

Original Research Article

Chemical Screening and Antimicrobial Activities of Rwandan traditional medicinal plant, *Urtica massaica* Mildbr (Urticaceae)Maniriho O^{1*}, Nkurunziza JP², Ayodele AE^{1,3}, Benimana F⁴, Murhula HP¹, Farhan HFA¹, Nimbeshaho F¹, Cyiza F⁵¹Department of Pharmacognosy, Medicinal Plants Research and Drug Development Programme, Pan African University/University of Ibadan, Ibadan, Nigeria²Department of Chemistry, School of Sciences, College of Science and Technology, University of Rwanda, Rwanda³Department of Botany, Faculty of Science, University of Ibadan, Ibadan, Nigeria⁴Department of Food and Biotechnology, Faculty of Medical Biology, South Ural State University, Chelyabinsk, Russia⁵Department of chemistry, University of Bandung, Indonesia**Article History**

Received: 21.02.2021

Accepted: 26.03.2021

Published: 27.04.2021

Journal homepage:<https://www.easpublisher.com>**Quick Response Code**

Abstract : Chemical screening and antimicrobial activities of stems and roots of Rwandan *Urtica massaica* Mildbr. (Urticaceae) were investigated. Chemical screening revealed that majority of bioactive secondary metabolites was present at higher concentration in stem than in root. Antimicrobial activities of both stem and root bark methanolic extracts, SBME and RBME, were followed by Agar Disc diffusion and Broth Macro-dilution methods; Inhibition zones, Minimum Inhibition Concentration, MIC and Minimum Bactericidal Concentration, MBC; against the tested microbial strains. Values of inhibition zone for SBME against *E. coli* were 21.667±1.529 to 28.000±2.851mm. RBME inhibited *E. coli* from 19.667±1.734 to 25.667±1.023mm. *Salmonella* sp were fully resistant to RBME. SBME inhibited *Salmonella* sp from 7.167±0.208 to 24.067±2.503mm but inhibited *S. aureus* from 17.333±1.335 to 21.667±0.334mm. RBME against *S. aureus* showed 15.000±0.578, 17.000±0.578 and 20.667±0.334mm inhibition zones respectively. MIC values of SBME were 6.250±1.562mg/mL (*E. coli*), 10.156±4.752mg/mL (*Salmonella* sp) and 6.250±1.563mg/mL (*S. aureus*). MIC of RBME were 7.813±1.563mg/mL (*E. coli*) and 7.813±1.563mg/mL (*S. aureus*) though it was found inactive against *Salmonella* sp. MBC values of SBME were 12.500±3.125mg/mL (*E. coli*), 19.531±10.156mg/mL (*Salmonella* sp) and 12.500±3.125mg/mL (*S. aureus*). MBC values of RBME were 15.625±3.125mg/mL (*E. coli*) and 15.625±3.125mg/mL (*S. aureus*). The results indicated greater antimicrobial activities of stem than root of *U. massaica*, thus corroborating with the fact that majority of bioactive phytochemicals were highly present in SBME than in RBME. *U. massaica* stem should be considered as natural antimicrobial potential to treat infectious diseases.

Keywords: *Urtica massaica* stem, root, phytochemical screening, bioactive phytoconstituents and antimicrobial activities.

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1. INTRODUCTION

Medicinal plants have been used in traditional health care systems since prehistoric times and are still the most important health care source for the most of the world's population. The World Health Organization (WHO) has estimated that more than 75% of the world's total population depends on herbal drugs for their primary healthcare needs [1]. Pathogenic microorganisms absolutely infect human and develop infectious diseases [2]. A variety of microorganisms threaten a human health. The ability of humans to control and prevent infectious diseases is continuously challenged due to the enormous diversity of microbial pathogens combined with their ability to evolve and adapt to changing environment [3]. The most common bacterial pathogens which cause

infectious diseases include *Escherichia coli*, *Salmonella* spp., *Aeromonas* spp., *Pseudomonas* spp., *Streptococcus pyogenes*, *Klebsiella* spp., and *Staphylococcus aureus* etc [4, 5].

The use of medicinal plants in the treatment of infectious diseases is an old age practice; and several natural products derived from plants are used for treatment of numerous human diseases for thousands of years. Medicinal plants are used in many parts of the world as healing for variety of human ailments [6]. The traditional use of plant for the treatment of infectious diseases remain the most affordable and easily accessible source of treatment particularly in the primary healthcare systems of developing countries [7]. All parts of a plant can be

utilized for medication purpose like roots, bark, woody stems, leaves, flowers, fruits, resin and seeds. For many species, different parts of the same plant are used to produce different remedies for various diseases [8].

In the case of *Urtica massaica* Mildbr. (Urticaceae), Rwandan traditional medicine practitioners use its stem and root alone and/or in mixtures with other plant species to treat numerous ailments including bruises, fractures, venereal diseases, stomach problems, skin infections, bladder complications, cough and headache [9, 10-11].

Considering the Rwandan traditional medicinal uses of this plant, the objective of this work was to screen the phytoconstituents and investigate antibacterial activities of *Urtica massaica* stem and root barks.

2. MATERIALS AND METHODS

2.1 Chemical reagents

All reagents and chemicals utilised in this work were of analytical grade (ANALAR).

2.2 Collection of plant materials and Herbarium authentication

The stem and root barks of *Urtica massaica* were collected from the wild areas surrounding Volcanoes National Park of Rwanda (VNPR) of Karambi village/Bugarama cell/Kabatwa sector/Nyabihu District, Northern Province, Rwanda. The area's geographical coordinates taken by GPS (at 3m of accuracy) were Latitude: (S01°34.351⁰ and E029°25.495⁰); (S01°34.401⁰ and E029°25.480⁰); (S01°34.365⁰ and E029°25.696⁰) and Altitude: 2362m. The pressed plant sample was identified and authenticated by Professor Elias BIZURU, a professional botanist at National Herbarium of Rwanda. The specimen was kept under the collector's collection, M.O 1(Maniriho Olivier 1), and preserved for future reference and other identification issues.

2.3 Plant material handling and Extraction procedures

The collected stem and root barks of *Urtica massaica* were garbed, washed with distilled water to remove any insect materials, clay and mud, chopped and then air-dried at room temperature. Dried plant materials were mechanically ground using pestle and mortar. The two grinding machines (RETSCH SM100 and RETSCH SM200, Germany) were later used to obtain the desired powder size. The obtained dry powder was then weighed and stored in airtight polyethylene containers.

The crude extract of air-dry powdered stem and root barks of *Urtica massaica* was prepared by cold maceration using 80% methanol as an extracting solvent. This was prepared by weighing 270 grams of

coarsely powdered plant materials (stem and root barks separately) using sensitive digital analytical balance (AHAUS Corp. Pine Brook NJ, USA) and soaked in a clean flask containing methanol (80%) in distilled water.

It was then kept for a period of seven days accompanied with intermittent shaking using mini orbital shaker. The entire mixture was filtered through a funnel plunged with muslin cloth two times. The remaining residue or marc was re-macerated twice for a total of six days with a fresh methanol. The marc was then pressed, and the resulting solution after successive filtration was evaporated using a rotary evaporator set at 40⁰C to remove the solvent methanol. Finally, the concentrated root bark and stem bark methanol extracts (RBME and SBME) were placed in an oven at 37-40⁰C until completely dried and solidified.

The dried powder of extracts was weighed in air tight container and then kept in dark place in refrigerator (0-4)⁰C for further analyses.

2.4 Phytochemical screening

In this work, the qualitative phytochemical investigations of the crude extracts of *Urtica massaica* stem and root barks were carried out using the following standard tests as described in research documents [12-14].

2.4.1 Alkaloids

About 0.5g of extract were stirred with 5mL of 1% aqueous hydrochloric acid on a water bath; 1mL of the filtrate was then treated (into separate test tubes) with few drops of Dragendorff's reagent, Wagner's reagent, and Tannic acid test (tannic acid solution) respectively.

The formation of a Reddish Brown precipitate, Yellow precipitate and Brownish precipitate respectively indicated the presence of alkaloids.

2.4.2 Sterols and Steroids

To 0.5g of extract in 2mL distilled water was added 1 mL of acetic anhydride. 2-3drops of Conc.H₂SO₄ were added. The coloration of deep green color indicated the presence of Steroids (Liebermann-Burchard Test).

About 0.5g of extract was dissolved in 2 ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of the test tube. Test tube was shaken for few minutes. The development of red colour in chloroform layer (lower layer) and greenish-yellow in acid layer (upper layer) indicated the presence of sterols (Salkowaski test).

2.4.3 Terpenoids

To 5mg of extract and each solvent fraction, add 5mL of acetic anhydride and 2-3 drops of conc. H₂SO₄. A deep red coloration was a positive test for Terpenoids.

2.4.4 Glycosides

To 5mg of extract, and each solvent fraction as well, dissolved 5mL distilled water was added aqueous NaOH (20%). Formation of Yellow colour showed the positive test for Glycosides.

2.4.5 Flavonoids

General Test: To 0.2g of the extract were added 5 ml of ethanol and 3 drops of FeCl₃. A dark green colour indicated the presence of flavonoids.

Shinoda Test: To 5mg of extract was added 1-2 magnesium turnings and 1-2 drops of concentrated HCl was added. Formation of a pink colour testified the presence of flavonoids.

2.4.6 Anthocyanins

To 0.2g of extract was added 2 mL of distilled water and warmed for 5 minutes and allowed to cool. Then 2mL of 20% HCl were added and observed for colouration. On addition of 2 mL NH₄OH solution, the pinkish red colouration which turned to bluish violet is indicative of the presence of anthocyanins.

2.4.7 Coumarins

To 0.02g of extract was added 2 mL of distilled water and warmed for 5 minutes and allowed to cool. Then, 2mL of 10% NaOH was added to the aqueous extract. The formation of yellow color indicated the presence of coumarins.

2.4.8 Phenols/Tannins

Ferric Chloride Test: Crude extract 0.5g was added to 20 mL distilled water by boiling for 10 minutes and filtered while hot. It was then allowed to cool and 1 mL FeCl₃ reagent (5%) added to the filtrate. An intense coloration ranging from Blue-Black, Green or Blue-Green indicated the presence of phenols and tannins.

Lead acetate Test: To 10mg of extract is added 1 mL of 1% Lead acetate solution. The formation of precipitate indicated the presence of tannins and phenolic compounds.

2.4.9 Saponins

Foam Test: Extract (1mg) is diluted with 20 mL distilled water. This is well shaken in a graduated cylinder for 10 minutes. The formation of foam to a length of 1mL is indicative of the presence of saponins.

2.5 Evaluation of Antimicrobial activity

2.5.1 Culture media and microorganisms

Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Blood Agar Base (BA Base) and Manitol

Salt Agar Base (MSA) manufactured by Himedia Laboratories (Mumbai, India) were used for bacterial cultivation.

Following the manufacturer's guidelines with some modification, all bacterial media preparation and inoculum standardization were accordingly performed [15].

The test bacterial strains were clinical isolates and were kindly provided by the Department of clinical research and training, University Teaching Hospital (CHUB), Butare, Rwanda. These were *Staphylococcus aureus* and *Streptococcus pyogenes* (Gram positive) and *E. coli*, *Salmonella* sp, *Klebsiella pneumonia*, *Acinetobacter* sp and *Proteus morganis* (Gram negative). The standard antibiotic discs used as positive controls were Erythromycin (15µg/Disc) for *Staphylococcus aureus*, Ampicillin (10µg/Disc) for *Streptococcus pyogenes*, Ciprofloxacin (5µg/Disc) for *E. coli*, *Salmonella* sp, *Klebsiella pneumonia*, *Proteus morganis* and *Acinetobacter* sp. The standards discs were manufactured by BioLab Inc., Budapest, Hungary.

2.5.2 Sterility proofing of the extracts

The method by Mounyr [16] was modified to check the sterility of the extracts. About 2mL of the extract were put into 10 ml of Muller Hinton broth then incubated at 37°C for 24 hours. The clearness or absence of turbidity of the broth after incubation signified that the extracts were sterile.

2.5.3 Susceptibility testing and determination of zone of inhibition

Agar disc diffusion method was used for susceptibility testing and determining zone of inhibition of plant extracts against test microorganisms [17]. Three different concentrations (150 mg/mL, 300 mg/mL, and 600 mg/mL) for each extracts (RBME and SBME) were prepared by dissolving 150 mg, 300 mg, and 600 mg of extract with 1 mL of their respective 20% dimethyl sulfoxide DMSO [18].

Thirty microlitres (30 µL) of each of three final concentrations for each extract was then used to impregnate the sterilized 6 mm blank discs (Whatman N^o 1 filter paper). Dimethyl sulfoxide-loaded discs were used as negative controls for both extracts RBME and SBME accordingly. All impregnated discs were ensured to be fully dried at room temperature in a bio-safety cabinet hood for a time prior to the application on bacterial lawn. The impregnated discs with plant extracts were manually placed on the completely dried inoculated Agar by using sterile forceps. The discs were pressed gently to ensure uniform contact with agar surface. Furthermore, No more than nine disks (including negative and positive control discs) were placed on a 100 mm Petri-plate for ensuring about equidistance to each other to avoid overlapping of inhibition zone [17, 19].

Then, the Petri-plates were inverted and placed undisturbed in a bio-safety cabinet at room temperature for 2 hours. Finally, they were incubated for 24 hours at $35\pm 2^{\circ}\text{C}$ [16].

The inhibition zone's diameter around either the treated discs or the control discs was measured for the sensitivity of the investigated bacteria to the plant crude extracts. Their diameters were measured to the nearest whole millimeter as judged by a naked eye using a ruler. All tests were carried out three times to ensure the reliability, and the average of the three replicates for each extract and control discs were calculated [19].

Hence, complete zone of inhibition was measured in millimeter for extracts and antibiotic standard discs. The mean zone of inhibition and standard error of the mean (Mean \pm SEM) was calculated for methanol extracts and standard discs as well.

The scale of measurement was (disc diameter included): ≥ 20 mm: zone of inhibition is strongly inhibitory; $< 20-7$ mm: zone of inhibition is moderately/mildly inhibitory; and < 7 mm means no inhibitory [20].

2.5.4 Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined by Broth Macro-Dilution Method [16, 21]. The stock concentration 600mg/mL was prepared by weighing 3600 mg of extract (RBME and SBME) and dissolving in 6 mL of 20% DMSO. Two-fold serial dilution procedure was performed by transferring 1mL of extract concentration from stock into three 1st tubes of the three rows of ten tubes each already containing 1mL of sterilized liquid growth medium. Two-fold serial dilutions were set up with 300, 150, 75, 37.5, 18.75, 9.375, 4.688, 2.344, 1.172 and 0.586 mg/mL in descending order from column number 1 (1st column) to column number 10 (10th column) of the three rows. 1 mL of the standardized bacterial suspension was added to each tube resulting in recommended final cell count of about 5×10^5 CFU/mL. Three additional tubes each one at the end of each row were used as: the first for sterility proofing of extract (tube containing growth medium and Extract), the second for Negative Control (tube containing growth medium, Solvent and particular bacterial isolate suspension) and the third for Positive Bacterial Growth Control (tube containing growth medium and particular bacterial isolate suspension). All the tubes were

incubated overnight (for 24 hours) at 37°C in an incubator (Binder BF 260 GmH, Tuttingen, Germany).

On the next day, by comparing with the tube of Positive Bacterial Growth Control, the lowest concentration of extract dilution showing no macroscopical growth (absence of turbidity) of a test microorganism was recorded as the MIC value of the extract. Absence of turbidity in sterility control tube indicated the absence of contaminants in extracts. Negative control tube showed that the solvent had no impact on the tested organism growth. The tests were performed in triplicate in aseptic conditions for each test organism.

2.5.5 Determination of Minimum Bactericidal Concentration (MBC)

MBC of the plant extracts on the tested microorganisms was performed according to method highlighted in Clinical Laboratory Standards Institute's document M26-A [15] and research work by Mounyr [16]. Briefly, a loop full of samples from all test tubes that showed no visible growth of bacteria in MIC assay was sub-cultured into antibiotic free agar medium in incubator at 37°C for 18-24 hours. After incubation period, the least concentration of the plant extract which killed 99.9% of the colony formation was recorded as MBC value of the extract. Triplicate tests were accordingly carried out in aseptic conditions [16].

3. RESULTS AND DISCUSSIONS

3.1 Chemical screening

Preliminary chemical screening of crude extracts (RBME and SBME) of *U. massaica* revealed the presence of different phytochemical compounds that are summarized in table 1.

RBME contained Alkaloids (high concentration), Coumarins (high concentration), Saponins (moderate concentration), Anthocyanins (moderate concentration), Terpenoids (low concentration), Phenols and Tannins (low concentration), Flavonoids (low concentration). Phytosterols, Steroids and Glycosides are absent in RBME.

In SBME, phytochemical components like Saponins, Phenols and Tannins, Flavonoids, Phytosterols and Steroids were present in high concentration. Those in moderate concentration were Glycosides and Terpenoids while Coumarins and Alkaloids were present in low concentration. Only Anthocyanins were absent in SBME.

Table-1: Phytochemical constituents in crude extracts of *Urtica massaica* (RBME and SBME)

Phytochemical components	Chemical screening tests	Positive confirmation sign test	RBME	SBME
Alkaloids	Dragendorf's test	Reddish-brown precipitate	+++	+
	Tannic acid test	Brownish precipitate	+++	+
	Wagner's test	Yellow precipitate	+++	+
Saponins	Foam or Froth test	Persistent foam	++	+++
	Haemolysis test	Haemolysis of red blood cells	++	+++
Sterols	Salkowski test	Red color in Chloroform layer	-----	+++
Steroids	Liebarman-Buchard test	Deep green color	-----	+++
Terpenoids	Acetic Anhydride + Conc.H ₂ SO ₄	Deep red color	+	++
Phenol/Tannins	Ferric Chloride (5% FeCl ₃) test	Blue-black, green or blue-green	+	++
	Lead acetate test	White precipitate	+	+++
Flavonoids				
	Shinoda test	Pink color	+	+++
Anthocyanins	Warmed & cooled Aqueous solution + 20% HCl + NH ₄ OH	Pinkish red to Bluish red	++	-----
Coumarins	Warmed & cooled Aqueous solution + 10% NaOH solution	Yellow color formation	+++	+
Glycosides	Reaction of Aqueous solution with 20% NaOH solution	Yellow color formation	-----	++

RBME and SBME: Root Bark and Stem Bark Methanol Extracts respectively

+++ : Present in high concentration, ++ : Present in moderate concentration, + : Present in low concentration and --- : Absent

Results of preliminary chemical screening revealed, in general, that stem bark methanol extract (SBME) is richer in bioactive phytochemical compounds than root bark methanol extract (RBME) as shown in Table 1. Ghosh [22] reported that Alkaloids, Saponins, Phenols, Tannins, Terpenoids and Flavonoids in general are known to have activities against pathogens and therefore aid the antimicrobial activities of medicinal plants through various mechanisms. Yenjai [23] reported Flavonoids as exerting many biological effects including antimicrobial activity.

3.2 Antibacterial screening

Results of antibacterial screening of the stem and root bark extracts of *Urtica massaica* were measured in terms of zones of inhibition, MIC and MBC. Among the tested bacteria, only three of them (two gram-negative: *Escherichia coli*, *Salmonella* sp, and one gram-positive: *Staphylococcus aureus*) were significantly sensitive to the plant extracts (RBME and SBME) with inhibition zone values greater than 7mm as described by Clinical Laboratory Standards Institute [15]. Osuntokun and co-workers reported that the resistance of Gram-negative bacteria towards antibiotic agents might relatively be caused by the hydrophilic surface of their outer membrane rich in lipopolysaccharides molecules, posing barrier to the penetration of numerous antibiotic compounds [24, 25-26].

This study revealed that the stem and root bark extracts, at different concentrations, exhibited various antibacterial activities against the susceptible tested strains (Table 2 and 3). Against *E. coli*, SBME at concentrations 150, 300 and 600 mg/mL exhibited higher inhibitory effect with average inhibition zones ranging from 21.667±1.529 to 28.000±2.851 mm while RBME at similar concentrations showed lower inhibitory ability with average inhibition zones ranging from 19.667±1.734 to 25.667±1.023 mm. In the case of *Salmonella* sp, SBME at 150 mg/mL concentration showed no inhibition while at concentrations 300 and 600 mg/mL it exhibited inhibitory effect with 7.167±0.208, and 24.067±2.503 mm respectively. Moreover, *Salmonella* sp manifested its resistance to RBME at all investigated concentrations. The positive control, Ciprofloxacin (5µg/Disc), inhibited the growth of *Salmonella* sp and *E. coli* with 30.000±0.000 mm zone of inhibition (Table 1).

In this study the standard antibiotic disc, Erythromycin (5µg/Disc), inhibited the growth of *S. aureus* in solid growth medium with 29.667±0.334 mm zone of inhibition. However, SBME, at concentrations 150, 300 and 600 mg/mL, showed inhibitory effect against the growth of *S. aureus* with zones of inhibition ranging from 17.333±1.335 to 21.667±0.334 mm respectively (Table 1). In the same vein, RBME exhibited lower inhibition against *S. aureus* with range

of inhibition zones from 15.000 ± 0.578 to 20.667 ± 0.334 mm (Table 1). The study showed that *U. massaica* stem bark (SBME) has greater antibacterial potential than root bark (RBME). This might be attributed to higher concentration of bioactive secondary metabolites present in the stem bark methanol extract (SBME).

According to American Society for Microbiology [17] and a research study carried out at University Sidi Mohamed, Morocco by Mounyr [16], only bacterial strains inhibited, in agar disk diffusion assay, by the plant extracts with inhibition zone greater or equal to 7 mm were further investigated for the determination of concentrations, MIC and MBC (mg/mL) as shown in Table 2 and 3 respectively.

The more sensitive/susceptible the bacterium is; the lower is the concentration of the extract required for inhibiting the growth of the test microbial organisms [16]. MIC values of SBME (Table 2) were 6.250 ± 1.562 mg/mL against *E. coli*, 10.156 ± 4.752 mg/mL against *Salmonella sp* and 6.250 ± 1.563 mg/mL against *S. aureus*. Although, RBME was found inactive against *Salmonella sp* as in Agar disc diffusion method, its MIC values obtained were 7.813 ± 1.563 mg/mL against *E. coli* and 7.813 ± 1.563 mg/mL *S. aureus* (Table 3). Furthermore, MBC values obtained were 12.500 ± 3.125 mg/mL for SBME against *E. coli*, 19.531 ± 10.156 mg/mL for SBME against *Salmonella spp* and 12.500 ± 3.125 mg/mL for SBME against *S. aureus*. But RBME exhibited the same MBC value, 15.625 ± 3.125

mg/mL, against both *E. coli* and *S. aureus* while it proved ineffective against *Salmonella sp* as it was similarly found in Broth Macro-Dilution Method for MIC investigation (Table 3).

In this study, MIC and MBC values of RBME were lower than those of SBME against all sensitive tested micro-organisms. This might be attributed to the higher content of secondary metabolites present in SBME than in RBME. It is believed that bioactive compounds contributed to the observed antimicrobial activity of the selected medicinal plant parts because Mbata [27] stated that tannins and phenols possess antibacterial activities.

The findings indicated greater antimicrobial activities of stem bark than root bark of the studied plant, *Urtica massaica*. These findings are consistent with the findings of Dorota [28] who found that for *Urtica* plants, (*U. plaviflora* and *U. dioica*), the stem barks in *n*-butanol and methanol showed greater antimicrobial activities than the root barks.

This study revealed that the values of inhibition zones of plant extracts against susceptible tested strains were inversely proportional to their MIC and MBC values. This suggests the reproducibility of the experiments and the consistent potency of antimicrobial activities against the susceptible test bacteria.

Table-2: Antimicrobial activities (in terms of Inhibition zone values in mm) of *U. massaica* extracts against tested bacteria (by Agar disc diffusion method)

Bacteria	Extract	Zone of inhibition (mm)			+control
		at 150 mg/ml	at 300 mg/ml	at 600 mg/ml	
<i>Escherichia coli</i>	SBME	21.667 ± 1.529	23.000 ± 1.203	28.000 ± 2.851	30.000 ± 0.000
	RBME	19.667 ± 1.734	23.667 ± 2.030	25.667 ± 1.023	
	Ciprofloxacin (5µg/Disc)	-	-	-	
<i>Salmonella sp</i>	SBME	N S	7.167 ± 0.208	24.067 ± 2.503	30.000 ± 0.000
	RBME	N S	N S	N S	
	Ciprofloxacin (5µg/Disc)	-	-	-	
<i>S. aureus</i>	SBME	17.333 ± 1.335	19.000 ± 0.578	21.667 ± 0.334	29.667 ± 0.334
	RBME	15.000 ± 0.578	17.000 ± 0.578	20.667 ± 0.334	
	Erythromycin (15µg/Disc)	-	-	-	

Each value is the average of 3 replicates \pm SEM (n=3). N S: Not sensitive to plant extracts (RBME and SBME) where RBME and SBME: Root Bark and Stem

Bark Methanol Extracts respectively. +Control: Positive control (Ciprofloxacin 5µg/Disc and Erythromycin 15µg/Disc).

Table-3: Antimicrobial activities (MIC and MBC in mg/mL) of *U. massaica* extracts against tested bacteria (using Broth Macro-Dilution method and Sub-culturing method respectively)

Test Bacterial organisms				
Plant Extracts	Concentration of extracts	<i>E. coli</i>	<i>Salmonella sp</i>	<i>S. aureus</i>
SBME	MIC (mg/mL)	6.250±1.562	10.156±4.752	6.250±1.563
	MBC (mg/mL)	12.500±3.125	19.531±10.156	12.500±3.125
RMBE	MIC (mg/mL)	7.813±1.563	N S	7.813±1.563
	MBC (mg/mL)	15.625±3.125	N S	15.625±3.125

Each value is the average of 3 replicates±SEM (Mean±SEM). N S: Not sensitive to plant extracts (RBME and SBME) where RBME and SBME: Root Bark and Stem Bark Methanol Extracts respectively.

4. CONCLUSION

It is concluded from this study that *Urtica massaica* plays an important role in the treatment of different microbial infectious diseases in Rwanda and this is because the chemical compositions of its parts used are active against microorganisms that cause diseases. Our findings relatively provide a scientific support for the traditional use of *Urtica massaica* stem and root barks as treatment for ailments caused by bacterial pathogens.

Further bioactivity guided studies and in vitro/in vivo bioassays of antimicrobial activity along with toxicity studies are needed for fractionation, isolation and characterization of bioactive compounds present in both stem and roots of this Rwandan *Urtica massaica* before it is used for commercialization in the form of pharmaceutical medicine. However, more solvents should be used to extract the plant prior to determining other compositions and concentration of chemical components of the plant, and more tested organisms should be used for the antimicrobial activities.

ACKNOWLEDGEMENT

This study was funded by the grants from Pan African University Life and Earth Sciences/University of Ibadan, Nigeria. The authors wish to express their appreciations to all the technical staff of both the Department of Pathology/Bacteriology Laboratory/Clinical Training and Research at Centre Hospitalier Universitaire de Butare (CHUB) and Department of Pharmacy, College of Medicine and Health Sciences, University of Rwanda, Rwanda.

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Cite This Article: Maniriho O *et al* (2021). Chemical Screening and Antimicrobial Activities of Rwandan traditional medicinal plant, *Urtica massaica* Mildbr (Urticaceae). *EAS J Pharm Pharmacol*, 3(2), 56-63.