

Original Research

Assessment of Antimicrobial Activity of Ethanolic and Aqueous Extracts of *Aesculus hippocastanum* L. (Horse Chestnut) Bark against Bacteria Isolated from Urine of Patients Diagnosed Positive to Urinary Tract Infections

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Abstract

The search for new antimicrobials is essential to address the worldwide issue of antibiotic resistance. The present work aimed at assessing the antimicrobial activity of *Aesculus hippocastanum* L. (horse chestnut) bark against bacteria involved in urinary tract infections (UTIs). Bioactive compounds were extracted from *A. hippocastanum* bark using water and ethanol as solvents. The extracts were tested against 10 clinical uropathogenic strains including five Gram-positive and five Gram-negative bacteria. *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 25922 were used as reference bacteria. The susceptibility to antibiotics was assessed using the Kirby Bauer disc diffusion method and the antibacterial activity of the extracts was evaluated using the well diffusion method. The Minimum inhibitory concentration (MIC) and the minimum bactericidal concentrations (MBC) were assessed by the microdilution method. *A. hippocastanum* bark possessed a dry matter content of 65.73%. The aqueous extract (AE) and ethanolic extract (EE) showed a volume yield of 77.77% and 74.07% (v/v), and a mass yields of 13.4% and 24.3% (w/w) respectively. All the bacteria were susceptible to amoxiclav, imipenem and ceftriaxone but the clinical strains were resistant to at least one antibiotic. *Kocuria rizophila* 1542 and *Corynebacterium* spp 1638 were the most resistant bacteria both with multidrug resistance index of 0.45. Except AE on *Proteus Mirabilis* 1543 and *Enterococcus faecalis* 5960 (0 mm), both AE and EE were active against all the microorganisms tested with inhibition diameters (mm) which ranged from 5.5–10.0 for AE and 8.0–14.5 for EE. The MICs of EEs varied from 1–4 mg/mL while those of AEs varied from 4–16 mg/mL. The ethanolic extracts (EE) were overall more active than the aqueous ones. The *A. hippocastanum* bark extracts had overall weak antibacterial activity (MIC ≥ 0.625 mg/mL) and bacteriostatic potential (MBC/MIC ≥ 16) on both Gram-positive and Gram-negative bacteria.

Keywords: *Aesculus hippocastanum* L.; horse chestnut; antimicrobial; uropathogens; urinary tract infections; herbal medicine

1. Introduction

Urinary tract infections (UTIs) are very common infections and occur at least once in a lifetime. These infections are serious public health issues and are responsible for nearly 150 million disease cases every year worldwide [1]. UTIs are defined as any infection, commonly of bacterial origin, which occurs in any part of the urinary system [1]. When UTIs are localized in urethra, they are called urethritis, cystitis (when localized in the bladder), pyelonephritis (infection of the kidneys), and vaginitis (infection of the vagina) [2,3]. UTIs are more likely to occur in women, because, compared to men, their urethra is shorter and there is relative proximity between the urethra and the anus [4]. In addition, the prevalence of UTIs among sexually active young women has been reported to vary from 0.5 to 0.7 person per year, while this incidence rate among young men was only 0.01 [5]. Otherwise, the so-called uropathogenic bacteria is responsible for 80–90% of UTIs

but other germs such as *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Streptococcus*, and *Enterococcus faecalis* are sometimes also involved [6]. The UTIs are easily managed with antibiotics [7] such as trimethoprim-sulfamethoxazole (TMP-SMX), nitrofurantoin, or fosfomycin for 3–5 days [8] and sometimes by cephalosporins (fluoroquinolones; cefixime) and β -lactams (amoxicillin-clavulanate) [9].

However, unfortunately, resistance to antibiotics, which spares no area, has made UTIs more difficult to treat. Several recent studies have reported a considerable increase in antibiotic resistance in uropathogens and an unprecedented number of multidrug-resistant germs worldwide [10,11]. To respond to this dangerously worrying situation, research teams from all over the world are permanently evaluating the use of potential alternatives to antibiotics such as nanoparticles [12], probiotics [13,14], phage



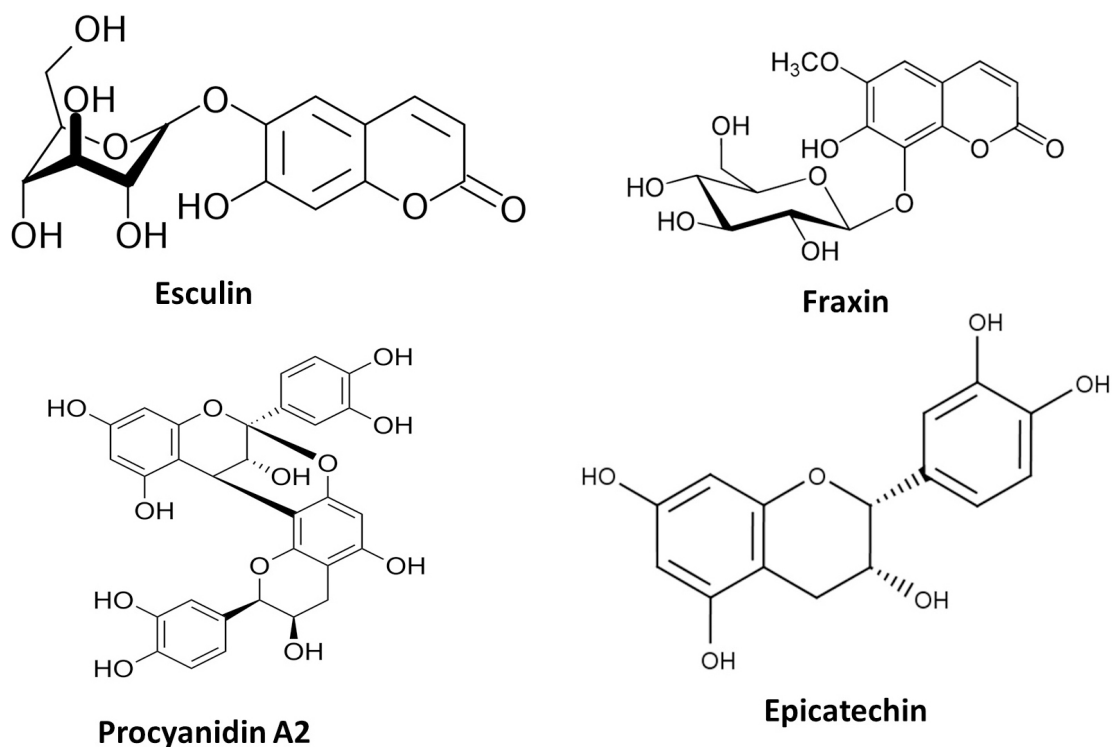


Fig. 1. Structures of the major constituents of the *A. hippocastanum* bark [21].

therapy [15], antimicrobial peptides [16] and medicinal plants [17,18]. Among these alternatives, medicinal plants seem to offer the most credible evidence [19], considering that plants have been used for millennia in the treatment and prevention of various diseases, including bacterial infections and some of these herbal remedies have proven to be effective in preventing and treating UTIs [19]. In this context, certain plants such as *Aesculus hippocastanum* which are very widespread (and therefore available) but scarcely utilized deserve to be investigated for their antimicrobial properties.

A. hippocastanum, commonly known as horse chestnut and buckeyes, is a rapidly growing ornamental tree belonging to the family Hippocastanaceae [20]. Trees of this plant are grown up to 30 m in height and 1 m thick with short stem having a rounded crown. *A. hippocastanum* is highly adapted in polluted environment and is commonly used for the treatment and prevention of many diseases [20]. Its composition rich in esculin (over 17%), fraxin (over 7%) and proanthocyanidins (over 6% of epicatechin and over 5% of procyanidin A2) (Fig. 1, Ref. [21]) gives it anti-inflammatory, vasoprotective, antidiabetic, anti-inflammatory, gastroprotective, neuroprotective, anticancer, hepatoprotective and antibacterial properties [20,21].

Thus, the aim of this work was to assess the antibacterial properties of ethanolic and aqueous *A. hippocastanum* bark extract against some bacteria involved in urinary tract infections.

2. Materials and Methods

2.1 Vegetal Material

The plant material used in this study was the bark of *Aesculus hippocastanum*. The plant was collected in June 2021 near the Medical Institute of the Peoples' Friendship University of Russia (MG23 + 9H Obrouchevski, Moscow, Russia). After harvest, the plant was taken directly to the laboratory where it was dried at 37 °C until the weight was constant. The dry matter content was calculated; the plant was grinded and the powders with particle sizes lower than 1 mm were stored in a sterile airtight container until further use.

2.2 Bacterial Strains

The microorganisms used for the screening of antimicrobial activity consisted of five Gram positive bacteria and 5 Gram negative. The five Gram + were *Kocuria rhizophila* 1542, *Enterococcus avium* 1669, *Staphylococcus simulans* 5882, *Corynebacterium spp* 1638 and *Enterococcus faecalis* 5960 while the five Gram—were *Proteus mirabilis* 1543, *Morganella morganii* 543, *Citrobacter freundii* 426, *Acinetobacter baumannii* 5841 and *Achromobacter xylosoxidans* 4892. *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 25922 were used as standard Gram + and Gram - respectively. All strains were provided by the Department of Microbiology and Virology of the Peoples' Friendship University of Russia.

Table 1. Interpretation criteria for antibiotic sensitivity [10].

		Antibiotics/limits of inhibition diameters (mm)										
		CIP	CAZ	AMC	CTR	TR	TE	NIT	AMP	IMP	CAC	FO
Interpretation	R	d ≤15	d ≤14	d ≤13	d ≤13	d ≤13	d ≤14	d ≤13	d ≤13	d ≤13	d ≤14	d ≤12
	I	16–20	15–17	14–17	14–20	14–15	15–18	14–17	14–16	14–15	15–17	13–15
	S	d ≥21	d ≥18	d ≥18	d ≥21	d ≥16	d ≥19	d ≥18	d ≥17	d ≥16	d ≥18	d ≥17

AMC, Amoxycillin; AMP, Ampicillin; CZ, Cefazolin; CAC, Cefazolin/clavulanic acid; CAZ, Ceftazidime; CTR, Ceftriaxone; CIP, Ciprofloxacin; FO, Fosfomycin; IMP, Imipenem; NIT, Nitrofurantoin; TE, Tetracyclin; TR, Trimethoprim.

2.3 Chemicals and Media

Dimethyl sulfoxide (DMSO) was purchased from BDH Laboratories, VWR International Ltd., USA. We also used BHIB (Brain Heart Infusion Broth) (HiMedia™ Laboratories Pvt. Ltd., India), Muller Hinton Agar (MHA HiMedia™ Laboratories Pvt. Ltd., India), Sabouraud Dextrose Broth (SDB, HiMedia™ Laboratories Pvt. Ltd., India) and all other reagents and chemicals used were of analytical grade.

2.4 Extraction of Active Compounds

Ethanol solution (80%, v/v) and distilled water were used as solvents because they have been reported to be efficient solvents for the extraction of bioactive compounds in the medicinal plants used in this study. As we described in our previous investigation [18] thirty grams (30 g) of vegetal material was weighed and added to 270 mL of the solvent in separate conical flasks. The flasks were covered tightly and were shaken at 200 rpm for 24 h and 25 °C in a shaker incubator (Heidolph Inkubator 1000 coupled with Heidolph Unimax 1010, Germany). The mixtures were then filtered by vacuum filtration, using Whatman filter paper № 1 then concentrated at 40 °C in rotary evaporator (IKA RV8) equipped with a water bath IKA HB10 (IKA Werke, Staufen, Germany) and a vacuum pumping unit IKA MVP10 (IKA Werke, Staufen, Germany). To avoid losses, the extracts were collected when the volumes were small enough and placed in petri dishes previously weighed and then incubated open at 40 °C until complete evaporation. The final dried crude extracts were weighed. Extraction volume and mass yield were determined using the following formulas:

$$\text{Volume yield (\%)} = \frac{\text{Volume of the extract after filtration (ml)}}{\text{Initial solvent volume (ml)}} \times 100$$

$$\text{Mass yield (\%)} = \frac{\text{Mass of extracted plant residues (g)}}{\text{Mass of plant raw sample (g)}} \times 100$$

2.5 Preparation of Antimicrobial Solution

For each plant extract, the crude extract was dissolved in the required volume of DMSO (5%, v/v) to achieve a concentration of 128 mg/mL. The extracts were sterilized by microfiltration (0.22 µm) and the solution obtained was used to prepare the different concentrations used in the analytical process.

2.6 Inoculum Preparation

Bacteria were cultured for 24 h at 37 °C in 10 mL of BHI broth. After incubation, the cells were collected by centrifugation (7000 g, 4 °C, 10 min), washed twice with sterile saline, resuspended in 5 mL of sterile saline to achieve a concentration equivalent to McFarland 0.5 using DEN-1 McFarland Densitometer (Grant-bio).

2.7 Evaluation of Susceptibility of the Test Bacteria to Antibiotics

The modified Kirby–Bauer's disk method described in our previous study [22] was used to study the antibiotic sensitivity of the bacterial strains, and the following eight antibiotics disks were used: amoxicillin, 30 µg/disk; ampicillin, 25 µg/disk; cefazolin, 30 µg/disk; cefazolin/clavulanic acid, 30/10 µg/disk; 30 µg/disk; ceftriaxone, 30 µg/disk; ciprofloxacin, 30 µg/disk; Fosfomycin, 200 µg/disk; imipenem (IMP), 10 µg/disk; nitrofurantoin, 200 µg/disk; tetracyclin (TE), 30 µg/disk and trimethoprim, 30 µg/disk. The inhibition diameters were measured and interpreted then referred to the Clinical & Laboratory Standards Institute [23]. Resistance R, Intermediate I, and Sensitive S interpretations were obtained automatically using algorithms written in Excel software [Microsoft Office 2016 MSO version 16.0.13628.20128 (32 bits), USA] with the parameters described in Table 1 [10].

2.8 Screening of Antibacterial Activity

2.8.1 Assessment of Antimicrobial Activity Using Well Diffusion Method

The well diffusion method previously described [18] was used to assess the antimicrobial activity of the extracts. Briefly, 15 mL of sterile Muller Hinton Agar (for bacteria) was poured into petri dishes and 100 µL of each microorganism were spread. Wells with a capacity of 20 µL were drilled on the culture medium and 20 µL (at 128 mg/mL) of

each plant material was added. The sterile DMSO 5% used to prepare the extracts was used as a negative control and all the trials were done in triplicate. After incubation at 37 °C for 24 h, the inhibition diameters were measured.

2.8.2 Determination of Minimum Inhibitory Concentrations (MIC)

MIC is the lowest concentration of antibacterial agent that completely inhibits the visible bacterial growth. The MIC of the extracts was determined using the microbroth dilution method as previously described without any modification [24]. Briefly, 100 μ L of broth (BHIIB) was added to all the wells of sterile U-bottom 96-well microplates and extract preparations (128 mg/mL) were subjected to serial twofold dilution. Each column represented one type of extract and a single strain. DMSO 5% was used as a negative control. For each test well, 10 μ L of the respective inoculum was added (with turbidity equivalent to a 0.5 McFarland scale). Finally, the plates were covered and incubated at 37 °C for 24 h and after incubation, MIC was considered the lowest concentration of the tested material that inhibited the visible growth of the bacteria.

2.8.3 Determination of Minimum Bactericidal Concentration (MBC)

MBCs were determined by subculturing the wells without visible growth (with concentrations \geq MIC) on MHA plates. Inoculated agar plates were incubated at 37 °C for 48 h and MBC was considered the lowest concentration that did not yield any microbial growth on agar.

2.8.4 Tolerance Level

Tolerance level of tested bacterial strains against aqueous and ethanolic extract was determined using the following formula:

$$\text{Tolerance} = \text{MBC/MIC}$$

The characteristic of the antibacterial activity of extracts was determined by the tolerance level indicating the bactericidal or bacteriostatic action against the tested strains. When the ratio of MBC/MIC is ≥ 16 , the antibacterial efficacy of the test agent is considered as bacteriostatic, whereas MBC/MIC ≤ 4 indicates bactericidal activity [25].

3. Results and Discussion

3.1 Dry Matter and Extraction Yield

The dry matter, water content, ethanolic (EE) and distilled water extract (AE) yields of *Aesculus hippocastanum* bark are presented in Table 2. We found that *A. hippocastanum* bark possessed a dry matter content of 65.73%. Dry matter is an indicator of the amount of constituents (excluding water) in the sample tested. The drying allowed the removal of water from the plant in order to standardize the extraction and to make this work reproducible if authors from other regions project to work with the same plant. Furthermore, we obtained an extraction volume yield of 74.07%

with distilled water as the solvent and 77.77% with ethanol. The difference in extraction volume yields can be explained by losses during the extraction process. Indeed, as reported by [18] we noticed that the filtration of ethanolic extracts was faster (less than 5 minutes for 300 mL) compared to the aqueous extract which took much longer (more than 50 minutes on average for 300 mL) and required in average 6 filter changes. Despite the filtration, the EAs still looked cloudy while the EEs were completely clear. This residue cloudiness could therefore explain the higher mass yield in AE (24.3%) compared to EE (13.4%). Several authors [17,18,26,27] reported observations similar to our findings while others [28,29] found opposite results. Therefore, as Arsene *et al.* [18] pointed out in their previous work, the extraction performance depends on several factors, including the method used, extraction time, the solvents, and the equipment used. In addition, Mouafo *et al.* [17] reported that the high yields of phytoconstituent does not necessarily imply better antimicrobial activity and this was further assessed in the present work by evaluating the antimicrobial activity of each of the extracts using the well diffusion method and the determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC).

Table 2. Dry matter, water content, volume and mass extraction yield of *A. hippocastanum* bark.

	Aqueous extract	Ethanolic extract
Volume yield (%)	74.07	77.77
Mass yield (%)	24.3	13.4

Dry matter of the fresh bark of *A. hippocastanum* = 65.73%
(Water content = 34.27%).

3.2 Susceptibility of the Tested Clinical Strains to Antibiotics

The susceptibility of uropathogenic tests to eleven (11) antibiotics was evaluated by the Kirby Bauer disc method, the Multidrug resistance Index (MDR) was calculated, and the results were reported in Table 3. As shown in Table 3, no bacteria were resistant to amoxiclav, imipenem and ceftriaxone while 7/12 were resistant to ampicillin, 6/12 to trimethoprim, 5/12 to tetracycline and ceftazidime/clavulanic acid, 4/12 to ceftazidime, 3/12 to fosfomycin, 2/12 to nitrofurantoin and 1/12 to ciprofloxacin. Interestingly, both standard bacteria (*E. coli* ATCC 25922 and *S. aureus* ATCC 6538) were sensitive to all antibiotics while the clinical strains were resistant to at least one antibiotic. Among clinical strains, MDRs ranged from 0.09 to 0.45. *K. rizophilia* 1542 and *Corynebacterium* spp 1638 were the most resistant bacteria with MDRs of 0.45 each. The results observed with these clinical strains are similar to those reported in other studies [10,11]. While

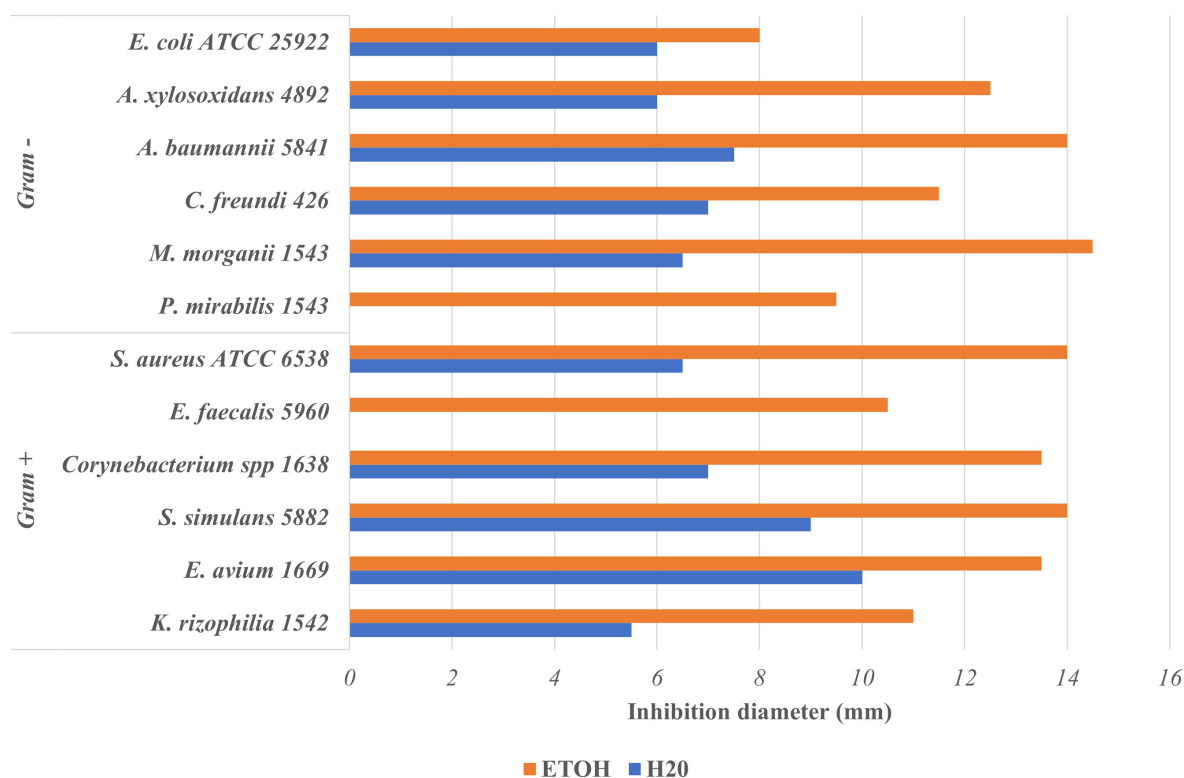


Fig. 2. Inhibition diameter of ethanolic and aqueous *Aesculus hippocastanum* bark extract on tested uropathogenic bacteria.

the strongest resistance was observed on ampicillin (which is an antibiotic from the β -lactams family, from the group A of the penicillins), no resistance was observed in amoxiclav which is a derivative of amoxicillin (antibiotic of the beta-lactam family, of the aminopenicillin group) of the same family, with the only difference that the latter is combined with clavulanic acid (β -lactamase inhibitor). This shows that antibiotic adjuvants can also play an important role in combating resistant germs [30]. Furthermore, similarly to our findings, other studies have reported low resistance of uropathogens to imipenem and ceftriaxone [11,31]. In addition, the sensitivity of all strains to amoxiclav, imipenem and ceftriaxone demonstrates that these 3 antibiotics are effective on a wide range of bacteria, including clinical strains with high resistance to other antibiotics. Therefore, these antibiotics which have kept a good activity against various microorganisms should be carefully used and administered only under prescription and after a prior antibiogram.

3.3 Inhibition Zone of the Extracts Against the Tested Bacteria

Fig. 2 presents the inhibition diameters of ethanolic (EE) and aqueous (AE) extract of *Aesculus hippocastanum* bark on the tested microorganisms. Except AE on *Proteus Mirabilis* 1543 and *Enterococcus faecalis* 5960 (0 mm), both AE and EE were active on all microorganisms tested with inhibition diameters (mm) which ranged from 5.5–10.0 for AE and 8.0–14.5 for EE.

The ethanolic extracts (EE) were overall more ac-

tive than the aqueous ones. Consequently, this means that ethanol extracts more compounds with antimicrobial properties compared to water although we found above that the extraction yields with water was higher than that with ethanol. Several authors have reported that compounds with antimicrobial activity such as flavonoids, polyphenols, tannins and alkaloids are generally insoluble in water but soluble in ethanol [32,33]. Other authors such as Arsene *et al.* [18], Mouafo *et al.* [17] and Evbuomwan *et al.* [34] also pointed out that ethanol extracts more antimicrobial compounds from plant materials as opposed to water. In addition, we found that extracts of *A. hippocastanum* bark were both active against Gram + bacteria as against Gram - bacteria. To our knowledge, the antibacterial properties of this plant have not yet been investigated but our findings suggest that *A. hippocastanum* bark has constituents exhibiting a broad-spectrum antimicrobial.

Most of the research conducted on *A. hippocastanum* focused on its properties to regulate circulatory system imbalances and relieve attacks of hemorrhoids [19]. In a study conducted by Owczarek *et al.* [19] on the composition of *A. hippocastanum* bark, it has been reported that this plant presents mainly two groups of compounds: coumarins and proanthocyanidins. Among the coumarins there was mainly esculin (over 17%) and fraxin (over 7%) while among the proanthocyanidins, the authors found epicatechin (over 6%) and procyanidin A2 (over 5%) (Fig. 1) [19]. Owczarek *et al.* [19] finally concluded that, in total, about 40% of the *A. hippocastanum* bark extract could be attributed to simple

Table 3. Susceptibility to antibiotics of the test uropathogenic bacteria.

		NIT	TE	CTR	AMC	FO	CAZ	IPM	CAC	CIP	AMP	TR	MDR
Gram +	<i>K. rizophilia</i> 1542	10 ± 0 (R)	13 ± 0 (R)	22 ± 0 (S)	22 ± 1 (S)	28 ± 2 (S)	10 ± 0 (R)	23 ± 1 (S)	6 ± 0 (R)	30 ± 1 (S)	13 ± 1 (R)	21 ± 2 (S)	0.45
	<i>E. avium</i> 1669	21 ± 1 (S)	6 ± 0 (R)	30 ± 4 (S)	25 ± 3 (S)	31 ± 3 (S)	23 ± 1 (S)	27 ± 4 (S)	24 ± 2 (S)	15 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	0.36
	<i>S. simulans</i> 5882	23 ± 1 (S)	20 ± 2 (S)	19 ± 1 (S)	27 ± 3 (S)	38 ± 4 (S)	11 ± 0 (R)	34 ± 3 (S)	11 ± 0 (R)	26 ± 2 (S)	6 ± 0 (R)	20 ± 2 (S)	0.27
	<i>Corynebacterium spp</i> 1638	20 ± 0 (S)	32 ± 4 (S)	32 ± 5 (S)	36 ± 2 (S)	14 ± 1 (R)	6 ± 0 (R)	27 ± 2 (S)	6 ± 0 (R)	26 ± 1 (S)	9 ± 0 (R)	10 ± 0 (R)	0.45
	<i>E. faecalis</i> 5960	21 ± 1 (S)	11 ± 0 (R)	22 ± 1 (S)	27 ± 1 (S)	31 ± 3 (S)	17 ± 1 (I)	24 ± 2 (S)	6 ± 0 (R)	32 ± 3 (S)	6 ± 0 (R)	6 ± 0 (R)	0.36
	<i>S. aureus</i> ATCC 6538	22 ± 1 (S)	32 ± 3 (S)	30 ± 2 (S)	35 ± 4 (S)	38 ± 2 (S)	25 ± 1 (S)	28 ± 3 (S)	25 ± 2 (S)	32 ± 0 (S)	20 ± 1 (S)	31 ± 2 (S)	0.00
Gram -	<i>P. mirabilis</i> 1543	19 ± 1 (S)	6 ± 0 (R)	30 ± 4 (S)	25 ± 2 (S)	31 ± 0 (S)	23 ± 2 (S)	27 ± 1 (S)	24 ± 1 (S)	30 ± 2 (S)	16 ± 1 (I)	6 ± 0 (R)	0.18
	<i>M. morganii</i> 1543	15 ± 0 (I)	6 ± 0 (R)	33 ± 2 (S)	17 ± 1 (I)	13 ± 0 (R)	23 ± 1 (S)	22 ± 0 (S)	23 ± 1 (S)	22 ± 2 (S)	10 ± 0 (R)	6 ± 0 (R)	0.36
	<i>C. freundii</i> 426	21 ± 1 (S)	30 ± 3 (S)	27 ± 2 (S)	35 ± 1 (S)	40 ± 2 (S)	12 ± 0 (R)	37 ± 1 (S)	10 ± 0 (R)	30 ± 2 (S)	6 ± 0 (R)	22 ± 2 (S)	0.27
	<i>A. baumannii</i> 5841	26 ± 2 (S)	16 ± 0 (I)	27 ± 3 (S)	35 ± 1 (S)	32 ± 3 (S)	19 ± 2 (S)	34 ± 2 (S)	17 ± 1 (I)	30 ± 1 (S)	21 ± 2 (S)	14 ± 0 (R)	0.09
	<i>A. xylosoxidans</i> 4892	6 ± 0 (R)	11 ± 0 (R)	23 ± 2 (S)	36 ± 4 (S)	6 ± 0 (R)	16 ± 0 (I)	32 ± 3 (S)	16 ± 1 (I)	20 ± 2 (I)	20 ± 1 (S)	6 ± 0 (R)	0.36
	<i>E. coli</i> ATCC 25922	21 ± 3 (S)	30 ± 2 (S)	24 ± 1 (S)	20 ± 1 (S)	40 ± 4 (S)	18 ± 1 (I)	32 ± 2 (S)	16 ± 1 (I)	24 ± 0 (S)	26 ± 0 (S)	24 ± 0 (S)	0.00

AMC, Amoxycillin; AMP, Ampicillin; CZ, Cefazolin; CAC, Cefazolin/clavulanic acid; CAZ, Ceftazidime; CTR, Ceftriaxone; CIP, Ciprofloxacin; FO, Fosfomycin; IMP, Imipenem; NIT, Nitrofurantoin; TE, Tetracycline; TR, Trimethoprim; R, Resistant; I, Intermediate; S, Sensitive.

Table 4. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of ethanolic and aqueous *Aesculus hippocastanum* bark extract on tested uropathogenic bacteria.

		Aqueous extract			Ethanolic extract		
		MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
		(mg/mL)	(mg/mL)		(mg/mL)	(mg/mL)	
Gram +	<i>K. rizophilia</i> 1542	16	>64	-	4	>64	-
	<i>E. avium</i> 1669	4	>64	-	2	64	32
	<i>S. simulans</i> 5882	16	>64	-	2	16	8
	<i>Corynebacterium spp</i> 1638	64	64	1	4	32	8
	<i>E. faecalis</i> 5960	4	64	16	2	64	32
	<i>S. aureus</i> ATCC 6538	16	64	4	2	16	8
Gram -	<i>P. mirabilis</i> 1543	8	>64	-	2	64	32
	<i>M. morganii</i> 1543	2	>64	-	1	64	64
	<i>C. freundii</i> 426	16	64	4	2	32	16
	<i>A. baumannii</i> 5841	4	>64	-	2	64	32
	<i>A. xylosoxidans</i> 4892	4	>64	-	2	64	32
	<i>E. coli</i> ATCC 25922	16	>64	-	2	16	8

phenolics compounds detectable by LC-PDA. Unfortunately, due to limited resources we were unable to assess the composition of our extracts in order to compare with the data in the literature. However, we can hypothesize that the antimicrobial properties of *A. hippocastanum* bark can be attributed to all its components or specifically to esculin [35], to fraxin [36] or proanthocyanidins [37] because these compounds (from other plants) have been reported to have antimicrobial properties. Notwithstanding our findings, further investigations are required to confirm or refute our hypothesis.

3.4 Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations

After evaluating the antibacterial properties of aqueous (AE) and ethanolic (EE) extracts of *Aesculus hippocastanum* bark against the uropathogens tested using the well diffusion, we investigated the minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of the two extracts. MIC and MBC are two very important elements in the search for new antimicrobials and respectively provide the minimum concentrations required to inhibit or kill the microorganism tested. Table 4 presents the MIC, the MBC, and the MBC/MIC ratio of our 2 extracts on the uropathogens investigated. Similarly to the inhibition diameters, ethanolic extracts (EE) showed the best antimicrobial activity with the lowest MIC and MBC. The MICs of EEs varied from 1–4 mg/mL while those of EAs varied from 4–6 mg/mL. Almost all the MBCs of AEs were indeterminate (>64 mg/mL) while those of EE were successfully determined.

With water as a solvent, the highest antimicrobial activity was observed against *E. faecalis* 5960, *S. aureus* ATCC 6538 and *C. freundii* 426. Although the MIC and MBC of AE were high against these bacteria, AE was found to be bactericidal against *S. aureus* ATCC 6538 and *C. freundii* 426 since the MBC/MIC ratio was 4 for both bacteria. Indeed, Mondal *et al.* [25] reported that when the ratio MBC/ MIC is ≥ 16 , the antibacterial efficacy of the test agent is considered as bacteriostatic, whereas MBC/MIC ≤ 4 indicates bactericidal activity. Similarly, although the MICs of EEs were relatively low, the MBCs of this extract were quite high, and we concluded that the antibacterial activity was overall bacteriostatic (MBC/ MIC ≥ 16) against most bacteria except against some Gram-positive bacteria such as *S. simulans* 5882, *Corynebacterium* spp 1638 and *S. aureus* ATCC 6538. This difference in the activity (although not significant) of EE between Gram positive and Gram negative can be ascribed to the cell wall structure of Gram - bacteria, which differs from the structure of Gram + bacteria with a thin layer of peptidoglycan, the presence of a periplasm, and ease of exchange on the plasma membrane [18]. Furthermore, according to the classification of Kuete [38], the different extracts could be considered as displaying a weak antimicrobial activity independently of the

extraction solvent and the tested strain as they scored a MIC value higher than 0.625 mg/mL. Therefore, further studies are needed to evaluate the antibacterial activity of *A. hippocastanum* bark extracts with other solvents and to assess their synergy with other antibiotics, as the weak antimicrobial activity observed here does not allow us to recommend our plant extracts as such for a possible use in the fight against antibiotic resistance and the management of urinary tract infections.

4. Conclusions

The search for new antimicrobials is essential for overcoming antibiotic resistance in the management of bacterial diseases including urinary tract infections (UTIs). In this study we evaluated the antibacterial properties of aqueous and ethanolic extracts of *Aesculus hippocastanum* bark against ten (10) clinical uropathogenic bacteria and two (2) standard bacteria. The results showed that, except against few bacteria, the extracts had overall weak antibacterial activity (MIC ≥ 0.625 mg/mL) and bacteriostatic potential (MBC/MIC ≥ 16) on both Gram positive and Gram-negative bacteria. Therefore, studies with other solvents (such as methanol, acetone or chloroform), other extraction techniques, and synergy tests with conventional antibiotics are needed to conclude on a potential better antimicrobial activity of this plant.

Abbreviations

UTI, Urinary tract infection; MIC, Minimum Inhibitory Concentration; MBC, Minimum Bactericidal Concentration; EE, Ethanolic extract; AE, Aqueous Extract; MDR, Multidrug Resistance.

Author Contributions

MMJA & PIV designed the research study. KYK & MMJA performed the research. MMJA analyzed the data. KYK, MMJA, VGE, MVA, MMA, AAA and PIV wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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