NATIONAL RESEARCH CENTRE
AL-BUHOUTH ST.DOKKI,CAIRO-EGYPT

المركز القومى للبحوث
الدقى - القاهرة - جمهورية مصر العربية

Results of antimicrobial assay

Company : Zyst Technology LLC

Date : 26/2/2024

1. Preliminary Remarks

In accordance with your request from Zyst Technology LLC Company for manufacturing of sanitary ware on the 26th of February 2024 9 for antimicrobial investigation for Acidic water sample with pH 0.84 against six waterborne microorganisms.

2. Methods

The reference microorganisms used were:

- *E. coli* (ATCC 25922)
- *Salmonella* Typhimurium (ATCC 14028)
- *Pseudomonas aeruginosa* (ATCC 15442) as Gram-negative bacteria
- *Staphylococcus aureus* (ATCC 6538)
- *Enterococcus faecalis* (ATCC 29212) as Gram-positive bacteria
- *Candida albicans* (ATCC 10231)

-Two concentrations of the acidic water sample were tested: the original concentration and a dilution of 1:9 (1 mL of original sample mixed with 9 mL of water). The inhibitory effect of both concentrations was estimated using the well diffusion assay and macro-dilution assay.

3. Results

- The results of antimicrobial effect of Two concentrations of acidic water sample against the mentioned microorganisms were represented in the below table in zone of inhibition using Well diffusion assay and the form of $\log_{10}$ reduction as Colony Forming Unit (CFU)/mL.

3.1. Well agar diffusion assay

The results presented in Table 1 demonstrate the antimicrobial effect of acidic water samples against various microorganisms using the well agar diffusion assay. The table shows the diameters of the zone of inhibition for both the original acidic water sample and a diluted sample (1:9 ratio) against *E. coli*, *S.* Typhimurium, *Ps. aeruginosa, S. aureus, E.faecalis*, and *C. albicans*.

The original and diluted samples both exhibit antimicrobial activity against Gram-negative bacteria (*E. coli, S.* Typhimurium, *Ps. aeruginosa*), Gram-positive bacteria (*S. aureus, E.faecalis*), and yeast (*C. albicans*). However, the original concentration of the acidic water sample demonstrates a higher antimicrobial effect compared to the diluted sample (1:9 ratio) across all tested microorganisms.

Table 1. Antimicrobial action and diameters of zone of inhibition of tested acidic water sample (1= original, and 2= diluted water 1:9) against ***E. coli, S. Typhimurium, Ps. aeruginosa, S. aureus, E.faecalis,*** and ***C. albican.***

Tested microbes	Acidic water sample Inhibition zone diameters (mm)	
	Original	Diluted in 1:9 ratio
E. coli	25±0.16	7±0.28
S. Typhimurium	24±0.23	8±0.37
Ps. aeruginosa	26±0.42	8±0.19
S. aureus	22±0.17	6±0.15
E.faecalis	21±0.32	8±0.22
C. albicans	18±0.44	7±0.53

In summary: it can be concluded that, the tested "original and diluted 1:9 samples have antimicrobial effect against Gram negative and Gram positive bacteria, and yeast. Further, results displayed that the original concentration of acidic water sample was more effective in antimicrobial action comparing with diluted (1:9) sample. These findings suggest that the original acidic water sample may possess stronger antimicrobial properties, indicating its potential as a disinfectant or antimicrobial agent.

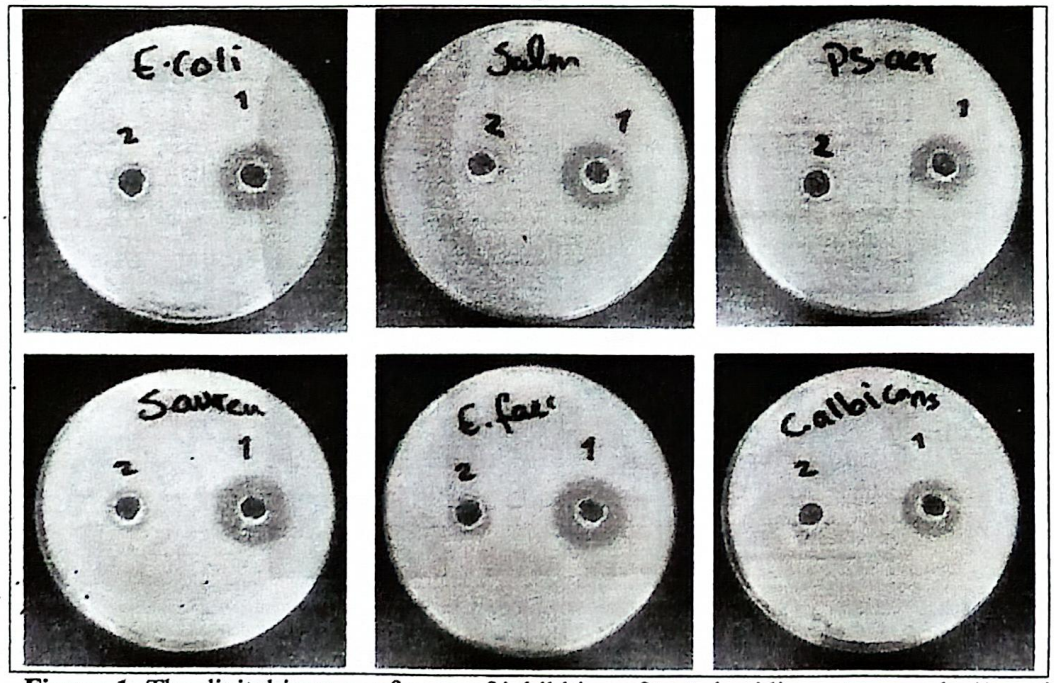

Figure 1. The digital images of zone of inhibition of tested acidic water sample (1= original, and 2= diluted water 1:9) against *E. coli, S.* Typhimurium, *Ps. aeruginosa, S. aureus, E.faecalis,* and *C. albican.*

Tel.: (+202) 33371615 - 33371433 - 33377164 Fax : 33370931 www.nrc.sci.eg P.C. 12622

- Inhibitory and Time killing effect

The results presented in Table 2 provide information on the log reduction and removal percentage of various microorganisms after exposure to the original concentration of acidic water for different time intervals (0-60 min). At the zero-point time, the initial count of microorganisms (measured in CFU/mL) for *E. coli, S.* Typhimurium, *Ps. aeruginosa, S. aureus, E.faecalis,* and *C. albicans* was relatively high. As the exposure time increased, the count of microorganisms decreased significantly, reaching zero at 15 min and remaining at zero for the subsequent time intervals (30, 45, and 60 min). The log reduction (L.R) values indicate the magnitude of reduction in microbial count after exposure to the acidic water. The L.R values consistently remained high (ranging from 6.14 to 6.61) across all tested microorganisms and time intervals, suggesting a substantial reduction in microbial population. Furthermore, the removal percentage (R%) values indicate the effectiveness of the acidic water in eliminating microorganisms. The R% values reached 100% for all microorganisms after 15 min of exposure and remained at 100% for the subsequent time intervals. These findings indicate that the original concentration of acidic water demonstrated strong antimicrobial activity, leading to a significant reduction and complete removal of the tested microorganisms within a short time frame.

Table 2. Log reduction of the tested microorganisms after exposure to original concentration of acidic water for various times (0-60)

Time, min	CFU/mL	Tested microorganisms					
		E. coli	*S.* Typhimurium	*Ps. aeruginosa*	*S. aureus*	*E.faecalis*	*C. albicans*
Zero point	Count	3.6×10^6	4.1×10^6	2.9×10^6	3.7×10^6	1.8×10^6	1.4×10^6
5	Count	1.2×10^2	2.5×10^2	1.8×10^2	4.7×10^2	5.4×10^2	7.3×10^2
	L.R	4.47	4.215	4.207	3.919	3.523	3.283
	R%	99.99	99.99	99.99	99.98	99.97	99.94
15	Count	0	0	0	0	0	0
	L.R	6.55	6.61	6.46	6.56	6.22	6.14
	R%	100	100	100	100	100	100
30	Count	0	0	0	0	0	0
	L.R	6.55	6.61	6.46	6.56	6.22	6.14
	R%	100	100	100	100	100	100
45	Count	0	0	0	0	0	0
	L.R	6.55	6.61	6.46	6.56	6.22	6.14
	R%	100	100	100	100	100	100
60	Count	0	0	0	0	0	0
	L.R	6.55	6.61	6.46	6.56	6.22	6.14
	R%	100	100	100	100	100	100

CFU= colony forming unit; L.R= Log reduction; R%= removal percentage

Tel.: (+202) 33371615 - 33371433 - 33377164 Fax : 33370931 www.nrc.sci.eg P.C. 12622

In Table 3, the initial count of microorganisms (measured in CFU/mL) at the zero-point time, for *E. coli*, *S.* Typhimurium, *Ps. aeruginosa*, *S. aureus*, *E.faecalis*, and *C. albicans* was relatively high. As the exposure time increased, the count of microorganisms decreased, but not as significantly compared to the original concentration of acidic water. The log reduction (L.R) values indicate the magnitude of reduction in microbial count after exposure to the diluted acidic water. The L.R values ranged from 1.1967 to 2.15, indicating a moderate reduction in microbial population. Furthermore, the removal percentage (R%) values indicate the effectiveness of the diluted acidic water in eliminating microorganisms. The R% values ranged from 93.64% to 99.31%, suggesting a moderate level of microbial removal.

These findings suggest that the diluted concentration (1:9) of acidic water exhibits some antimicrobial activity, leading to a reduction in microbial count and removal of the tested microorganisms. However, the efficacy appears to be lower compared to the original concentration of acidic water.

Table 3. Log reduction of the tested microorganisms after exposure to diluted concentration (1:9) of acidic water for various times (0-60).

Time, min	CFU/mL	Tested microorganisms					
		E. coli	*S.* Typhimurium	*Ps. aeruginosa*	*S. aureus*	*E.faecalis*	*C. albicans*
Zero point	Count	3.6×10^6	4.1×10^6	2.9×10^6	3.7×10^6	1.8×10^6	1.4×10^6
5	Count	2.5×10^4	3.7×10^4	5.8×10^4	7.5×10^4	6.1×10^4	8.9×10^4
	L.R	2.15	2.04	1.69	1.69	1.47	1.1967
	R%	99.31	99.09	98	97.97	96.61	93.64
15	Count	1.7×10^3	2.9×10^3	4.2×10^3	5.3×10^3	4.8×10^3	1.3×10^4
	L.R	3.326	3.1504	2.839	2.844	2.574	2.032
	R%	99.95	99.93	99.89	99.85	99.73	99.07
30	Count	2.4×10^2	3.7×10^2	2.8×10^2	5.6×10^2	7.3×10^2	8.1×10^2
	L.R	4.176	4.044	4.015	3.797	3.392	3.237
	R%	99.99	99.99	99.99	99.98	99.96	99.94
45	Count	0	0	0	0	0	0
	L.R	6.55	6.61	6.46	6.56	6.22	6.14
	R%	100	100	100	100	100	100
60	Count	0	0	0	0	0	0
	L.R	6.55	6.61	6.46	6.56	6.22	6.14
	R%	100	100	100	100	100	100

CFU= colony forming unit; L.R= Log reduction; R%= removal percentage

Tel.: (+202) 33371615 - 33371433 - 33377164 Fax : 33370931 www.nrc.sci.eg P.C. 12622

4. Conclusion

The antimicrobial investigation of acidic water samples demonstrated strong efficacy against a range of microorganisms. The original concentration of acidic water exhibited higher antimicrobial activity compared to the diluted sample (1:9 ratio), as evidenced by larger zones of inhibition and higher log reduction values. Both concentrations showed effectiveness against Gram-negative bacteria, Gram-positive bacteria, and yeast. The original concentration of acidic water resulted in complete removal of all tested microorganisms within 15 min of exposure, while the diluted sample showed a moderate reduction in microbial count.

Tel.: (+202) 33371615 - 33371433 - 33377164 Fax : 33370931 www.nrc.sci.eg P.C. 12622