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Antibacterial Effect of Cinnamon Oil against Uropathogenic Multidrug Resistant

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Cinnamon is known for its antimicrobial activity and the aim of this study was to investigate the effect of its essential oil against ten of the multidrug-resistant uropathogenic by agar well diffusion assays. The diameters of the inhibition zone to the Cinnamon oil were 27 mm for S. aureus, 24 mm for *E coli*, 20 mm for *P.aeruginosa,* 22 mm for *K.pneumoniae*, 23 mm for *E.aerogenes & P. mirabilis*, 24 mm for *E. faecalis*, and 27 mm for *N.gonorrhoeae*, *A.baumanni, S.epidermis.* In this study, the antimicrobial effects of the cinnamon essential oil are evaluated by determining the minimum inhibitory concentration (MIC), the inhibition zone, and minimum bactericidal concentration (MBC). Cinnamon was the most effective agent in inhibiting *A.baumanni, N.gonorrhoeae, S. epidermis, E. faecalis* and *E. coli* with the lowest MIC (0.0313%) while *S.aureus, E. aerogenes* and *P. aeruginosa* with MIC (0.125%) while *P. mirabilis* was 0.0625% in our study. The MBC was 0. 25% for *A.baumanni,* 0.5 % for *N, gonorrhoeae*, *S. epidermis, E.* faecalis, and *K. pneumonia,* while 1% *S.aureus, E. aerogenes, P. mirabilis, E. coli,* and *P. aeruginosa.*

Keywords: Antimicrobial; cinnamon oil; agar well diffusion; MIC & MBC; uropathogenic.

1. INTRODUCTION

Cinnamon is a common spice that has been used for centuries by different cultures around the world. Cinnamon oil is extracted from the shredded cinnamon tree's dried inner bark. It is native to Sri Lanka and India's Malabar Coast. Jamaica and Brazil are also home to this species

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[1]. There has been a constant increase in the search for alternative and efficient compounds from cinnamon oil for multidrug-resistant bacteria, aimed at partial or total replacement of antimicrobial chemical additives where is Cinnamon oil has been shown to have numerous health benefits, especially as an anti-inflammatory. Its phytochemical elements, such as phenolic and volatile compounds, are primarily responsible for this [2]. Various types of extraction methods are used to obtain cinnamon oil, which are solvent extraction, ultrasonic extraction, hydro-distillation, shaking, and stirring with organic solvents [3]. The chief significance of the above the study was therefore to test the antimicrobial activity of cinnamon oil against uropathogenic bacteria where is urinary tract infection (UTI) is the second most common infection next to respiratory tract in human body. The disease affects people of all ages and both gender, about 150 million people are diagnosed with UTI yearly. As well as *Enterobacteriaceae*, gram-negative facultative anaerobic bacilli cause UTIs. The most common of these bacteria is *Escherichia coli* which forms about 90% of all Urinary tract infections. The other one is *Klebsiella* and *Proteus,* additionally*, Pseudomonas* that cause a complicated infections, especially in women. *Staphylococci* may cause 5-10% of UTIs in many populations. *E. coli* usually causes a child's first infection [4]. Staphylococcal infections, especially those due to *Staphylococcus saprophyticus* are common causes of urinary tract infection among female adolescent.

2. MATERIALS AND METHODS

2.1 Essential Oils Distillation

Cinnamon commercial essential oils (purity ≥98%) was purchased from Bluray Nutritional Products runs under the brand name Bluray Nutritional. Essential oils (EO) were obtained by water-steam distillation for 6 hours by Clevenger apparatus at 100°C for 6 hours.

2.2 Antimicrobial Activity Test

Agar Well Diffusion Method (AWD): Bacterial suspension (1-2x 10^6cells/mL.) was spreaded on Mueller Hinton agar plates using sterile cotton swab, then wells with a diameter of 6 mm were made on the surface and filled with (20µl), of cinnamon Standard Ciprofloxacin (20µl) were added to the 5mm well on agar plates. The treated plates with *E.coli, P.aeruginosa, K.pneumoniae, P.mirabilis, E.aerogenes, E.faecalis, A.baumanni, N.gonorrhoeae, S.aureus and S.epidermis* were incubated at 37°C for 24hrs. After incubation the treated plates were observed for zone of inhibition around the wells were recorded in millimeters [5].

2.3 Determination of Minimal Inhibitory and Bactericidal Concentrations

To facilitate the dilution of the oil and the reading of the results, Brain-Heart Infusion broth supplemented with 0.15% agar was used [6,7]. The micro dilution broth technique was performed as follows: double serial dilutions of selected EOs were prepared ranging from 1%, 0.5%, 0.25%, 0.125%, 0.0625%, 0.03125% & 0.015625% in 280µl tryptone broth. Standard - Ciprofloxacin (8µg): 8, 4, 2, 1, 0.5, 0.25 and 0.125 µg in 280 µl tryptone broth. Control- Tryptone broth was inoculated with respective cultures and without a test sample. Then, the plate was incubated at 35°C for 20–24 hr under aerobic conditions. After incubation O.D @ 590 nm was measured using plate reader Resazurin (0.015%) was added to all wells (30 µL per well) and further incubated for 2–4 h for the observation of colour change. On completion of the incubation, columns with no colour change (blue Resazurin colour remained unchanged) were scored as above the MIC value. Metabolism of Resazurin by active bacterial cells leads to reduction of Resazurin (Purple-blue) to resorufin (pink-colorless) pink color. Determine MIC as Minimum concentration of drug giving 50% inhibition of OD as compared with control. Turbidity indicates growth of the microorganism and the MIC is the lowest concentration where no cell metabolism is observed visually. To determine the MBC, the dilution representing the MIC and at least two-four of the more concentrated test product dilutions are platted (0.1mL) on SCDA plates and incubated at 37°C for 24hrs and enumerated to determine viability of cells CFU/mL. The dilution concentration that produces no growth is recorded as the MBC. The MBC is the lowest concentration that demonstrates a pre-determined reduction (such as <99.9%) in CFU/mL when compared to the MIC.

3. RESULTS AND DISCUSSION

Cinnamon essential oil was screened for their antibacterial activity by two techniques. The target cultures used were *Escherichia coli, Pseudomonas aeruginosa, Klebsiella*

pneumonia, Proteus mirabilis, Enterobacter aerogenes, Enterococcus faecalis, Acinetobacter baumanni, Neisseria gonorrhoeae, Staphylococcus aureus and Staphylococcus epidermis. Our research exhibited a strong variation in the anti-bacterial activity selected essential oil against ten bacterial species is summarized in Table 1 and Plate 1. The results revealed that the selected essential oil showed antibacterial activity with varying magnitudes. Cinnamon has been employed for centuries as food preservatives and as medicinal plants due to their antioxidant and antimicrobial activities. Nowadays, many reports confirmed the antibacterial, antifungal, antiviral, and anticarcinogenic properties of spice plants [8]. In the present study, the antibacterial effect of cinnamon oil against some important uropathogens studied and their potencies were qualitatively assessed by the presence or the absence of inhibition zone diameter. The results indicated that the essential oil of cinnamon had substantial antibacterial activity against *E.coli, P.aeruginosa, K.pneumoniae, P.mirabilis, E.aerogenes, E.faecalis, A.baumanni, N.gonorrhoeae, S.aureus, and S.epidermis* (≥20 mm inhibition zone diameter). (Table 2 and plat 2). The results of the present study agree with those reported by Braga et al. [9], Bayoub et al., [10] who stated that against Gram-positive and Gram-negative bacteria. In our study was find the results of showed antimicrobial activity against all of the bacterial strains used in this study: *Staphylococcus aureus* with an inhibitory zone of 27 mm , *E coli* with an inhibitory zone of 24 mm P.aeruginosa with an inhibitory zones of 20 mm, *K.pneumoniae* with an inhibitory zone of 22 mm, *P.mirabilis* with an inhibitory zone of 23 mm, *E.aerogenes* with an inhibitory zone of 23 mm, *E.faecalis* with an inhibitory zone of 24 mm, *A.baumanni* with an inhibitory zone of 27 mm, *N.gonorrhoeae* with an inhibitory zone of 27 mm, and *S.epidermis* with an inhibitory zone of 27 mm.

Table 1. Inhibitory activity of test cinnamon oil against uropathogens

Z	Test Compounds	Conc.per well	Zone of inhibition (mm)
E.coli	Ciprofloxacin (Std)(20µl)	2µg	21
	Cinnamon oil(20µl)	2mg	24
P.aeruginosa	Ciprofloxacin (Std)(20µl)	2µg	20
	Cinnamon oil (20µl)	2mg	20
K.pneumoniae	Ciprofloxacin (Std)(20µl)	2µg	19
	Cinnamon oil (20µl)	2mg	22
P.mirabilis	Ciprofloxacin (Std)(20µl)	2µg	22
	Cinnamon oil (20µl)	2mg	23
E.aerogenes	Ciprofloxacin (Std)(20µl)	2µg	19
	Cinnamon oil (20µl)	2mg	23
E.faecalis	Ciprofloxacin (Std)(20µl)	2µg	23
	Cinnamon oil (20µl)	2mg	24
A.baumanni	Ciprofloxacin (Std)(20µl)	2µg	19
	Cinnamon oil (20µl)	2mg	27
N.gonorrhoeae	Ciprofloxacin (Std)(20µl)	2µg	20
	Cinnamon oil (20µl)	2mg	29
S.aureus	Ciprofloxacin (Std)(20µl)	2µg	22
	Cinnamon oil (20µl)	2mg	27
S.epidermis	Ciprofloxacin (Std)(20µl)	2µg	21
	Cinnamon oil (20µl)	2mg	27

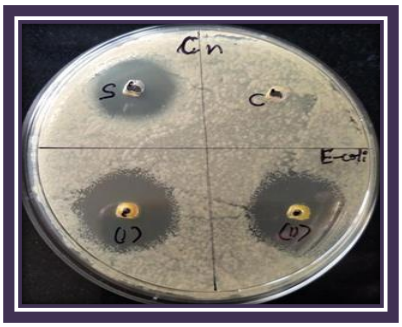

Plate 1 (a) - *E.coli*

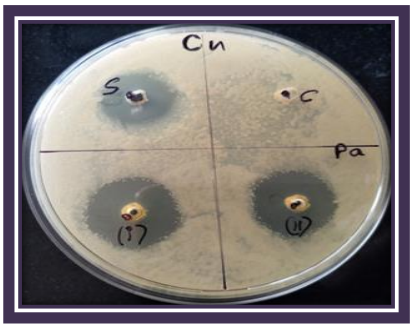

Plate 1 (b) - *P.aeruginosa*

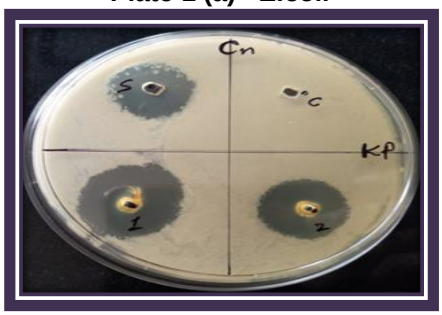

Plate 1 (c) - *K.pneumoniae*

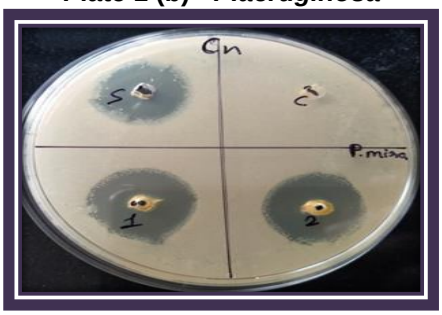

Plate 1 (d) - *P.mirabilis*

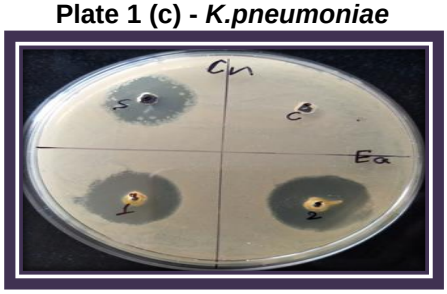

Plate 1 (e) - *E.aerogenes*

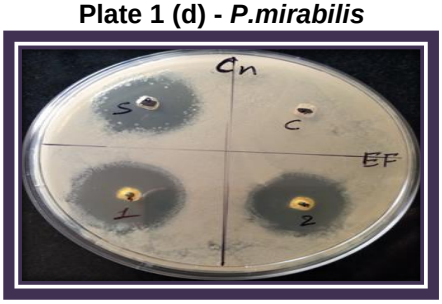

Plate 1 (f) - *E.faecalis*

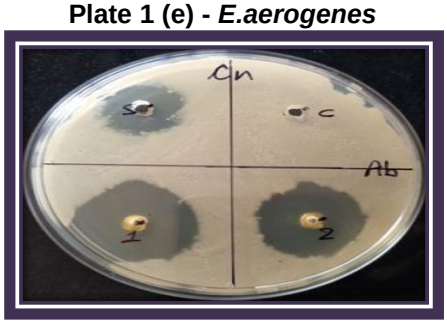

Plate 1 (g) - *A.baumanni*

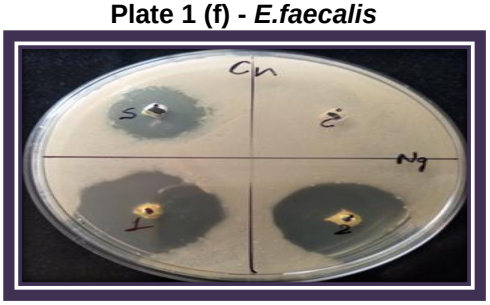

Plate 1 (h) - *N.gonorrhoeae*

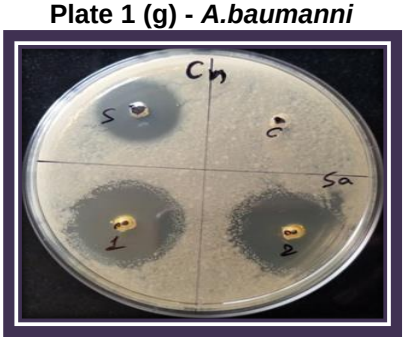

Plate 1 (i) - *S.aureus*

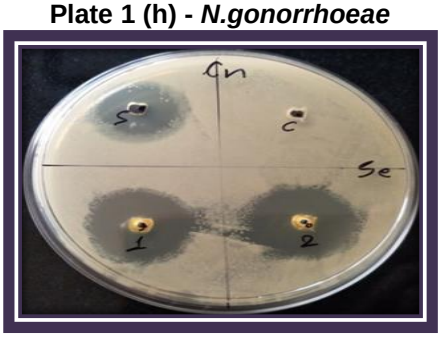

Plate 1 (j) - *S.epidermis*

Plate 1 (a-j). Inhibitory activity of cinnamon oil against test organisms S – Standard (Ciprofloxacin); C - Control (distilled water)

Table 2. Minimum inhibitory activity of samples against uropathogenic bacteria

Test Organism	OD & %	Cinnamon oil concentration %								
		0.0000	**0.0156**	**0.0313**	**0.0625**	**0.1250**	**0.2500**	**0.5000**	**1.0000**	**MIC %**
E.coli	OD at 590nm	0.5335	0.3591	0.2601	0.0929	0.0297	0.0085	0.0021	0.00019	0.0313%
	% Inhibition	0.0000	32.6898	51.2390	82.5867	94.4330	98.4049	99.6109	99.9765	
P.aeruginosa	OD at 590nm	0.5835	0.4725	0.3816	0.3130	0.2446	0.1405	0.0098	0.0034	0.125%
	% Inhibition	0.0000	19.0315	34.6041	46.3625	58.0891	75.9195	98.3171	99.4173	
K.pneumoniae	OD at 590nm	0.7033	0.4809	0.2809	0.0858	0.0404	0.0158	0.0028	0.0006	0.0313%
	% Inhibition	0.0000	31.6224	60.0597	87.8004	94.2557	97.7534	99.6006	99.9111	
P.mirabilis	OD at 590nm	0.6434	0.4812	0.3441	0.2603	0.0690	0.0102	0.0102	0.0102	0.0625%
	% Inhibition	0.0000	25.2129	46.5185	59.5431	89.2757	98.4162	99.7760	99.9501	
E.aerogenes	OD at 590nm	0.6492	0.5207	0.4805	0.3715	0.2527	0.1527	0.0270	0.0001	0.1250%
	% Inhibition	0.0000	19.7936	25.9858	42.7757	61.0752	76.4787	95.8410	99.9809	
E.faecalis	OD at 590nm	0.6985	0.4352	0.2649	0.0904	0.0413	0.0069	0.0007	0.0005	0.0313%
	% Inhibition	0.0000	37.9652	62.0759	87.0580	94.0873	99.0150	99.8933	99.9329	
A.baumanni	OD at 590nm	0.6981	0.4060	0.3010	0.2060	0.0251	0.0030	0.0025	0.0010	0.0313%
	% Inhibition	0.0000	41.8421	56.8787	70.4913	96.4045	99.5770	99.6405	99.8575	
N.gonorrhoeae	OD at 590nm	0.6865	0.4408	0.3080	0.0899	0.0469	0.0079	0.0019	0.0001	0.0313%
	% Inhibition	0.000	35.7902	55.1347	86.9046	93.1682	98.8536	99.7170	99.9836	
S.aureus	OD at 590nm	0.6745	0.4079	0.2776	0.0710	0.0297	0.0071	0.0002	0.0001	0.0313%
	% Inhibition	0.0000	39.5256	58.8436	89.4737	95.5967	98.9474	99.9703	99.9703	
S.epidermis	OD at 590nm	0.6855	0.4189	0.2886	0.0820	0.0307	0.0082	0.0003	0.0002	0.0313%
	% Inhibition	0.0000	38.8972	57.8993	88.0379	95.5251	98.8083	99.9621	99.9752	

Table 3. Minimum inhibitory activity of standard (Ciprofloxacin) against uropathogenic bacteria

Test Organism	OD & %	Std (Cipro) Conc. 8µg/well								
		0	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**MIC%**
E.coli	OD at 590nm	0.533	21.326	34.360	52.445	63.767	81.446	90.582	97.657	0.5%
	% Inhibition	0.000	21.326	34.360	52.445	63.767	81.446	90.582	97.657	
P.aeruginosa	OD at 590nm	0.541	0.433	0.349	0.241	0.215	0.171	0.089	0.034	0.5%
	% Inhibition	0.000	20.054	35.582	55.445	60.342	68.482	83.582	93.715	
K.pneumoniae	OD at 590nm	0.685	0.586	0.422	0.350	0.291	0.202	0.079	0.039	1%
	% Inhibition	0.000	14.359	38.417	48.866	57.546	70.470	88.533	94.376	
P.mirabilis	OD at 590nm	0.642	0.463	0.374	0.296	0.196	0.089	0.045	0.013	0.5%
	% Inhibition	0.000	27.852	41.708	53.897	69.483	86.191	93.033	97.974	
E.aerogenes	OD at 590nm	0.667	0.631	0.496	0.295	0.254	0.157	0.051	0.020	0.5%
	% Inhibition	0.000	5.424	25.697	55.769	61.912	76.551	92.433	97.048	
E.faecalis	OD at 590nm	0.724	0.620	0.526	0.361	0.225	0.087	0.068	0.025	0.5%
	% Inhibition	0.000	14.432	27.413	50.146	68.955	87.985	90.637	96.534	
A.baumanni	OD at 590nm	0.695	0.657	0.612	0.505	0.335	0.211	0.109	0.023	1%
	% Inhibition	0.000	5.413	11.863	27.296	51.828	69.565	84.308	96.743	
N.gonorrhoeae	OD at 590nm	0.666	0.613	0.517	0.349	0.269	0.134	0.092	0.013	1%
	% Inhibition	0.000	7.944	22.471	47.658	59.637	79.886	86.205	97.989	
S.aureus	OD at 590nm	0.772	0.651	0.551	0.340	0.268	0.181	0.088	0.045	0.5%
	% Inhibition	0.000	15.693	28.642	55.950	65.311	76.602	88.580	94.160	
S.epidermis	OD at 590nm	0.686	0.560	0.464	0.337	0.173	0.088	0.053	0.016	0.5%
	% Inhibition	0.000	18.379	32.313	50.896	74.803	87.203	92.231	97.639	

Table 4. Minimum Bactericidal Concentration (MBC) of Samples against test organisms

Organisms	**Cinnamon oil Test conc. (%)**					**MBC**
	1.00	**0.50**	**0.25**	**0.125**	**0.0625**	
A.baumanni	-	-	-	+	+	0.25
N.gonorrhoeae	-	-	+	+	+	0.5
S.aureus	-	+	+	+	+	1
S.epidermis	-	-	+	+	+	0.5
E.faecalis	-	-	+	+	+	0.5
E.aerogenes	-	+	+	+	+	1
P.mirabilis	-	+	+	+	+	1
K.pneumoniae	-	-	+	+	+	0.5
E.coli	-	+	+	+	+	1
P.aeruginosa	-	+	+	+	+	1

The effect of Cinnamon against tested pathogenic bacteria using Minimum Inhibitory Concentration (MIC) as the lowest concentration of an antimicrobial to prevent the visible growth of bacteria and Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium. The results of *in vitro* antibacterial activity by crude Cinnamon at five concentrations (1, 50, 25, 125, and .00625 conc. (%) against *E. coli, P. aeruginosa, K. pneumonia, P.mirabilis, E. aerogenes, E. faecalis, A. baumanni, Neisseria gonorrhoeae, S. aureus* and *S.epidermis* by broth dilution technique are summarized in Table 2.

We carried out antibiotic susceptibility testing to determine the activity of cinnamon essential oil against growing uropathogens. As shown in Table 2, Cinnamon was the most effective agent in inhibiting *A. baumanni*, *N.gonorrhoeae, S. epidermis*, *E. faecalis* and *E. coli* with the lowest MIC (0.0313%) while *S. aureus, E.aerogenes* and *P. aeruginosa* with MIC (0.125%) while *Proteus mirabilis* was 0.0625% in our study. The MBC was 0. 25% for *A.baumanni,* 0.5 % for *N.gonorrhoeae, S. epidermis, E. faecalis, and K. pneumonia, while 1% S. aureus, E. aerogenes, Pr. mirabilis, E. coli,* and *P.aeruginosa.*Therefore the growth of uropathogenic was efficiently suppressed by Cinnamon oil at the concentration of 0.03%., 0.0625% and 0.125. Clinical drug Ciprofloxacin included as a control inhibited the growth of uropathogenic growing cells with MIC of 1 and 0.5 µg/mL (Table 3).

Therefore, Cinnamon were highly effective against uropathogenic bacteria more Ciprofloxacin. The main reason for inhibiting the growth of bacteria is the active constituents in cinnamon oil, the variation in the concentration of the active compounds in each extract contribute to prevent the normal growth of the pathogenic bacteria [11,12]. Essential oil are potential source of novel antimicrobial activity especially pathogenic bacteria, cinnamon essential oil contain cinnamaldehyde and eugenol as major component [13]. Cinnamon oil also contain other active compounds which participate in antimicrobial activity as alkaloids, terpens, Cumarine and flavones [14]. The MIC results in this study agreed with [15], who mentioned that cinnamon oil gave (MIC) against some pathogenic bacteria *(such as E. coli, K pneumonia, S. aureus , P aeroginosa, Proteus spp and Brucellaspp)* and observed that gram positive bacteria was more sensitive than gram negative tocinnamon oil. The result of this study were corresponding with [16,17], they reported that S. aureus, E. coli and salmonella typhimurium were inhibited by essential oil of cinnamon. Friedman et al., [18] found that essential oil of cinnamon was active against E. coli and Campylobacter jejuni. In another study [19] recorded that essential oil of cinnamon showed high antibacterial activity against *S. aureus.* Trajano et al. [20] analyzed antimicrobial activity of 11 essential oils, including cinnamon, mint, black pepper, rosemary and ginger, against 10 strains of Gram-positive and Gram-negative bacteria. They reported that among the oils tested, cinnamon showed greater inhibitory act.

According to [21] natural compounds such as essential oils can act on bacterial cells by disintegration of the cell membrane by destabilising the proton force, electron flow, active transport and coagulation of cell contents. However, considering that essential oils have many different groups of chemical compounds, the antibacterial activity may be related to its composition and the mechanism of action cannot be assigned to a specific mechanism. Besides, there may be other targets in the cell, not only the cytoplasmic membrane. The direction for proper use of essential oil may be closely related to its composition. High concentrations can denature proteins and low concentrations can interfere with the activity of enzymes involved in the energy production of the cell [22]. Silva et al. [23] analyzed the effect of essential oils on E. coli and Salmonella spp. isolated from humans and ATCC cultures. Cinnamon essential oil showed the lowest MIC values for the studied microorganisms thereby confirming its high inhibitory activity. The active compounds present in the essential oil of cinnamon such as eugenol and cinnamaldehyde are responsible for causing damage to the structure of bacterial cell wall and has the capacity to interfere with the synthesis of certain bacterial enzymes [24].

4. CONCLUSION

The antimicrobial effects of the cinnamon essential oil was evaluated by determining the minimum inhibitory concentration (MIC), the inhibition zone, and minimum bactericidal concentration (MBC). Cinnamon have strong effective agent in inhibiting A. baumanni, N.gonorrhoeae, S. epidermis, E. faecalis and E. coli with the lowest MIC (0.0313%) while S. aureus, E.aerogenes and P. aeruginosa with MIC

(0.125%) while Proteus mirabilis was 0.0625% in our study. The MBC was 0. 25% for

A.baumanni, 0.5 % for N.gonorrhoeae, S. epidermis, E. faecalis, and K. pneumonia, while 1% S. aureus, E. aerogenes, Pr. mirabilis, E. coli, and P.aeruginosa. Therefore, the growth of uropathogenic was efficiently suppressed by Cinnamon oil at the concentration of 0.03%., 0.0625% and 0.125. Clinical drug Ciprofloxacin included as a control inhibited the growth of uropathogenic growing cells with MIC of 1 and 0.5 µg/mL. From the result we can conclude that cinnamon oil showed better inhibitory activity against uropathogenic multidrug resistant

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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