Research Paper

Determination of minimum inhibitory concentrations of 2-(2-nitroviny1) furan

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ABSTRACT

The minimum inhibitory concentration of the synthesized compound 2-(2-Nitroviny1) Furan was determined due to its potency towards some pathogens. The compound inhibited all the fungal isolates used in the research at concentrations of $5 \times 10^{-1}$ mg/ml and $5 \times 10^{-2}$ mg/ml respectively. It was also active towards Rhizoctonia solani and Drechslera oryzae at concentration of $5 \times 10^{-3}$ mg/ml but only D. oryzae at concentration of $5 \times 10^{-2}$ mg/ml. The activity of the compound diminished with decrease in concentration. The inhibition zones for 2-(2-Nitroviny1) Furan are 15 mm (Bacillus cereus), 17.5 mm (Shigella dysenteriae), 19 mm (Bacillus subtilis), 19 mm (Pseudomonas syringae), 19 mm (Pseudomonas fluorescens), 20 mm (Staphylococcus epidermidis), 20 mm (Xanthomonas manihotii), 19 mm (Candida albican) and 20 mm (Geotrichum albidus) respectively. The results clearly indicated that among the concentrations ($5 \times 10^{-1}$ to $5 \times 10^{-6}$ mg/ml) used for the study, the MIC of 2-(2-Nitroviny1) Furan at $5 \times 10^{-2}$ mg/ml and $5 \times 10^{-2}$ mg/ml were the most effective concentrations and as well the minimum inhibitory concentrations. 2-(2-Nitroviny1) Furan is a very good antimicrobial agent that would be very useful in medical and agrochemical industries.

Key words: 2-(2-Nitroviny1) Furan, minimum inhibitory concentration, pathogens, potency.

INTRODUCTION

The discovery of the resistance of microorganisms against antibiotics is an increasingly problematic clinical concern. This has brought about the search for new antimicrobial substances that led to the discovery of nitroviny1furan and its derivatives.

The compound, 2-(2-Nitroviny1) Furan is a lipid soluble substance with molecular formula C$_6$H$_5$NO$_3$, an average mass of 139.108795 Da and a boiling point of 190°C (RSC Database, 2013). It is a derivative of furan (heterocyclic) that has been discovered to have antimicrobial properties, against skin related microbial infections.

The in vitro activity of the 2-(2-Nitroviny1) Furan was tested against trypomastigotes of trypanosome cruzi grown in a rat myoblast cell line. Minimal parasiticidal concentration and mean inhibitory concentrations were determined according to Buckner’s method. The result showed that the test compounds had an in vitro parasiticidal activity superior to that of benznidazole, which proves its antitrypanosomaisis activity (Buckner et al., 1996).

Minimum inhibitory concentration (MIC) determination is important to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents (Andrews, 2001; Turnidge, et al., 2003). Fungi cause significant qualitative and quantitative loss of stored food stuffs rendering them unfit for human consumption by altering their nutritive value and producing mycotoxins. Consequently, about five billion people in developing countries are at the risk of chronic exposure to aflatoxins through contaminated foods (Shephard, 2003; Williams et al., 2004). The work at hand therefore focuses on the determination of minimum inhibitory concentration of the synthesized nitro-compound that is, 2-(2-Nitroviny1) Furan in order to guide whoever that wishes to incorporate it as an active ingredient in their products.
MATERIALS AND METHODS

Extraction of Furfural from Sugar cane bagasse

2-(2-Nitrovinyl) Furan was synthesized at Department of Chemical Sciences, Fountain University, Osogbo. Furfural was extracted from sugar cane bagasse using sulphuric acid (H2SO4) and sodium chloride through distillation process. The distillate was neutralized with sodium hydroxide and the furfural was separated with chloroform using separating funnel (Hoydonckx et al., 2007).

Condensation of Furfural with Nitromethane

Knoevenagel condensation method with sodium butoxide as the base was utilized (Tietze et al., 2005). Sodium metal (0.13 g) was first reacted with normal butanol (20 ml) in a round bottom flask; then nitromethane (0.01 mol) and freshly distilled furfural (0.01 mol) were added which was shaken for 3 min and the reaction was stopped immediately with diluted hydrochloric acid (1:1), thus the crystal was filtered.

Microbial cultures

A combination of bacteria, filamentous fungi and yeast obtained from Phytopathology Section of Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Ondo state were used for the analysis. The bacteria include Bacillus subtilis, Bacillus cereus, Pseudomonas syringae, Pseudomonas fluorescens, Staphylococcus epidemidis, Xanthomonas manihoti and Shigella dysenteriae. The filamentous fungi include Fusarium solani, Sclerotium rolfsii, Rhizoctonia solani, Pyricularia oryzae, Drechslera oryzae while the yeast comprises of Candida albican and Geotrichum albids. Cultures were tested for purity by standard microbiological methods. The bacterial and fungal cultures were maintained on nutrient agar and potato dextrose agar slants at 4°C for further analysis.

Preparation of the diluent

Stock solution (solution A) of the compound was prepared by dissolving 0.5 g of the crystal in 10 ml of distilled water. Serial dilution of the stock solution was done to obtain solutions B, C, D, E and F respectively.

Determination of antibacterial activity

The antimicrobial activity of diluents (5×10⁻¹ to 5×10⁻⁶) was analyzed using the agar well diffusion method according to Murray et al. (2004). The Nutrient agar (NA) was poured onto the Petri plates with an inoculum size of 10⁶ colony forming units (cfu)/ml of bacteria. The wells were made in the NA plates using borer, with a diameter of 6 mm. Different extract concentrations between 5×10⁻¹ to 5×10⁻⁶ with streptomycin as positive control were dispensed into the wells, allowed to diffuse for 45 min and incubated at 37°C for 24 h. The analysis was carried out in triplicate and the sensitivity of the microbial species to the extract was determined by measuring the diameter of the inhibitory zones around the wells (including the diameter of the well).

Determination of antifungal activity

2- (2-Nitrovinyl) Furan diluents of concentrations 5×10⁻¹ to 5×10⁻⁶ with Benlate (0.05 mg/ml) as positive control and distilled water as negative control were mixed with sterile molten potato dextrose agar (PDA) using pour plate method. The poured plates were inoculated with selected fungal disc (5 mm). PDA plates were incubated at 27°C for 7 days and the mycelial inhibition was determined using the following formula (Albuquerque et al., 2006):

\[
\text{Percentage of mycelial inhibition} = \frac{dc - dt}{dc} \times 100
\]

Where dc = average diameter of fungal colony in control sets, dt = average diameter of fungal colony in treated sets.

Statistical analysis

Results calculated from triplicate data were expressed as means±standard deviations. The data was compared by least significant difference test using Statistical Analysis System (ver. 9.1).

RESULTS AND DISCUSSION

Acid hydrolyses of hemicelluloses from sugar cane bagasse to xylose led to the synthesis of five-member ring furfural by removing molecules of water (Figure 1). The purity of the furfural extracted was identified by thin layer chromatography and its boiling point (160°C). The retention factor (Rf = 0.5) was similar to that of furfural collected from the Department of Chemistry, Federal University of Technology, Akure, Nigeria. The nuclear magnetic resonance spectrum (NMR) of 2-(2-Nitrovinyl) Furan is shown on Figure 2.

A yellow crystal with retention factor (Rf) 0.88 that melted at 70°C precipitated out when distilled water was added after stopping the reaction with diluted HCl (Figure 1). The nuclear magnetic resonance spectrum also confirmed the result with the 2 methylene substituents...
appearing at δ 6.5 and δ 6.9 respectively and the furan ring appeared between δ 7.5 and δ 7.8 (Figure 2). The compound has shown good activity towards all the fungal isolates at concentrations of $5\times10^{-1}$ mg/ml and $5\times10^{-2}$ mg/ml respectively (Table 1). It was also active towards *R. solani* and *D. oryzae* at a concentration of $5\times10^{-3}$ mg/ml but only towards *D. oryzae* at a concentration of $5\times10^{-2}$ mg/ml. The activity of the compound diminished with decrease in
Table 1. Zones of inhibition of selected fungi isolates.

<table>
<thead>
<tr>
<th>Concentrations of 2-(2-Nitrovinyl) Furan (mg/ml)</th>
<th>Percentage mycelial growth inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sr</td>
</tr>
<tr>
<td>Solution A (0.5)</td>
<td>100.00</td>
</tr>
<tr>
<td>Solution B (0.05)</td>
<td>64.00</td>
</tr>
<tr>
<td>Solution C (0.005)</td>
<td>5.06</td>
</tr>
<tr>
<td>Solution D (0.0005)</td>
<td>2.53</td>
</tr>
<tr>
<td>Solution E (0.00005)</td>
<td>2.53</td>
</tr>
<tr>
<td>Solution F (0.00005)</td>
<td>NI</td>
</tr>
<tr>
<td>Benlate</td>
<td>NI</td>
</tr>
</tbody>
</table>

Legend: Sr = Sclerotium rolfsii, Rs = Rhizoctonia solani, Po = Pyricularia oryzae, Do = Drechslera oryzae, Fs = Fusarium solani, NI = No inhibition.

Table 2. Zones of inhibition of selected yeast and bacteria isolates.

<table>
<thead>
<tr>
<th>Concentrations of 2-(2-Nitrovinyl) Furan (mg/ml)</th>
<th>Zones of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ps</td>
</tr>
<tr>
<td>Solution A (0.5)</td>
<td>19</td>
</tr>
<tr>
<td>Solution B (0.05)</td>
<td>13</td>
</tr>
<tr>
<td>Solution C (0.005)</td>
<td>NI</td>
</tr>
<tr>
<td>Solution D (0.0005)</td>
<td>NI</td>
</tr>
<tr>
<td>Solution E (0.00005)</td>
<td>NI</td>
</tr>
<tr>
<td>Solution F (0.00005)</td>
<td>NI</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>NI</td>
</tr>
</tbody>
</table>

Legend: Ps=Pseudomonas syringae, Xm = Xanthomonas manihoti, Bs = Bacillus subtilis, Bc = Bacillus cereus, Se = Staphylococcus epidemidis, Sd = Shigella dysenteriae, Pf = Pseudomonas fluorescens, Ca = Candida albicans, Ga = Geotrichus albidum, NI = no inhibition.

Concentration. Kalembo and Kunicka (2003) reported that aromatic plants have been widely used in folk medicine because of their properties due to presence of volatile oils and have been empirically used as antimicrobial agents. In recent years, there has been a growing interest in Knoevenagel condensation products because of their significant biological activity (Tietze et al., 2005) just like the compound in question. The inhibition zones for 2-(2-Nitrovinyl) Furan are 15 mm (B. cereus), 17.5 mm (S. dysenteriae), 19 mm (B. subtilis), 19 mm (P. syringae), 19 mm (P. fluorescens), 20 mm (S. epidemidis), 20 mm (X. manihoti), 19 mm (C. albicans) and 20 mm (G. albidus) respectively (Table 2). The results clearly indicated that among the concentrations (5×10⁻¹ to 5×10⁻⁶ mg/ml) used for the study, the MIC of 2-(2-Nitrovinyl) Furan at 5×10⁻¹ mg/ml was excellent compared to the standards. Furfural and its 5-substituted derivatives were chosen as being synthetically versatile molecules with a reactive carbonyl group and significance for their biological activities (Rabarova et al., 2004). The antimicrobial activity of nitroaromatic compounds is related to an enzymatic reduction of the nitro group in vivo, yielding toxic species. Nitro compounds are reductively metabolized to form highly potent cytoxins and also act as radiosensitizers of hypoxic cells (Adams et al., 1990). Despite its rare occurrence in natural products, the nitro group is present in various synthetic substances like the compound in question 2-(2-Nitrovinyl) Furan that are used as antibacterial, antiparasitic, trypanocides, fungicides, vasodilators, tranquillizers and antiviral (Naylor et al., 1990).

Conclusion

2-(2-Nitrovinyl) Furan as antimicrobial agent would be useful in medical and agrochemical for the production of fungicide, fumigant and bactericide due to its ability to inhibit microbial growth at concentrations compared to the standard antimicrobial agents used.

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REFERENCES


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