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Comparative Antimicrobial Activity of Commercial Disinfectants with Naphtholics

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Abstract: Studies were carried out to determine the disinfectant property of naphthol and its derivatives. The sensitivity of some clinical organisms as compared with the activity of some selected commercial disinfectants was tested. The methods employed for assessing the efficacy of disinfectants in this study are Minimal Inhibitory Concentration (MIC) Test and Capacity Use Dilution Test. The clinical organisms used for the tests are *Pseudomonas aeruginosa, Salmonella typhi and Proteus mirabilis* while the commercial disinfectants used are Dettol (Chloroxylenol), Savlon (Cetrimide/chlorhexidine mixture) and TCP (Trichlorophenol) and the Naphtholics are alpha naphthol and 2-amino-1,4-naphthoquinonimine hydrochloride. Dettol showed highest antibacterial activity against all the test organisms. Savlon's antibacterial activity was high against the test organisms except *Pseudomonas aeruginosa*. TCP showed low activity against all the test organisms while Purified ∝-naphthol and its derivative, 2-amino-1, 4-naphthoquinonimine hydrochloride were found to exhibit disinfecting properties, with the derivative showing more antimicrobial activity than ∝-naphthol. The compounds have bactericidal effect against the test organisms used in this study.

Key words: Naphthol, commercial disinfectants, antibacterial activity, test organisms, sensitivity

INTRODUCTION

Ever since the identification of microorganisms as the causative agents of infectious diseases, various methods have been devised in reducing the population and prevalence of these organisms. The various methods embarked upon include chemotherapy, immunization, sterilization and disinfection (Kim *et al*., 2007).

Disinfection, as defined by The European Committee For The Standardization of disinfectants is the selective elimination of certain undesirable organisms in order to prevent their transmission, achieved by action on their structure or metabolism, irrespective of their functional state (Block, 1991).

Disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications (Olowe *et al*., 2004). In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections (Rutala, 1995). The main object of disinfection is to reduce the count of pathogenic organisms in a potential source of infection to below that required to cause infection (Gerald and Denvar, 1999). Chemical agents used in disinfection are referred to as disinfectants and the three main types of disinfection available are

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cleaning, heating and disinfection with chemical agents (Geo *et al*., 2004). Most disinfectants are highly effective against pathogenic organisms and their effect can either be bacteriostatic or bactericidal (Ascenzi, 1996).

The activities of disinfecting agents are affected by many factors like concentration, time of action, pH, temperature, formulation as well as phenol content (Gerald and Denvar, 1999).

Disinfectants take time to act, they are greatly inactivated by excess organic matter and they show higher activity at adequate concentrations (Olowe *et al*., 2004). The activity of disinfectants is also affected by presence of hard water during dilution (Rutala, 1995). Naphthol compound is a derivative of Naphthalene (Reynolds, 1982). 1-Naphthol and 2-Naphthol are prepared from the respective naphthalene sulphonic acids or by diazotizing the naphthylamines (Goksu *et al*., 2005). They have been found to be useful as starting materials for the production of certain pharmaceuticals and perfume ingredients, for instance, 2-hydroxymethyl-1-naphthol (TAC) and other derivatives have been found to exhibit cytotoxic and antimicrobial activity (Ai *et al*., 1995). They resemble Phenols in chemical properties but they are more reactive (Gerald and Denver, 1999). Alpha naphthol and 2-amino-1,4-naphthoquinonimine hydrochloride were used to carry out this study. This study therefore examines the disinfecting effect of Alpha naphthol and 2-amino-1,4-naphthoquinonimine hydrochloride on some pathogenic test organisms compared to some selected commercially available disinfectants.

MATERIALS AND METHODS

This research work was carried out between February and May, 2006. Alpha-naphthol was purified and various derivatives were synthesized from it and obtained from the Department of Chemistry, University of Ilorin, Kwara state, Nigeria. The disinfectant activity/sensitivity of some clinical isolates to alpha- Naphthol ($C_{10}H_8O$) and-2-amino-1, 4-naphthoquinonimine hydrochloride ($C_{10}H_9N_{2O}Cl$) were tested. The test organisms used were clinical isolates collected from the Department of Microbiology, University of Ilorin Teaching Hospital. These are *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus mirabilis*.

The commercial disinfectants used for this research work were purchased from reputable Pharmaceutical stores in Ilorin and Lagos metropolis. They are Dettol (chloroxylenol), Savlon (Cetrimide/chlorhexidine) and TCP (Trichlorophenol).

Preparation of Yeast Suspension

A Five percent yeast solution was prepared as described for the Chick-Martin test (Martin, 1969), by weighing 2.5 g of commercial dried yeast to 47.5 mL of distilled water and was autoclaved. Two percent of the solution was used.

Preparation of Standard Hard Water

Standard hard water of 300 Parts per Million (PPM) hardness was prepared by adding 17.5 mL of 10% (W/V) solution of $CaCl.6H_2O$ and 5 mL of 10% (W/V) solution of $MgSO_4.7H_2O$ to 3000 mL of distilled water (Maurer, 1973).

Broth cultures of the test organisms were prepared in peptone water.

Minimal Inhibitory Concentration (MIC) Test

The Minimal Inhibitory Concentration (MIC) is the highest dilution which fails to show growth.

In this test, 10 doubling dilutions of the disinfectant in nutrient broth were prepared starting with 1:20 dilution and were then inoculated with 0.2 mL of culture and incubated at 32°C for 27 h. For naphthol compound and its derivative, 1 in 10 dilution was used. This test was carried out for all the organisms and test disinfectants.

Capacity Use Dilution Test

In the Capacity Use Dilution test, successive volumes of a suspension of a test organism, at standard time intervals were added to the disinfectants diluted in standard hard water solutions.

After each addition of the organisms, portions of the mixture were removed and cultured for survivors.

The test organism to be used was prepared in a suspension not less than 10^8 organisms per mL of the organism. The preparation is made up in broth to simulate clean conditions and in yeast/horse serum to simulate dirty conditions.

Dilutions of the disinfectants are made up in the standard hard water at 3 strengths:

- The use dilution as recommended by the manufacturer
- Twenty five percent weaker than the recommended use dilution and
- Twenty five percent stronger than the recommended use dilution.

The three strength dilutions for the disinfecting agents were made as follows:

Disinfectant	25% weaker	Recommended dilution	25% stronger dilution
Dettol	0.4/10 mL	1:20	0.6/9 mL of water
Savlon	12/250 mL	16/250 mL	20/250 mL of water

For α-naphthol, 0.20 g was dissolved in 1% tween 80 to make 0.05 g mL^{-1} of α-naphthol solution. Two milliliter of the solution and 1 mL of sterile standard hard water was used as recommended dilution.

Three milliliter of α-naphthol solution is used as 25% stronger dilution and 1 mL of α-naphthol solution plus 2 mL standard hard water as 25% weaker dilution.

For 2-amino-1, 4-naphthoquinonimine hydrochloride, 0.25 g of it was dissolved in 10 mL of 1% between 80. The three strength dilution was made as for α-naphthol above.

RESULTS AND DISCUSSION

Antibacterial activities of Savlon, Dettol, TCP, alpha-Naphthol and 2-amino-1,4-naphthoquinonimine hydrochloride against the test organisms i.e., *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella typhi* (Table 1).

MIC of the active ingredients in each disinfectant, naphthol and naphthoquinone were found against the test bacteria species. The active ingredients in Savlon are Cetrimide (3.0% w/v) and Chlorhexidine gluconate mmu(0.3% W/V).

In Dettol, the active ingredient is Chloroxylenol (4.8%w/v).

Trichlorophenol (TCP) has 0.4%w/v chlorine and 0.63%w/v phenol as the active ingredients.

For alpha-Naphthol and 2 amino-1,4-naphthoquinonimine hydrochloride the naphtholics are the active ingredients.

Table 1: Minimum inhibitory concentration of commercial disinfectants and naphtholics on some bacteria

	Disinfectants	Savlon		Dettol	TCP		Naphthol	Naphthoquinone
Test organisms	Active ingredients	Cetrimide	Chlorhexidine gluconate	Chloroxylenol (g)	Chlorine (g)	Phenol (g)	Alpha-naphthol	2-amino-1,4-naphthoquinonimine hydrochloride
P. aeruginosa		7.5×10^{-8}	7.5×10^{-9}	1.2×10^{-7}	1×10^{-4}	1.58×10^{-4}	2.0×10^{-4}	2.5×10^{-8}
S. typhi		7.5×10^{-10}	7.5×10^{-11}	1.2×10^{-8}	1×10^{-5}	1.58×10^{-5}	2.0×10^{-4}	2.5×10^{-8}
P. mirabilis		7.5×10^{-9}	7.5×10^{-10}	1.2×10^{-3}	1×10^{-4}	1.58×10^{-4}	2.0×10^{-6}	2.5×10^{-10}

The results of the capacity use dilution test with *P. aeruginosa*. Savlon and Dettol gave satisfactory results after first incremental additions in 25% weaker and recommended dilutions. In 25% stronger dilutions, they gave satisfactory results (antibacterial activity) after the first and second incremental additions. α-Naphthol and 2-amino-1,4-naphthoquinonimine hydrochloride showed bactericidal activity after the first incremental addition in 25% weaker dilution and after the first second and third incremental additions in the recommended and 25% stronger dilutions (Table 2).

The use capacity dilution test with *S. typhi*. In 25% weaker dilution, all the commercial disinfectants failed the test and α-naphthol gave satisfactory results only after the first incremental addition. In the recommended dilution, Savlon and Dettol were effective only after the first incremental addition, while α-naphthol was effective after the 1st, 2nd and 3rd incremental additions. In 25% stronger dilution, Savlon and Dettol were effective after the first and second incremental additions while α-naphthol was effective after the 1st, 2nd and 3rd incremental additions (Table 3).

The use capacity dilution test with *Proteus mirabilis* as the test organism (Table 4.) revealed that Savlon, Dettol and ∝-naphthol did not give satisfactory results in 25% weaker dilution i.e., they were inactive after the first, second and third incremental additions of suspension of *Proteus* except ∝-naphthol which was effective after the first incremental addition. Savlon was active after the 1st and 2nd incremental addition in the recommended and 25% stronger dilutions but was not active after the third incremental addition in both dilutions.

Dettol was effective only after the 1st addition of *Proteus* in the recommended dilution and after the 1st and 2nd incremental additions in 25% stronger dilution. At 18 and 28 min in the recommended dilution and 28 min in 25% stronger dilution, Dettol was no more effective against *Proteus mirabilis*. ∝-naphthol was effective after the 1st and 2nd incremental additions but ineffective after the 3rd incremental addition in the recommended and 25% stronger dilutions.

Table 2: Capacity use dilution test with *P. æruginosa* use capacity test at different dilutions

Disinfectant	Time (min)	25% weaker	Recommended dilution	25% stronger
Savlon	8	- - - - +	- - - - -	- - - - -
	18	- + + + +	- + + + +	- - - - +
	28	+ + + + +	+ + + + +	- - + + +
Dettol	8	- - - + +	- - - + +	- - - - -
	18	- + + + +	- - + + +	- - - - +
	28	+ + + + +	- + + + +	- - + + +
∝-naphthol	8	- - - + +	- - - - +	- - - - -
	18	- - - + +	- - - - +	- - - - -
	28	- - - + +	- - - + +	- - - - -
2-amino-1,				
4-naphthoquinonimine hydrochloride	8	- - - - +	- - - - -	- - - - -
	18	- - - + +	- - - + +	- - - - -
	28	- - - + +	- - - + +	- - - + +

- = No microbial growth; + = Microbial growth

Table 3: Capacity use dilution test with *S. typhi* use capacity test at different dilutions

Disinfectant	Time (min)	25% weaker	Recommended dilution	25% stronger
Savlon	8	- - + + +	- - - - -	- - - - -
	18	- + + + +	- - - + +	- - - - +
	28	+ + + + +	+ + + + +	- - - + +
Dettol	8	- - + + +	- - - - +	- - - - +
	18	- + + + +	- - + + +	- - - + +
	28	+ + + + +	- + + + +	- - + + +
∝-naphthol	8	- - - + +	- - - - -	- - - - -
	18	- - + + +	- - - - -	- - - - +
	28	+ + + + +	- - - + +	- - - - +
2-amino-1,				
4-naphthoquinonimine				
hydrochloride		N.D	N.D	N.D

N.D = Not Done; - = No microbial growth; + = Microbial growth

Table 4: Capacity use dilution test with *Proteus mirabilis* Use capacity test at different dilutions

Disinfectant	Time (min)	25% weaker	Recommended dilution	25% stronger
Savlon	8	- - + +	- - - -	- - - - -
	18	- + + +	- - - +	- - - + +
	28	+ + +	+ + + +	- + + +
Dettol	8	- - + + +	- - - + +	- - - - +
	18	- + + + +	- - + + +	- - - + +
	28	+ + + + +	- + + + +	- - + + +
∝-naphthol	8	- - - + +	- - - - +	- - - - +
	18	- - + + +	- - - + +	- - - + +
	28	+ + + + +	- + + + +	- - + + +
2-amino-1, 4-naphthoquinonimine hydrochloride		N.D	N.D	N.D

N.D. = Not done; - = No microbial growth; + = Microbial growth

The result of this study clearly showed that most disinfectants are effective when used at correct concentrations. This is in agreement with the findings of Kaarina *et al*. (2000). Disinfectants like Savlon and Dettol were effective against many pathogenic organisms, especially when the number of cells present were not disinfected in the presence of excess organic matter as had been observed by Olowe *et al*. (2004).

From the MIC test carried out, all the test organisms were sensitive to the different disinfectants and naphtholic compounds, but the rate of sensitivity vary with each organism and each dinsinfecting agent.

The highest MIC (i.e., lowest activity) of Savlon was recorded for *Pseudomonas aeruginosa* while the lowest MIC (highest activity) was recorded for *S. typhi* (Table 1).

Chawner and Gilbert (1989), Langsrud and Sundheim (1997), had observed that *Pseudomonas* sp. was able to develop adaptive resistance to some disinfectants, especially Savlon or Clorhexidine solution. *P. aeruginosa* however showed high sensitivity to Dettol (Chloroxylenol) and naphthoquinone.

S. typhi was highly sensitive to all the disinfectants especially Savlon, Dettol and TCP as Lowest MIC (highest activity) were recorded against the organism.

α-Naphtol and naphthoquinone exhibited highest activity against *Proteus mirabilis* although their activities against *P. aeruginosa* and *S. typhi* was also very high.

The MIC of TCP was high (low activity) for all the test organisms, MIC being recorded as 10^{-4} and 10^{-5} g for chlorine and 1.58×10^{-4} g and 1.58×10^{-5} g phenol. The survival of pathogenic organisms in TCP solution can be attributed to the ability of some organisms to use aromatic compounds as the carbon source (Gerald and Denver, 1999).

∝-naphthol and its derivative used in this study showed high antimicrobial activity against each of the test organisms, the disinfecting activities of naphthoquinonimine hydrochloride being similar to that of Dettol and ∝-naphthol more effective than TCP.

The most resistant among the test organisms used was *Pseudomonas*.

At very low concentrations, pathogenic organisms were able to survive in the disinfectant solutions. This was also observed by Kaarina *et al*. (2000).

From the results of capacity use dilution tests it is found that disinfectants take time to act and their activity is greatly affected by the amount of organic matter present and concentrations of the disinfectants This corroborates the findings of Olowe *et al*. (2004). The test organisms are all pathogenic organisms, disinfectants like Dettol and Savlon are active against them. This is in agreement with the findings of Rutala (1995), that disinfectants are useful in the control of nosocomial infections.

Savlon and Dettol failed the use capacity test in 25% weaker dilution when the test was carried out with *Pseudomonas aeruginosa* as the test organism. This is because the test was not satisfactory after the second incremental addition of *Pseudomonas* suspension. The failure of the test was due to

the presence of large numbers of organisms at 18 and 28 min. Savlon and Dettol were found to have satisfactory activity against *Pseudomonas aeruginosa* after the second incremental addition of its suspension in 25% stronger dilutions. However, in 25% stronger dilution, *Pseudomonas* was able to grow after the third incremental addition.

∝-Naphthol and 2-amino-1, 4-naphthoquinonimine hydrochloride were highly active against *Pseudomonas aeruginosa* in the three dilutions after each of the three incremental additions. These naphtholic compounds were less inactivated by organic matter and even hard water and increase in the number of organisms present had little effect on their activity. The satisfactory result obtained from test could be attributed to the fact that the compounds are in contact with the microbial cells and penetration of the compounds was effective.

The results of the antimicrobial activity of Dettol and Savlon against *Salmonella typhi* were satisfactory after the first incremental additions in the recommended dilutions and after first and second incremental additions in 25% stronger dilutions. Alpha naphthol gave a satisfactory result at recommended and 25% stronger dilutions

The action of Savlon was satisfactory in the recommended and 25% stronger dilutions against *Proteus mirabilis* while Dettol gave a satisfactory result only in the 25% stronger dilution. ∝-Naphthol gave a satisfactory result against *Proteus* in the recommended and 25% stronger dilutions.

2-amino-1, 4-naphthoquinonimine hydrochloride was found to be stronger and more active against microbes than ∝-naphthol and this is also pronounced from the results of the tests carried out in this study. The results obtained in this study have clearly shown that ∝-naphthol and its derivative used are highly active against gram negative organisms. Savlon and Dettol on the other hand have relatively low activity against gram negative bacteria while TCP generally has little activity against pathogenic organisms.

There are correlations between MIC and the capacity use dilution results. In both tests Savlon and Dettol showed lower activity against the organisms while the naphtholics were more active against organisms.

Savlon and Dettol were however sensitive to hard water and organic matter and large number of organisms and they are therefore rendered inactive when these materials are in excess. The naphtholics were found to be very active against pathogenic organisms even in the presence of these organic materials.

Comparing the MIC of Phenol assisted by chlorine (TCP) with that of naphtholics, Naphthoquinonimine hydrochloride was found to have lower MIC (higher activity) than TCP while the MIC of TCP was slightly lower (higher activity) than that of ∝-naphthol.

CONCLUSIONS

From the result obtained in this study, it can be concluded that disinfectants have broad activity against pathogenic organisms like *Pseudomonas auruginosa*, *Proteus mirabilis* and *Salmonella typhi*. Nosocomial infections and other infectious diseases can be reduced greatly by the use of disinfectants. This could only be achieved when the disinfectants are adequately diluted and in clean environments free from organic matter and other materials such as salts and hard water. When disinfectants are over diluted, their effectiveness will be reduced and this can make Pathogenic organisms build resistance to such disinfectants.

Purified ∝-naphthol and its derivative, 2-amino-1, 4-naphthoquinonimine hydrochloride were found to exhibit disinfecting properties, with the derivative showing more antimicrobial activity than ∝-naphthol. The compounds are active against pathogenic organisms i.e., the test organisms used in this study.

It is therefore recommended that ∝-naphthol and 2-amino-1, 4-naphthoquinonimine hydrochloride be considered for use as disinfecting agents.

It may also be suggested that the use dilution of these disinfectants i.e., Savlon and Dettol be increased to 25% higher than the present recommended dilution so as to reduce the resistance of pathogenic organisms, especially *Pseudomonas* sp. and hence prevent nosocomial infections.

TCP which had a very low activity i.e., high MIC against all the test organisms is recommended to be used only as concentrated solution for disinfection purposes.

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