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Antimicrobial activity of Dibenzalacetone ($C_{17}H_{14}O$)

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Abstract

Dibenzalacetone was synthesized via the base catalysed condensation reaction between acetone and benzaldehyde. This study was done to evaluate the antimicrobial activity of the synthesized compound dibenzalacetone ($C_{17}H_{14}O$) against four human pathogenic microorganisms: *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginiosa* and the fungus *Candida albicans.* The highest area of zone of inhibition of 68.23 mm^2 was induced against *Candida albicans* and the lowest AZOI of 37.55 mm^2 was induced against *E.coli*. *Staphylococcus aureus* was totally resistant to the drug, in that zero zone of inhibition was induced against dibenzalacetone. The order of antimicrobial potency followed the sequence: *Candida albicans > Klebsiella pneumoniae > Pseudomonas aeruginosa > Staphylococcus aureus*. However, its antimicrobial potency was lowered than that of standard synthetic drug, Ampicillin and Ketoconazole.

Keywords: Dibenzalacetone; Antimicrobial activity; Synthesized compound; Pathogenic; Microorganism

1. Introduction

Antimicrobial activity is the process of inhibiting or eliminating a particular disease causing microbe. There are various antimicrobial agents and are typically classed as an antibiotic, antifungal, or antiviral, each having its own mode of action to suppress the infection [1-3]. Examples of antibiotic compounds include penicillin, tetracyclines, aminoglycosides, azoles (ketoconazole, miconazole etc.) (Smith, 1991; Bertram, 2018). However, bacteria and fungi develop resistance to antimicrobials over a period of time. It's a global problem.[4-8] Antimicrobial resistance (AR or ABR) due to indiscriminate use of commercial antimicrobial drugs used for the treatment of infectious diseases and is a global concern [4-8] . It may also be due to physicians over prescribing antibiotics or using a broad spectrum antibiotics in place of a narrow spectrum.

As a result bacterial strains developed a range of mechanisms to protect against the drug. These include the production of enzymes that will destroy certain prime selective groups in the drug molecule that are necessary for it to act. For example, bacteria produce the enzyme, β-lactamase that will destroy the β-lactam ring of penicillin. The latter is necessary for the antibacterial effect of the drug, penicillin. Other methods bacteria have evolved to protect against the drug, include an increase efflux of the drug across the bacterial membrane, the protection of bacterial ribosomes via processes such as methylation, induced by the enzymes methylase, decrease influx of the drug across bacterial membrane. All these processes are facilitated by indiscriminate use of selected antibiotics. Antibiotic resistance is mostly induced against synthetic antibiotics.

The resistance of bacteria to antibiotics was noticed almost as soon as antibiotics came into existence; however, it is only in the most recent years that extremely dangerous resistant strains of bacteria emerged [9]. The cost attached to

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treating an infection that is resistant to common antibiotics is high and requires the patient to be in the hospital for a lengthy period of time, as compared to treating an infection that is not resistant to common antibiotics [10]

Solutions to the antibiotic resistance problem include reducing the use of antibiotics by promoting the use of alternative products. There will be no one quick fix to replace all uses of antibiotics, but we can drastically cut down on the usage by combining various alternatives. In addition, continue the search for more selective antimicrobials. Also, to resort to herbal natural antimicrobials. The latter seem to suffer less antimicrobial resistance, in comparison to the synthetic analogue. In this regard, the continued rise in herbal medicinal research in the Caribbean is anticipated [11-12].

In Guyana, like the rest of the world, there is a need for the safe use of antimicrobials. Also, to continually search for new antimicrobials. Thus, the aims and objectives of this research were

- To test the antimicrobial effectiveness of the synthesized compound dibenzalacetone against the four human pathogenic microorganisms: *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginiosa,* and the fungus *Candida albicans.*

Specific Objectives were

- To identify the level of effectiveness of dibenzalacetone against each human pathogenic microorganism and fungus used in this study.
- To determine whether dibenzalacetone can potentially be used as an alternative treatment for antibiotic resistant bacteria.

It was hypothesize that dibenzalacetone will show significant antimicrobial activity against the four human pathogenic microorganisms: *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginiosa,* and the fungus *Candida albicans.*

Dibenzalacetone ($C_{17}H_{14}O$) is an organic compound synthesized from the reaction between acetone and benzaldehyde, using sodium hydroxide as a base. It is a pale yellow compound often used in commercial sunscreen due to its ability to absorb ultraviolet light, as a ligand in organometallic chemistry, and as an anti-inflammatory agent[13]. The synthesis of dibenzalacetone is done by the condensation of acetone with two equivalents of benzaldehyde. The aldehyde's carbonyl is more reactive than the ketone; therefore, it tends to react more swiftly with the anion of the ketone to give a β-hydroxyketone, which easily undergoes base catalyzed dehydration. Monobenzalacetone or dibenzalacetone is produced, depending on the relative quantities of the reactants[14]. Dibenzalacetone is expected to display a relatively good antimicrobial activity due to the ease at which its conjugated system is oxidized [15].

Over the years there has been an increase number of articles in the literature on the use of synthesize compounds as antimicrobial agents in pursuit of better and selective antimicrobial agents to prevent antimicrobial resistance.

`Synthesis and Biological Activities of Chalcones Derived from Nitroacetophenone" were looked at[16]. Nitrochalcones were synthesized by reacting nitroacetophenones and benzaldehydes, and the synthesized compounds were then tested against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescence, Staphylococcus aureus, Aspergillus niger, Candida albicans* and *Trichophyton rubrum*. Of all the compounds tested, (E)-3-(2-methoxyphenyl)-1-(3-nitrophenyl)prop-2-en-1-one showed the strongest antimicrobial activity with a minimum inhibitory concentration (MIC) value of 20 μg/mL against *Pseudomonas fluorescence*.

Heterocyclic compounds derived from dibenzalacetone were tested for their antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli.* To compare the effectiveness of the antimicrobial activity of each heterocyclic compound, the zones of inhibition were compared to that of a standard drug (Ampolix), whose zone of inhibition was 38 mm and 37 mm for *Staphylococcus aureus* and *Escherichia coli,* respectively. It was found that the heterocyclic compound (dibenzalacetone + 2,4-dinitrophenylhydrazine / semicarbazide) showed the most significant antimicrobial activity, that being 38 mm and 36 mm for *Staphylococcus aureus* and *Escherichia coli,* respectively[17].

The antimicrobial activity of some aromatic halogenohydroxy aldehydes and ketones against four bacteria and two fungi was reported. 3,5-dichloro-2,4-dihydroxy benzaldehyde and 3,5-dichloro-2,4-dihydroxy acetophenone were the most effective and in some cases showed equal inhibition as compared to the standard drugs used in the study; streptomycin and fluconiazol [18].

The "synthesis and antibacterial activity of some Uinoxaline-1,4-dioxide Derivatives". The bacteria used for testing was grown in macrophages *in vitro.* The results showed a good antibacterial activity. This factor combined with the low potency and toxicity of the compounds deemed the compounds "valid leads for synthesizing new compounds that possess better activity"[19].

The synthesis and antimicrobial activity of Bis-4,6-sulfonamidated 5,7-Dinitrobenzofuroxans has been reported. In this study the bacteria *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*, as well as the fungi *Aspergillus niger* and *Candida albicans,* were the microorganisms used. It was found that compounds studied exhibited potent antimicrobial activity[20].

2. Material and methods

2.1. Storage of Materials

The human pathogenic microorganisms used for the antimicrobial activity were collected at GPHC and stored at the University of Guyana Turkeyen Campus, in a cool, safe environment away from any cross contaminants. The prepared plates containing the bacterial colonies were incubated for 24 hrs and 48 hrs at 35 ^{o}C.

2.2. Synthesis of Dibenzalacetone [21-23]

In a 250 ml conical flask, 5 g of KOH pellets was dissolved in 50 ml of water and 40 ml of ethanol was added. Separately, 5.1 ml of benzaldehyde and 1.9 ml of acetone was prepared. Whilst swirling the contents of the 250 ml conical flask, the mixture of benzaldehyde and acetone was added in one lot and shaken frequently, this was allowed to cool to room temperature for about 30 minutes. The precipitate was filtered using a Buchner funnel and flask and washed thoroughly with distilled water, the solid was then dried with the pump for about 10 minutes and the weight of the crystals were recorded. Recrystalization was done using ethanol, the new weight was recorded, and the Melting Point was determined.

2.3. UV-Vis Spectroscopy

In order to proceed with the UV-VIS analysis, 0.118g of dibenzalacetone was placed into a 50 ml volumetric flask and made up to the mark with acetonitrile. From this, 3 serial dilutions were done resulting in the following concentrations of dibenzalacetone: 1.0 x 10^{-3} M, 1.0 x 10^{-4} M and 1.0 x 10^{-5} M. The blank (acetonitrile) was placed into a cuvette and placed into the spectrophotometer and the lid was closed; a value for the absorbance was recorded. The third dilution made was selected and the absorbance between the wavelengths 200 nm to 800 nm was measured. The wavelength at the maximum absorbance value was recorded.

2.4. Preparation of Mueller-Hinton (MH) Agar

23 grams of the powdered media (Muller Hinton) was dissolved in 1000 ml sterile distilled water in a conical flask. The weighed amount was mixed properly then allowed to dissolve by heating over a water bath. The conical flask was then plugged with cotton wool and wrapped in aluminum foil and autoclaved at 121°C for 15 minutes.

2.5. Antimicrobial Activity [24-25]

A fresh culture of bacteria and fungus were grown using Mueller-Hinton (MH) agar and left to reproduce for 24 hours, the Kirby-Bauer Disc Diffusion Susceptibility test was then carried out.

Preparation of Mueller-Hinton plate: The MH agar was poured into 45 petri dishes to a depth of 1cm and left to cool to room temperature in the incubator at 35 ^{o}C. A sterile inoculating loop was used to touch approximately 5 colonies of the selected microorganism to be tested, this was then suspended in 2ml sterile saline and the turbidity of this solution was adjusted to a 0.5 McFarland standard [25]. A sterile swab was placed into the inoculum tube and rotated, this was then placed on the dry surface of the MH agar plate by streaking the swab over the entire agar surface and placed into an incubator for no more than 15 minutes before the next step. The appropriate antimicrobial-impregnated discs were placed on the surface of the agar, using forceps to dispense each antimicrobial disc one at a time. Once all discs were in place, the lids were put back on, the plates were then inverted and placed in a 35°C air incubator for 24 hours (bacteria) and 48 hours (fungus) respectively. The diameter of zone of inhibition (DZOI) and Area of Zone of inhibition (AZOI) were subsequently computed.

2.6. Data Analysis

The data gathered from the laboratory testing was used to identify the effectiveness of dibenzalacetone against each human pathogenic microorganism, a cross reference of the inhibition zones of dibenzalacetone with that of ampicillin and ketoconazole was done. The data was placed into tables and graphs which were then analyzed using an analysis of variance (ANOVA); one way without replication. Recommendations were made based on the data gathered from each of these tests.

3. Results

3.1. Synthesis of Dibenzalacetone

Figure 1 Synthesized dibenzalacetone crystals

3.2. UV-vis Analysis of the Synthesized Dibenzalacetone

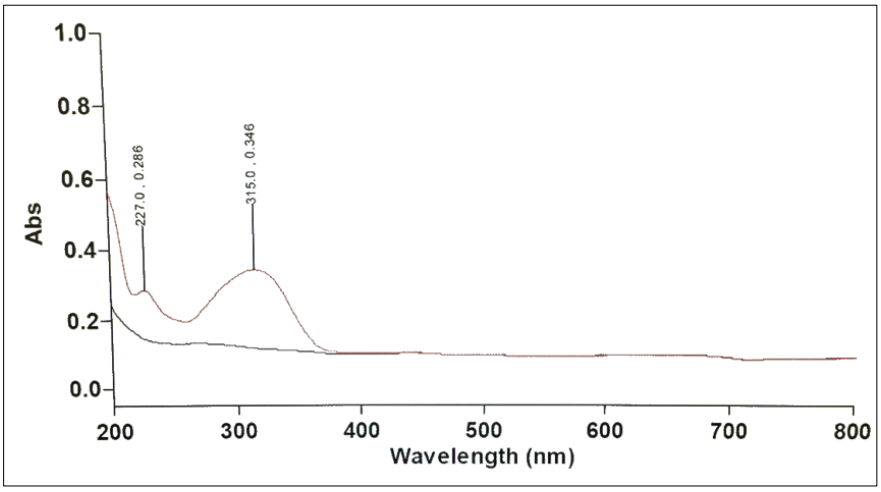


Figure 2 UV-vis spectrum of the synthesized Dibenzalaceton

Table 1 Antimicrobial activity of Dibenzalacetone

Dibenzalacetone	

Name of organism	Concentration (mg/ml)	Mean diameter zone of inhibition (mm)	Area zone of inhibition (mm2)	Average Area of Zone of Inhibition, AZOI
Staphylococcus aureus	1.5	0	0	0.0
	2.5	0	0	
	3.5	0	0	
Klebsiella pneumoniae	1.5	7.60	45.36	49.6
	2.5	8.83	61.24	
	3.5	7.33	42.20	
Escherichia coli	1.5	5.33	22.31	37.55
	2.5	7.33	42.20	
	3.5	7.83	48.15	
Pseudomonas aeruginosa	1.5	6.83	36.64	40.74
	2.5	6.50	33.18	
	3.5	8.17	52.42	
Candida albicans	1.5	8.83	61.24	48.34
	2.5	9.67	73.44	
		3.5	10.33	

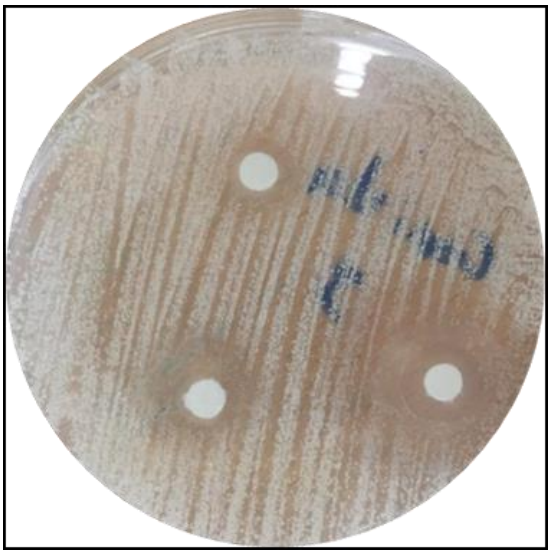
Table 2 Antimicrobial activity of Ampicillin

Ampicillin				
Name of organism	**Concentration (mg/mL)**	**Mean diameter zone of inhibition (mm)**	**Area zone of inhibition (mm2)**	**Average Area of Zone of Inhibition**
Staphylococcus aureus	1.5	24.00	452.39	579.07
	2.5	27.67	601.32	
	3.5	29.50	683.49	
Klebsiella pneumoniae	1.5	7.17	40.38	55.15
	2.5	8.67	59.04	
	3.5	9.17	66.04	
Escherichia coli	1.5	21.17	351.99	487.49
	2.5	24.33	464.92	
	3.5	28.67	645.57	
Pseudomonas aeruginosa	1.5	0	0	0.00
	2.5	0	0	
	3.5	0	0	
Candida albicans	**1.5**	0	0.0	0.00
	2.5	0	0.0	
	3,5	0	0.0	

Table 3 Antimicrobial activity of Ketoconazole

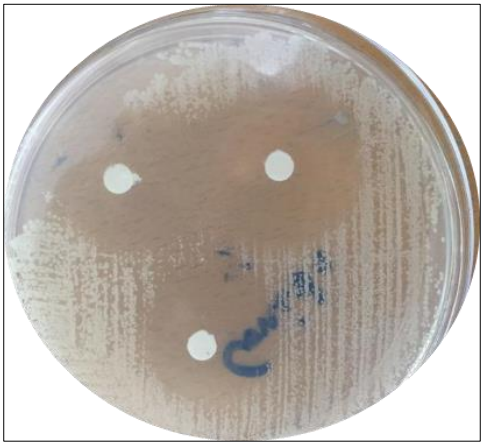
Ketoconazole	

Name of organism	Concentration (mg/mL)	Mean diameter zone of inhibition (mm)	Area zone of inhibition (mm^2)	Average Area of Zone of Inhibition, AZOI
Staphylococcus aureus	1.5	9.67	73.44	217.68
	2.5	13.33	139.56	
	3.5	23.67	440.03	
Klebsiella pneumoniae	1.5	7.33	42.20	44.28
	2.5	8.00	50.27	
	3.5	7.17	40.38	
Escherichia coli	1.5	12.33	119.40	210.04
	2.5	6.17	29.90	
	3.5	8.83	61.24	
Pseudomonas aeruginosa	1.5	8.17	52.42	68.13
	2.5	9.67	73.44	
	3.5	10.00	78.54	
Candida albicans	1.5	20.00	314.16	577.42
	2.5	28.33	630.35	
	3.5	31.67	787.75	



Dibenzalacetone: AZOI induced against *C. albicans*

Ampicillin: AZOI induced against *E.coli*



Ketoconazole against *C. albicans*

Figure 3 Comparative antimicrobial activity of Dibenzalacetone

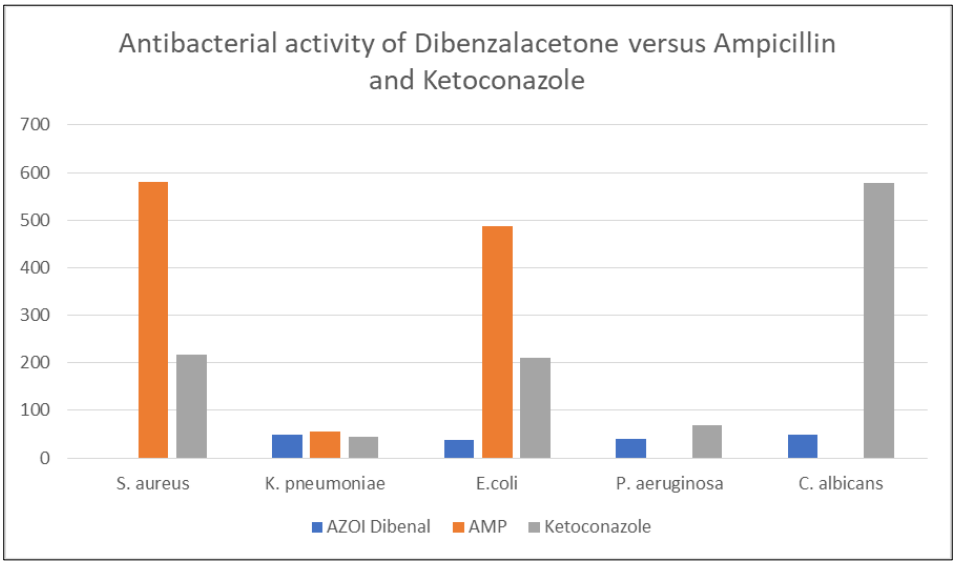


Figure 4 Antibacterial activity of Dibenzalacetone versus Ampicillin and Ketoconazole

Table 4 ANOVA results

	Source of Variation		
	F	*P-value*	*F crit*
Dibenzalacetone vs. Ampicillin	6.759	0.0147	4.196
Dibenzalacetone vs. Ketoconazole	6.260	0.0185	4.196

4. Discussion

4.1. Synthesis and Characterisation of Dibenzacetone

First, the compound dibenzalacetone was synthesized and characterized. It was synthesized via the reaction of two moles of benzaldehdye with one mole of acetone, Figure 5.0. The mechanism is shown in Fig. 6.0. The first step involves the formation of a carbanion from acetone using NaOH. The mono carbanion is formed first. This carbanion, acting as a

nucleophile attacks the carbonyl group of benzaldehyde to form an addition adduct. Another carbanion is then formed from this adduct at the acetyl end of the acetone fragment. This then attacks the second mole of benzaldehyde to form another addition product. Two moles or molecules of water are then lost to produce the condensation product, dibenzalacetone. Fig. 1.0. shows dibenzalacetone to be a yellow compound. It was characterized using UV-VIS spectroscopy and melting point apparatus. From the UV/Vis spectrum, Fig. 2.0., the maximum absorbance value of the synthesized compound was found to be 315 nm, with an absorbance of 0.32 and the melting point was found to be 109.5 ^{o}C. This suggests a *trans* form of dibenzalacetone was synthesized. In the literature *trans* dibenzalacetone has a maximum absorbance value of 330 nm and a melting point of 110-111 ^{o}C [26]

Dibenzylideneacetone is used as a sunscreen component and as a ligand in organometallic chemistry. It's a bright yellow solid insoluble in water but soluble in ethanol. Sunlight causes the compound to be converted to a cyclobutene mixture in a [2+2] cycloaddition[27].

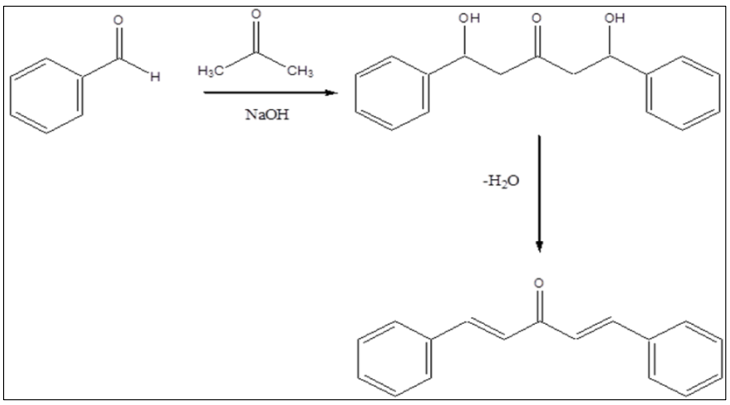


Figure 5 Aldol Condensation Reaction between Benzaldehyde and Acetone Forming Dibenzalaceton

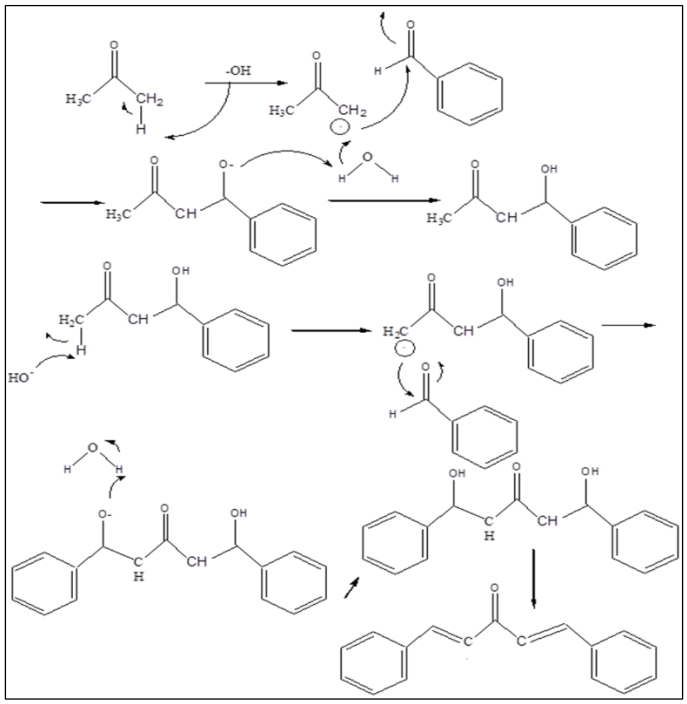


Figure 6 Mechanism involved in the synthesis of dibenzalacetone

Antimicrobial activity of Dibenzalacetone was investigated using the Disk Diffusion Assay. Here the diameter of Zone of Inhibition, DZOI and Area of Zone of Inhibition, AZOI are indicative of the compound anti-microbial potency. The larger

the DZOI and AZOI, greater is the Antimicrobial activity. The results indicates that *Staphylococcus aureus* was the most susceptible organism, inducing zero zone of Inhibition. *Klebsiella pneumoniae, Eschericia coli, Pseudomonas aeruginosa, Candida albicans* were all susceptible pathogens, with the highest average AZOI of 49.6 mm^2 induced by *Klebsiella. Pneumoniae* and the lowest AZOI induced by *Eschericia.coli*. The order of susceptibility of pathogen to dibenzalacetone follows the sequence: *Klebsiella Pneumoniae > Candida albicans > Pseudomonas aeruginosa > Eschericia coli*, Table 1.0.

The reference compound, Ampicillin showed significantly larger diameter and area of zone of inhibition than that of dibenzalacetone against all pathogens, with the exception against *Pseudomonas aeruginosa* and *Candida albicans*, Table 2.0. The latter was highly resistant against dibenzalacetone, inducing zero zone of inhibition. The AZOI range from 0.00 mm^2 to 579.07 mm^2. The reference compound, Ketoconazole, an antifungal agent showed zone of inhibition against all pathogens. However, was more effective against *Candida albicans* with a zone of Inhibition of 577.42 mm^2,Table 3.0. Fig. 3.0 shows the comparative antimicrobial disc diffusion assay of dibenzalacetone versus bacterial standard ampicillin and reference standard nystatin. The corresponding graph is shown in Fig. 4.0.

A one-way ANOVA analysis was carried out on the data to compare the AZOI of Dibenzalacetone vs. Ampicillin and Dibenzalacetone vs. Ketoconazole and it was found that the. The P value for both ANOVA tests (Dibenzalacetone vs. Ampicillin & Dibenzalacetone vs. Ketoconazole) were less than 0.5, i.e F statistic value was higher than the F critical value for both "Dibenzalacetone vs. Ampicillin" and for "Dibenzalacetone vs. Ketoconazole", indicating that AZOI induced by Dibenzalacetone is not comparable with that of either Ampicillin or Ketoconazole. In addition, the Null hypothesis is rejected. The *Null Hypothesis: Dibenzalacetone will not show significant antimicrobial activity against the four human pathogenic microorganisms: Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginiosa, and the fungus Candida albicans.*

5. Conclusion

Dibenzalacetone displayed antimicrobial activity against three human pathogenic microorganisms: *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginiosa*, and the fungus *Candida albicans*, with the most potent antimicrobial activity being against *Candida albicans* followed closely by *Klebsiella pneumoniae*. Dibenzalacetone showed no antimicrobial activity against *Staphylococcus aureus.* Thus, Dibenzalacetone appears to be antifungal in nature.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Both authors declare that there is no conflict of interest in conducting the experiment

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