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INVESTIGATION OF THE INTERACTION BETWEEN XANTHINES AND COMMERCIAL ANTIMICROBIALS ON SELECTED MICROBES

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Abstract: - Since the 1940s, antimicrobials have been used to combat microbial infections and sickness enormously. However, due to the overuse and abuse of antimicrobials, antimicrobial resistance has been exacerbated resulting in side effects. The microbes have developed resistant mechanisms towards antimicrobials making these drugs less effective (Soni, 2014). Antimicrobial resistance reduces the effectiveness of an antimicrobial in curing a disease or condition. In order to combat the increasing rate of antimicrobial resistance, new strategies are needed; hence researchers are looking into using combination therapy to combat AMR. This research envisages treating infections with different combinations and/or sets of drugs rather than individual drugs. Nucleoside analogues are commonly used for treating viral and fungal infections, as well as for treating cancers, however, a significant number of clinically approved derivatives of both pyrimidines and purines have been shown to have antibacterial activity. In view of this, this experiment will be conducted to determine the drug interactions between the methylxanthines and antimicrobials to find out their potential antimicrobial properties in an attempt to repurpose these drugs. The method employed includes drug preparation and antibiotic/fungal preparations, determination of their minimum inhibitory concentration and the determination of their drug interactions with the antimicrobials by disc diffusion bioassay. For the results, it is envisaged that interactions between the xanthine-based drugs and the antibiotics or antifungals may potentiate the activity, have no significant effect on the activity and/or suppress the activity of antibiotics and antifungals against the selected bacterial and fungal culture.

Keywords: commercial antimicrobials, xanthines, minimum inhibitory concentration, Nucleoside analogues.

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1.0 Introduction

Drugs used to treat diseases caused by microorganisms are termed antimicrobials. These agents kill microbes or stop their growth. They have been categorized according to the microbes they act against. Antimicrobials used against bacteria are known as antibiotics, those used against fungi are known as antifungals and those against viruses are antiviral (Singh, 2016**).** The most unremarkably best-known antimicrobial is antibiotics, which eliminate bacteria or halt bacterial expansion.

After Alexander Fleming discovered penicillin in 1928 (Fleming *et al.*, 1945), the role of antibiotics became known and was used by all. Since the 1940s, antimicrobials have been used to combat microbial infections and sickness enormously. However due to the gross use and abuse of antimicrobials, antimicrobial resistance has been exacerbated and resulting side effects. The microbes have developed resistant mechanisms towards antimicrobials making

these drugs less effective (Soni, 2014).

Drug resistance or antimicrobial resistance (AMR) is the ability of pathogens to evolve or grow in the presence of an antimicrobial that would normally inhibit their activity and proliferation, and therefore no longer respond to the drug that was previously administered as a cure. Antimicrobial resistance reduces the effectiveness of an antimicrobial in curing a disease or condition (Blower *et al*, 1998). Now and then, microbes can cause this resistance by producing enzymes that modify the active drug, synthesizing a pseudo-target site against which the drug has no effect or reducing drug accumulation by changing membrane permeability to antibiotics (Baptist, 2001). These activities can be curtailed when patients stop taking antibiotics for self-limiting infections, doctors also stop giving unnecessary antibiotic prescriptions and patients follow and complete antibiotic prescriptions.

Multi-drug resistance (MDR) or multiresistance is AMR shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories. It occurs by accumulation of genes on resistance plasmids with each evolving for resistance to a specific agent and or by the action of multidrug efflux pumps, each of which can pump out more than one type of drug. Multidrug-resistant species (MDR) are considered a health threat to humans and animals (Hall, 2004). AMR does not only develop in bacteria ideally but may also develop in fungi, parasites, and viruses (Goldmann 1996).

To determine the susceptibility of bacteria to antibiotics or fungi to antifungals, an antibiotic sensitivity test is used. Some of these tests include Disc Diffusion Methods, Kirby-Bauer Method, Stoke's Method and E-Test. Antibiotic Assay measures the amount of antibiotic in the blood or other body fluids of patients. The trough and peak levels of drug are estimated by two assays namely the Bioassay and Immunochemical assay (Murray *et al.*, 2004).

Thus in order to combat the AMR, researchers developed strategies such as combinational therapies, which increases the treatment efficacy of microbial infection. Whenever two or more drugs are being taken, there is a chance that there will be an interaction among the drugs. Drug interactions may lead to an increase or decrease in the beneficial or the adverse effects of the given drugs. When a drug interaction increases the benefit of the administered drugs without increasing side effects, both drugs may be combined to increase the control of the condition that is being treated. For example, the combination of penicillin with streptomycin for enterococcal infections and a combination of rifampin isoniazid-pyrazinamide in the treatment of tuberculosis (Worthington, 2013).

Methylxanthines are a special class of medications or drug compounds derived from the xanthine-purine base (Bell et al., 1998). Xanthine is naturally derived from plants and to some extent from animals. They are involved with and found in the central nervous system and other peripheral tissues and aid in the antagonization of adenosine receptors; A1, A2A, A2B and A3 and phosphodiesterase inhibition (Wong & Ooi, 1985). Methylxanthines have previously been known to have an impact or effect on microbes by antagonization of the adenosine receptors and phosphodiesterase inhibition.

This research is aimed to assess the effect of combination therapy of methylxanthines and antimicrobials on known MDRs using antibiotic susceptibility tests.

1.1. RATIONALE

Antimicrobials came into existence to help fight infection caused by microbes but antimicrobial resistance (AMR) emerged naturally over time, usually by genetic modifications. AMR has been exacerbated by the overuse and abuse of antimicrobials. This has led to a stage where there was a need to come up with strategies to help combat AMR. There are many strategies to help the cause but combination therapy has shown promising effectiveness. Methyl xanthines have previously shown to have some effects to microbes and may serve as a promising outlook as an agent in the development of combination therapies.

1.2. HYPOTHESIS

Xanthine used in the treatment of infectious agents resistant to conventional antimicrobials will enhance or suppress the activity of conventional antibiotics or anti-fungal drugs on bacteria or fungi.

AIM

To examine the effect of the combination of xanthine such as (Caffeine, paraxanthine, IBMX, theobromine, theophylline, pentoxifylline) and antimicrobials (antifungals and antibiotics) on multi drug resistant (MDR) strains of *M. smeg, Ery A, Ery B, C. albicans, Saccharomyces cerevisiae, E. coli and S. aureus.*

1.3. SPECIFIC OBJECTIVES

- To determine the MICs of xanthine compounds using antibiotic susceptibility tests.

- To examine the effect of the combination of xanthines (caffeine, paraxanthine, IBMX, theobromine, theophylline, pentoxifylline) and antifungals or antibiotics against some selected microbial strains (*M. smeg, Ery A, Ery B, C. Albicans, Saccharomyces cerevisiae, E. coli and S. aureus*) using disc diffusion method.

2.0 LITERATURE REVIEW

2.1. Background Information

Antimicrobials are agents that kill microbes or stop their growth. Antimicrobials used against bacteria are known as antibiotics, those used against fungi are known as antifungals, and those against viruses are antiviral and so on (Singh, 2016**).** Antimicrobial agents are active medicines acquired from microorganisms to prevent and cure bacterial infections (Lewis, 2013). Approximately 75% of antibiotics currently in clinical use are extracted from soil or water-isolated actinobacteria (Trujillo, 2008).

Paul Ehrlich, a German physician, concluded that, "it must be possible to create substances that can kill certain bacteria selectively without harming other cells" which is how Sir Alex Fleming and others started working on antibiotics (Fleming et al., 1945). To date, the persistent use of antibiotics has resulted in the ineffectiveness of antibiotics, leading to a global increase in drug resistant bacteria (Singh, 2016). The 1930s to the 1960s was the "golden age" of antibiotics that made them very successful without any problems. Sadly, this era ended due to the advent of resistant microbes that antibiotic discovery researchers were unable to pace up with. The development of antibiotic resistance stemmed mainly from the inability to identify new antibiotics and the non-medicinal

use of antibiotics. (Rossolini et al, 2014).

2.1.1. Antimicrobial Resistance

Antimicrobial Resistance (AMR) is prevalent in every country. AMR is the ability of microbes to evolve or grow in the presence of a drug that would normally kill them and thus no longer respond to a drug that previously eradicated them. Antimicrobial resistance threatens a resurgence of life-threatening bacterial infections and the potential demise of many aspects of modern medicine. Despite intensive drug discovery efforts, no new classes of antibiotics have been developed into new medicines for decades, in large part owing to the stringent chemical, biological and pharmacological requisites for effective antibiotic drugs.

Recently, many microbial infections have become resistant to antimicrobial treatment rendering them ineffective, as they no longer respond to them, resulting in multidrug resistance. The rise and spread of resistant bacteria is a major health problem and a specific challenge for both science and medicine (Julian Davies, 1975). Center for Disease Control and Prevention (CDC) (2013) evaluated antimicrobial resistance infections based on different aspects: clinical effects, economic impact, incidence, 10- year incidence prediction, transmutability, availability of appropriate antibiotics, and prevention barriers.

Microbes are able to cause this resistance periodically by developing enzymes that either change the active drug, synthesize a pseudo-target site against which the drug has no impact or reduce drug accumulation by decreasing the membrane permeability to antibiotics (Odonkor et al., 2012). Taking antibiotics without a doctor's prescription as well as administering antibiotics needlessly for the treatment of usual viral illness, such as common cold, fosters antimicrobial resistance (Hamilton-Miller, 1984. Since the discovery and subsequent widespread of antimicrobials, a number of pathogenic viruses, bacteria, protozoa and helminths have evolved various mechanisms to make them immune to some and, in certain instances, almost all antimicrobials (Martinez & Baquero, 2000). Using the right antibiotic or determining the susceptibility of bacteria to antibiotics is done by antibiotic sensitivity testing. Some of the tests are Disc Diffusion Methods, Kirby-Bauer Method, Stoke's Method and E-Test (Wagner et al., 1975).

2.2. Some Clinically Relevant Microbes That Have Shown High Resistance (TestOrganisms)

Numerous organisms have been identified as fast evolving and resistant strains worldwide; some of them namely, *M. smeg, Ery A, Ery B, C. Albicans, Saccharomyces cerevisiae, E. coli and S. aureus have been selected as* the test model organisms for this project.

2.2.1. Mycobacterium smegmatis, Erythromycin-resistant Mycobacteriumsmegmatis A & B

Over the years, *M. tuberculosis* has developed resistance to anti-tuberculosis drugs designed against it. Moreover, this was aided by the sophisticated nature of the techoic acids in its cell walls. Moving forward new anti- tuberculosis drugs are urgently needed in combating the TB pathogen (Rattan, 1998). The non-pathogenic fast growing *Mycobacterium smegmatis* has been identified as the research organismto help arrest this problem. (Gillespie, 2002). *M. smegmatis* belongs to the genus*Mycobacterium* as originally *Mycobacterium smegmatis*, to which many pathogenic mycobacteria, including but not limited to M. tuberculosis, causative agent of tuberculosis, and *M. leprae*, causative agent of leprosy all belonged to (Gupta etal.,2018; Oren and Garrity, 2018). Djuretic (2002) reported about the use of *M. Smegmatis* as a substitute for *M. tuberculosis* or *M.leprae* in molecular biology research studies. However, inherent resistance to antibiotics and host persistence have been observed to be two contrasting and distinct characteristics of the Mycobacterialspecies.

Phenotypic correlations between these two distinct characteristics have been observed in vitro and in vivo. Work done by Espina*l et al.,* (2001) reports that Mycobacterium tuberculosis persists in a persevering state during concealed infection, and subsequently shows a lower antibiotic resistance ability compared to bacterium duringactive growth. The significant detail about the perseverance of M.tuberculosis is its ability to survive within an infected *M.tuberculosis* patient and accelerate to an acute condition in the patient (Chaturvedi et al, 2007).

Continuous exposure to antibiotics promotes its acclimatization with the antibiotic by sub populations of the mycobacterium, and eventually leaving their drug susceptible associates extinct. Drug resistance in M. tuberculosis is mostly tied to erythromycin-resistant Mycobacterium smegmatis A and B (Ery A and B). Ery A and B boast of durability and viability in the presence of erythromycin even in high dose concentrations. In effect, the erythromycin antibiotic has lost efficacious power against M. smegmatis. WHO upon consultations with other regulatory bodies adopted the term MDR for any strain who is able to resist at least two most potent anti-TB drugs in Rifampicin and Isoniazid. Fortunately, for the purpose of research and drug advancements, the aggressively growing phenotype of *M.smegmatis* was maintainedin these MDR strains, further buttressing their position as blueprint-model organisms for the in vitro screening of novel drugs designed against MDR strains of Mycobacterium tuberculosis (Arthur et al., 2019).

2.2.2. Saccharomyces cerevisiae

Saccharomyces cerevisiae peculiar to the yeast strain is another aggressively evolving microbe, which relatively boasts of a simple eukaryotic prototype system, which has been studied extensively, and the understanding of its genetic make-up forming the premise of recent advancements in functional genomic technologies. Interestingly, *S. cerevisiae* yeast, which has been used over the years in the food industry especially for bakery products, has also been verified to cause different forms and caliber of invasive infections, mostly after its usage as a probiotic for the treatment of diarrhea mostly emanating from the use of an antibiotic.

Angrave and Avery (200), reported that, acute opportunistic infections caused by the *Saccharomyces cerevisiae* have been reported in sick individuals with persistent diseases such as cancer and immunosuppressed systems manifesting as fungemia, endocarditis, urinary tract infections, esophagitis, pneumonia, skin infections andperitonitis. Understanding of the fungi's MDR draws inference and conclusions from studies conducted in non-pathogenic yeast, *S.cerevisiae*, in which the phenomenon of multi drug resistant phenotype is called Pleiotropic Drug Resistance (Alarco *et al.,* 1997). Key to this resistance is the conservation of gene functions between the yeast and humans, due to our frequent use of the microbe in our day-to-day activities. This gives rise to over 40 percent of single gene determinants of human heritable diseases having yeast homologs (Finch, 1980). *Saccharomyces cerevisiae* presents a useful insight into genetics of eukaryotic cell

biology, which would otherwise be problematic to achieve with higher eukaryotes with complex and cell systems and metabolic routes. (Winzeler, 1999),

2.2.3 *Candida albicans*

Candida albicans (monilia) is a fungus that is normally part of the human microflora and hence commonly live in or on our bodies. It can be located in the gastrointestinal tract, mouth and the vagina. It is the most prevalent cause of fungal infections in people such as candidiasis and urinary tract infection (Collin *et al.,* 1999). It is one of the few species of the genus *Candida* that causes the human infection candidiasis that result from an overgrowth of the fungi. There are only few antifungals like fluconazole that are available now to treat diseases caused by *C. albicans*. Resistance can be either intrinsic or acquired. A timely diagnosis of the disease is perilous to better treatment and management of patients with these infections. Reduction of unnecessary use of antifungals via antifungal stewardship is critical to limit multidrug resistance emergence in fungi (Masala, 2003).

2.2.4. Escherichia coli

Escherichia coli (*E. coli*) is a gram-negative anaerobe predominant in the flora of the human and animal intestine and hence in food. Some strains, however, overtime have been able to develop the ability to cause illness in the urinary, gastrointestinal and central nervous systems (Feng *et al.,* 2016). They are able to cause both intestinal and extra intestinal infections. Antimicrobial resistance of this foodborne microbe to several antibiotics is of major concern to public health and researchers. The occurrence of the multiple drug resistant bacteria and newly developing strains has been attributed to the indiscriminate use of antimicrobials and the memory function of *E. coli* to adapt to and resist antimicrobials (Edgar *et al.,* 1997). *E. coli* is a ubiquitous microbe capable of forming a biofilm, which is an important and critical virulence factor in diseases and development of antimicrobial resistance (Pitout *et al*, 2008).

2.2.5. Staphylococcus aureus

Staphylococcus aureus (S. aureus) is a gram-positive bacterium, a widespread human microbe that causes a wide range of symptomatic diseases (Foster, 1996). It is a bulging cause of many infections *such as* pneumonia, cellulitis, bacteremia etc. It is also considered as a well-known antibiotic resistance bacteria due to its ability to develop adaptive countermeasures against multiple antibiotics (Giersing *et al*., 2016). Particularly, methicillin-resistant S. aureus (MRSA) strains are the main cause of antibiotic-resistant nosocomial infections all over the world.

At the outset, the pathogenicity of the antibiotic-resistant strains was underestimated, and is still sometimes questioned, but today most authorities consider MRSA a serious threat, especially given current preoccupation with cost-effectiveness within the health service. Approximately one-third of the population in the world is colonized with *S aureus*, primarily in the nose and throat. Since the 1950s, methicillin-resistant *S aureus* (MRSA) has primarily been a nosocomial infection in many institutions such as hospitals, community centers, and nursing homes. MRSA can spread directly between individuals or indirectly via fomites. Hospital-associated MRSA is often acquired within the hospital setting and is one of the many infections exhibiting increased antimicrobial resistance. *Staphylococcus aureus* and particularly methicillinresistant *S. aureus* (MRSA) is one example of an antimicrobial resistant pathogen found in livestock and apparently able to transfer to humans (Fowler, 2004).

2.3. Drug-Drug Interaction to Microorgamsims

Drug interactions may make the drug less effective, cause side effects or increase the action of a particular drug (Helms, 2006). Some interaction with the medications can be even dangerous, thus the patient should read the label whenever using a non-prescription or prescription medication and take time to learn about drug interactions as this is important to the patient's wellbeing (Zhou *et al.,* 2004). With a bit of knowledge on drugs, one can scale back the risk of unquestionably harmful drug reactions and facet effects. Interactions between the drugs fall into three broad categories:

Drug-drug/compound interactions occur when two or more drugs react with each other. Drug-drug interactions occur once two or additional medication react with one another. This drug interaction may cause the patient to experience an unexpected side effect (Goldberg *et al.,* 1996).

Drug-food/beverage interactions result from medication reacting with foods or beverages. For example, mixing alcohol with some drugs may cause one to feel tired or slow your reactions (Massarella *et al.,* 1989).

Drug-condition interactions may occur when an existing medical condition makes certain drugs potentially harmful. For example, a patient with high blood pressure could experience an undesirable reaction if he or she takes a nasal antihistamine (Longo, 2012). This therefore inspired the combination therapy since it can have an effect on the receptor function and physiological control process. Although these interactions can go sideways, its beneficial pharmacokinetic interaction is impressive.

2.3.1. Combination Therapy

It is extensively known that many MDR strains have become and are still becoming more resistant to new and modern antimicrobial drugs. In order to combat the increasing rate of antimicrobial resistance, new development strategies are needed, hence researchers are looking into using the combination therapy to combat AMR. Combination therapy is amongst one of the suggested way to increase treatment efficacy, to prevent the development of drug resistance and to reduce the duration of treatment of a microbial infection or disease (Worthington, 2013). Combinations of antimicrobials and or antimicrobials with non-antimicrobial activityenhancing compounds offer an industrious strategy to address the enormous widespread of antibiotic and antifungal resistant strains. This is what shifted the mind of research to look into methyl xanthine, a nucleobase base polymer (Fischbach, 2011). A lot of nucleosides and their synthetically modified analogues have been shown to have antimicrobial activity ranging from moderate to good against different bacterial and fungal strains. These compounds target several key processes of bacterial and fungal cells such as nucleoside metabolism and cell wall, nucleic acid, and protein biosynthesis and many others although these are not well characterized and may therefore represent opportunities to discover new drugs with unique mechanisms of action (Jessica & Lamont, 2019).

2.3.2. Nucleoside Analogues (Methylated Xanthines)

Nucleoside analogues are commonly used for treating viral and fungal infections, as well as for treating cancers, however, a significant number of clinically approved derivatives of both pyrimidines and purines including halogenated, thiolated, and azolated compounds have been shown to have antibacterial activity. In recent studies, such compounds have shown potential in treating bacterial infections.

Methylxanthines such as theophylline and dyphylline are potent bronchodilator agents and are widely used as a treatment for patients with acute asthma and bronchitis. These therapeutic agents have shown antibacterial activities against some bacterial pathogens (Jessica & Lamont, 2019). Caffeine, theophylline, pentoxifylline, IBMX, paraxanthine, pentoxifylline, and theobromine are the common known pharmaceutically active methylxanthines. Caffeine is the coffee's dominant methylxanthine; theobromine is abundant in chocolate where the ratio of theobromine to caffeine varies widely but usually higher than one; and theophylline is the primary methylxanthine in tea (Barnes, 2005).

Purines can have negative influence on the sporulation rate of bacterial cells and their morphological development. This is because the action of purine on the metabolic pathways of bacteria may range from harmful to beneficial, depending upon the species of bacteria known as they block the adenosine receptors and this is exactly how methyl xanthines work. The similarities between purine alkaloids and nucleic acids in structure encourage the use of these compounds as antimicrobial agents (Martinez & Baquero, 2000).

To progress in the quest of eradicating antimicrobial resistance, methylxanthines have been combined with antimicrobials such as antibiotics and antifungals. They are a special class of drug compounds derived from nucleoside analogues (xanthine) purine base (Bell et al., 1998). Xanthine is naturally derived from plants, to some extent from animals and are involved with, and found in the central nervous system and other peripheral tissues. They aid in antagonization of adenosine receptors; A1, A2A, A2B and A3 and in phosphodiesterase inhibition (Wong & Ooi, 1985). Nowadays the intake and use of methyl xanthine-containing products is undeniably widespread and very common.

3.0 MATERIALS AND METHOD

3.1. MATERIALS

3.1.1. Model Strains

- *Mycobacterium smegmatis*
- *Saccharomyces cerevisiae*
- *Erythromycin M. smegmatis resistance strain A and B*
- *Candida albicans*
- *E. coli*
- *S. aureus (MRSA & MSSA)*
- *A. faecalis*

3.1.2. Model Phenotypic Drugs

- Caffeine
- Paraxanthine
- Theobromine
- Theophylline
- IBMX
- Pentoxifylline

3.1.3. Media

- MHA
- Yeast extract
- Peptone
- Dextrose
- Nutrient broth
- Agar
- Middlebrook 7h10 Agar
- Middlebrook 7h9 broth
- Glycerol
- Tween80

3.2. METHODOLOGY

3.2.1. Media Preparation

The MDR strains in this research will be cultured under in vitro conditions. Middlebrook 7H10 agar base (Sigma Aldrich) , yeast peptone dextrose agar, MHA and Middlebrook 7H9 broth base (Sigma Aldrich) will be used for preparing agar plates and liquid broth, respectively, according to the manufacturer's instructions. The M7H10 agar base is supplemented with 0.085%NaCl and 0.5%dextrose and those for the bioassays had the xanthines added in concentrations whilst the M7H9 broth base was supplemented with 0.085% NaCl, 0.44% glycerol, and 0.25% Tween 80. To prepare the media; the powder form of the medium is rehydrated. The agar medium is then stirred the medium is then distributed into tubes and then autoclaved to sterilize it. The agar medium is also autoclaved for plate production and then poured into sterile petri dishes.

3.2.2. Minimum inhibitory Concentration determination of Xanthines

Serial dilution of the xanthines will be made and impregnated unto cut disc. The impregnated xanthine disc is then placed on the agar plate as shown in figure 1.0. The disc is then incubated for about 24-48 hours. The zone of inhibition will be measured and the data collected will be analyzed. It is to note that the step will be repeated for the other five xanthines analogs selected for this research.

3.2.3. Drug Susceptibility Assay

Disc diffusion or the Kirby–Bauer test is one of the classic microbiology techniques used for determining antimicrobial resistance around the world. Prior to a drug susceptibility assay, organisms will be cultured for at least 24 hours. Quality control test will then be performed on the cells and then transferred into broth. Thus, the bacterial cultures that are obtained will be diluted to an optical density at 600 nm (OD600)of 0.7 in 50 mL of freshly prepared M7H9 broth base and incubated at 30 °C for 24 h with

shaking. The 24 h, day two cultures will be diluted to OD600 of 0.7 and will be used for the uniform inoculation (spreading) of M7H10 agar plates.

Xanthine will be prepared at preferred concentration and streaked on the agar plate. It will be allowed to dry and then cells will be streaked onto the fresh M7H10 agar plates. The cells that grow on the agar plates can be confirmed using acid-fast staining. The inoculated broths will be incubated at 30 °C for 24 h with shaking (160 rpm). Paper discscontaining the indicated amount of antibiotic will be placed on the inoculated plates and the plates will be incubated at 30 °C for 48 h. The zone of inhibition (in mm) around each antibiotic disc will be measured and used to ascertain the susceptibility profile ofthe organism to the panel of antibiotics. The concentration of the antibiotic at the edge of the disk is high and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory to the microorganism, allowing it to grow freely. A clear circular zone of no growth in the immediate vicinity of a disc indicates susceptibility to that antimicrobial (Tendencia, 2004). This principle will be used to investigate the effect of the combination therapy between the drugs made from nuclear-based polymer used to treat the model microorganisms and antifungals or antibiotics activity of these selected antibiotic discs.

3.3. EXPERIMENTAL DESIGN

3.3.1. Determination of Mic of Each Xanthine

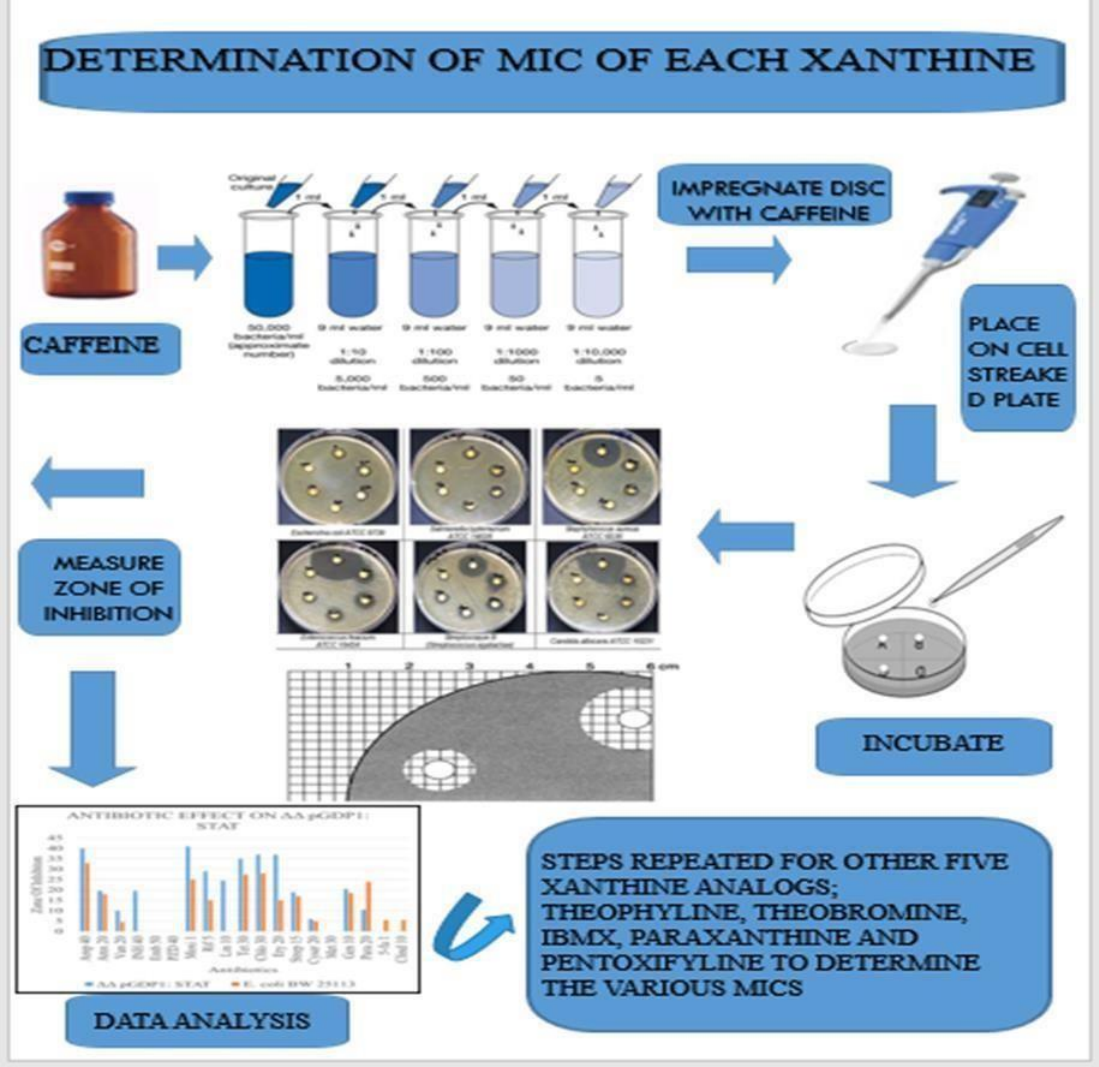

***Figure 1.0.*:** Graphical representation of the determination of the MICs of the six MethylXanthines.

3.3.2. Bioassay Procedure of Drug-Compound Interaction

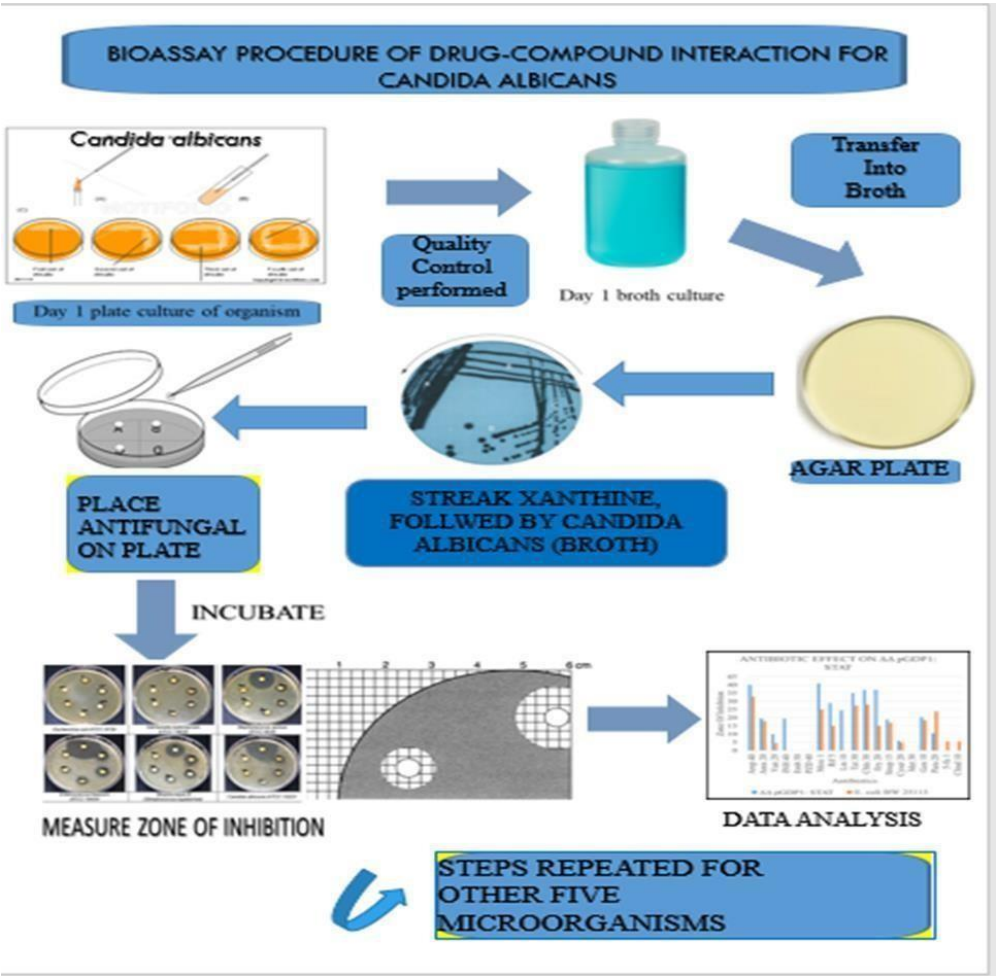

***Figure 2.0.*:** Graphical representation of Bioassay procedure of drug-compound interactionfor *candida albicans.*

4.0 EXPECTED OUTCOMES

Combination of the Xanthine based drugs and the antimicrobial (antibiotics orantifungals) may:

· Potentiate the activity of antibiotics and antifungals against the selected bacterial andfungal cultures (less resistant)

· Have no significant effect on the activity of antibiotics and antifungals against theselected bacterial and fungal cultures.

· Suppress the activity of antibiotics and antifungals against the selected bacterial andfungal cultures (more resistant).

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