RESEARCH PAPER

Antibacterial And Antifungal Activities of The Oxidised Product Of 3-Methylindole By Potassium Bromate

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Abstract

The kinetic studies of oxidation of 3-Methylindole (3-MI) by potassium bromate ($KBrO_3$) have been carried out in ethanol medium. The final product was identified as 3-methyloxindole by IR and NMR spectral studies and then examined for biological activity. The antibacterial activity of the synthesized product was determined by agar diffusion method using nutrient agar medium. The antifungal activity of the synthesized product was evaluated by agar diffusion method using potato dextrose agar.

Keywords: Antibacterial activity, Antifungal activity, 3-MI, Oxidation, Potassium bromate

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Introduction

Indoles are important precursors for other substances made within the human body used in lifestyle and medical applications. The oxidation of 2,3-dimethylindole by peroxodisulphate anions (PDS) to give 3-methylindole-3-carbaldehyde have been already reported in the literature 1. Oxidation of indoles has received much attention due to the involvement of their resulting products in significant biological processes 2. Peroxomonosulfuric acid a derivative of hydrogen peroxide when one of the hydrogens of the latter is replaced by sulfuric acid group.3-5 The oxidation of indole into isatin using PMS 6 in acetonitrile medium and oxidation of indole-3-acetic acid (IAA) into 2-hydroxy indole-3-methanol 7 has been reported in the literature. Studies on the oxidation of organic compounds and inorganic complexes by peroxodisulfate have been reported 8-9. The main objectives of the present study are to ascertain the reactive species of the substrate and oxidant elucidate a plausible mechanism, identify the oxidation products and evaluate the kinetic and biological activity of the product.

Potassium bromate has been used as an oxidant in acidic media 10-11. The product of bromate oxidation is bromide which can be safely recycled, thus making bromate oxidations environmentally being compared to metal ion oxidations. Although bromate itself a strong oxidizing agent, having a redox potential of 1.45V 12. Bromate oxidations sometimes even involve oscillation reactions 13-15. Hence, the chemistry of bromate ion in an aqueous acid medium is of considerable interest, given its importance in mechanistic chemistry.[16]

Materials and Methods

Prepation of 3-methyl-2-oxaindole:

Oxidation of 3-MI was done by mixing $3X10^{-3}$ mol/dm^3 of 3-MI, $2x10^{-2}$ mol/dm^3 of potassium bromate, 0.1 H_2SO_4 and calculated quanities of sodium perchlorate, mercuric acetate, acetic acid were added in a reaction vessel and kept it at room temperature for 24 hours and the reaction mixture was poured on ice cold water and recrystallised using ethanol water mixture. The final product was analysed by the spectral studies.

Preparation of 24 hours broth culture:

The nutrient broth medium was prepared and 5 ml broth was transferred to each sterile test tube, closed with cotton plug. All the test tubes were then sterilized at 121˚C for 15 min, 15 lbs in autoclave. A loop full of bacterial stock culture was inoculated to appropriate labelled tube. Then the tubes were incubated at 370˚C for 24 hours. The Sabouraud's dextrose broth medium was prepared and 5 ml broth was transferred to each sterile test tube, closed with cotton plug. All the test tubes were then sterilized at 121˚C for 15 min, 15 lbs in autoclave. A loop full of fungal stock culture was inoculated to appropriate labelled tube. Then the tubes were incubated at room temperature for 48 hours.

In vitro evaluation of antibacterial activity:

The antibacterial activity of synthesized product was evaluated by agar diffusion method using nutrient agar. The 24 hour broth cultures of the test organisms were used for the assay. A sterile cotton swab was dipped into the standardized bacterial culture. The swab was evenly inoculated in three directions over the entire surface of the agar plate to obtain the uniform inoculums. Wells were made on the seeded plates using a sterile borer. The plates were allowed to dry for 3-5 minutes after which 30 µl of the extracts at various concentrations and the controls were dispensed into each well using micropipette. The plates were incubated at 37˚C for 24 hours. The zone of inhibition surrounding the well was measured and compared with control. Ampicillin was used as a reference antibacterial agent. A set assay medium containing only inoculated medium was kept as negative control and likewise solvent controls were also done simultaneously.

In vitro evaluation of antifungal activity:

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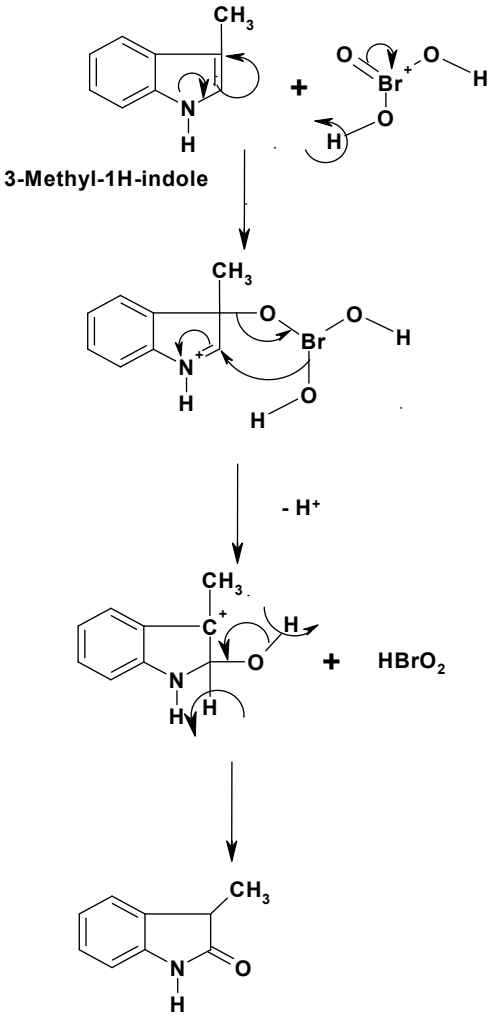
The antifungal activity of the synthesized product was evaluated by agar diffusion method using potato dextrose agar. The 48 hour broth cultures of the test organisms were used for the assay. A sterile cotton swab was dipped into the stock fungal culture. The swab was evenly inoculated in three directions over the entire surface of the agar plate to obtain the uniform inoculums. Wells were made on the seeded plates using a sterile borer. The plates were allowed to dry for 3-5 minutes after which 30 µl of the extracts at various concentrations and the controls were dispensed into each well using micropipette. The plates were incubated at room temperature for 48 hours. The zone of inhibition surrounding the well was measured and compared with control. Amphotericin B was used as a reference antifungal agent. A set assay medium containing only inoculated medium was kept as negative control and likewise solvent controls were also done simultaneously.

Collection of clinical pathogens:

Bacteria as Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Bacillus subtilis and fungi as Candida albicans, Aspergillus flavus, Aspergillus niger and Aspergillus oryzae were the microorganisms used and they were obtained from the Microbiology Laboratory of the Thanjavur Medical College Hospital, Thanjavur. These microorganisms were identified and confirmed by Microbiologists, Department of Microbiology, Thanjavur Medical College, Thanjavur.

Product analysis:

The final product obtained was isolated and characterised. To the solution of 3-MI in ethanol (0.06 mol dm^{-3}) was added to $KBrO_3$ (0.125 mol dm^{-3}) in water. The $[H^+]$ and percentage of acetic acid was maintained as in the regular kinetic runs. The reaction mixture was kept aside at room temperature for a day, so that the substrate was completely converted into product. The reaction mixture was poured into ice cold water. A solid mass obtained was filtered and washed several times with doubly distilled water and dried. The product was identified as 3-methyl-2-oxaindole and it is confirmed from its HNMR and FT-IR spectra. FT-IR (KBr) 3406, 1714 and 1675 cm^{-1}, 1H NMR (DMSO) ppm = 6-8 (m, 5H, ArH, NH), 3.3 (s, 3H, C-H).The mechanism of the product formed is as follows.



Measurement of zone of inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the sample were measured using a millimeter scale. The diameter sizes in mm of the zone of inhibition are shown in the table 1 and 2

It has been observed that the product 3-mrthyl-2-oxaindole exhibits inhibitory activity against the fungal pathogen Aspergillus flavus, Aspergillus niger and Candida albicans in figure 1 and table 1. The zone of inhibition is found to increase on increasing the concentration. The product also shows inhibitory activity against the bacterial pathogen Staphylococcus aureus in figure 2 and table 2.

Table.1 Antifungal activity of sample: Zone of inhibition

S. No.	Name of the Atifungal pathogen	Zone of inhibition at different concentration of 3-MI (mm)			
		50 µg/ml	100 µg/ml	150 µg/ml	Standard (30 µl) (Nystatin)
1.	Candida albicans(mm)	6 ± 0.02	9 ± 0.04	14 ± 0.08	9 ± 0.04
2.	Aspergillus oryzae (mm)	3 ± 0.02	6 ± 0.01	18 ± 0.09	19 ± 0.01
3.	Aspergillus niger (mm)	4 ± 0.04	7 ± 0.05	17 ± 0.06	14 ± 0.06
4.	Aspergillus flavus (mm)	3 ± 0.04	8 ± 0.08	19 ± 0.01	19 ± 0.05

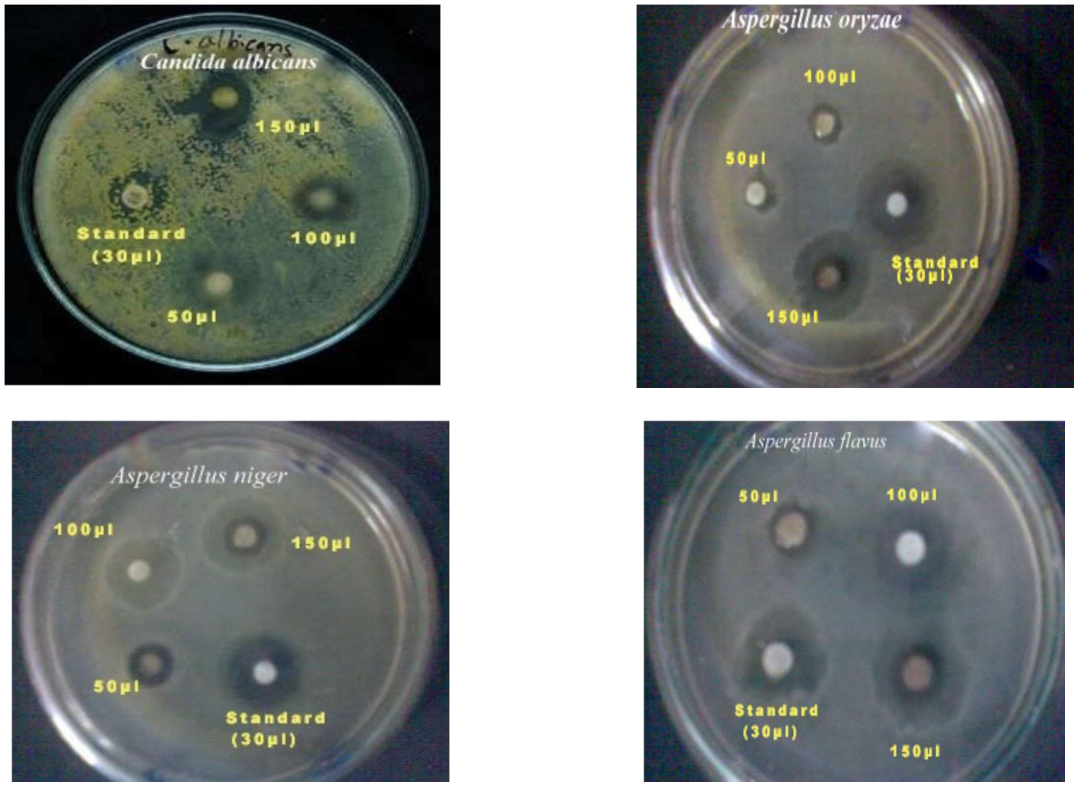


Figure 1: Antifungal activity of oxidised product of 3-MI

Table.2 Antibacterial activity of sample: Zone of inhibition

S. No.	Name of the Atibacterial pathogen	Zone of inhibition at different concentration of 3-MI (mm)			
		50 µg/ml	100 µg/ml	150 µg/ml	Standard (30 µl) (Chloromphenical)
1.	Bacillus subtilis (mm)	-	5 ± 0.01	8 ± 0.04	11 ± 0.01
2.	Klebsiella pneumonia (mm)	3 ± 0.04	8 ± 0.03	14 ± 0.06	10 ± 0.05
3.	Staphylococcus auerus (mm)	2 ± 0.05	5 ± 0.07	14 ± 0.08	12 ± 0.01
4.	Escherichia Coli (mm)	-	4 ± 0.04	7 ± 0.04	7 ± 0.05

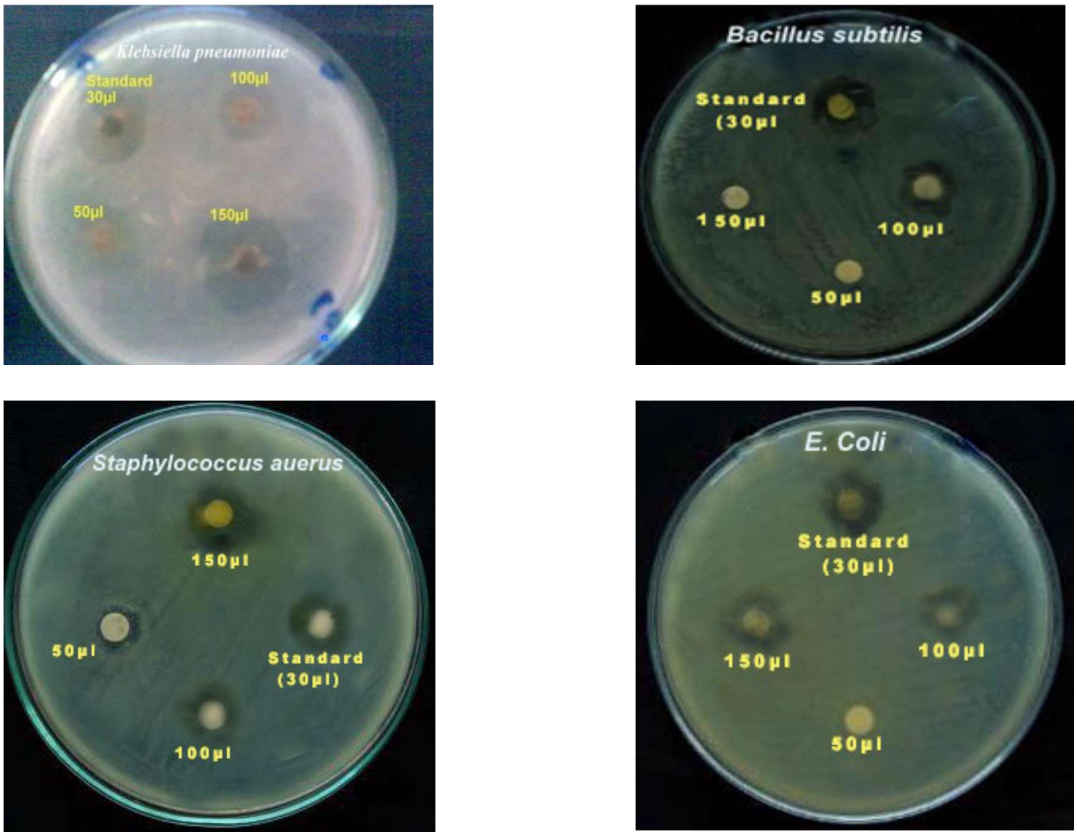


Figure 2: Antibacterial activity of oxidised product of 3-MI

Conclusion

Oxidising agents such as potassium bromate provide strengthening of dough during the manufacture of yeast-leavened products. As a result, oxidising agents are used to provide greater loaf volume, enhance symmetry and to maintain the quality of yeast-leavened product. It has been observed that the product 3-methyl-2-oxindole shows good inhibitory activity against the fungal pathogen Aspergillus flavus, Aspergillus niger and Candida albicans than the standard Nystatin. The product also shows good inhibitory activity against the bacterial pathogen Staphylococcus auerus than the standard Chloromphenical and shows same inhibitory activity against Escherichia coli. We hope that our discussion will prove helpful in further development and progress of the pharmacological applications of our oxidized product.

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