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Antimicrobial Activity of *Warburgia ugandensis* on Selected Standard Organisms that Cause Urinary Tract Infections

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: The antimicrobial effect of *Warburgia ugandensis* has been recognized for many years in developing countries especially in East Africa. However, limited investigations have focused on its effect on microorganisms causing urinary tract infection.
Objective: To determine the antimicrobial activity of *Warburgia ugandensis* on selected standard microorganisms that cause urinary tract infections i.e. *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 10231), *Proteus mirabilis* (ATCC 25933), and *Staphylococcus aureus* (ATCC 25923).
Methods: *Warburgia ugandensis* stem bark was obtained from Tooro Botanical centre and were shade dried. The aqueous and ethanolic extracts were prepared and evaluated for phytochemical

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components using semi qualitative phytochemical screening techniques. The antimicrobial activity on three bacteria; *Staphylococcus aureus, Escherichia coli*, *Proteus mirabilis* and one fungus *Candida albicans* was tested by agar well diffusion and broth dilution which to obtain the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the plant extracts respectively.

Results: Phytochemical screening showed the presence of tannins, flavonoids, alkaloids, saponins and terpenoids in both the aqueous and ethanolic extracts. The bacteria were susceptible to the aqueous extract which caused significant inhibition of microbial growth with the highest activity observed on *Staphylococcus aureus* (MIC 0.49mg/ml), while less susceptibility to the ethanolic extract was observed with *Staphylococcus aureus* having an MIC of 1.95mg/ml and so was MBC/MFC, in which *Staphylococcus aureus* had an MBC of 7.81mg/ml. *Candida albicans* showed the same pattern with more susceptibility to the aqueous extract (MFC 15.63mg/ml) as compared to the ethanolic extract (MFC 62.5mg/ml)

Conclusion: The aqueous and ethanolic extracts of *Warburgia ugandensis* exhibit antimicrobial effect against the selected urinary tract infection causing organisms. The aqueous extract showed antimicrobial activity in both agar well diffusion and broth dilution methods. This study further shows the potential of *Warburgia ugandensis* being a novel source of modern drugs with further studies and these results provide some new perspectives on the traditional uses of *Warburgia ugandensis* in treating urinary tract infections.

Keywords: Warburgia ugandensis; Minimum Inhibitory Concentration; Minimum Bactericidal Concentration; Minimum Fungicidal Concentration; Antimicrobial activity; Staphylococcus aureus; Escherichia coli; Proteus mirabilis; Candida albicans.

1. INTRODUCTION

Warburgia ugandensis plant extracts have shown a significant activity against most common infection causing pathogens. According to the study by Mbwambo et al. on Antimicrobial activity and cytotoxic activities of fresh leaf extracts of *Warburgia ugandensis*, both the freeze dried and air dried ethanolic leaf extracts showed antimicrobial activity against standard microorganisms. Reference- antimicrobial and cytotoxic activities of fresh leaf extracts of *warburgia ugandensis Warburgia ugandensis* Sprague (Family Canellaceae), commonly known as “Ugandan Greenheart tree”, is an evergreen plant, which is mainly distributed in Eastern and Southern Africa. The plant is found in the following countries; Kenya, Uganda, Ethiopia and some parts of western Africa” [1]. “For generations, traditional healers have been using *Warburgia ugandensis* extracts made of bark, roots or leaves to treat different kinds of diseases/ailments like malaria, tuberculosis, skin diseases, ulcers, lung problems or intestinal worms, head ache, body pains, fever, hernia, to name a few. “The wood of this plant is also used for timber, firewood, poles, charcoal, stools, carvings and spoons [2,3].

“Urinary tract infections (UTIs) are amongst the most common human infections globally. It has been estimated that nearly 800 million people (equating to approximately 11% of the global population) develop at least one UTI in any given year” [4,5]. “They are substantially more common in women than in men, with the prevalence in women estimated to be approximately five times higher in females than in males” [6]. It is expected that more than half of female population of the world will contract at least one UTI in their lifetime, with a substantial proportion experiencing recurrent infections.

“Urinary tract infections are classified as either complicated or uncomplicated. Complicated infections occur in people with underlying conditions or abnormalities in any part of the genitourinary tract, making the infection more serious and more challenging to treat than uncomplicated infections. In contrast, uncomplicated UTIs are classified as infections occurring in the absence of comorbidities or other anatomical urinary tract and renal abnormalities” [7]. (Reference)- Complicated Urinary Tract Infections Ayan Sabih, Stephen W. Leslie.

There can be notable differences between the infectious agents responsible for uncomplicated and complicated UTIs. The vast majority of these pathogens are normal flora of the gastrointestinal or vaginal microflora, thereby increasing the chances that they cause UTIs. For both classes of UTI, uro-pathogenic *Escherichia coli* are the

leading infective agent, accounting for approximately 75 and 65% for uncomplicated and complicated UTIs, respectively" [7]-(Reference)-Urinary tract infections: epidemiology, mechanisms of infection and treatment options

"Notably, complicated UTI-causative pathogens are linked to increased rates of antimicrobial resistance. (reference)- Causative pathogens and antibiotic resistance in community-acquired urinary tract infections in central South Africa. Therefore, the development of effective therapies to treat these conditions is vital, not only to decrease the effects of these infections, but also to slow the development of further antibiotic-resistant bacterial strains" [7].

In Uganda, antimicrobial resistance (AMR) is a threat that needs to be addressed to minimize its associated negative effects. A study by Gerald Turyatunga on the prevalence of bacterial pathogens associated with urinary tract infection among patients attending Kan medical consult clinic found out the overall prevalence at 63.3% with children aged (1-10) having a prevalence of 5.8%. (reference)- the prevalence of bacterial pathogens associated with UTI among patients attending Kam Medical consult clinic, Uganda.

It is estimated that AMR could lead to 10 million deaths globally per year by 2050 and a USD $100 trillion economic loss if no action is taken [5]. There is need to supplement the current antimicrobial treatment regimen to minimize development of antimicrobial resistance reference-Strategies to Combat Antimicrobial Resistance.

The medicinal components in *Warburgia ugandensis* plant could be a solution [6]. The plant is readily available and accessible to the indigenous communities; however, little is known about its activity on common UTI causing pathogens. The understanding of antimicrobial activity of *Warburgia ugandensis* on these prevailing urinary tract pathogens could become an alternative herbal treatment option to supplement on the current antimicrobial treatments.

2. MATERIALS AND METHODS

2.1 Study Design

This was a laboratory-based study in which the active components of *Warbugia ugandensis* were extracted by boiling in hot water and the present phytochemicals determined. The *Warbugia ugandensis* aqueous and ethanolic extracts were used to determine the antimicrobial activity against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 25933), and *Candida albicans* (ATCC 10231).

2.2 Study Area

Warburgia ugandensis plant was obtained from Tooro Botanical centre which is located in Kabarole district approximately 1.5 kilometres from Forty portal town. The standard organisms used were obtained from the Microbiology Laboratory of Mbarara University of Science and Technology (MUST). The extraction of the plant extract and the antimicrobial testing were carried out in the Pharmaceutical Chemistry and Microbiology Laboratories of Mbarara University of Science and Technology respectively.

Plant material: The bark of *Warburgia ugandensis* was harvested and collected in its mature state by the botanist during the dry season, who identified the plant based on its features and the plant voucher attached on the stem bark indicating its full name.

2.3 Preparation of the Extract

The stem barks which were harvested by debarking the tree using a sharp-edged machete were cleaned using a hard brush to remove dirt and soil particles. They were then chopped into small pieces which were then air dried for 3 weeks until they were completely dry.

Aqueous Extraction (decoction): The dried stem barks were ground using a motor and a pestle and sieved using 250nm sieve to obtain a fine powder. 500g of the dried stem bark powder were weighed and added to 2600 ml of boiling distilled water and the mixture boiled for 50 minutes. The decoction was cooled and then filtered with a muslin cloth and then with cotton wool using a Buchner funnel. The filtrate was then concentrated using a rotary evaporator (model; RV 10 D S99, manufactured by IKA) and the resultant concentrate was freeze dried to obtain a powder. The dried extract was further crushed and then sieved using a 700nm sieve and later a 250nm sieve so as to obtain a finer powder.

Ethanolic Extraction (Maceration): The dried stem barks were ground using a motor and a

pestle and sieved using 250nm sieve to obtain a fine powder. 500g of the dried stem bark powder was then weighed and added to 2000 ml of absolute ethanol in a glass maceration container. The container was closed and agitation was done once daily for 7 days. After the 7 days of extraction, the extract was then filtered using a muslin cloth and then with cotton wool using a Buchner funnel. The filtrate was concentrated using a rotary evaporator with a revolution of 95 revolutions per minute for 3 hours at 50^{o}C. A paste like substance was then obtained and it weighed 119.3g. The resultant concentrate was freeze dried to obtain a powder. The dried extract was further crushed and then sieved using a 700nm sieve and later a 250nm sieve so as to obtain a finer powder.

2.4 Phytochemical Screening

Two grams of powder each of the ethanolic and aqueous extract of *Warburgia ugandensis* stem bark was dissolved in 20 ml of absolute ethanol and 20 ml of distilled water respectively to form stock solutions which were used for phytochemical screening.

The preliminary phytochemical analysis of the prepared plant extracts was carried out using standard methods as below; phenols or tannins; 2mls of each extract were added to 2mls of iron (iii) chloride solution. Flavonoids; 2mls of sodium hydroxide were added to 2mls of the extract. Alkaloids; 2mls of hydrochloric acid were added to the extract to convert it to a salt, then followed by 5 drops of Dragendoff's reagent. Saponins; 2mls of distilled water were added to each extract, shaken vigorously and then left to stand for 30 minutes. Terpenoids; 2mls of each extract were mixed with 2mls of chloroform and then 3mls of concentrated sulphuric acid added to the sides of the test tube. Reducing sugars; 2mls of Benedict's solution were added to 2mls of each extract in a test tube. The resulting solution was heated in a water bath for 5 minutes and the colour change observed.

2.5 Microbiological Assay

The organisms used were standard strains of *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 25933), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231) which were obtained from MUST Microbiology laboratory. The agar well diffusion method was used to determine the Minimum Inhibitory Concentration (MIC) for both the Ethanolic and aqueous extracts of *Warburgia ugandensis* against the standard strains. The broth dilution method was used to determine the Bactericidal Concentration (MBC) and the Fungicidal activity (MFC) for both the Ethanolic and aqueous extracts of *Warburgia ugandensis* against the standard bacterial and fungal organisms respectively.

Preparation of bacterial culture suspension: Upon sub-culturing the reference strains on Nutrient agar, a colony of each organism was emulsified in 1.5 mL of distilled water. The density of the bacteria culture suspension to be used for the tests was adjusted for the McFarland standard 0.5 (1.5×10^8 Colony Forming Units/ml).

Determination of MIC by agar well diffusion: Serial dilutions were made to be dispensed on culture plates inoculated with the standard organisms. The Minimum Inhibitory Concentration (MIC) was established by measuring the zone of clearance observed on the culture plate after subjecting standard organisms to the different dilutions of the extracts in agar wells. The lowest concentration of the extracts for which clearance was observed was then taken as the MIC. For both the aqueous and ethanolic extracts, the zone of clearance at different dilutions of the extract reflected organism's susceptibility and resistance patterns to the *Warburgia ugandensis* extract. *Escherichia coli, Staphylococcus aureus, Proteus mirabilis* and *Candida albicans* had different MICs as shown in the Table 4 of the results.

Preparation of working solutions: Serial dilutions of aqueous and ethanolic extracts of *Warburgia ugandensis* were made using Nutrient Broth using a stock solution of 500mg/ml concentration to obtain 11 fold dilutions (250mg/mL, 125mg/mL, 62.5mg/mL, 31.25mg/mL, 15.63 mg/mL, 7.81mg/mL, 3.91mg/mL, 1.95mg/mL,0.98mg/mL, 0.49mg/mL, and 0.24mg/mL respectively [8].

Preparation of agar well diffusion plates: Two sterile plates of MHA were used in each test. One of these plates was inoculated with the test organism; the other was left un inoculated and served as a check for media sterility [9]. Using a sterile cotton swab, the inoculum onto the Mueller Hinton Agar was made to form a microbial lawn. Using the bottom of pipette tips, wells were dug, 8 mm diameter into the Mueller

Hinton Agar (5 wells for each plate). 100µl volume of the different *Warburgia ugandensis* extract dilutions were dispensed into the wells including the positive control which was a known antibiotic Ciprofloxacin (≤0.25mcg/ml), known antifungal Fluconazole (0.125-64µg/ml) and phosphate buffered saline as the negative control [10]. The extract was allowed to diffuse and thereafter was incubated in an upright position at 37^{o}c for 24 hours. After 24hrs, the plates were read for zones of inhibition and measured using a millimetre ruler. The plates were inverted and incubated for more 24 hours to ensure maximum clearance and another reading of zone diameter was recorded. *Candida albicans* was incubated for 72 hours to ensure optimum growth of the plated organisms. The procedure was repeated 3 times on the same organism using the same extracts to get the average.

Determination of Minimum Bactericidal Concentration (MBC) by broth Dilution method: Serial dilutions of aqueous and ethanolic extracts of *Warburgia ugandensis* were made using Nutrient Broth using a stock solution of 500mg/ml concentration to obtain 11 fold dilutions (250mg/mL, 125mg/mL, 62.5mg/mL, 31.25mg/mL, 15.63 mg/mL, 7.81mg/mL, 3.91mg/mL, 1.95mg/mL,0.98mg/mL, 0.49mg/mL, and 0.24mg/mL respectively). 200µl of the different standard microbial and fungal suspensions were dispensed into each tube and incubated for 48hours at 37^{o}C. Subcultures of the suspensions from the respective bottles were made on Mueller Hinton Agar and chocolate Agar plates incubated at 37^{o}C for 24hrs hours. Plates were read and the highest dilution which gave no growth on the Agar plates was recorded as the MBC

2.6 Quality Control

The appropriate temperatures and revolutions (for the rotary evaporator) for the extraction procedures were 50^{o}C and 95 revolutions per minute for the ethanolic extract respectively. The stem bark extracts were also stored in dark amber bottles to prevent deterioration caused by ultra violet light on the active substances.

The culture plates were stored at 2-8^{o}C and autoclaved for sterility at 121^{o}C for 15 minutes, the colour and pH of the media was checked and each new batch of agar was tested with control strains for example *Enterococcus faecalis* (ATCC 29212 or 33186).

2.7 Data Analysis

The data collected which included; zone diameter of inhibition, MIC and MBC of both the ethanolic and water extract of *Warburgia ugandensis* was entered into Microsoft Excel 13, which was used to obtain the mean diameter for the zones of inhibition.

3. RESULTS

3.1 Extracts

The aqueous extract obtained weighed 34.7g and the ethanolic extract weighed 38.6g.

3.2 Phytochemistry Findings

The phytochemical screening of the *Warburgia ugandensis* aqueous and ethanolic extract found a positive reaction to tannins, flavonoids, alkaloids, saponins and terpenoids which were all abundant in the aqueous extract while the ethanolic extract had an abundance of flavonoids and alkaloids with moderate tannins and terpenoids and less pronounced saponins and no detectable reducing sugars (Table 1).

3.3 Antimicrobial Activity

Both the aqueous and ethanolic extract demonstrated antimicrobial activity. However, it was more pronounced in the aqueous extract which showed larger zones of inhibition as shown in Table 2 and Table 3. The aqueous extract showed more antimicrobial activity against *Staphylococcus aureus* with a zone inhibition diameter of 30 mm followed by *Proteus mirabilis, Escherichia coli and Candida albicans* with 28mm, 27mm and 27mm diameter respectively as shown in Table 2.

3.4 Minimum Inhibitory Concentration

Both the aqueous and ethanolic extract showed low MIC. However, the aqueous extract had lower MIC values for example, the MIC for the aqueous extract against *Proteus mirabilis* was the lowest at 0.24 mg/ml followed by 0.49 mg/ml against *Staphylococcus aureus* whereas the highest MIC of 0.98 mg/ml for the aqueous extract was against *Escherichia coli* and *Candida albicans.* The ethanolic extract however showed higher MIC values the highest being 62.5 mg/ml against *Escherichia coli* followed by an MIC of 31.25 mg/ml against *Proteus mirabilis* while the

lowest MIC values (more antimicrobial activity) were 15.63 mg/ml and 1.95mg/ml against *Candida albicans* and *Staphylococcus aureus* respectively.

3.5 Minimum Bactericidal Concentration

Both the aqueous and ethanolic extract showed low concentration of MBC. However, the aqueous extract had lower MBC values for example, the MBC of the *Warburgia ugandensis* aqueous extract was 7.81mg/ml, 62.5mg/ml, 125mg/ml, 15.63mg/ml against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 25933) and *Candida albicans* (ATCC 10231) respectively; While the MBC of the *Warburgia ugandensis* ethanolic extract was 7.81mg/ml, 125mg/ml, 250mg/ml, 62.5mg/ml against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 25933) and *Candida albicans* (ATCC 10231) respectively.

Table 1.Phytochemical constituents of *Warburgia ugandensis* aqueous and ethanolic extracts

Constituents	Aqueous extract	Ethanolic extract
Tannins	+++	++
Flavonoids	+++	+++
Alkaloids	+++	+++
Saponins	+++	+
Terpenoids	+++	++
Reducing sugars	+++	-

Key: High (+++), Moderate (++), Low (+), Absent (-)

Table 2. Diameter of Zone of inhibition (mm) due to activity of aqueous *Warburgia ugandensis* plant extract (standard deviation ±1.2)

	Negative control(mm)	Diameter of Zone of inhibition due to activity of Aqueous extract(mm)	Positive control (mm)
Staphylococcus aureus	0	30	29
Escherichia coli	0	27	32
Proteus mirabilis	0	28	38
Candida albicans	0	27	28

Table 3. Diameter of Zone of inhibition (mm) due to activity of ethanolic *Warburgia ugandensis* plant extract (standard deviation ±2.1)

	Negative control(mm)	Zone diameter of inhibition due to activity of Ethanolic extract	Positive control(mm)
Staphylococcus aureus	0	20	28
Escherichia coli	0	15	35
Proteus mirabilis	0	18	35
Candida albicans	0	15	33

Table 4. Minimum Inhibitory Concentration (mg/ml) of *Warburgia ugandensis* plant against standard organisms

	***Staphylococcus aureus* (ATCC 25923)**	***Escherichia coli* (ATCC 25922)**	***Proteus mirabilis* (ATCC 25933)**	***Candida albicans* (ATCC 10231)**
Aqueous extract	0.49	0.98	0.24	0.98
Ethanolic extract	1.95	62.5	31.25	15.63

Table 5. Minimum Bactericidal Concentration (MBC) (mg/ml) of *Warburgia ugandensis* plant extract

	***Staphylococcus aureus* (ATCC 25923)**	***Escherichia coli* (ATCC 25922)**	***Proteus mirabilis* (ATCC 25933)**	***Candida albicans* (ATCC 10231)**
Aqueous extract	7.81	62.5	125	15.63
Ethanolic extract	7.81	125	250	62.5

4. DISCUSSION

The phytochemical screening of *Warburgia ugandensis* ethanolic and aqueous extracts showed the presence of tannins, flavonoids, alkaloids, saponins and terpenoids which match those reported by Denis Okello et al, 2018. Concentration of tannins, flavonoids, alkaloids, and terpenoids in the *Warburgia ugandensis* extracts was high which are believed to be responsible for the antimicrobial activity [11].

As the need for the use of medicinal herbs especially, in the rural communities increases, there is a great need for studies that will assist in safe and effective use of herbal formulations [4]. In this study, the antimicrobial activity of both aqueous and ethanolic *Warburgia ugandensis* extracts on four standard organisms was determined.

The results of this study indicated that the aqueous extracts had a moderate to high antimicrobial activity while the ethanolic extracts had a low to moderate activity on the test organisms as shown by the MIC and MBC/MFC results in Tables 4 and 5. This agrees with a study carried out by D Olila that also concluded that aqueous *Warburgia ugandensis* extracts were more effective than the ethanolic extracts on the test organisms [12], unlike the study done by Njire, Bundabula and Kiiru which showed that alcoholic extracts had more activity than the aqueous extracts [13,14]. The antimicrobial activity is also in agreement with the study done by Yibeltal merawie Betseha on the antimicrobial activity of crude and semi-purified fractions of *Warburgia ugandensis* against six bacteria and one fungus where *Shigella boydii* and *Staphylococcus aureus* were the most susceptible to both extracts -Reference-Antimicrobial activity of crude and semi-purified fractions of *Warburgia ugandensis* against some pathogens.

The difference in the antimicrobial activity of the extracts could be attributed to the fact that during phytochemical analysis there was variation in the phytochemical component concentration. The aqueous extract contained a higher concentration of tannins, flavonoids and terpenoids which have been associated with the antimicrobial activity of this plant [15,16].

5. CONCLUSION

The aqueous and ethanolic extracts of *Warburgia ugandensis* have considerable effect on the pathogens causing urinary tract infection. The aqueous extract has better antimicrobial activity in both agar well diffusion and broth dilution method. This study shows the potential of *Warburgia ugandensis* for further study to be used as a drug in the management of urinary tract infections. The results from this study will also provide some new perspectives on the traditional uses of *Warburgia ugandensis* in treating urinary tract infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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