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Research Article
Antimicrobial and Antioxidant Efficacy of *Acokanthera oblongifolia* Hochst. (Apocynaceae)

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Abstract

Background and Objective: *Acokanthera oblongifolia* is an evergreen medicinal shrub used for snakebites, itches, wounds and internal worms and the relief of itchy conditions and other skin disorders by the Mpondo and Xhosa tribes in South Africa. The objective of this study was to investigate the efficacy of the plant extracts against selected pathogens that cause human skin disorders and evaluation of the antioxidant capability for validation of folk uses of the plant. **Materials and Methods:** The agar diffusion and micro-dilution methods were used to determine the antimicrobial activities of the extracts against selected bacteria and fungi. The data were subjected to one way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was used to determine significant differences ($p<0.05$) among treatment means. **Results:** The highest antibacterial activity (inhibition zone diameter >19 mm) was obtained with the acetone extract against *Pseudomonas aeruginosa*, *Shigella sonnei*, *Shigella flexneri*, *Bacillus cereus*, *Streptococcus pyogens* and *Bacillus subtilis;* by the ethanol extract against *B. cereus.* None of the extracts was active against the tested fungi, apart from the acetone extract which showed strong inhibitory activity against *Candida glabrata.* The ethanol extract showed a higher ABTS scavenging than those of gallic acid and BHT at concentrations lower than 0.15 mg mL^{-1}. **Conclusion:** The *in vitro* antibacterial activity of the acetone extract of *A. oblongifolia* against the tested pathogens has provide scientific evidence to justify the ethnomedicinal use of *A. oblongofolia* against skin disorders in the study area and also indicate that the plant is a potential source for the development of antimicrobial and antioxidant agents.

Key words: *Acokanthera oblongifolia*, skin ailments, ethnomedicinal investigation, free-radical scavenging activity, antimicrobial activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants have been used by mankind for the management of diverse diseases since antiquity[1]. According to the World Health Organisation, about 20,000 plant species are used as medicines[1,2]. The widespread usage of medicinal plants in many cultures of the world could be attributed to a number of reasons including affordability and limited availability of Western medicine as well as the popular believe that herbal medicines are less toxic since they are natural[1]. Natural products, whether standardised plant extracts or pure compounds provide opportunities for the development of new drug leads partly due to their unmatched chemical diversity[3,4]. Natural products and related drugs are used to treat 87% of infections caused by bacteria, immunological disorders and cancer cases and about 25% of prescribed drugs in the world originate from medicinal plants[5,6].

Acokanthera oblongifolia Hochst. (Apocynaceae) is a common medicinal plant used for the treatment of various diseases in the Eastern Cape Province of South Africa. It is an ornamental shrub with densely clustered white flowers located in leaf axils. The plant can grow to a height of 3 m as a shrub and up to 6 m as a small tree. The dark-green leaves are elliptic, opposite and leathery in texture. Because all parts of the plant are toxic, it has been used as arrow poison and related species in other parts of the world have been reported as causing deaths[7]. The ripe berries may cause salivation, vomiting and abdominal pain when ingested. The milky the sap is irritant to the skin and eyes[8]. Hence the plant is also known as poison bush, bushman's poison, or poison tree because of its toxicity[7]. The leaf or wood decoction of the plant is used in local medicinal treatments for snakebites, itches, wounds and internal worms and an ointment made from the root scrapings of *Acokanthera oblongifolia* is used for the relief of itchy conditions and other skin disorders by the Mpondo and Xhosa tribes of the Eastern Cape Province of South Africa[8]. In view of the importance of *A. oblongifolia* in ethnopharmacology and the dearth of scientific literature of the *in vitro* medicinal potential of *A. oblongifolia*, the present study was undertaken to investigate the efficacy of the acetone and ethanol extracts of the plant against selected pathogens that cause human skin disorders and evaluation of the antioxidant capability for validation of folk uses of the plant. This study has provided new evidence necessary for the scientific validation of the claimed medicinal potential of *A. obolongifolia* extracts and a basic understanding of the plant's efficacy which may lend further support to the widespread use of traditional medicine in health care systems in South Africa.

MATERIALS AND METHODS

This study was carried out from December 2015 to February 2017 in the Amathole District, Eastern Cape, South Africa.

Extraction procedure: The leaves of *A. oblogifolia* were sterilized with 70% analytical grade ethanol and then washed with distilled water. Dried, pulverised leaf samples were shaken for 24 h in ethanol and acetone within separate conical flasks placed on an orbital shaker. The solutions were filtered through Whatman No. 1 filter paper in a Buchner funnel and the filtrates were dried in a rotavapor at 40°C under reduced pressure. A 20 mg mL^{-1} stock solution was formed by dissolving the extracts in their respective solvents of extraction.

Microorganisms: Antibacterial studies were conducted using five Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis, Bacillus cereus* and *Bacillus subtilis*) and six Gram-negative bacteria (*Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Shigella sonnei, Shigella flexneri* and *Klebsiella pneumonia*). Antifungal activities were evaluated using *Candida krusei* and *Candida glabrata*. The choice of these microbes was attributed to their significance as pathogens that cause human skin disorders. The microbes were American Type Culture Collection (ATCC) purchased from Total Laboratory, South Africa.

Antimicrobial susceptibility assays: Nutrient agar and Sabouraud Dextrose Agar (SDA) media were prepared as per the recommendations of the manufacturer and poured into sterilized disposable petri dishes under aseptic conditions. The labeled plates were inoculated with 100 µL of 0.5 Mcfarland solutions of the respective organisms and loaded with extracts of *A. oblongifolia* (50 mg mL^{-1}) into 6 mm wells. Positive controls for fungi and bacteria were nystatin and gentamycin respectively. The plates were incubated at 37°C and the zones of inhibition were recorded after 24 h. The Minimum Inhibitory Concentration (MIC) of the plant extracts was determined using 96 well microtitre plates[9]. The least concentration of plant extract that killed the microbes was considered as MIC.

Assay of DPPH scavenging activity: The DPPH radical-scavenging activity of the test extracts was examined as previously described by Erukainure *et al.*[10]. Different extract concentrations (0.025-0.5 µg mL^{-1}) were added to equal volumes of methanolic solution of DPPH (100 µM) and the mixture was left for 30 min under darkness at room

temperature. Vitamin C and Rutin were used as standard controls. Three replicates were made for each test sample. The Absorbance (A) was measured after 30 min at 518 nm and the percentage antioxidant activity was calculated according to the Eq. 1:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of the control-Absorbance of extract or standard}}{\text{Absorbance of the control}} \times 100 \quad (1)$$

The IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals. The IC_{50} values were calculated by linear regression of plots, where the abscissa represented the concentration of the tested plant extracts and the ordinate the average percent of scavenging capacity from three replicates.

ABTS scavenging activity: For the ABTS assay, an aqueous solution of ABTS (7 mmol L^{-1}) was made and $ABTS^+$ was produced by reacting the ABTS stock solution with 2.45 mmol L^{-1} potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use[11]. The $ABTS^+$ stock solution was diluted with 80% methanol to an absorbance of 0.70±0.02 at 734 nm. After the addition of 4.85 mL of diluted $ABTS^+$ to 0.15 mL of 10-fold diluted samples (final concentration 0.025-0.5 μg mL^{-1} of each extract were added, at an equal volume dry material), the absorbance reading was taken 6 min after the initial mixing. Gallic acid and BHT were used as positive controls. The ABTS activities of the samples were calculated according to the Eq. 2[11]:

$$ABTS^+ \text{ scavenging activity (\%)} = \frac{\text{Absorbance of the control-Absorbance of the sample}}{\text{Absorbance of the control}} \times 100 \quad (2)$$

Reducing power: Different extracts concentrations (0.025-0.05 μg mL^{-1}) prepared in distilled water were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (1% w/v)[4]. The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 mL of trichloroacetic acid (10% w/v). This was then centrifuged at 3000 rpm for 10 min and the supernatant (2.5 mL) was mixed with an equal volume of distilled water and 0.5 mL of $FeCl_3$ (0.1% w/v) and the absorbance (at 700 nm) was read. Vitamin C and gallic acid were used as positive controls.

Statistical analysis: All tests were done in triplicates and the zones of inhibitions were subjected to one way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was used to determine significant differences among treatment means. Minitab program (version 12 for windows) was the statiscal software that was used. p-values<0.05 were regarded as significant[3].

RESULTS

Antimicrobial assays: The inhibition diameters and the MICs of the extracts and positive controls are shown in Table 1. The acetone extract of *A. oblongifolia* showed the most potent antibacterial activity (inhibition zone diameter >19 mm)

Table 1: Inhibition zone diameters and MICs of *A. oblongifolia* extracts against the test bacteria and fungi

	Ethanol		Acetone		Positive control	
Microorganisms	ZI (mm)	MIC (mg mL^{-1})	ZI (mm)	MIC (mg mL^{-1})	ZI (mm)	MIC (mg mL^{-1})
Gram negative bacteria						
Salmonella typhimurium	12±1.1	5.00	17±1.7	2.50	25±1.0	0.01
Escherichia coli	17±2.1	2.50	16±1.1	2.50	25±0.7	<0.01
Pseudomonas aeruginosa	15±1.1	2.50	20±2.0	0.01	24±2.1	0.01
Shigella sonnei	15±1.1	2.50	20±1.0	0.01	24±2.6	<0.01
Shigella flexneri	12±1.0	5.00	20±1.3	0.01	24±1.1	<0.02
Gram positive bacteria						
Enterococcus faecalis	15±1.1	5.00	23±1.7*	0.01	24±1.4	<0.10
Bacillus cereus	20±1.1	0.01	20±1.4	0.01	24±1.6	<0.01
Streptococcus pyogens	15±2.0	5.00	20±1.2	0.01	23±2.1	<0.01
Bacillus subtilis	15±1.2	5.00	20±2.0	0.01	22±1.2	0.02
Klebsiella pneumoniae	15±1.1	5.00	15±1.5	5.00	24±2.2	<0.01
Staphylococcus aureus	15±1.3	5.00	15±2.0	5.00	24±0.6	<0.10
Fungal strains						
Candida krusei	N.A.	N.A.	N.A.	N.A.	21±1.2	0.02
Candida glabrata	N.A.	N.A.	20±1.3	0.02	20±1.7	0.02

Values are Mean±SD of triplicates experiments, N.A.: Not active, *Not significantly different form positive control (p<0.05), ZI: Zone of inhibition, MIC: Minimum inhibitory concentration

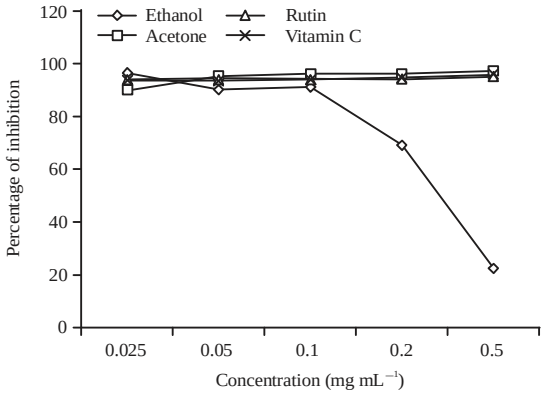

Fig. 1: DPPH scavenging activity of *A. oblongifolia* extracts

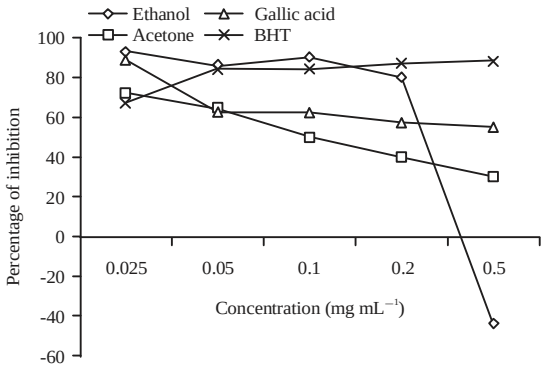

Fig. 2: ABTS scavenging activity of *A. oblongifolia* extracts

Table 2: Scavenging activity (mg mL^{-1}) of the ethanol and acetone extracts of *A. oblongifolia*

Parameters	Reducing power (mg mL^{-1})	DPPH (mg mL^{-1})	ABTS (mg mL^{-1})
Ethanol	0.16	0.15	0.12
Acetone	0.18	0.12	0.15
Vitamin C	0.19	0.13	ND
BHT	ND	ND	0.11
Gallic acid	0.14	ND	0.14
Rutin	ND	0.13	ND

ND: Not determined

against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Shigella sonnei*, *Streptococcus pyogens* and *Bacillus subtilis.* The acetone extract strongly inhibited *Candida glabrata* but was not active against *Candida krusei.* The ethanol extract was active against *B. cereus* with an inhibition diameter of 20±1.1 mm but was not active against any of the tested fungi.

The least antibacterial activity was exerted by the ethanol extract against *Salmonella typhimurium* and *Shigella flexneri* with zones of inhibition of 12±1.1 and 12±1.0 mm, respectively.

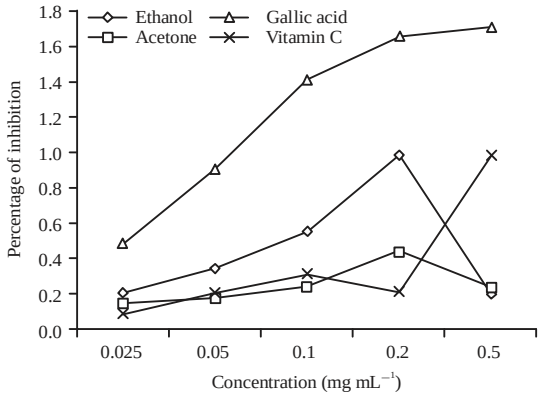

Fig. 3: Reducing power of *A. oblongifolia* extract

Results of antioxidant assays: The concentration required to attain 50% radical-scavenging effect (IC_{50}) was determined from the results of a series of concentrations tested and are presented in Table 2. A lower IC_{50} value corresponds to a larger scavenging activity.

DPPH radical-scavenging activity: Scavenging activity was expressed as percentage of inhibition of DPPH free radical (Fig. 1). Higher % inhibition indicates better scavenging activity or antioxidant potential. The results of the DPPH assay also showed that the scavenging activity of the acetone extract was higher than that of the ethanol extract and was not significantly different from those of vitamin C and rutin.

ABTS scavenging activity: The ABTS scavenging activity of the ethanol extract of *A. oblongifolia* was higher than those of the acetone extract, BHT and Gallic acid, except at concentrations greater than 0.05 mg mL^{-1} (Fig. 2). The least DPPH scavenging activity was exhibited by the acetone extract which decreased in a dose-dependent manner.

Reducing power assay: The reducing power of both the ethanol and acetone extracts of *A. oblongifolia* increased with increasing concentration up till a concentration of 0.2 mg mL^{-1}, after which there was a sharp fall in reducing power at concentrations greater than 0.2 mg mL^{-1} (Fig. 3). The reducing power of the ethanol extract was higher than that of vitamin C within the range of 0.025-0.25 mg mL^{-1}, while the least reducing power was exhibited by the acetone extract.

DISCUSSION

In the current study, the acetone extract of *A. oblongifolia* proved to be active against *Pseudomonas aeruginosa, Shigella sonnei*, *Shigella flexneri*, *Bacillus cereus*, *Streptococcus pyogens*, *Bacillus subtilis* and *Candida glabrata*. Although the antimicrobial activity of *A. oblongifolia* extracts has not been reported in previous scientific literature, studies by various authors have documented the *in vitro* medicinal potential of other members of the genus *Acokanthera.* Chaurasia and Sharma[12] reported that the methanol, acetone and chloroform extracts of *Acokanthera oppositifolia* showed significant antibacterial activity ($p = 0.02$) against two human pathogenic bacteria: *E. coli* (gram negative) and *B. subtilis* (gram positive) by disk diffusion assay. The range of antibacterial activity varied from 17 ± 0.73-25 ± 0.8 mm in *E. coli* and 18 ± 0.16-24 ± 0.14 mm zone of inhibition in *B. subtilis.*

The antimicrobial activity of different plant extracts against pathogens that cause skin disorders in humans have previously been reported by other authors. Nesy and Mathews[13] assessed the antimicrobial and antioxidant activity of *Thevetia neriifolia* flowers against seven pathogenic microbes implicated in human skin diseases. A poor antimicrobial activity was observed at lower doses (1.25-2.5 mg per well), but at medium concentration (5 mg per well), *S. aureus* was significantly inhibited ($p = 0.03$) in a manner comparable to standard Gentamicin. The methanolic extract of *Tabernaemontana heyneana* showed limited *in vitro* antibacterial activity against *Staphylococcus aureus* and *Proteus vulgaris*[14]. The polyphenol extract of the edible flower of *Sesbania grandiflora* reportedly inhibited *Shigella, exneri, S. aureus* and *E. coli*; *S. aureus* was the most susceptible with a minimum inhibitory concentration of 0.013 mg mL^{-1} [15]. The ethanol extract of the flower of *Catharanthus roseus* was reported to possess wound healing activity in Sprague Dowley rats[16]. The antimicrobial activity of plant extracts against microbes implicated in human skin disorders were attributed to the presence of polyphenolics like kaemferol, quercetn, kaempferol and quercetin 7-o-galactoside[17].

Infectious diseases of the skin and mucous membranes are common in areas characterised by lack of potable water, poor sanitation and little hygienic food habits. Many health centers in the Eastern Cape Province of South Africa are often run by nurses who have very limited training in diagnosing dermatologic conditions[18]. This has led to an uproar in the prevalence of skin diseases such as dermatitis, scabies, acne, urticaria and prurigo. These diseases are either under-treated or over-treated with antibiotics leading to great disability[19]. These limitations have fostered the search of better antimicrobials and much attention is now being directed towards medicinal plants.

The human skin is exposed to many environmental pro-oxidants including solar radiation, pollutant chemicals, drugs, food additives and cosmetic products, which generate Reactive Oxygen Species (ROS). Natural skin antioxidant molecules such as vitamin E, glutathione, catalase, vitamin C, etc. can neutralise the deleterious effects generated by reactive oxygen species. However, when this homeostatic mechanism is overwhelmed, the resultant increase in reactive oxygen species may cause inflammatory skin diseases[20]. Although synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene are used to treat ROS-mediated disorders, the health risks associated to synthetic antioxidants has motivated the search for safer antioxidants from plants.

In this study, ABTS scavenging activities of the acetone and ethanol extracts were comparable to the standard controls. Adedapo *et al.*[21] reported the presence of phenolics in *A. oppositifolia* and that the ABTS activity of the methanol extract was not significantly different to that of BHT. These studies taken together suggest that the antioxidant activity *A. oblongifolia* could be attributed to the presence of phenolics. The antioxidant activity of polyphenols is mainly due to their redox properties which neutralise free radicals, quench singlet and triplet oxygen and decompose peroxides[21].

The antibiotic and free-radical scavenging effects of *A. oblongifolia* extracts as revealed in this study implies that the plant is a potential source of leads compounds that may be used for the development of alternative remedies for the treatment of skin ailments. However, taking into consideration the reported irritancy of the milky sap of *A. oblongifolia* to the skin and eyes[8], the dosage and mode of application of the sap should be treated with caution. Hence, *In vitro* toxicity studies of *A. oblongifolia* extracts are highly recommended.

CONCLUSION

In this study, the acetone extract of *A. oblongifolia* showed the most potent antibacterial activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Shigella sonnei*, *Streptococcus pyogens* and *Bacillus subtilis*. The acetone extract strongly inhibited *Candida glabrata* but was not active against *Candida krusei.* The ethanol extract was active against *B. cereus* but was not active against any of the tested fungi. The DPPH scavenging activity of the acetone extract was similar to those Rutin and Vitamin C. The ABTS

scavenging activity of the ethanol extract of *A. oblongifolia* was higher than those of the acetone extract, BHT and gallic acid, except at concentrations greater than 0.15 mg mL^{-1}.

SIGNIFICANCE STATEMENT

This study discovers the antibiotic and free-radical scavenging effects of *A. oblongifolia* extracts that can be beneficial in the search of safer, effective and alternative remedies for the treatment of skin ailments.

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