

Original Research Article

Antimicrobial activity of *Warburgia ugandensis* on selected standard organisms that cause urinary tract infections

Abstract

Background: The antimicrobial effect of *Warburgia ugandensis* has been recognized for many years in developing countries especially in Uganda (East Africa). However, limited investigations have been focused on its effect on Urinary tract infection causing organisms.

Objective: To determine the antimicrobial activity of *Warburgia ugandensis* on selected standard organisms 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 which is located in Kabarole district a few kilometres from Fort portal town and were shade dried. The aqueous and ethanolic extracts were prepared using the decoction extraction technique and evaluation of the phytochemicals was performed using semi quantitative 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 were used 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 extract of *Warburgia ugandensis*. All the bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*) were susceptible to the aqueous extract which caused significant inhibition of microbial growth. While all the bacteria were less susceptible to the ethanolic extract when compared to the standard susceptibility ranges of ciprofloxacin. The standard organisms were more susceptible to the aqueous extract with the highest activity observed on *Staphylococcus aureus* (ATCC 25923) which had an MIC of 0.49mg/ml. The same trend was observed in the ethanoilic extract where *Staphylococcus aureus* (ATCC 25923) had an MIC of 1.95mg/ml

compared to *Escherichia coli* (ATCC 25922) which had 62.5mg/ml. A similar trend was observed also with the MBC/MFC. *Candida albicans* also showed more susceptibility on aqueous extract than the ethanolic.

Conclusion: The aqueous and ethanolic extracts of *Warburgia ugandensis* exhibit antimicrobial effect against the selected urinary tract infection causing organisms. The aqueous extract shows antimicrobial activity in both agar well diffusion and broth dilution method. 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.

Key words: *Warburgia ugandensis*, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, Minimum Fungicidal Concentration, antimicrobial activity. *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Candida albicans*.

Introduction

Warburgia ugandensis Sprague (Family Canellaceae), commonly known as “Uganda Green Heart Tree”, is an evergreen plant, which is mainly distributed in East and South Africa. For generations, traditional healers were 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, to name a few. *Warburgia ugandensis* more pharmacognosy description family is also known as **Ugandan greenheart**, and is a species of evergreen tree native to Africa. The plant is found in the following countries; Kenya, Uganda, Ethiopia and some parts of western Africa (D. Olila, Olwa, & Opuda-Asibo, 2001). The wood of this plant is used for timber, firewood, poles, charcoal, stools, carvings, spoons, treatment of many human diseases including stomach-ache, fever, colds, headache, stomach-ache, hernia, malaria, toothache, intestinal problems, generalized body pains, fatigue, constipation (Lovett, Ruffo, Gereau, & Taplin, 2006; Van Wyk, 2008).

Urinary tract infections (UTIs) are amongst the most common human infections globally. Indeed, 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 [1, 2]. They are substantially more common in women than in men, with the prevalence in women estimated to be approximately five times higher than in males [3]. Indeed, 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.

Interestingly, there can be notable differences between the infectious agents responsible for uncomplicated and complicated UTIs. The vast majority of these pathogens are normal components 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.

Notably, complicated UTI-causative pathogens are linked to increased rates of antimicrobial resistance. 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.

In Uganda, Antimicrobial resistance (AMR) is a threat that needs to be addressed to minimize its associated negative effects. 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 (Bassetti et al., 2017). There is need to supplement the current antimicrobial treatment regimen to minimize development of Antimicrobial resistance. The medicinal components in *Warburgia ugandensis* plant could be a solution (Dilbato, Begna, & Joshi, 2019). 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.

MATERIALS AND METHODS

Study Design

This study was Laboratory based in which the actives of *Warburgia ugandensis* were extracted by boiling in hot water and the present phytochemicals determined. The *Warburgia 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).

Study area

Warburgia ugandensis plant was obtained from Tooro Botanical centre which is located in Kabarole district approximately 1.5 kilometres from Fort portal town. A variety of plant species especially medicinal plants are grown there. However, it can be found in other areas in Uganda which include the districts of Kabale, Mpigi, Iganga, and Moroto (Gumisiriza, Birungi, Olet, & Sesaaazi, 2019). The standard organisms we used were obtained from Microbiology Laboratory of Mbarara University of Science and Technology.

The study was carried out at Mbarara University of Science and Technology where the extraction process was done from the Pharmaceutical Analysis/Chemistry Laboratory of Mbarara University of Science and Technology. The antimicrobial studies were carried out in the Microbiology laboratory of Mbarara University of Science and Technology. Microbiology laboratory of Mbarara University serves a population of over four million people in its catchment area comprising the districts of Mbarara, Bushenyi, Ntungamo, Kiruhura, Ibanda, Buhweju, Rubirizi, Mitooma and Isingiro. The hospital also receives patients from Kabale, Masaka, Fort Portal and neighbouring countries like Rwanda and Tanzania. In south- western Uganda, majority of the population are living below the poverty line thus *Warburgia ugandensis* plant is a good treatment option for them if proven effective.

Plant material

Warburgia ugandensis plant extracts have been reported to show some antimalarial, antifungal, and antimicrobial properties. The stem bark was used in this study because it accumulates more phytochemical compared to other parts of the tree (Okello, Komakech, Matsabisa, & Kang, 2018). The bark of the plant was obtained from Tooro Botanical centre which grows different species of plants and local herbs for research and commercial purposes.

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 away from direct sunlight 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 was then 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 mucilin cloth and then with cotton wool using a Buchner funnel. The filtrate was then concentrated using a rotary evaporator 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. The aqueous extract obtained weighed 34.7g.

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 per day for 7 days. After the 7 days of extraction, the extract was then filtered using a mucilin 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 50⁰C. 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. The aqueous extract obtained weighed 38.6g.

Phytochemical screening

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

The preliminary phytochemical analysis of the prepared plant extracts were carried out using standard methods that is; Tannins [Ferric Chloride test], Amino acids [Ninhydrin test], alkaloids [Drangendorffs test], saponins [Frothing test], terpenoids [Chloroform and

concentrated H₂SO₄], glycosides [Benedict's test], flavonoids [Ammonia test] and reducing sugars [Benedict's test].

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.

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.

Tests organisms and media: The following culture media was used; Mueller Hinton agar and Nutrient agar. The following microbial organisms were used, *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 10231), *Proteus mirabilis* (ATCC 25933), and *Staphylococcus aureus* (ATCC 25923) and they were obtained from Mbarara University microbiology laboratory. Ciprofloxacin (≤ 0.25 mcg/ml) and Fluconazole (0.125-64 μ g/ml) was used as control for the bacterial and fungal test 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).

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 (neat, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048 giving concentrations of 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 (Mwitari, Ayeka, Ondicho, Matu, & Bii, 2013).

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 not inoculated and served as a check for media sterility (Mekonnen, 2010). 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, known antifungal Fluconazole and phosphate buffered saline as the negative Control (Khumalo, Sadgrove, Van Vuuren, & Van Wyk, 2019). The extract was allowed to diffuse and there after we incubated in an upright position at 37⁰c 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 and another reading of zone diameter was recorded. 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 (neat, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048 giving concentrations of 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 37⁰C. Subcultures of the suspensions from the respective bottles were made on Mueller Hinton Agar and chocolate Agar plates incubated at 37⁰C for 24hrs hours. Plates were read and the highest dilution which gave no growth on the Agar plates was recorded as the MBC

Quality control.

The appropriate temperatures and revolutions (for the rotary evaporator) for the extraction procedures were 50⁰C 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 2-8⁰C and autoclaved for sterility at 121⁰c 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). Ciprofloxacin (≤ 0.25 mcg/ml) and Fluconazole (0.125-64 μ g/ml) were used as positive controls for the bacterial and fungal test organisms, respectively while phosphate buffered saline was used as the negative control.

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.

Results

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 (table 1).

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

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

Key: High (+++), Moderate (++), Low (+).

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 30mm followed by *Proteus mirabilis*, *Escherichia coli* and *Candida albicans* with 28mm, 27mm and 27mm diameter respectively as shown in table 2.

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

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

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

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

Note: Susceptibility range for ciprofloxacin are

Sensitive ≥ 31 mm, Intermediate (21-30) mm, Resistant ≤ 20 mm

Minimum Inhibitory Concentration

Both the aqueous and Ethanolic extract showed low concentrations of 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 0.49mg/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.

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

	<i>Staphylococcus aureus</i> (ATCC25923)	<i>Escherichia coli</i> (ATCC25922),	<i>Proteus mirabilis</i> (ATCC25933),	<i>Candida albicans</i> (ATCC10231),
Aqueous extract	0.49	0.98	0.24	0.98
Ethanollic extract	1.95	62.5	31.25	15.63

Note: Standard MIC range for; Ciprofloxacin (positive control) is 0.12 µg/mL to 1 µg/mL, Fluconazole (positive control) is 0.03mg/ml to 16mg/ml

Minimum Bactericidal Concentration

Both the aqueous and Ethanollic 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* ethanollic 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 5: Minimum Bactericidal Concentration (MBC)(mg/ml) of *Warburgia ugandensis* plant extract

	<i>Staphylococcus aureus</i> (ATCC25923)	<i>Escherichia coli</i> (ATCC2592)	<i>Proteus mirabilis</i> (ATCC259)	<i>Candida albicans</i> (ATCC10231)
Aqueous extract	7.81	62.5	125	15.63
Ethanollic extract	7.81	125	250	62.5

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 and his colleagues in 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 (Okello et al., 2018).

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 (Adiukwu, Amon, & Nambatya, 2011). In this study, the antimicrobial activity of both aqueous and ethanolic *Warburgia ugandensis* extracts on four standard organisms was determined. The MIC was determined using the agar well diffusion method and the MBC/MFC was determined using the broth dilution method.

The results of this study indicated that both aqueous and ethanolic extracts of *Warburgia ugandensis* had an antimicrobial activity on the test organisms but with higher susceptibility of organisms to the aqueous extract than the ethanolic extract as shown by the MIC and MBC/MFC results in table 4 and table 5. This is in agreement 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 (D Olila & Opuda-Asibo, 2001) unlike the study done by Njire, Bundabula and Kiiru which showed that alcoholic extracts to have more activity than the aqueous extracts (Njire, Budambula, & Kiiru, 2021). The aqueous extract of *Warburgia ugandensis* showed remarkable activity against all the test organisms with the highest activity at a MIC value of 0.24 mg/ml against *Proteus mirabilis* and MIC of 0.49 mg/ml against *Staphylococcus aureus*. The aqueous extract had the least activity against *Escherichia coli* and *Candida albicans* with MIC value of 0.98 mg/ml which agrees with the study done by Yibeltal merawie Betseha on the antimicrobial activity of crude and semi-purified fractions of *Warburgia ugandensis* against some pathogens. The ethanolic extract of *Warburgia ugandensis* followed a similar trend and showed the highest activity on *Staphylococcus aureus* with MIC of 1.96 mg/ml and the least activity on *Escherichia coli*, *Proteus mirabilis* and *Candida albicans* with MIC of (62.5, 31.25 and 15.63) mg/ml respectively (Merawie, Sahile, Moges, & Husen, 2013).

Ethanol extract of *Warburgia ugandensis* had a lower MIC and MBC/MFC in comparison to the aqueous extract against tested microbial strains. The difference 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 (Okello & Kang, 2019).

Conclusion: The aqueous and Ethanolic extracts of *Warburgia ugandensis* have considerable effect on the Urinary tract infection causing pathogens. The aqueous extract has better antimicrobial activity in both agar well diffusion and broth dilution method. This study shows the potential of *Warburgia ugandensis* which may be adopted for treatment 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.

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