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SPECTRAL AND PHARMACOLOGICAL ACTIVITY OF 3, 5-DIMETHYL PYRAZOLIUM CHLOROCHROMATE AND PHENOXYACETIC ACID

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ABSTRACT

This study presents the empirical findings related to the preparation and antimicrobial efficacy of two specific compounds, namely phenoxyacetic acid and 3, 5- dimethyl pyrazolium chlorochromate. The structural identification of these compounds was accomplished by analyzing their spectral data. Subsequently, the antibacterial and antifungal properties of these compounds were evaluated against various microorganisms including *Bacillus Subtilis, Staphylococcus Aureus, Micrococcus, Enterococcus Faecalis, Klebsiella Pneumoniae, Salmonella, Enterobacter, Moraxella, Aspergillus Flavus, Mucor, Trichophyton,* and *Microsporum* using the disc diffusion technique.

Keywords: 3, 5- Dimethyl Pyrazolium Chlorochromate, Phenoxyacetic acid, Spectral Data, Disc Diffusion Method, Biological Activity.

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INTRODUCTION

The intrinsic molecular architecture of the fundamental form of heterocyclic scaffold possesses profound implications for a diverse array of pharmacological and biological applications.[1-3] The countless novel medications are being created with the aid of methodologies based on heterocyclic and non-heterocyclic system.[4-7] The ability of these heterocyclic compounds plays a critical role in determining their kinetic properties and behavior of various organic substrates.[8-17] The utilization of antimicrobial medications has played a crucial role in safeguarding human lives against the detrimental effects of pathogenic bacteria and fungi, thereby mitigating the prevalence of infectious diseases.[18-19] Pharmacists are tasked with formulating a diverse array of antimicrobial agents to combat the dissemination of these infectious ailments effectively. By the comprehensive analysis of existing literature, we have identified two specific compounds, namely 3, 5- dimethyl pyrazolium chlorochromate (DmpzHCC), and phenoxyacetic acid (PAA), which have been prepared and evaluated for their biological efficacy in contradiction of a spectrum of positive-gram and negative-gram bacteria, along with some fungi.

EXPERIMENTAL

Materials and Methods

Sigma Aldrich chemicals occupies a pivotal function in the preparation sequence of phenoxyacetic acid, and 3, 5- dimethyl pyrazolium chlorochromate. The investigation into the melting points of these aforementioned compounds was conducted meticulously, utilizing the Thomas Hoover capillary method. Furthermore, the UV-visible spectra were methodically recorded and analyzed employing the advanced PerkinElmer Lambda 365 Instrument. The analysis of the FTIR spectra was carried out using the FTIR Bruker Alpha spectrometer, allowing for precise and accurate spectral data to be obtained. Further characterization was performed using NMR spectral data on a Bruker Avance 400 MHz FT-NMR spectrometer, which included PMR and CMR studies. Additionally, GC-MS investigation was implemented on a SHIMADZU/QP2020 Instrument.

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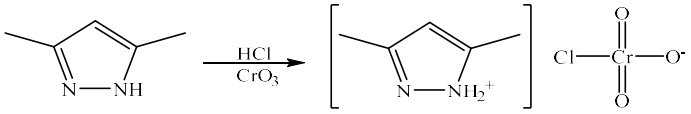
General Procedure

Preparation of 3, 5- Dimethyl Pyrazolium Chlorochromate (DmpzHCC)

One gram of chromium trioxide was dissolved in 0.5 ml of water, and subsequent introduction of 2 ml of 12 M HCl was performed with agitation at the standard ambient temperature. Consequently, there was the production of a solution displaying a vivid red-orange hue, which was then subjected to cool within an ice bath environment. It was at this juncture that the temperature of the experimental amalgamation reached the threshold of 0 ^{0}C, the portion-wise introduction of 0.96 g of 3, 5- dimethyl pyrazole over 20 minutes. Throughout an hour of continuous stirring of the reaction mixture, the formation and subsequent isolation of an orange crystalline substance occurred. This particular compound was subsequently subjected to filtration under vacuum conditions, followed by a thorough washing utilizing hexane, a drying process, and ultimately storage within an airtight container located in a freezer. The resultant compound is 3, 5- dimethyl pyrazolium chlorochromate[20] (m.p. 56 ^{0}C) (Scheme-1).

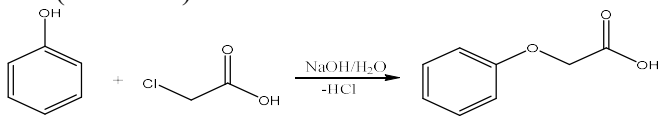


3,5- Dimethyl Pyrazole — 3, 5- Dimethyl Pyrazolium Chlorochromate

Scheme-1: Preparation of 3, 5- Dimethyl Pyrazolium Chlorochromate

Preparation of Phenoxyacetic Acid (PAA)

The mixture is comprised of 4 mL of 40% NaOH, to which 1.5g of phenol was subsequently introduced. Following this, 3 mL Chloroacetic acid was solubilized in gentle heat water, and incorporated into the aforementioned mixture, which was then continuously stirred for a duration of 1.5 h within the temperature range of 75 ^{0}C to 85 ^{0}C. Subsequently, 40% hydrochloric acid was introduced, and the resulting solution underwent vacuum fractionation utilizing C_6H_6 as the designated solvent. The resultant white solid is phenoxyacetic acid [21] (m.p. 98.6 ^{0}C). Further, it was subjected to a recrystallization process involving a mixture of ethanol and water (Scheme-2).



Phenol — Chloroacetic acid — Phenoxyacetic acid

Scheme-2: Preparation of Phenoxyacetic acid

Antimicrobial Screening of DmpzHCC and PAA

The solution of 3, 5- dimethyl pyrazolium chlorochromate and phenoxyacetic acid was exactly prepared at varying concentrations by dissolving them in a medium comprising dimethyl sulphoxide solvent. Subsequently, this solution was subjected to screening to evaluate its antibacterial and antifungal properties against the microorganisms, including *Bacillus Subtilis, Staphylococcus Aureus, Micrococcus, Enterococcus Faecalis, Klebsiella Pneumoniae, Salmonella, Enterobacter, Moraxella, Aspergillus Flavus, Mucor, Trichophyton*, and *Microsporum,* using the agar disc diffusion experiment.[22-23] All materials utilized in this experiment underwent sterilization at a temperature of 249.8^{0}F for 25 minutes under a pressure of 15 psi. The bacterial inoculums employed contained the microbial counts of 1×10^5 to 1×10^6 *CFU/ml*. After the initialization, the agar plate was subjected to an incubation period of 20 to 28 hours, maintained at a temperature of 98.6^{0}F for gram-positive and gram-negative bacteria, and at 77^{0}F for fungi. Gentamycin was employed as the standard reference, against which the inhibitory zone was meticulously examined and associated with control samples. Microbial strains utilized in this study were diligently held on nutrient agar slopes at a temperature of 39.2°F.

The outcomes of the antibacterial and antifungal screenings of 3, 5-dimethyl pyrazolium chlorochromate and phenoxyacetic acid revealed varying levels of zone inhibition in the microorganisms under examination. With an increase in the concentrations of 3, 5-dimethyl pyrazolium chlorochromate and phenoxyacetic acid from 25 µl to 100 µl, the inhibitory zones against both bacteria and fungi also expanded. Within this research, gentamycin was utilized as the standard antibiotic for comparative analysis with the aforementioned compounds.

RESULTS AND DISCUSSION

Spectral Studies of DmpzHCC

The UV-visible spectrum revealed two significant peaks: a strong peak at 227.55 nm corresponding to the 3, 5- dimethyl pyrazolium cation, and a moderate-to-broad peak at 361.65 nm indicative of the charge transfer transition within the chromate group. The IR spectrum of DmpzHCC exhibits absorption bands in the following ranges: 3227.56, 3137.68, 1602.70, 1502.01, 1384.51, 1042.75, 944.12, 825.78, and 440.58 (Fig.-1). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.286 (2H, s, H-3 and H-5), 6.344 (^{1}H, s, H-4), 2.577 (6H, s, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) = 146.91 (C-3 and C-5), 107.59 (C-4), 11.50 (CH_3). 3, 5- dimethyl pyrazolium chlorochromate, $C_5H_8N_2H[CrO_3Cl]$: The detected MS peak at (m/z) = 282 (100% abundance). Here, DmpzHCC is solvated with CH_3OH and H_2O, (calculated molecular weight = 232.5818 g/mol) (Fig.-3).

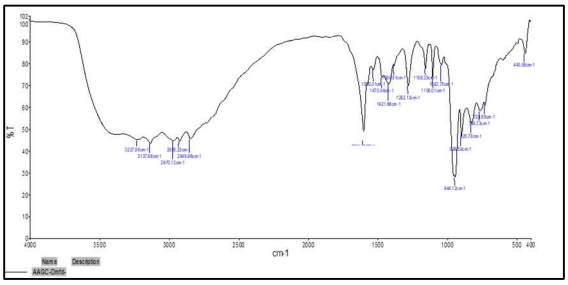


Fig.-1: Infrared Absorption Spectral Data of DmpzHCC

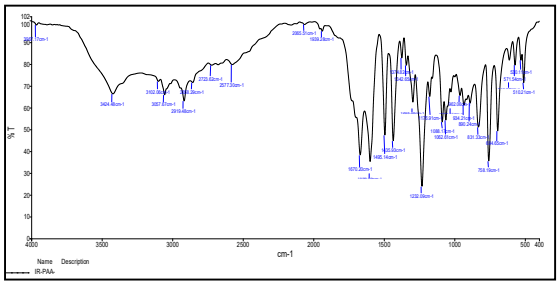


Fig.-2: Infrared Absorption Spectral Data of PAA

Spectral Studies of PAA

A prominent absorption peak was detected at 215 nm in the UV-Vis region corresponding aromatic benzene ring, and 276 nm represents the n-π* transition of the (C=O) group and aromatic ring. The IR spectral analysis of phenoxyacetic acid reveals characteristic absorption bands occurring within the following wavenumber ranges: 3424.48, 3057.67, 1670.20, 1232.09, 1062.61, 962.06, 890.24, and 694.65 (Fig.-2). The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) displays: δ (ppm) = 10.71, 10.56 (br, COOH), 7.32-7.24 (m, H-3, H-4, H-5), 6.90-7.03 (m, H-2, H-6), 4.67 (s, $-CH_2$). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) = 174.94 (-COOH), 157.43 (C-1, phenoxy), 129.72, 122.12, 114.66 (C-2 to C-6, phenoxy), 64.78 ($-CH_2$). Phenoxyacetic acid, $C_8H_8O_3$: Calculated molecular weight = 152.1578 g/mol, MS peak observed at m/z = 152 (90% abundance) (Fig.-4).

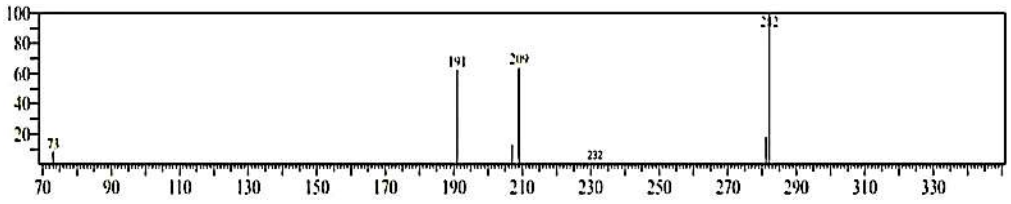


Fig.-3: Mass Spectral Data of DmpzHCC

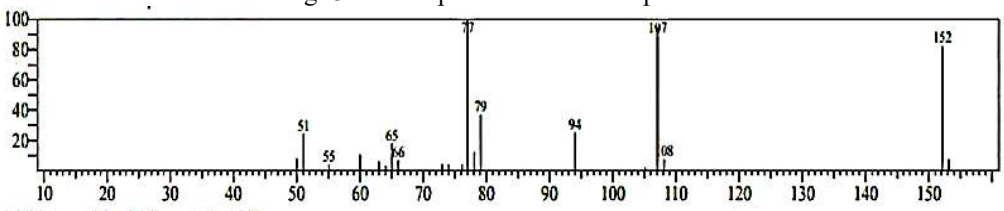


Fig.-4: Mass Spectral Data of PAA

Zone of Inhibition of DmpzHCC

The zone of inhibition values for 3, 5- dimethyl pyrazolium chlorochromate (DmpzHCC) were determined at concentrations ranging from 25 µl to 100 µl against various microorganisms (Table-1). Concerning DmpzHCC, the positive-gram bacteria, *Micrococcus, Staphylococcus Aureus,* and *Bacillus Subtilis* exhibited higher efficacy than *Enterococcus Faecalis* at 100 µl, in contrast to the standard antibiotic at the same concentration. Additionally, the gram-negative bacteria *Moraxilla, Klebsiella Pneumonia*, and

Salmonella demonstrated greater efficacy than *Enterobacter* at 100 µl compared to gentamycin at the same concentration. Furthermore, the fungal strains *Mucor, Trichophyton,* and *Microsporum* displayed increased efficiency than *Aspergillus Flavus* at 100 µl compared to the standard antibiotic at the same concentration. A comparative graph (Fig.-5) illustrates the microbial activity of 3, 5- dimethyl pyrazolium chlorochromate versus the standard control, gentamycin, at a concentration of 100 µl, highlighting the differences in antimicrobial efficacy against various microorganisms.

Table-1: Antimicrobial Activity of 3, 5- Dimethyl Pyrazolium Chlorochromate (DmpzHCC)

S. No	Organism	DMSO Extract 100µl Added and Zone of Inhibition (mm/ml)				
		25µl	50µl	75µl	100µl	Control
1	*Bacillus Subtilis*	15	20	25	30	21
2	*Staphylococcus Aureus*	15	20	24	30	20
3	*Micrococcus*	20	25	30	35	18
4	*Enterococcus Faecalis*	10	15	19	23	22
5	*Klebsiella Pneumoniae*	30	34	36	30	20
6	*Salmonella*	20	24	27	30	15
7	*Enterobacter*	10	14	17	21	25
8	*Moraxilla*	17	21	24	29	20
9	*Aspergillus Flavus*	19	13	17	20	26
10	*Mucor*	24	29	34	37	16
11	*Trichophyton*	20	23	27	30	14
12	*Microsporum*	17	20	24	28	16

Zone of Inhibition of PAA

The inhibitory effects of phenoxyacetic acid (PAA) on microorganisms were investigated by determining zone of inhibition values at concentrations spanning 25-100 µl (Table-2). Regarding phenoxyacetic acid, it was observed that the positive-gram bacteria, *Micrococcus, Staphylococcus Aureus*, and *Bacillus Subtilis* exhibited enhanced effectiveness when exposed to a dosage of 100 µl, surpassing the efficacy of the standard antibiotic gentamycin administered at the equivalent concentration. Similarly, the gram-negative bacteria *Klebsiella Pneumonia*, *Salmonella,* and *Enterobacter* displayed superior efficacy at the same 100 µl dosage compared to gentamycin at an equivalent concentration. Moreover, the fungal strains such as *Aspergillus Flavus, Mucor, Trichophyton,* and *Microsporum* showcased heightened efficiency when subjected to 100 µl of phenoxyacetic acid in comparison to the standard control at the same concentration. The antimicrobial potency of phenoxyacetic acid is compared to that of gentamycin, a standard control, at a concentration of 100 µl, in a graphical representation (Fig.-6) that highlights the variations in their inhibitory effects against diverse microbial species.

Table-2: Antimicrobial Activity of Phenoxyacetic Acid (PAA)

S. No.	Organism	DMSO Extract 100 µl Added and Zone of Inhibition (mm/ml)				
		25 µl	50 µl	75 µl	100 µl	Control
1	*Bacillus Subtilis*	15	18	22	26	21
2	*Staphylococcus Aureus*	18	24	30	33	20
3	*Micrococcus*	10	18	22	25	18
4	*Enterococcus Faecalis*	10	13	15	17	22
5	*Klebsiella Pneumoniae*	10	15	20	25	20
6	*Salmonella*	10	13	16	19	15
7	*Enterobacter*	15	21	25	32	25
8	*Moraxilla*	07	10	13	15	20
9	*Asprgillus Flavus*	15	20	25	30	26
10	*Mucor*	15	20	25	30	16
11	*Trichophyton*	10	16	23	25	14
12	*Microsporum*	10	15	20	24	16

Therefore, this data suggests that 3, 5-dimethyl pyrazolium chlorochromate, and phenoxyacetic acid may have potential as an effective agent in contradiction of a diversity of microbial strains, both positive and negative-gram, as well as fungal pathogens. The systematic examination of both compounds was conducted

through procedures screened for efficacy against twelve microbial strains. As the activity increased, there was a corresponding increase in the concentration of the compounds.[24] It is fascinating to remember the analogous behavior observed in solution-based reactions driven by chemical kinetics, where an increase in reactant concentration accelerates the reaction rate.[25] Similarly, there appears to be a possible connection between concentration and biological activity.

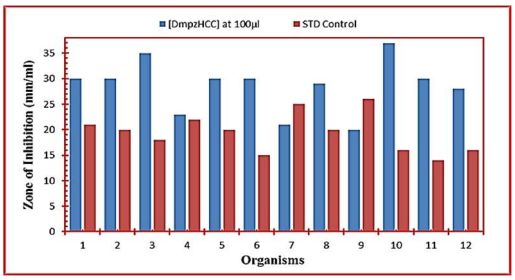


Fig.-5: Antibacterial Activity of DmpzHCC. (Numbers presented in Table-1)

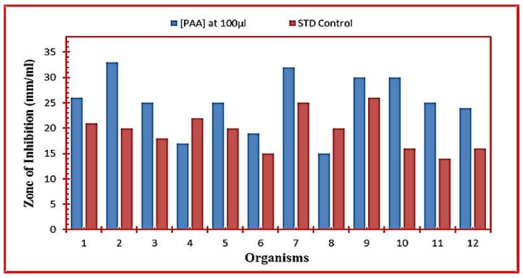


Fig.-6: Antibacterial Activity of PAA (Numbers presented in Table-2)

CONCLUSION

3, 5-dimethyl pyrazolium chlorochromate and phenoxyacetic acid were successfully produced and identified through UV, IR, PMR, CMR, and MS spectroscopic techniques for analysis. Both compounds demonstrated good effectiveness against all tested organisms. The antimicrobial activity increased with increasing concentrations of 3, 5-dimethyl pyrazolium chlorochromate (DmpzHCC) and phenoxyacetic acid (PAA). This study revealed that *Staphylococcus Aureus,* a Gram-positive bacterium, revealed the highest susceptibility to both compounds at a concentration of 100 µl. In contrast, *Enterobacter*, a negative-gram bacterium, revealed the lowest susceptibility to DmpzHCC at 100 µl, but was more susceptible to PAA at the same concentration. Notably, *Aspergillus Flavus*, a fungal strain, demonstrated relatively low efficacy compared to other fungal strains in response to both compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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