Many synthesis compounds have been shown to possess bioactivity and several drugs based on antimicrobial complexes have been developed. There has been considerable interest in antimicrobial activity, in recent years, because of their potential applications. Recently, the search for novel antifungal and antibacterial compounds has received special attention as a result of an enhanced microbial resistance to current pesticides (Quiñones et al., 2000). In medicine, QACs such as benzalkonium chloride (BAC) and cetlypyridinium chloride (CPC) are still broadly used as antibiotics. The synthesis and antimicrobial activity of various QACs including imidazolium and pyrrolidinonium salts, (Demberelnyamba et al., 2004) 4,40-(a,x-polymethylenedithio) bis (1-alklypyridinium iodide) (Ohkura et al., 2005) and fluorinated bis-ammonium salts (Massi et al., 2003) are known in the literature. Synthesized 30-(3-aminopyrazolium) Cephalosporins-based QACs which show good antibacterial activity with Gram-positive and Gram-negative bacteria
While plant extracts (Rojas et al., 1992; Kandil et al., 1994) and isolated pure natural products (Batista et al., 1994; Barre et al., 1997) have been used for antimicrobial activities, there are few reports of aldols as antimicrobial agents (Silva et al., 1996; Pernak et al., 2004). Furthermore, antibacterial activity of the carbazolophanes 1-6b was assayed against Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi and Staphylococcus aureus at different concentrations (Anthonysamy et al., 2008).

This present study outlines the antimicrobial activity of \( \alpha, \beta \)-unsaturated carbonyl compound (aldols) via Agar diffusion and Poison plate techniques. Aldols (1) and (2) were obtained by base catalysed condensation of acetophenone with benzaldehyde and salicylaldehyde with acetophenone respectively. After work up, compounds (1) and (2) were obtained as a yellow and red solids in 85 and 92% yield respectively. The synthesis and antimicrobial activity under in vitro conditions are reported in the present investigation.

**Materials and Methods**

**Reagents and materials**

Aldols were synthesized and solvents were purchased from Sigma-Aldrich. The antibiotic Ciprofloxacin, Mueller Hinton agar, agar plates and microbial discs were purchased from the International Pharmacy Association in Guyana. Aldols were made up to the appropriate concentration at 1mg/mL of dichloromethane in a 25 ml round bottom flask and were stored under aseptic conditions.

**Antibacterial activity assay**

The human pathogenic bacteria *Staphylococcus aureus* ATCC 25923 was used in this study and which was obtained from the microbiology laboratory at John's campus, University of Guyana, Berbice, South America. The strain was maintained on Nutrient Agar (NA) consisting of the following (Beef extract 1.0 g; Yeast extract 2.0 g; Peptone 5.0 g; NaCl 5.0 g; Agar 15.0 g; Distilled H\(_2\)O 1000 mL; pH 7.2.) in slants or Petriplates at room temperature (28 ± 2°C). The antibacterial activity of the aldols compounds against human pathogen was evaluated by the agar diffusion method (Huang and Hoes, 1976). About 1 ml of inoculum of each test pathogen was added to the molten Nutrient Agar (NA) medium and poured into sterile Petriplates under aseptic conditions. After solidification, a 5-mm well was made in the centre of each plate using a sterile cork borer. Each compound was dissolved in 10% DMSO to get different concentrations and filter sterilized using 0.22µ filter paper. Each well received 50 µl solution of each compound and the plates were incubated at room temperature. Filter sterilized DMSO (10%) was used as control. After 48 h, the appearance of inhibition zone around the well was observed.

**Antifungal activity assay**

The human pathogenic fungi *Candida albicans* ATCC 1023 used in this study was obtained from the the microbiology laboratory at John's campus, John's, University of Guyana, Berbice, South America. The biological screening of aldols unsaturated carbonyl compound was performed there itself. The pathogens of *Candida albicans* ATCC 1023 was maintained on potato dextrose agar (PDA) containing the following (Potato 200 g; Dextrose 20 g; Agar 15 g; Distilled H\(_2\)O; 1 L; pH 6.5) in slants or Petriplates at room temperature (28 ± 2°C). Effect of unsaturated carbonyl compound on the growth of human pathogenic fungi minimum inhibitory

![Fig. 1. \( \alpha, \beta \)- unsaturated carbonyl compounds synthesized and predicted structure](image)

concentration (MIC) was determined for unsaturated carbonyl compound by Poison plate assay (Hoang and Hoes, 1976). The compound concentration range of 5-100 mg/ml in 10% DMSO was used in this study with carbendazim as positive control. Minimum inhibitory concentration (MIC) value was taken as the lowest concentration of unsaturated carbonyl compound.

RESULTS

The synthesis compound structures were elucidated by $^1$H NMR, $^{13}$C NMR, DEPT-135 and IR. The structure was predicted by the analysis of spectroscopic techniques and the compound name is Aldols 1 and 2 (Fig 1).

Table 1. Effect of $\alpha$, $\beta$-unsaturated carbonyl compounds (Aldols) (1 mg/ml) on Random check, Diffusion Plate and Poison Plate using Candida albicans

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Random check</th>
<th>Poison Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Random check</td>
<td>Area of inhibition (mm²)</td>
</tr>
<tr>
<td></td>
<td>ED$_{50}$</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.5 mm±1.56</td>
<td>132.67±3.02</td>
</tr>
<tr>
<td>2</td>
<td>7.7 mm±1.08</td>
<td>186.34±2.12</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8.5 mm±1.76</td>
<td>198.40±1.89</td>
</tr>
</tbody>
</table>

Table 2. Effect of $\alpha$, $\beta$-unsaturated carbonyl compounds (Aldols) (1 mg/ml) on Random check, Diffusion Plate and Poison Plate using Staphylococcus aureus

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Random check</th>
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<tr>
<td>2</td>
<td>7.7 mm±1.45</td>
<td>186.17±1.95</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8.1 mm±1.08</td>
<td>197.15±1.27</td>
</tr>
</tbody>
</table>

Values are mean of three replicates with ± standard deviation at *P<0.05 and **P<0.01, respectively.
Effect of Aldols compounds on *Candida albicans*

We used the synthesis compounds Aldols 1 and 2 to check antimicrobial activity. The results show compound 2 had more antifungal activity than compound 1 for the random, diffusion and poison plate techniques (Table 1).

**DISCUSSION**

Compounds (1) and (2) are Aldols and were synthesised through the base catalysed condensation of acetophenone with benzaldehyde and salicylaldehyde with acetophenone respectively. Antimicrobial activities of both compounds were investigated using the Agar disc/well diffusion and poison plate methods. The zone of inhibition (mm) is quoted at the ED50 value and as the area of inhibition (mm2). ED50 is the effective dose concentration of the sample required to kill 50% of the pathogen growth. The zone of inhibition in mm at ED50 was calculated and converted into area of inhibition, mm2. First, a random check of both compounds antimicrobial activity was investigated using both methods. Table 1 was followed with careful microbial triplicate experiments for both techniques. Results indicate under similar aseptic conditions, samples (1) and (2) induce a larger zone of inhibition against the fungus, *Candida albicans* than against the bacteria, *Staphylococcus aureus* for both diffusion and poison plate methods (Jagessar and Mohamed, 2006) For instance, for the diffusion plate method, sample 1 induce zone of inhibition of 63.6 mm2 (ED50= 4.5 mm) compared with a zone of inhibition of 32.2 mm2 (ED50 = 32.15 mm2) against the fungal and bacterial species respectively.

In comparison, for the poison plate, sample 2 induce zone of inhibition of 132.67 mm2 and 36.29 mm2 for fungal and bacterial species respectively. It is noteworthy that a smaller zone of inhibition was observed against the bacterial species in comparison to the fungal species. Thus, both Aldols are more antifungal in activity as contrary to their bactericidal effects. The larger zone of inhibition observed for compound 2 in both cases may be attributed to the hydroxyl substituent attached to the phenyl ring at position 2. The carbonyl compound has already reported in plant pathenges. The toxicity of the ω, β unsaturated carbonyl compounds (ω, β UCCs) (patulin, penicillic acid, parasorbic acid, tulipalin and plumbagin) towards *Pythium* sp. (Jagessar et al., 2007) group F was neutralized by the addition of an excess of cysteine. This suggests that the mode of action of these compounds could be due to a binding of the ω, β UCCs to sulphhydryl groups in enzymes or other macromolecules. Alcohol dehydrogenase (ADH), an enzyme with a sulphhydryl group at the active site, was assayed spectrophotometrically and all the, β UCCs inhibited ADH (Larsen and Olson, 2008). The hydroxyl substituent may further augment the
disintegration or disruption of the bacterial and fungal cell wall. Similarly Kondo et al. (1990) reported that Streptovitacin A synthetic compounds showed moderate growth inhibition against fungi and lettuce seeds.

The results revealed that both antibacterial and anti fungal studies, compound (2) is the more active or potent. This indicates that the presence of the hydroxyl group on phenyl ring of compound 2 might be responsible. Thus, future design of antibiotic and antifungal drugs can take this structure activity relationship into consideration.

In conclusion, of these tested microbes, the fungi Candida albicans highly inhibited by both Aldols compounds. Furthermore, Aldols-2 had potential inhibition than Aldols-1 for both fungal and bacteria. However, further studies are required to determine their potential against a wide range of human pathogens and its mode of action. Syntheses of other similar dicationic carbazolophanes and their antibacterial activity as well as molecular recognition towards various biologically important anions are under investigation.

ACKNOWLEDGEMENTS

This research was financially supported by a small grant to Dr. R.C. Jagessar from the Royal Society of England and the University of Guyana, Turkeyen. The use of the Microbiology Laboratory at John’s Science Centre, Berbice Campus, University of Guyana is acknowledged.

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