their health needs. The use of plants or plant extracts has long been used in different indigenous cultures in all parts of the world for the treatment of diseases[9]. Herbal medicines are gaining popularity because of several advantages such as fewer side effects, better patient tolerance and acceptance due to its long history of use[10]. The emergence of drug resistant strains has complicated the treatment of these infectious diseases. These complications have demanded the search for new antimicrobial substances from various sources. Plant extracts possess active compounds that act positively against sexually transmitted pathogens thus may be a good source for new active agents. The prevention of the spread of STDs, alongside with early detection and appropriate therapy, has the potential of reducing infections that might damage the reproductive tract. According to the WHO global survey, there are three common challenges faced in traditional medicine; the lack of sharing gathered information, lack of safety monitoring of herbal medicines and also the lack of methods to evaluate their safety and efficacy. In this study, ten plant species were selected for investigation based on their ethnomedicinal uses in the treatment STI's and related infections. Crude ethanol extracts of the selected plant species were tested against six micro-organisms; five bacteria and one fungal strain. These microorganisms have been reported to be associated with causing many sexually transmitted diseases (STDs) and urinary tract infections (UTIs).

MATERIAL AND METHODS

Plant material

Plants were selected on the basis of their ethnomedicinal uses in the treatment of sexually transmitted diseases (Table 1). All the plant material (bulbs, roots and seeds)

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were collected from the Jongilanga community in Mpumalanga. Voucher specimen of each plant species were prepared and identified by Mr Calvin Mophuting at the HGJW Schweickerdt herbarium of the University of Pretoria.

Preparation of plant extracts

Plants parts were air dried and grounded into fine powder. The powdered plant materials were then dissolved in ethanol and vigorously shaken for 72 hr using a Labcon 3086U machine at moderate speed. The extract was filtered using a vacuum system and concentrated to dryness under reduced pressure using a Rotavapor. The plant extracts were stored in polytops at 4º C environment before tested for different assays.

Microbial strains

The microorganisms used in this study include *Escherichia coli* (ATCC 8739), *Klebsiella oxytoca* (ATCC 700324), *Klebsiella pneumoniae subsp. pneumoniae* (ATCC 13883) and *Staphylococcus aureus* (ATCC 9144) grown at 37°C on nutrient Agar. *Neisseria gonorrhoeae* ATCC 49226 was grown in chocolate agar (GC) under anaerobic conditions in a jar with anaerocult A (Merck SA (Pty) Ltd.), at 37°C for 72 hours. Sabouraud Dextrose Agar medium (SDA) (Merck SA (Pty) Ltd.) was used for the culturing of *Candida albicans* (ATCC10231) and incubated at 25°C for 24 hours under aerobic conditions.

Antimicrobial assay

The minimum inhibitory concentration (MIC) of the plant extracts was determined using the broth micro-dilution assay in 96-well microtitre plates as described by Eloff[19], with slight modifications. Briefly, 50 mg of each plant extract was weighed in 200 mL Eppendorf tubes. Each plant extract was dissolved in 100 µL of 10% DMSO and 900 µL of nutrient/MH broth, to make a final concentration of 50 mg/mL. One hundred microliters of each dissolved extract and positive control ciprofloxacin (0.1mg/ml) were serially diluted (two fold) with broth in triplicate down a 96-well micro-plate for all the microbial strains. Twenty-four-hour microorganism's cultures were inoculated in sterile broth and prepared to a density of 1.5 x 10^8 colony forming units (CFU) per mL (CFU/mL) corresponding with the 0.5 McFarland Standard. Inoculated broth (100 µL) was added to each well. The plates (96-well) were covered and incubated for 24 hours at 37 ºC. Microbial growth in the wells was determined based on the visual colour change in the wells after the addition of 50 µl Presto blue dye, a pink colour change indicated growth. The MIC values were recorded as the lowest concentration of extract showing a blue well.

Antioxidant assay

The free radical scavenging activity was determined by using DPPH (2, 2-diphenyly-1-picrylhydrazyl), following the methods as described by Adebayo[16] with a few modifications. For each tested sample, a dilution series was prepared in a 96-well microtiter ELISA plate, using distilled water as a dilution medium. All samples were tested in triplicate. The ten 2mg ethanol-extracted plant samples were tested at concentrations ranging from 500–3.91 µg/mL. Ascorbic acid (Vitamin C) was used as a positive control and ethanol was used as a solvent control (blank). Colour controls for the samples were used. DPPH was added to each test plate to determine colour change, for the presence of antioxidants. Upon the reduction of DPPH by the presence of an antioxidant, the DPPH changes from purple to colourless. After 30 min of incubation time at room temperature, the radical scavenging activity was determined by reading off absorbencies with an ELISA- plate reader at 515 nm. The IC_{50} (50%) inhibitory concentration of DPPH or turning colourless by an extract was determined using the GraphPad Prism Version 4.0. This is the amount of antioxidant that is necessary to decrease the initial absorbance of DPPH by 50%.

Cytotoxicity assay

Cytotoxicity of selected plant extracts was determined as previously described by Sharma[20] 2014. About 100 000 Vero cells were seeded onto a microtiter plate and incubated for 24 hours to allow the cells to attach to the bottom of the plate. The extracts and positive control (Actinomycin D) were then added to the cells to give the final concentrations of extracts and actinomycin ranging from 400-3.13 and 0.013 to 0.0001 µg/ml, respectively. The plates were then incubated at 37º C in a 5% CO_2 humidified atmosphere for 72 hr. Following incubation, the toxicity effects of the samples was determined by adding 50 µL of XTT reagent [1 mg/mL XTT with 0.383 mg/mL N-methyl dibenzopyrazine methyl sulphate (PMS)] d to cells in the plates and incubated for 3 hr. After incubation, the absorbance of the colour was spectrophotometrically quantified using an ELISA plate reader, which measured the optical density at 490 nm with a reference wavelength of 690 nm. The assay was carried out in triplicate. Cell viability was assessed by comparing optical densities of samples with those of the negative control (DMSO control). The EC_{50} values (concentration of sample that causes 50% cell death) were analysed using the GraphPad Prism Version 4.0, (Statistical program).

RESULTS AND DISCUSION

Sexually transmitted and urinary tract infections are highly prevalent in many rural areas of developing countries due to poor sanitary conditions and lack of proper hygiene. Most people in these communities depend on traditional healers and medicinal plants to treat std's because they are too shy to talk to unknown western doctors or they don't have access to modern medical facilities. Traditional healers also claimed their medicine are more effective and cheaper than western medicine[21,22]. Several plant extracts have showed huge potential as effective measure in the treatment and management of sexually transmitted diseases including AIDS[23].

Antimicrobial activity

Table 2 present antimicrobial activity results of the plant extracts. All the plant extracts showed a broad-spectrum activity against all the tested microorganisms. Most of the extracts had anti-microbial activity between the concentration ranges of 12.5 to 0.4 mg/mL. Extracts of *A. karroo* had a good antimicrobial activity and indicated the lowest MIC value against *Klebsiella oxytoca* (0.8 mg/ml), *Neisseria gonorrhoeae* (0.8 mg/ml) and *Staphylococcus*

Table 1: The list of selected plant species and their traditional medicinal uses. Also shown are the different plant parts used and the secondary compounds found in each plant species

Scientific name and family name	Voucher number	Plant part used	Medicinal uses	Secondary compound (s)	Biological activity
Abrus precatorius L. Fabaceae	Mophuting 119334	Aerial parts and seeds	Kidneyproblems, blood urine	Alkaloids, flavonoids, glycosides, triterpene	Antifungal and antibacterial activity[11]
Acacia karroo Fabaceae	Mophuting 119360	Roots	STD's	Anthroquinones, saponins, tannins	Antibacterial activity[12]
Blepharis sub volubilis subs. Sub volubilis Acanthaceae	BC129	Whole plant	Urinary tract infections	Flavonoids, saponins, steroids, tannins	*
Diospyros mespiliformis Ebenaceae	Mophuting 117182	Roots leaves	Urinary tract infections	Flavonoids, tannins terpenoids	Antimicrobial activity[13]
Ipomoea crassipes Hook Convolvulaceae	BC162	Roots	HIV	*	*
Jatropha zeyheri Euphorbiaceae	BC24 (B1)	Bulb	Testicle sores	Alkaloids, diterpenes, saponins	Antibacterial activity[15]
Peltophorum africanum Fabaceae	BC40 (B1)	Roots	STD's	Flavonoids	Antiinflammatory activity[16]
Rhoicissus tridentata subsp. cuneifolia Vitaceae	49 (B1)	Roots	Bladder infections	Alkaloids, flavonoids, tannins	Antifungal activity, antioxidant[17]
Senna petersiana Fabaceae	BC08 (B1)	Roots	Bladder problems	Anthraquinone, glycosides	Antibacterial, antifungal activity[18]
Ximenia caffra Olacaceae Olacaceae	BC63 (B1)	Roots	HIV	Alkaloids	Antibacterial activity[12]

Table 2: The MIC (mg/mL) values of ten plant species tested against different microorganisms

Plant species	*Candida albicans*	*Escherichia coli*	*Klebsiella oxytoca*	*Klebsiella pneumoniae*	*Neisseria gonorrhoeae*	*Staphylococcus aureus*
Abrus precatorius L.	6.3	12.5	12.5	12.5	6.3	6.3
Acacia karroo	1.6	1.6	0.8	1.6	0.8	0.4
Blepharis sub volubilis subs. Sub Volubilis	0.8	0.8	1.6	1.6	12.5	0.8
Diospyros mespiliformis	0.8	3.1	1.6	1.6	0.8	3.1
Ipomoea crassipes Hook	<12.5	<12.5	<12.5	<12.5	<12.5	<12.5
Jatropha zeyheri	0.8	0.8	1.6	12.5	12.5	0.8
Peltophorum africanum	1.6	1.6	1.6	1.6	12.5	1.6
Rhoicissus tridentata subsp. cuneifolia	0.8	0.8	1.6	3.1	6.3	0.8
Senna petersiana	3.1	1.6	1.6	1.6	6.3	1.6
Ximenia caffra	3.1	<12.5	<12.5	<12.5	<12.5	<12.5

aureus (0.4 mg/ml). *R. tridentata*, *B. volubilis* and *J. zeyheri* extracts all showed promising activity by exhibiting MIC values of 0.8 mg/mL against *C.andida albicans*, *Escherichia coli* and Staphylococcus *aureus*. *A. precatorius, D. mespiliformis and S. pertesiana* exhibited moderate antimicrobial activity. Plant extracts of *I. crassipes* did not show activity against all the microorganisms, while *X. caffra* only showed activity against *C. albicans*. Previous studies have showed that plants produce secondary metabolites which have the ability inhibit the growth of microorganisms. These phytochemicals are now can be used in drug discovery for the treatment of infectious dieseases[24-26].

Antioxidant activity

The antioxidant activities of the plant extracts in the study were determined by using the free radical 2, 2-diphenyly-1-picrylhydrazyl (DPPH). The free radical scavenging activity of the plant extracts indicated the presence of secondary compounds which can donate electron and reacted with free radicals to covert and terminate radical

Table 3: The antioxidant activity (IC_{50} values) of ten tested plant extracts

Plant species	IC_{50} values (µg/mL)
Ascorbic acid	1.44
Abrus precatorius L.	2.25
Acacia karroo	0.83
Blepharis sub volubilis subs. Sub volubilis	11.77
Diospyros mespiliformis	1.21
Ipomoea crassipes Hook	10.41
Jatropha zeyheri	1.10
Peltophorum africanum	2.24
Rhoicissus tridentata subsp. cuneifolia	0.06
Senna petersiana	3.31
Ximenia caffra	9.21

Table 4: The cytotoxicity of extracts against Vero cell lines expressed in 50% inhibitory concentration (IC_{50}) and the values of regression squared

Plant extract	IC_{50} (µg/mL)	R^2
Actinomycin D	0.009	0.07
Abrus precatorius L.	118.5	0.05
Acacia karroo	115.2	0.08
Blepharis subvolubilis subs. Subvolubilis	83.35	0.06
Diospyros mespiliformis	89.53	0.08
Ipomoea crassipes Hook	100.1	0.09
Jatropha zeyheri Sond.	123.4	0.09
Peltophorum africanum	133.3	0.01
Rhoicissus tridentata subsp. cuneifolia	88.5	0.09
Senna petersiana	70.83	0.05
Ximenia caffra	91.0	0.08

chain reaction. The IC_{50} of the extracts ranged from 11.77–0.06 µg/mL, while Vitamin C (positive control) had an IC_{50} value of 1.44 µg/mL. The lowest IC_{50} values were observed from *Acacia karroo* and *Rhoicissus tridentata subsp. cuneifolia* with IC_{50} values of 0.83 µg/mL and 0.06 µg/mL respectively (Table 3). The plant extracts of *Diospyros mespiliformis* and *Jatropha zeyheri* also exhibited IC_{50} values lower than that of vitamin C of 1.21 µg/mL and 1.10 µg/mL, respectively. The highest radical scavenging activities were observed from *Blepharis sub volubilis subs. Sub volubilis, Ipomoea crassipes* Hook and *Ximenia caffra* having IC_{50} values of 11.77 µg/mL, 10.41 µg/mL, and 9.21 µg/mL, respectively.

Cytotoxicity of selected plant extracts

The plant extracts of *A. precatorius, A. karoo, I crassipes, J. zeyheri* and *P. africanum* showed low toxicity with 50% viability of cells (EC_{50}) at concentrations values of greater than 100 µg/mL (Table 4). The Lower cytotoxity of *P. africanum* extract was recently reported by Adebayo[16] with an EC_{50} of 103 µg/mL, similar to our findings. The plant extract of *Senna petersiana* was found to be the most toxic among the tested plant extracts with an EC_{50} value of 70.83 ± 0.05 µg/mL, whereas *B. subvolubilis, D. mespiliformis, R. tridentate* and *X. caffra* showed moderate toxicity with an EC_{50} value of 83.35±0.06, 89.53±0.08, 88.5±0.09 and 91.0±0.08 respectively. Actinomycin D which was used as a positive control exhibited an IC_{50} value of 0.00932 µg/mL.

CONCLUSION

Our results indicated that, all the plant extracts exhibited significant antimicrobial and antioxidant activities and also suggest that all the extracts have little or low cytotoxicity against Vero cells. *Acacia karroo* and possibly *Rhoicissus tridentata* showed the most promising activities and have the potential to be developed as antimicrobial agents in the treatment of STDs and UTIs. The results of this study may lend credence and support to anecdotal claims for the use of the selected plant species to treat sexually transmitted diseases. However, further work is required to explore other pharmacological activities and isolation of bioactive compounds.

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