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Antimicrobial activity of crude and semi-purified fractions of *Warburgia ugandensis* against some pathogens

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PEER REVIEW

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Comments

This study is a valuable research works at which authors have demonstrated the antimicrobial properties of the crude and semi-purified fraction of the leaves and heartwood of *W. ugandensis*. The results are interesting and suggestes that the heartwood petroleum ether fraction has better antimicrobial efficacy against fungal infection.
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ABSTRACT

Objective: To investigate *in vitro* antimicrobial activities of leaves and heartwood crude and semi-purified fractions of *Warburgia ugandensis* (Canellaceae) (*W. ugandensis*) on some pathogens.

Methods: Crude and semi-purified fractions of the leaves and heartwood of *W. ugandensis* were prepared. Six bacteria [*Klebsiella pneumoniae* (*K. pneumoniae*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Shigella boydii* (*S. boydii*), *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumonia*] and one fungus (*Candida albicans*) were tested by agar well diffusion and broth dilution method to determine minimum inhibitory concentration (MIC).

Results: *S. boydii* and *S. aureus* were found to be the most susceptible bacterial isolated in agar well diffusion and broth dilution method of both the crude and petroleum ether extracts, while *K. pneumoniae* was the most resistant bacterium isolated under the same condition except in chloroform fraction. *K. pneumoniae* had shown MIC value of 10 mg/mL in the leaves and heartwood in both the crude and petroleum ether extract. *S. boydii* and *S. aureus* had shown the MIC value of 1.0 mg/mL in the crude extract for the both leaves and heartwood; Whereas the petroleum ether semi-purified fraction had shown 0.5 mg/mL in the heartwood. In the crude extract, *E. coli* and *P. aeruginosa* exhibits similar MIC value of 1.75 mg/mL. In semi purified petroleum ether extract, *E. coli* had MIC value of 1.0 mg/mL; Whereas *P. aeruginosa* had shown no change in crude extract. *Candida albicans* revealed equal MIC value of 1.0 mg/mL for the both crude and semi-purified fractions of the leaves and heartwood.

Conclusions: The crude and semi-purified fractions of *W. ugandensis* have considerable effect on pathogens. Semi-purified petroleum ether fraction has better antimicrobial activity in both agar well diffusion and broth dilution method. This study further shows the potential of *W. ugandensis* for further study in order to be use as a modern drug.

KEYWORDS

Antimicrobial activity, Leaves and heartwood, Agar well diffusion, Broth dilution method, Minimum inhibitory concentration, *Warburgia ugandensis*

1. Introduction

The medicinal quality of plants has been known and exploited by man for centuries. A large number of modern drugs have been isolated from traditional herbal plants[1]. Numerous secondary metabolites obtained from plants, with previously unknown pharmacological activities, have been extensively investigated as a source of medicinal agents[1,2]. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics led to investigation on the antimicrobial activity of medicinal plants. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic agents has led to the screening of several

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medicinal plants for their potential antimicrobial activity, such as vincristine (antitumor drug) and digitalis (heart regulator) [3,4], which were all originally discovered through research on plants. The majority of people in the developing countries still rely on traditional medicines for their primary health care[5,6]. Ethiopia is a tropical country which is endowed with rich plant biodiversity, and there are many plant species found in this country for medicinal purposes, and it is often reported[7].

Warburgia ugandensis (Canellaceae) (*W. ugandensis*) is a medicinal plant, traditionally used as herbal medicine for a wide range of diseases in some parts of Ethiopia[8]. The traditional medicinal practitioners used *W. ugandensis* to treat malaria, tuberculosis, bronchitis, pneumonia, hepatitis, tapeworm, gonorrhea, and asthma in Dollo Menna, Bale region of Ethiopia[8]. Previous studies have reported about its ablities to control *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum*, *Plasmodium knowlesi*, *Plasmodium berghei* and *in vitro* antileishmanial activity, *Leishmania major* promastigotes and antimicrobial activity[8-12]. Since no information is available to determine the activity of the semi-purified fractions of the leaves and heartwood against the standard strains of bacteria and fungi. Thus an attempt was made to evaluate the *in vitro* antimicrobial activity of crude and semi-purified fractions of the leaves and heartwood of *W. ugandensis* against some pathogens.

2. Materials and methods

2.1. Plant material

The leaves and heartwood of *W. ugandensis* were collected in January 2012 from Ethiopian Forest Research Center, Gurdsholla, Addis Ababa, Ethiopia. It is situated on North Latitude 9° 1′ 48″ and East Longitude 38° 44′ 24″, 2355 m above sea level. Additionally, this plant was identified by The National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia. Voucher specimens (01) were recorded in the Herbarium.

2.2. Extract preparation

Sample materials (leaves) were coarsely powdered and subjected to maceration with methanol, and the heartwood was chopped and dried. The dried heartwood was milled into fine powder and extracted with methanol in Soxhlet. It was dried at 40 °C by using Rota evaporator. Parts of the methanol extract concentrate was suspended in about 100 mL of distilled water and taken by petroleum ether by continuous vortexing for 30 min using separating funnel. The petroleum ether fraction was combined, evaporated and labeled as fraction I. The aqueous residue was taken and fractionated between water and *n*-butanol twice. The *n*-butanol layer was combined and evaporated to dryness (fraction II) and the remaining aqueous residue was suspended in chloroform and evaporated to dryness (fraction III). The aqueous residue that left after fractionation was filtered and dried in an oven (fraction IV).

2.3. Test organisms

Klebsiella pneumoniae (ATCC 700603) (*K. pneumoniae*), *Escherichia coli* (ATCC 25922) (*E. coli*), *Pseudomonas aeruginosa* (ATCC 27853) (*P. aeruginosa*), *Shigella boydii* (ATCC 9289) (*S. boydii*), *Staphylococcus aureus* (ATCC 25923) (*S. aureus*), *Streptococcus pneumoniae* (ATCC 49619) (*S. pneumoniae*) and a clinically isolated fungus *Candida albicans* (*C. albicans*) were used as a test organism. They were obtained from Biomedical and Laboratory Sciences Research Center, University of Gondar, Gondar; and Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia.

2.4. Standard antibiotics

Tetracycline and ketoconazole powder was used as control for the bacterial and fungal test organisms, respectively.

2.5. Antibacterial and antifungal tests

The antimicrobial tests were undertaken in three replications, at 37 °C for 24 h for bacterial isolate and at 37 °C for 48 h for fungal isolate.

2.6. Inocula preparation

The test organisms were grown in a nutrient agar medium and three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. Each isolate was grown in 100 mL broth (nutrient broth for bacteria isolate and Sabouroud's broth for fungal isolate) in Erlenmeyer flask on a rotary shaker (120 r/min) at 37 °C. Samples were taken every 2 h for a total of 12 h and the optical density was measured by spectrophotometer at 660 nm. After determining the active phase, inoculum preparation was standardized by inoculating bacterial strains from the exponential phase and standardized with 0.5 McFarland turbidity standard prepared by adding a 0.5 mL aliquot of 1.175% w/v $BaCl_2 \cdot 2H_2O$, added to 99.5 mL of 0.18 mol/L H_2SO_4 (1% v/v). The test fungus, *C. albicans* was grown in Sabouroud's broth at 37 °C for 48 h. The culture was diluted 1:100 in normal saline, followed by a 1:100 dilution in Sabouroud's broth. Approximately 10^3 CFU of yeast was contained in 0.1 mL of the final broth dilution, which was determined by plating onto potato dextrose agar. For comparison, a 0.5 McFarland standard containing 10^7 to 10^8 CFU/mL was used, and 0.1 mL portion of broth (10^3 CFU) was used as standard inoculum in agar well diffusion and broth dilution techniques.

2.7. Agar well diffusion

Bacterial strains were tested in Muller-Hinton agar medium by making a 6 mm well using a sterile borer. Inoculum from exponential phase of each bacterial isolates was centrifuged

using a vortex. The turbidity of the reconstituted organisms was adjusted to 0.5 McFarland standards. Both the standard and bacterial suspensions were agitated on a vortex mixer immediately prior to use. After inoculating the bacterial isolates, the plates were allowed to dry for 5 min after the crude, semi-purified extracts and the control were dispensed into each well. The plates were incubated at 37 °C for 24 h. Inhibition zone sizes were measured in millimeters compared to standard tetracycline (0.025 mg/mL). For *C. albicans*, the same method was used to make a well in potato dextrose agar. Broth will be expressed from the swabs by pressing and rotating the swabs firmly against the inside of the tube above the fluid level. The swab was then evenly streaked over the entire surface of the agar plate to obtain uniform inoculum. Each of the extract concentrations were applied into the well. All these activities were undertaken under aseptic condition. The plates were then incubated for 48 h at 37 °C to determine the inhibition zone. ketoconazole (25 mg/mL) were used as the standard drug.

2.8. Minimum inhibitory concentration (MIC)

The MIC of the extract was determined by broth dilution technique for fungus. Firstly, the ether extracts were prepared in different concentrations (0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 1.75, 2.5, 5.0, 10.0 and 25.0 mg/mL). Dissolve 1 mL of each extracts in sterile capped test tubes which containing 8.9 mL of microbial growth medium. Then, serial dilutions of the concentrations were inoculated with 0.1 mL of standard size of bacterial and fungal inoculum from the exponential phase. Two test tubes containing broth without antimicrobial agent were added in each test. One of these tubes was inoculated with the test organism, the other was left uninoculated and served as a negative control for media sterility. The test tubes were incubated for 24 h at 37 °C for bacterial isolates and 48 h at 37 °C for fungi. Lowest concentration of compound showing antimicrobial activity was recorded as MIC value.

2.9. Data analysis

The results were analyzed for crude and semi-purified fractionates of the plant and for each of two modern antibiotics. Data analysis was made by using Statistical Package for Social Sciences (SPSS) windows® software package in terms of the mean of the growth inhibition value obtained from each of the six bacterial strains and one clinical isolate of fungal strain.

3. Results

3.1. Agar well diffusion assay of the crude leaf and heart wood extracts

Growth inhibition was observed within all cases at 25.0 mg/mL concentration of the heartwood extract (Table 1 and Figure 1). The inhibition range varied from 15.7 to 22.3 mm diameter. Similarly, the leaves extract had shown growth inhibition at 25.0 mg/mL concentration for all tested organisms, and inhibition range varied from 0.0-22.3 mm diameter. *S. boydii* and *S. aureus* were the only bacterial species inhibited by 1.0 mg/mL concentration of the leaves extract (Table 1). The growth of *S. aureus* and *S. boydii* was inhibited at the lowest test concentration of 1.0 mg/mL of the leaves extract with inhibition diameters of 10.7 mm and 9.0 mm, and displayed 20.7 mm and 18.0 mm inhibition diameter respectively at a concentration of 25 mg/mL. *S. pneumoniae* and *E. coli* showed the highest inhibition zone (22.3 mm and 20.0 mm) at 25 mg/mL, respectively (Table 1).

3.2. Agar well diffusion assay of the petroleum ether leaf and heartwood extract

In petroleum ether semi-purified leaves extract of *W. ugandensis*, *S. boydii* and *S. aureus* have shown high sensitivity at a concentration of 1.0 mg/mL with inhibition diameter of 12.0 mm and 11.0 mm respectively. They also have the highest inhibition diameter of 21.3 mm and 20.0 mm at a concentration of 25.0 mg/mL. The most resistant strains were *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *S. pneumoniae*, with sensitivity to the 25.0 mg/mL were 10.3 mm, 12.7 mm, 17.3 mm and 11.3 mm respectively (Table 2). The highest inhibition diameter of strains against heartwood extract was observed by *S. pneumoniae* with a diameter of 30.0 mm at a concentration of

Table 1
The zone of inhibition of the crude leaf and heartwood extracts of *W. ugandensis* on test microorganisms using agar well diffusion method (mm).

Test organisms	Leaves				Heartwood				Control*
	1.0 mg/mL	5.0 mg/mL	10.0 mg/mL	25.0 mg/mL	1.0 mg/mL	5.0 mg/mL	10.0 mg/mL	25.0 mg/mL	
K. pneumonia	–	–	–	–	–	10.0	14.0	17.7	14.0
E. coli	–	13.3	15.0	20.0	12.3	15.7	19.7	20.3	23.0
P. aeruginosa	–	–	–	20.0	–	9.3	15.0	15.7	19.0
S. boydii	9.0	13.7	15.0	18.0	10.7	17.3	19.3	21.0	22.0
S. aureus	10.7	15.3	17.7	20.7	13.0	15.7	19.0	22.7	30.0
S. pneumoniae	–	15.3	20.3	22.3	10.0	15.7	18.3	20.3	26.0
C. albicans	8.7	10.3	12.3	13.0	8.7	10.7	15.0	20.0	20.0

*Control standard drugs were tetracycline (0.025 mg/mL) for bacterial strains and ketoconazole (25.0 mg/mL) for *C. albicans*; and all data were mean of triplicate values.

Table 2

The zone of inhibition of the petroleum ether extracts of the leaf and heartwood of *W. ugandensis* against test organisms using agar well diffusion method (mm).

Test organisms	Leaves				Heartwood				Controls*
	1.0 mg/mL	5.0 mg/mL	10.0 mg/mL	25.0 mg/mL	1.0 mg/mL	5.0 mg/mL	10.0 mg/mL	25.0 mg/mL	
K. pneumoniae	–	–	9.0	10.3	–	–	–	–	14.0
E. coli	–	–	10.0	12.7	10.3	11.0	15.7	21.3	23.0
P. aeruginosa	–	–	12.0	17.3	8.0	10.7	13.7	17.7	19.0
S. boydii	12.0	15.0	18.0	21.3	13.3	20.0	22.3	25.7	22.0
S. aureus	11.0	13.3	14.0	20.0	8.7	9.3	11.0	16.0	30.0
S. pneumonia	–	–	9.3	11.3	8.0	12.7	14.0	30. 0	26.0
C. albicans	8.3	10.7	15.0	20.7	10.7	10.0	13.7	25.0	20.0

*Control standard drugs were tetracycline (0.025 mg/mL) for bacterial strains and ketoconazole (25.0 mg/mL) for *C. albicans*, and all data were mean of triplicate values.

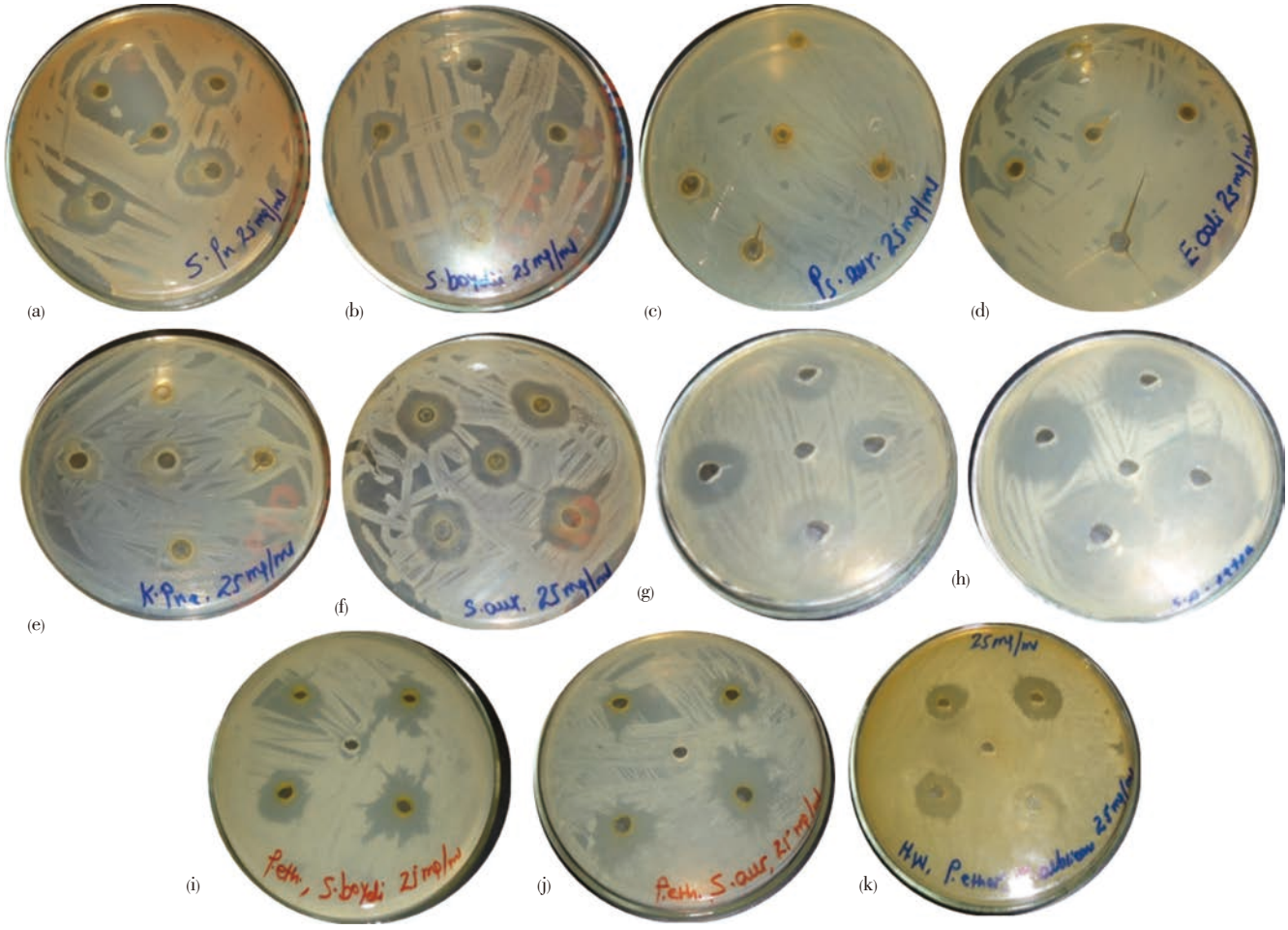

Figure 1. Antimicrobial activity tested in an agar well diffusion method.

a: *S. pneumoniae* with crude heartwood extract, b: *S. boydii* with crude heartwood extract, c: *P. aeruginosa* with crude heartwood extract, d: *E. coli* with crude heartwood extract, e: *K. pneumoniae* with crude heartwood extract, f: *S. aureus* with crude heartwood extract, g: *C. albicans* in crude heartwood extract, h: *S. aureus* in tetracycline, i: *S. boydii* in petroleum ether extracts heartwood extract, j: *S. aureus* in petroleum ether extracts heartwood extract, k: *C. albicans* in petroleum ether extracts heartwood extract.

25.0 mg/mL followed by *S. boydii*, *E. coli*, *P. aeruginosa*, and *S. aureus* with a diameter of 25.7 mm, 21.3 mm, 17.7 mm, and 16.0 mm respectively. *K. pnemoniae* was resistant to heartwood petroleum ether extract.

3.3. Agar well diffusion assay of leaf and heartwood extract in n–butanol

The *n*–butanol semi–fraction of *W. ugandensis* leaves did not show any activity against both the bacterial and fungal pathogens (Table 3). On the contrary, the heartwood had some activity against tested bacteria. The results showed that the most sensitive two strains were *E. coli* and *P. aeruginosa* that displayed inhibition diameter of 10.0 mm and 8.3 mm, respectively at a concentration of 1.0 mg/mL and the highest inhibition diameter were 19.3 mm and 16.7 mm at 25.0 mg/mL of the two sensitive bacteria. *S. pnemoniae*, *S. boydii* and *S. aureus* did not shown activity at the lowest concentration of 1.0 mg/

mL, but they were inhibited at 10.0 mg/mL and 25.0 mg/mL. *K. pneumoniae* was the most resistant bacteria which showed no inhibitory activity to the *n*-butanol fraction (Table 3).

Table 3
The antimicrobial activity of the *n*-butanol extracts of the heartwood of *W. ugandensis* against test organisms using agar well diffusion method (mm).

Test organism	Heartwood				Controls*
	1.0 mg/mL	5.0 mg/mL	10.0 mg/mL	25.0 mg/mL	
K. pneumoniae	–	–	–	–	14.0
E. coli	8.3	10.0	14.3	16.7	23.0
P. aeruginosa	10.0	14.0	14.5	19.3	19.0
S. boydii	–	–	8.5	10.1	22.0
S. aureus	–	8.3	10.0	12.0	30.0
S. pneumoniae	–	–	11.0	20.5	26.0
C. albicans	–	–	–	–	20.0

*Control standard drugs were tetracycline (0.025 mg/mL) for bacterial strains and ketoconazole (25.0 mg/mL) for *C. albicans*, and all data were mean of triplicate values.

3.4. Agar well diffusion assay of the chloroform extract of leaf and heartwood

The chloroform extract of *W. ugandensis* leaves had shown activity only to the *K. pneumoniae* with inhibition diameter of 14.7 mm at a highest concentration of 25.0 mg/mL, and 13.7 mm and 11.7 mm at 10.0 mg/mL and 5.0 mg/mL respectively (Table 4). With respect to the heartwood chloroform extract, *S. aureus*, *E. coli* and *P. aeruginosa* had shown sensitivity whereas *K. pnemoniae*, *S. boydii*, and *S. pnemoniae* were resistant at all concentration. From the sensitive bacteria *E. coli* was the most sensitive one with inhibition diameter of 15.0 mm to the lowest concentration 1.0 mg/mL and the highest inhibition diameter of 30.0 mm at the concentration of 25.0 mg/mL followed by *S. aureus* and *P. aeruginosa* having 22.7 mm and 18.7 mm at 25.0 mg/mL concentration, respectively (Table 4). *C. albicans* was sensitive to both leaves and heartwood extract with a diameter of 8.3 mm and 15.0 mm for the leaves; and 10.0 mm and 23.0 mm for the heartwood extract with 1.0 mg/mL and 25.0 mg/mL concentration, respectively.

3.5. MIC of the crude leaf and heartwood extract

S. aureus and *S. boydii* were highly susceptible to the MIC of 1.0 mg/mL of the leaf and heartwood extracts followed by *E. coli* and *P. aeruginosa* which were inhibited at the concentration of 1.75 mg/mL of both extracts (Table 5). *S. pneumoniae* was inhibited at 2.5 mg/mL of the leaves and heartwood extracts. *K. pnemoniae* was the most resistant bacteria having MIC value of 10.0 mg/mL.

Table 4
The inhibition zone of the chloroform extracts of the leaf and heartwood of *W. ugandensis* against test organisms using agar well diffusion method (mm).

Test organism	Leaves				Heartwood				Control*
	1.0 mg/mL	5.0 mg/mL	10.0 mg/mL	25.0 mg/mL	1.0 mg/mL	5.0 mg/mL	10.0 mg/mL	25.0 mg/mL	
K. pneumoniae	–	11.7	13.7	14.7	–	–	–	–	14.0
E. coli	–	–	–	–	15.0	20.7	27.0	30.0	23.0
P. aeruginosa	–	–	–	–	–	10.3	12.0	18.7	19.0
S. boydii	–	–	–	–	–	–	–	–	22.0
S. aureus	–	–	–	–	–	8.5	20.0	22.7	30.0
S. pneumonia	–	–	–	–	–	–	–	–	26.0
C. albicans	8.3	10.0	11.7	15.0	10.0	13.3	19.0	23.0	20.0

*Control standard drugs were tetracycline (0.025 mg/mL) for bacterial strains and ketoconazole (25.0 mg/mL) for *C. albicans*; and all data were mean of triplicate values.

Table 5
MIC of the crude leaf and heartwood extracts of *W. ugandensis* against pathogenic test microorganisms.

Test organism	Plant part	Concentrations (mg/mL)					
		0.5	1.0	1.75	2.5	5.0	10.0
K. pneumoniae	Leaves	+	+	+	+	+	β
	Heartwood	+	+	+	+	+	β
E. Coli	Leaves	+	+	β	–	–	–
	Heartwood	+	+	β	–	–	–
P. aeruginosa	Leaves	+	+	β	–	–	–
	Heartwood	+	+	β	–	–	–
S. boydii	Leaves	+	β	–	–	–	–
	Heartwood	+	β	–	–	–	–
S. aureus	Leaves	+	β	–	–	–	–
	Heartwood	+	β	–	–	–	–
S. pneumoniae	Leaves	+	+	+	β	–	–
	Heartwood	+	+	+	β	–	–
C. albicans	Leaves	+	β	+	–	–	–
	Heartwood	+	β	+	–	–	–

–: absence of growth, +: presence of growth and β: MIC.

There was variation in the MIC value of the heartwood and leaves crude extract in *P. aeruginosa* (1.75 mg/mL) in the heartwood and 2.5 mg/mL in the leaves. The MIC value of *C. albicans* was 1.0 mg/mL in both the heartwood and leaves (Table 5).

3.6. MIC semi-purified fraction of leaf and heartwood extract of petroleum ether

Within the bacterial isolate *S. boydii* and *S. aureus* were the most sensitive ones having MIC value of 0.5 mg/mL in the heart wood petroleum ether extract. In the leaves, *S. aureus* had MIC value of 1.0 mg/mL which is equivalent with the crude extracts where as *S. boydii* had the same MIC like the heartwood (0.5 mg/mL). *E. coli* (1.0 mg/mL) and *S. pneumoniae* also showed sensitivity at 1.0 mg/mL of the heartwood extract but on the leaves extract it had MIC value of 1.75 mg/mL while most resistant was *K. pneumoniae* (Table 6).

Table 6
MIC of the petroleum ether leaf and heartwood extracts of *W. ugandensis* against pathogenic test microorganisms.

Test organism	Plant part	Concentrations (mg/mL)					
		0.5	1.0	1.75	2.5	5.0	10.0
K. pneumonia	Leaves	+	+	+	+	+	β
	Heartwood	+	+	+	+	+	β
E. Coli	Leaves	+	β	−	−	−	−
	Heartwood	+	β	−	−	−	−
P. aeruginosa	Leaves	+	+	β	−	−	−
	Heartwood	+	+	β	−	−	−
S. boydii	Leaves	β	−	−	−	−	−
	Heartwood	β	−	−	−	−	−
S. aureus	Leaves	+	β	−	−	−	−
	Heartwood	β	−	−	−	−	−
S. pneumonia	Leaves	+	+	β	−	−	−
	Heartwood	+	β	−	−	−	−
C. Albicans	Leaves	+	β	−	−	−	−
	Heartwood	+	β	−	−	−	−

−: absence of growth, +: presence of growth and β: MIC.

4. Discussion

The plant based antimicrobial compounds have tremendous therapeutic potential as they can serve the purpose without or with very few side effects that are often associated with synthetic drugs. With regard to inter-bacterial differences in response to exposure to heartwood crude extract of *W. ugandensis*, the gram positive (*S. aureus* and *S. pnemoniae)* were the most sensitive bacteria with the highest inhibition diameter, followed by *S. boydii*, *E. coli*, and *K. pneumoniae*. The relatively resistant test microorganisms were *P. aeruginosa* with small inhibition diameters. *S. boydii* was the most sensitive bacterium to both leaves and heartwood crude extract of *W. ugandensis*. While, in another study of *E. coli* and *S. aureus* have given similar inhibition diameter in the freeze-dried ethanolic leaf extracts of *W. ugandensis*[13]. It was also reported that the stem bark extract inhibits *S. aureus* more than *E. coli*[14]. Additionally, the antibacterial activity of *W. ugandensis* is only detectable at a very high concentration of the extract in the agar well diffusion method[14]. While in this investigation it was possible to detect the antibacterial effect of the plant up to 1 mg/mL in both the leaves and heartwood extract. This might be due to the presence of *W. ugandensis* in different geographical areas or the type of extraction techniques. *C. albicans* was found to be sensitive to crude leaves extracts. Likewise, the heartwood extracts displayed larger inhibitory diameters. Freeze-dried ethanolic leaf extract of *W. ugandensis* showed inhibition of *C. albicans* with a diameter but the air dried leaf crude 80% ethanol extract have not shown the antifungal activity against *C. neoformans* and *C. albicans*[13]. In the present investigation the shed-dried leaves and heartwood extracts exhibits the antifungal activity even at lower concentration. The sundried leaves lose the volatile compound and hence activity may have lost. Air dried *W. salutaris* stem bark was also effective against *C. albicans*[15]. Similarly, the antifungal activity of *W. ugandensis* crude leaves extract of methanol was found against *Fusarium* spp[16].

The petroleum ether extract showed the highest diameter from other semi-purified fractionates. Earlier study indicated that the petroleum ether extract of the stem bark showed the highest activity in the agar well diffusion method[14]. The fractionated *W. ugandensis* leaf extract showed some bioactive fractions against *F. oxysporum*[16]. This result might indicate the active compound which is found in the leaves and heartwood is mainly non-polar in its nature like sesquiterpenes in the heart wood and unsaturated sterol/triterpens in the leaf. It was also reported that the hexane extract of *W. ugandensis* had the best activity against *L. major* promastigotes and amastigotes[10].

The leaf chloroform extract was sensitive only to *K. pneumonia*. Heartwood chloroform extract exhibited better antimicrobial activity against *S. aureus*, *E. coli* and *P.*

aeruginosa where as *K. pnemoniae*, *S. boydii*, and *S. pnemoniae* were found resistant. Further, both leaves and heartwood chloroform extract were found active against *C. albicans*. Perhaps, this might be due to the presence of more than one active compound which inhibits the growth of *C. albicans* and other bacterial pathogens in the different semi-purified fractionates.

The *n*-butanol semi-fraction of *W. ugandensis* heartwood was more effective in comparison to leaves extract against the studied pathogens. In heartwood extract, the most sensitive was *E. coli* while *K. pneumoniae* was the most resistant bacteria. Due to previous study, at 150 mg/mL concentration of *Croton zambesicus* stem bark extract, *S. aureus*, *P. aeruginosa*, *E. coli* displayed inhibition diameter of 30.2, 27.7, 29.8 mm, respectively[17]. While present study showed that at 25 mg/mL concentration of *W. ugandensis* heartwood, *S. aureus*, *P. aeruginosa*, *E. coli* displayed inhibition diameter of 12.0, 19.3, 16.7 mm, respectively. Comparison shows that the *n*-butanol extract of heartwood of *W. ugandensis* had better antimicrobial activity than *C. zambesicus* only at one sixth of the tested concentration[17].

The aqueous fraction of *W. ugandensis* leaves and heartwood did not show any antimicrobial activity against all bacteria and fungi. While, the water extract showed slightly higher activity than the ethanolic extract of stem bark in the agar well assay at the highest concentration than the present study[14]. There may be several factors which can reduce antimicrobial activity. The method of plant extraction technique is another factor which affects the antimicrobial activity of the medicinal plant. Aqueous extract may contain both water soluble and alcohol soluble compound and hence it may be more effective than either of them.

The heartwood crude extract had better MIC value than the crude leaves extract. The stem bark of *W. salutaris* had similar MIC value for *S. aureus* and *C. albicans*[15]. Leaf crude extracts of *W. ugandensis* inhibited *S. aureus* MIC of 2.0 mg/mL[18], but in the present investigation *S. aureus* showed the lowest MIC value in the heartwood (1.0 mg/mL) and leaves (1.75 mg/mL). With regard to *C. albicans*, the MIC value was 1.0 mg/mL in both the heartwood and leaves. The petroleum ether semi-purified fractions showed better antibacterial and antifungal activity in the agar well diffusion method. The most resistant was *K. pneumoniae*. Antimycobacterial activity of the bioassay guided fractions of the dichloromethane extracts of the stem bark of *W. ugandensis* was also reported[19], and compound 13 which was linoleic acid had displayed the most potent inhibitory activity against *Mycobacterium aurum*, *Mycobacterium fortuitum*, *Mycobacterium phlei* and *Mycobacterium smegmatis*[19]. Additionally, compound 5 which was muzigadial having the coloratadiene-dialdehyde structural feature displayed pronounced antimycobacterial activities[19]. Since most of the active compounds which are found in the stem bark are also found in the heartwood, it is possible to screen a pure compound by further fractionation of the different fractions of the heartwood to reduce the MIC value displayed in the present study. In case of *C. albicans*, the same MIC value was recorded to the crude leaves and heartwood extract. In another study, muzigadial, a pure compound, which can be extracted from the leaves of *W. ugandensis* had lower MIC value against *Aspergillus niger*, *Fusarium oxysporum* and *Alternaria passiflorae* than the present study[16]. This indicates further work is required to be undertaken to isolate the pure compound muzigadial and others which may have lower MIC value for the *C. albicans* and other pathogens.

It is concluded that the potential of *W. ugandensis* in modern drugs preparations is considered, and from the different semi-purified fractionates particularly the petroleum ether fraction have shown the best antimicrobial activity in both agar well diffusion and broth dilution method.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The emergency of single and multiple antibiotic resistance strain call for the search of an alternative agent with possible antimicrobial effect from natural products. Thereforeit is very important to study the pharmacological activity of the medicinal plant such as *W. ugandensis* as an alternative agent.

Research frontiers

This study explores antimicrobial activity of leave and heartwood extract of *W. ugandensis*. Research was conducted on leaves and barks but studies on leave and heartwood crude and semi purified fraction are not available.

Related reports

From the results obtained, it appears that the heartwood

extract had more pronounced effect on the *C. albicans*. Previous report[14]suggested that the antibacterial activity of *W. ugandensis* is only detectable at a very high concentration while in this investigation it was possible to detect the antibacterial effect up to 1 mg/mL in both the leaves and heartwood extract.

Innovations and breakthroughs

Data on the antimicrobial properties of the semi-purified fraction of the heartwood of *W. ugandensis* is limited. This study evaluates the antimicrobial efficacy of the crude and semi-purified fraction of leaves and heartwood against the tested organisms.

Applications

It is beneficial to know the potential application of *W. ugandensis* in medicine. The results of the present study suggest the use of the plant as an alternative for the treatment of candidiasis.

Peer review

This study is a valuable research works at which authors have demonstrated the antimicrobial properties of the crude and semi-purified fraction of the leaves and heartwood of *W. ugandensis*. The results are interesting and suggested that the heartwood petroleum ether fraction had better antimicrobial efficacy against fungal infection.

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