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Microbiological Activities Of Para - Bromophenoxy Acetic Acid

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Abstract: -Para-bromophenoxyacetic acid's antimicrobial activity showed promise as it inhibited the growth of various microorganisms, including bacteria and fungi. The compound's bromo group appeared to have enhanced its effectiveness against these microorganisms, resulting in larger lethal zones compared to the standard drug, gentamicin. The antimicrobial assay demonstrated notable inhibitory effects on Bacillus subtilis, Enterobacter, E. coli, Klebsiella pneumoniae, Candida albicans, and Trichoderma. The potential modes of action involved the disruption of cell membranes, interference with cell wall synthesis, inhibition of protein or nucleic acid synthesis, or induction of reactive oxygen species. The compound's antimicrobial properties provided an intriguing avenue for future research into novel treatments against microbial infections.

Keywords*: Para-bromophenoxyacetic acid, antimicrobial activity, microorganisms, bacteria, fungi, bromo group, lethal zone.*

1. Introduction

Para-bromophenoxyacetic acid is a synthetic chemical compound that has garnered significant interest in recent years due to its potential as an antimicrobial agent. The need for effective antimicrobial compounds has become increasingly crucial as the world faces growing challenges posed by antibiotic resistance and the prevalence of infectious diseases. In this context, exploring the antimicrobial properties of novel compounds like p-bromophenoxyacetic acid has become essential to identify new strategies for combating microbial infections[1, 2].The compound's unique chemical structure, which includes a bromo group, has sparked curiosity regarding its potential uses and applications. Initially known for its role as an herbicide and plant growth regulator, researchers have turned their attention to investigating its effects on microorganisms, such as bacteria and fungi. Understanding its microbial activities and mechanisms of action could pave the way for the development of new antimicrobial treatments and therapies [3]. Industrial development has brought about remarkable progress, but it has also led to the release of numerous potentially harmful substances into our environment. These pollutants find their way into the atmosphere, water bodies, and soils, posing serious threats to both the environment and human health [4, 5].

Over the last few decades, there has been a significant correlation between the rise in environmental pollutants and the increase in human diseases, especially those related to the immune system. Many technologies are used to overcome the ill effects of these chemical compounds [6, 10]. Phenoxyacetic acid derivatives, such as 2, 4-D, 2,4,5-T, 2,4-DB, MCPA, and silvex, have been extensively researched for their pharmacological and phytobiological activity. 2, 4-D, the first of the "phenoxy herbicides," showed remarkable selectivity in targeting broadleaf weeds and effective translocation throughout plants. These compounds play a crucial role in modern agriculture as powerful tools for weed control while being less harmful to desired crops. However, environmental concerns have led to the regulation and scrutiny of some of these herbicides. Responsible usage and further research are essential to harness their potential benefits while minimizing any adverse effects on the ecosystem and human health [11]. *Para*-bromophenoxyacetic acid, also known as 4-Bromophenoxyacetic acid, is a chemical compound with the molecular formula $C_8H_7BrO_3$. It is a synthetic compound and belongs to the family of phenoxyacetic acids. The structure of the compound consists of a phenyl ring (C_6H_5) attached to a carboxylic acid group (COOH) through an ether linkage (O) at the para position of the phenyl ring, which means the bromine atom (Br) is attached to the carbon atom adjacent to the carboxylic acid group.

Para-bromophenoxyacetic acid is a white crystalline solid exhibits a melting point range of approximately 160°C to 162°C. This compound is moderately soluble in water and can dissolve more readily in organic solvents like benzene and alcohol. *p*-bromophenoxyacetic acid is used in various applications, including as a herbicide and plant growth regulator. It can control the growth of weeds and other unwanted plants, making it useful in agricultural and horticultural practices. As a plant growth regulator, it may influence plant growth processes, such as seed germination, root development, and flowering.However, caution must be exercised during its handling and use, as it can be toxic if not managed properly. Its chemical reactivity allows it to participate in various reactions typical of carboxylic acids and phenols, such as esterification, hydrolysis, and substitution reactions. It is essential to consider the environmental impact and stability of p-bromophenoxyacetic acid to ensure responsible application and minimal harm to ecosystems. Proper storage in cool, dry conditions, adhering to safety guidelines, and following local regulations is crucial when dealing with this compound.

Compounds like p-bromophenoxyacetic acid are synthesized for a range of purposes that span various fields. In agriculture and horticulture, these compounds find utility as herbicides and pesticides, crucial for efficient weed and pest management to safeguard crop yields. In the realm of pharmaceuticals and medicine, their synthesis allows for the exploration of potential therapeutic effects, with researchers aiming to discover novel drugs to combat specific ailments. Moreover, these synthesized compounds serve as vital subjects of chemical research, shedding light on their reactivity, properties, and behaviour under diverse conditions, thus deepening our understanding of fundamental chemical principles. Industries benefit from such compounds, employing them in the manufacture of dyes, polymers, and other essential products [12]. If a synthesized compound displays promising traits, it may pave the way for commercial applications, potentially catalysing the development of innovative products and technologies. Collectively, the synthesis of compounds like *p*-bromophenoxyacetic acid fuels scientific progress, societal advancement, and the evolution of diverse industries.

1.1 Microorganisms

Microbes can have both positive and negative effects on living organisms and the environment. The negative effects of harmful microbes, such as pathogenic bacteria and fungi, include causing infectious diseases in humans, animals, and plants, food spoilage, environmental pollution, and antibiotic resistance. These harmful microbes can pose a threat to public health, agriculture, and ecosystems. To mitigate the negative effects of microbes, various measures can be taken, including the use of antimicrobial agents like the compound *para*-bromo phenoxyacetic acid.

In certain applications, such as agriculture or industrial processes, controlling the growth of harmful microbes is crucial to ensure food safety, prevent crop diseases, and maintain product quality. The use of antimicrobial compounds like *para*-bromo phenoxyacetic acid could be one approach to achieve this goal. However, it's essential to use such antimicrobial agents responsibly and judiciously to avoid unintended consequences, such as the development of antimicrobial resistance. Overuse or misuse of antimicrobial compounds can lead to the emergence of resistant strains of microbes, making infections and diseases more challenging to treat.

Therefore, while there may be a need to control harmful microbes using antimicrobial agents, it is equally important to consider their potential impact on the environment, human health, and microbial ecosystems. The use of these compounds should be supported by comprehensive research and regulations to strike a balance between effective microbial control and minimizing any adverse effects. Responsible and sustainable practices are essential in managing microbial populations and maintaining a healthy and balanced environment.

Para-bromo phenoxyacetic acid the major benzene metabolite, is a ubiquitous chemical in the environment due to its widespread application in human and industrial activities. This compound plays a vital role as the plant regulators, pesticides, herbicides, dyes, flavouring agents and as antimicrobial agent. The literature survey reveals that are very few reports on the antimicrobial activities of the some heterocyclic compounds. Therefore the scope of the present study was designed to show the antimicrobial activity of the heterocyclic compound of phenoxy acetic acid. *Bacillus subtilis, Enterobacter, Escherichia coli (E. coli), Klebsiella pneumoniae, Candida albicans*, and *Trichoderma* are microorganisms commonly found in various environments. Their effects on living organisms can vary significantly, depending on the specific species, strain, and the conditions in which they interact with the host organism. Here's an overview of their effects:

*Bacillus subtilis: Bacillus subtil*is is a beneficial bacterium known for its probiotic properties. In some cases, it is used as a probiotic supplement in animal feed to promote gut health and improve digestion. It may also have potential as a biocontrol agent against plant pathogens, helping to protect plants from certain diseases.

Enterobacter: Enterobacter species are a diverse group of bacteria found in various ecological niches. Some strains of Enterobacter can be beneficial, aiding in nutrient cycling and breaking down organic matter in the environment. However, certain pathogenic strains of Enterobacter can cause infections in humans and animals, leading to a range of health issues.

Escherichia coli (E. coli): While most strains of E. coli are harmless and part of the normal gut flora of humans and animals, some pathogenic strains can cause severe gastrointestinal infections. These pathogenic E. coli strains are associated with foodborne illnesses and can be harmful to human health.

Klebsiella pneumoniae: Klebsiella pneumoniae is a bacterium that can cause a range of infections, including pneumonia, urinary tract infections, and bloodstream infections. It is considered an opportunistic pathogen and can be particularly problematic for individuals with compromised immune systems.

Candida albicans: Candida albicans is a common yeast species that normally resides in the human body, especially in the gastrointestinal tract and on mucous membranes. While it is generally harmless in healthy individuals, it can cause infections, such as oral thrush and vaginal yeast infections, in certain situations, such as when the immune system is weakened or the microbial balance is disrupted.

Trichoderma: Trichoderma is a beneficial fungus known for its biocontrol properties. It acts as a natural antagonist to many plant pathogens and is used in agriculture as a biopesticide to protect crops from fungal diseases. It is generally considered safe for plants and does not have harmful effects on most other organisms.

This paper aims to delve into the antimicrobial activity of p-bromophenoxyacetic acid against various microorganisms and explore its potential as a promising candidate for fighting bacterial and fungal infections. By examining its inhibitory effects and the lethal zones it creates in comparison to standard antimicrobial drugs, we can gain insights into its efficacy. The findings from this research may have significant implications for addressing the global health challenge of antimicrobial resistance and contribute to the discovery of new antimicrobial agents to safeguard human and animal health.

2. Environmental Impact

To remove *p*-bromophenoxyacetic acid (*p*-Bromophenoxyacetic acid) from the environment, careful and environmentally responsible methods should be employed. Dilution and dispersal can be considered in cases of accidental releases into large bodies of water or soil, but this method is effective only for low initial concentrations. Biodegradation, utilizing naturally occurring microorganisms to break down the compound, can help reduce its presence in the environment. Additionally, advanced oxidation processes (AOPs) such as ozonation, UV photolysis, or Fenton's reagent can chemically degrade p-Bromophenoxyacetic acid into less harmful substances. Absorption and adsorption using materials like activated carbon or clay minerals can effectively remove the compound from water or soil. Soil remediation techniques like soil flushing, soil vapor extraction, or phytoremediation can address contaminated soil. In instances where complete removal is not achievable, containment and safe disposal of the contaminated material should be implemented to prevent further spread. It is essential to seek guidance from environmental experts and adhere to local regulations while selecting and implementing removal methods to ensure the protection of the environment and human health.

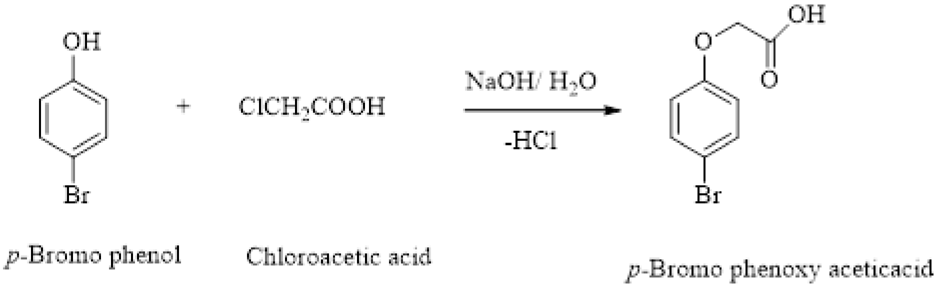
3. Agar Well Diffusion Method

The agar well diffusion method is a widely employed technique to assess the antimicrobial activity of various substances, including plant extracts and antibiotics, against microorganisms. In this method, nutrient agar plates are prepared as a growth medium for the microorganisms. The test microorganisms are cultured and spread uniformly on the agar surface. Wells of specific diameters are created, and the test substances are added to these wells. Following incubation, the test substances diffuse outward from the wells into the agar medium. If the substance possesses antimicrobial properties, clear zones of inhibition appear around the wells, indicating the inhibition of microbial growth. The diameter of the zones of inhibition is measured and compared to controls to evaluate the antimicrobial activity of the test substances. This method provides qualitative information about the presence or absence of antimicrobial activity, offering valuable insights into the potential efficacy of the tested substances against microorganisms.

4. Results and Discussion

4.1 Preparation of para-Bromophenoxyaceticacid ($C_8H_7 BrO_3$)

5.6 grams of chloroacetic acid were weighed and dissolved in 7.5 milliliters of warm water within a beaker. With precision, 7 milliliters of 12M hydrochloric acid (HCl) were added very slowly to the solution. Separately, 18 grams of sodium hydroxide were dissolved in distilled water. To this sodium hydroxide solution, 2 grams of p-Bromophenol were added drop by drop, and the resulting mixture was allowed to cool. Once cooled, the p-Bromophenol solution was cautiously added drop by drop into the chloroacetic acid solution contained in the beaker, all the while employing a magnetic stirrer. Over a span of two hours, the amalgamated solution was stirred within a temperature range of 70°C to 80°C. This process yielded a white solid precipitate. Subsequently, the solid was extracted using benzene. The purification of the obtained solid was achieved through recrystallization, involving the use of 2.4 milliliters of water and 0.5 milliliters of alcohol. The physical parameters, including the melting point, were measured, indicating a range of 160°C to 162°C for the final product. Impressively, the yield of this synthesized compound amounted to 92%, signifying the successful conversion of reactants into the desired product. However, the specific chemical identity of the resulting compound remains undisclosed based on the provided description.



4.2 Spectral Data

Para-bromo phenoxy acetic acid is a compound with significant functional groups, as indicated by its infrared (IR) spectrum. The IR peaks at 1300 to 1280 cm-1 and 1270 to 1260 cm-1 suggest the presence of ester (RCOOR') and ether (R-O-R') groups, respectively. The peak at 1235 to 1220 cm-1 indicates a phenol group (C6H5-OH) in the molecule. Additionally, the IR peak at 1200 to 1020 cm-1 corresponds to C-H bending vibrations, common in aromatic compounds. Lastly, the peak at 1180 to 1160 cm-1 indicates a C-Br stretching vibration, suggesting the presence of a bromine substituent (Br) attached to the aromatic ring. These functional groups are crucial in determining the compound's properties and potential applications in various fields, such as pharmaceuticals, agriculture, and chemical synthesis.

4.3 Antimicrobial Assay

In the anti-fungal assay, the agar well diffusion method was used, following the protocols established by Perez et al. and Baur et al. respectively [13 – 15]. The test organism's diluted inoculum (0.5 ml with 105 CFU/ml) was spread evenly on Potato Dextrose Agar (PDA) plates, creating a microbial lawn. Wells of 8 mm diameter were then carefully punched into the agar medium, and different concentrations of plant extracts were added to these wells for well diffusion. The plates were incubated at 38°C for 48 hours, providing the optimal conditions for fungal growth. After incubation, the anti-fungal activity was evaluated by measuring the diameter of the clear zones of inhibition around the wells, which indicate the extent to which fungal growth was inhibited by the plant extracts. The diameter of the zone of inhibition was recorded and compared to standard antibiotics for interpretation and assessment of the anti-fungal activity. A larger zone of inhibition suggests stronger anti-fungal properties of the tested plant extracts. The results obtained from this assay provide valuable information on the potential efficacy of the plant extracts against the test organism and can be used to guide further investigations in the field of antifungal research.

Table 1: The antibacterial and antifungal activity of the by the Dimethyl Sulphoxide extract on various species are depicted in the table.

SAMPLE	**DMSO Extract 100 µl added and Zone of inhibition (mm/ml)**				
	25 µl	**50 µl**	**75 µl**	**100 µl**	**Control**
Bacillus subtilis	12	14	16	18	20
Enterobacter	12	14	16	18	20
E.coli	14	16	18	20	20
Klebsiella pneumoniae	14	16	18	20	22
Candida albicans	10	12	14	16	20
Trichoderma	14	16	18	20	22

From the results it is evident that all the fungi and the bacteria are effective against the standard Gentamicin antibiotic drug. The activity is noted in the form of inhibition zone around formed around each well whose diameter measures the degree of inhibition. From the data it is confirmed that for each species the extend of inhibition varies. The inhibition zone diameter formed in DMSO extract against each species was found to be either less than or greater than or equal to that of standard antibiotics.

The compound proved to have good inhibition against the gram positive, gram negative bacteria and few fungi. The nature of the bromo group in the compound itself exhibits resistance towards the bacteria and fungi. The lethal zone is more than the standard drug taken for the test. For the species, *Bacillus subtilis* (18 mm/ml), *Enterobacter* (18 mm/ml), *E.coli* (20 mm/ml), *Klebsiella pneumonia* (20 mm/ml), *Candida albicans* (16 mm/ml), *Trichoderma* (20 mm/ml) than the gentamicin control

The outer membrane of cell walls of gram-negative bacteria was consisted of lipoprotein, lipid bilayer, and lipopolysaccharide. The lipid bilayer is similar to the cell membrane. In addition, gram-negative bacteria cell walls have periplasmic space, which contains a variety of enzymes such as protease, nuclease, and detoxification enzymes. Those enzymes play an important role in bacterial antidrug-resistant activity. The compound was more effective to destroy the growth of the bacterias and hence proved to be more resistant.

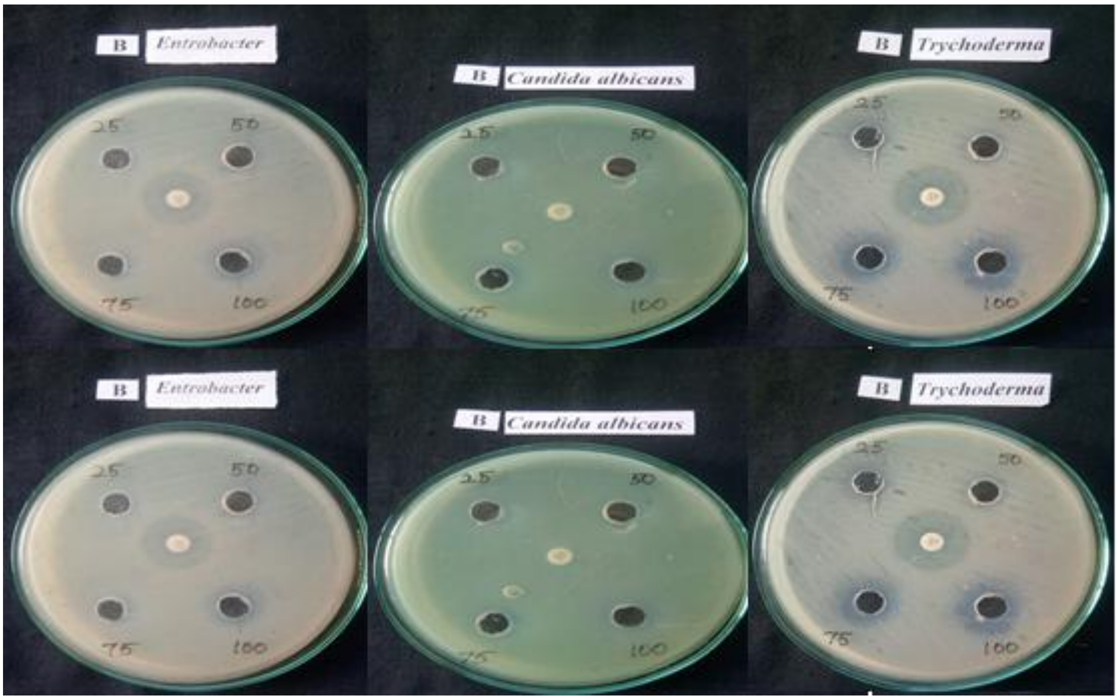


Figure 1: Inhibition Zone of microorganisms against P-bromophenoxyacetic acid

P-bromophenoxyacetic acid demonstrates potent antimicrobial activity against the tested microorganisms, comprising both bacteria and fungi. The presence of the bromo group in the compound appears to confer resistance towards these microorganisms, enabling it to interact effectively with their cellular structures or processes. Notably, the observed lethal zone, the zone of inhibition surrounding the wells containing the compound, exceeds that of the standard drug, gentamicin, and a widely used antibiotic for bacterial infections. This outcome signifies the compound's superior antimicrobial efficacy compared to the standard drug, effectively inhibiting the growth and survival of the tested microorganisms. Antimicrobial compounds generally exert their effects through various mechanisms, which could include:

Cell Membrane Disruption: Some antimicrobial agents can disrupt the integrity of the microbial cell membrane, causing leakage of cellular contents and ultimately leading to cell death.

Inhibition of Cell Wall Synthesis: Certain compounds target the synthesis of the bacterial cell wall or the fungal cell wall, weakening the structural integrity and causing cell lysis.

Inhibition of Protein Synthesis: Antimicrobials may interfere with bacterial or fungal protein synthesis, disrupting essential cellular processes and leading to cell death.

Inhibition of DNA/RNA Synthesis: Some compounds interfere with DNA or RNA synthesis, disrupting the microorganism's ability to replicate and propagate.

Generation of Reactive Oxygen Species: Certain antimicrobials can induce the production of reactive oxygen species within the microbial cell, causing oxidative damage and cellular destruction.

5. Conclusion

Para-bromophenoxyacetic acid exhibits potent antimicrobial activity against a range of microorganisms, including bacteria and fungi. The presence of the bromo group in the compound enhances its effectiveness, leading to larger lethal zones compared to the standard drug, gentamicin. However, the exact mechanism of action remains to be fully elucidated, and further research is required to understand its specific interactions with microbial cells. Despite this, the compound's antimicrobial properties hold promise for potential therapeutic applications in combating microbial infections. *P*-bromophenoxyacetic acid presents an intriguing avenue for ongoing research into developing new and effective treatments against various microbial pathogens.

References

[1] Bukowska, B. (2006). Toxicity of 2,4-Dichlorophenoxyacetic Acid – Molecular Mechanisms. Polish Journal of Environmental Studies, 15(3), 365-374.

[2] Trillas, M., Peral, J., & Domenech, X. (1996). Photocatalyzed degradation of phenol, 2,4-dichlorophenol, phenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid over supported TiO2 in a flow system. Journal of Chemical Technology and Biotechnology, 67(3), 237-242.

[3] Palaniappan, A. N., Udhayakumar, R., Srinivasan, S., & Raju, C. (2012). Kinetics of oxidation of phenoxyacetic acid by TPCC in aqueous acetic acid medium. Der Chemica Sinica, 3(3), 708-712.

[4] Anbarasu, K., & Ilavenil, K. K. (2018). Synthesis, Characterization and Pharmacological Activity of Phenoxy Acetic Acid and Pyrazinium Chlorochromate. Asian Journal of Chemistry, 30(10), 2238-2240, https://doi.org/p10.14233/ajchem.2018.21417

[5] Ilavenil, K. K., Kasthuri, A., & Anbarasu, K. (2022). Oxidation kinetics of Phenol, its derivatives and some para-substituted phenol by Pyrazinium chlorochromate in acidic medium: A Non-Linear Hammett Plots. Rasayan Journal of Chemistry, 15(3), 1660-1667, http://doi.org/10.31788/RJC.2022.1536920

[6] Senthilkumar, V., & Ilavenil, K. K. (2023). Artificial Neural Network Prediction of Optimal Phenylic Acid Adsorption using Lantana camara Activated Carbon. Oriental Journal of Chemistry, 39(1), 172-178, http://dx.doi.org/10.13005/ojc/390121

[7] Srinivasan, D., & Ilavenil, K. K. (2017). Biosorption of heavy metals and dyes using plant weeds - A review. International Journal of ChemTech Research, 10(7), 1038-1047

[8] Srinivasan, D., & Ilavenil, K. K. (2017). Bioremediation of dyes and heavy metal ions using Cynodon dactylon: A review. Journal of Chemical and Pharmaceutical Sciences, 10(1), 363-370

[9] Ilavenil, K. K., & Dhamodharan, M. (2016). Synthesis, structure, and characterization of Cr (III) and Fe (III) complexes of m-nitro benzaldehyde thiosemicarbazone. Journal of Chemical and Pharmaceutical Sciences, 9(1), 1458-1462.

[10] Ilavenil, K. K., Pandian, P., & Kasthuri, A. (2023). Adsorption study of removal of lead ions using Prosopis juliflora and prediction by artificial neural network modeling. Materials Today Proceedings, 72, 2344-2350, https://doi.org/10.1016/j.matpr.2022.09.402

[11] Moandai, A., & Radu, S. (2008). New Phenoxyacetic Acid Analogues with Antimicrobial Activity. Rev. Chim. (Bucuresti), 59(6).

[12] Anbarasu, K., & Ilavenil, K. K. (2019). Structural activity of unsaturated alcohols and oxidation towards pyrazinium chlorochromate. Oriental Journal of Chemistry, 35(1), 436-441, http://dx.doi.org/10.13005/ojc/350156

[13] Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single-disk method. American Journal of Clinical Pathology, 45(4), 493–496.

[14] Susceptibility test of Bauer-Kirby-Sherris-Turck. American Journal of Clinical Pathology, 1972, 57(5), 592–597.

[15] Perez, C., Paul, M., & Bazerque, P. (1990). Antibiotic assay by agar-well diffusion method. Society for Biology and Experimental Medicine, 15, 113-115.