University of Mohamed khidher Biskra

Faculty of exactes sciences and nature and life sciences

Depertement of nature and life sciences

Master thesis

Domaine: Nature and life sciences

Biological sciense

Applied biochemistry

Ref. : ……

Submitted by:

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At: Thuesday 9 July 2019

Theme

Evaluation of antibacterial and antifungal activities of Calcium hypochlorite *in vitro*

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Academic year : 2018/2019

Acknowledgments

My first thanks to ALLAH, who created me, sent me to this life and helped me in all the difficulties that I faced.

I would also like to thank my supervisor Abdel Wahab DEHIMAT, for his support and guidance. As well as i thank the biostatistic teacher Mr.CHERFAOUI Mouloud for his priceless information to releaze my statistic tests as I thank Mr. Abdelhamid FOUGHALIA, who accompanied me throughout the duration of my internship at the microbiology laboratory of the center for scientific and technical research on arid regions (STRCAR) in Biskra.

I am also very grateful to the veterinary doctor Mrs.TOUMI Mouna so that it is impossible to forget her assistance wich she has given me in order to accomplish this work, as I'm gratful to all the technician's laboratory of Al-Hadjeb Abdul Qadir, Saliaha, Sara and Alima for their understanding and assistance,I thank too all the employees of the administration Samaha, Mohsen, Fadila and SabrinaFor their kindness and respectful manner to deal with all students.I would like to thank also my dear friend BENHAMZA Ibtissem who standed with me and guided me with her priceless advices.

Without forget to thank all the teachers that i met without exception from my first classes of my primary school till now.In short, I would like to thank all the people who have appeared in my life, both as an incentive and as an obstacle.

Thank you all

Dedication

I dedicate this humble work to:

- My precious mother MIMOUNE Hadda ,
- My struggling father Saleh,
- My dear four sisters Soada,Soumia,Souad and Ahlem,
- My lovely tow brothers Djoubir and Mohamed Fouad ,
- My tow grandfather Abdul Ghani and Mansour – MIMOUNE-, may God have mercy on them.
- All my dear friends.

God bless all of you.

I love you from the deep of my heart

Loubna

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g: Gram.

mL: Mililiter.

L: Litre**.**

hr: Hour.

°C: Celsius.

nm: Nanometre

G+: Gram positive.

G−: Gram negative.

ATCC: American type culture collection.

NCTC: National Collection of Type Cultures.

pH: Potential hydrogen .

MHA: Mueller-Hinton agar.

MPO: Myeloperoxidase.

MBC: Minimum bactericidal concentration.

MIC: Minimal inhibitory concentration.

µg: Microgram .

µl : Microlitre .

Ca (ClO) $_2$: Calcium hypochlorite.

HClO: *Hypochlorousacid.*

ClO^-: Hypochlorite ion.

H^+: Hydrogen ion.

STRCAR: The center for scientific and technical research on arid regions.

SPSS: Statistical Package for the Social Sciences.

GN: Gentamicin.

ANOVA: ANalysis Of Variance.

Introduction

Everywhere on earth, microorganisms are abundant, even within our own human bodies. Bacteria are found all over our skin, in our mouths, up our noses, and within every part of our interior bodies. Outside of us, microorganisms including bacteria, viruses, fungi, and protozoa proliferate in every known environment, from the frozen arctic regions to the warm tropics. The possible presence of pathogenic organisms that have the ability to cause dangerus diseases threat human health (Pepper *et al.*, 2011; Britz *et al.*, 2012).

Water is an important way of infectious agents diffusion that has been loosing importance along the last century as different strategies, such as drinking water disinfection. The cholera and typhoid fever incidence and mortality decrease is a clear example of what has happened in the developed countries (Bald et al., 2004).

Disinfection is a process that deliberately reduces the number of pathogenic microorganisms(Marhaba, 2009),these microorganisms can also find their way into river systems when wastewater treatment plants overflow after heavy rainfall, thereby causing further contamination of surface water (Hutchison *et al.*, 2010).

Chlorine, the most common and widely applied water disinfection method in the world was first discovered in 1774 in its gaseous state by Mark Steele in Sweden (Lazarova and Bahri, 2004). In 1886, the first chlorine disinfection occurred when it was applied to combat a typhoid fever epidemic (Schoenen, 2002).

Chlorine exists in three common forms: chlorine gas, hypochlorite (sodium hypochlorite or calcium hypochlorite) and chlorine dioxide (Newman, 2004; Ivey and Miller, 2013).

Hypochlorites are commercially available in dry and liquid forms and are considered much safer than other chlorine sources such as chlorine gas and chlorine dioxide,Calcium hypochlorite, another form of hypochlorite, is available in the form of powder, tablets or granules (Lewis, 2010).

This allows the Calcium hypochlorite ($Ca(ClO)_2$)to be very effective against bacteria, algae, slime, fungi and other microorganisms (Newman, 2004).

The present work was under taken in the view the following objectives:

- To evaluate the antibacterial activity of calcium hypochlorite against ten bacterial strains.

- To measure the minimum inhibitory concentration (MIC) of calcium hypochlorite against ten bacterial strains.
- To measure the minimum bactericidal concentration (MBC) of calcium hypochlorite against ten bacterial strains.
- To evaluate antifungal activity of calcium hypochlorite against *Candida albicans*

This study is divided into two parts:

- The first part consists of a two bibliographics chapters the first one represents general backround on calcium hypochlorite and the second chapter is simple generalizations about biological activities (antimicrobial activities).
- The second partrepresents the experimental part of this study it includes two chapters the first one on the materiels used and the methodology followed. The second one includes the results obtained and discussed in this study.

We conclude this study with a conclusion.

Bibliographic part

- Chapter 1 -

Background about Calcium hypochlorite

1.1 The history of chlorination

In 1744, in Sweden, chlorine was first discovered. In 1835; it was used to remove bad odors from water. People believed thatthese odors emanated from the water responsible for diseases andtheir transmission at that time. However; chlorine's ability to clean up was not discovered. In 1890; chlorination began in Great Britain and then spread to the United States of America in 1908 and Canada in 1917. Today chlorination is the most widely used method in the world(Alobaied, 2013).

1.2 Calcium hypochlorite

Calcium hypochlorite ($Ca(ClO)_2$) is a member of the class of chemical compounds known as halogen oxyacid salts(San Su and Ogle, 2009).It is an alkaline white powder that was initially used for drinking water treatment and industrial sterilization(Souza *et al.*, 2018).

1.1 Physico-chemical properties of calcium hypochlorite

The physico-chemical properties of calcium hypochlorite have great affection on his fonctionality to destruct germs(Dutta and Saunders, 2012).The deffrents phisico-chemical properities of calcium hypochlorite are summerized in the Table 1 .

Table1. Physico-chemical properities of calcium hypochlorit.

Property	**Value**	**References**
Physical state and appearance	Solid with white or grayish-white powder by chlorine-like odor	O'Neil *et al.*, 2001
Relative density	2.35 g/cm3	Weast and Astle, 1984
Water solubility	Approximately 214 g/L (20 °C)	Mark et al., 1963
Melting point	Decomposes at 175 °C	

1.4 The mains fields of using calcium hypochlorite

- Calcium hypochlorite ($Ca(ClO)_2$)is distributed in a formulated granular solid product for the purpose of swimming pool chlorination(Clancey, 1975).
- Calcium hypochlorite ($Ca(ClO)_2$) is an excellent stain removal, it kills the bacteria responsible for malodour generation, ithas also oxidative properties against odorous

compounds such as sulphur; aldehydes and esters derivatives; highly-effective disinfectant action as Friedman it described (Friedman, 2004).

- Calcium hypochlorite ($Ca(ClO)_2$)in aqueous solution was tested as a disinfectant additive to type V dental stone (Twomey *et al.*, 2003).

1.5Advantages and disadvantages of calcium hypochlorite uses

Despite the widespread use of calcium hypochlorite ($Ca(ClO)_2$), there are some disadvantages that prevent its use in some cases Table 2shows some of its disadvantages and his advantages of using as disinfectant (Bloomfield, 1996).

Table 2. Adantages and disadvantages of calcium Hypochlorit uses.

Advantages	Disadvantages
•Very effective postharvest disinfectants of fungus and bacteria in fruits and vegetables. •Inexpensive in comparison to other disinfectants. • Easy availability. •It may be implemented in operations of any size or scale.	• May not be effective against parasites. • Taste and odor are unacceptable to some. • Contact time is required.

1.6 Interaction of calcium hypochlorite with water

The mode of action of Calcium hypochlorite ($Ca (ClO)_2$) in water is attributed to the dissociation of hypochlorous acid (HClO); which is formed when chlorine dissolves in water, into one hydrogen ion (H^+) and one hypochlorite ion (ClO^-) in(reaction 2). Since two hypchlorous acid (HClO) molecules are produced from one calcium hypochlorite ($Ca (ClO)_2$) molecule in (reaction1); this later is considered like a strong oxidant (Lewis, 2010).Hypochlorous acid is considered to be the most potent biocide in the aqueous mixture (Karsa, 2007).

$$Ca(ClO)_2 + 2H_2O \rightleftharpoons Ca(OH)_2 + 2HClO \quad \ldots\ldots 1$$

$$HClO \rightleftharpoons ClO^- + H^+ \quad \ldots\ldots 2$$

The sum of hypochlorous acid and hypochlorite ion { HClO+ClO-} concentrations is together known as '**free chlorine'** wich is more powerful as a bactericide than combined chlorine estimated that 25 times as much combined chlorine is needed to achieve the same degree of kill of bacteria as free chlorine in the same time(Brandt *et al.*, 2017).

1.6.1 Hypochlorous acid

Hypochlorous acid (HOCl) is a weak acid; as described by Winterbourn and Kettle(2000). It is the major strong oxidant; generated by neutrophil cells wich formed from the activation of phagocytes through myeloperoxidase (MPO)-mediated peroxidation of Cl^-using H_2O_2. Hypochlorous acid (HOCl) is responsible for the killing action of phagocytes against a variety of pathogens (Giles *et al.*, 2001; Winterbourn, 2002; Turell *et al.*, 2008).

1.6.2Hypochlorite ion

Hypochloriteion (ClO-) serves as a powerful antimicrobial agent in human immune system (Wang et al., 2016; Tang et al., 2018) .It plays critical roles in many biologically vital processes(Song et al., 2018).The activity of hypochlorite as a bactericide is slightly inferior to that of the hypochlorous acid,being almost 80 times less powerful(Instruments, 2010).

1.7 Factors relating to the disinfection efficiency of chlorine

Chlorine is the most widely used like water biocide; the amount which needs to be added is determined by temperature;time of contact and pH of water (Moran, 2018).

1.7.1 Temperature.

Low temperature causes delay in disinfection ; the rate of disinfection is significantly affectedby temperature; reducing as the temperature falls(Brandt *et al.*, 2017).

1.7.2Time of contact

The disinfecting effect of chlorine is not instantaneous andsufficient time must be allowed for the chlorine to kill organisms (Brandt *et al.*, 2017).

1.7.3 pH

The proportion of hypochlorous acid (HOCl)andhypochloriteion(OCl−) in a solution depends on pH of the water as the (Figure1)showes, between pH 3 and 6 the predominant species is hypochlorous acid (HOCl).

Within this pH range, the concentration ofhypochlorous acid(HOCl) is optimal,and its dissociation is minimal ;that's why this disinfectant is effective at low pH values ,whereas at higher pH, hypochlorite ion (OCl−)is formed (SHI *et al.*, 2009; Brandt *et al.*, 2017).

The dissociation of hypochlorous acid (HOCl) to the less microbicidal form hypochlorite ion (OCl−) depends on pH (Figure 1).

The disinfecting efficacy of chlorine decreases with an increase in pH that parallels to the conversion of hypochlorous acid (HOCl) into (OCl−) (McKeen, 2012)

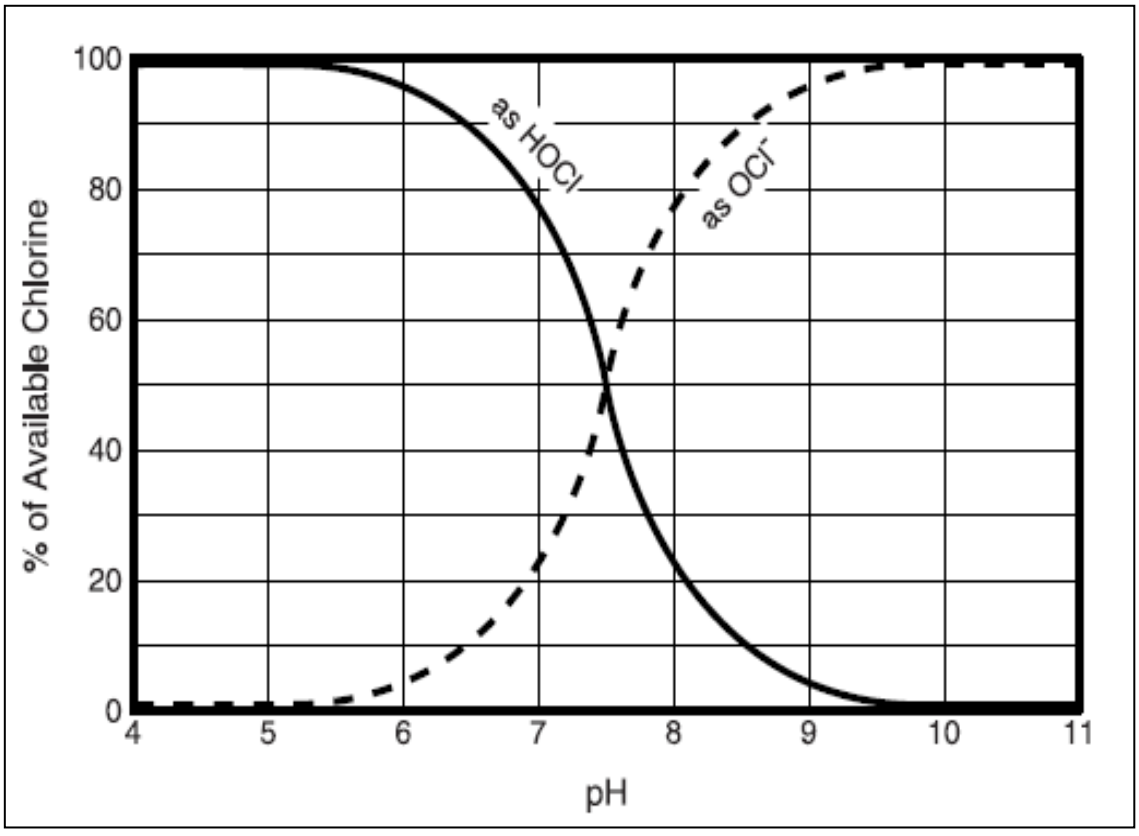

Figure 1. Relationship between Hypochlorous acide and Hypochlorite ion at various pH value(Instruments, 2010).

1.8 Antimicrobial activity of Calcium hypochlorite

Calcium hypochlorite ($Ca(ClO)_2$) shows antibacterial properties. It acts by oxidation of bacterial cell walls, reaction with the cell membrane, and induction of cell lysis and microbial death. Because chlorine remains a popular disinfectant owing to its ease of applicationand (Van Haute *et al.*, 2013; Mohammed, 2019).

Hypochlorous acid (HClO) molecules are neutral in charge and small in size; resumt from dissolution of calcium hypochlorite in water; these properties allow them to easily diffuse through the cell walls of bacteria; this changes the oxidation-reduction potential of the bacterian cell and inactivates triosephosphate dehydrogenase, an enzyme which is essential for the digestion of glucose, so the inactivation of this enzymes effectively destroys the microorganism's ability to function (Escudero-Oñate, 2014).

- Chapter 2 –

The strains to be tested

2.1 Antibacterial activity

Different methods of disinfection chemical, physical and photochemical are essential for the treatment of contaminated water.Calcium hypochlorite ($Ca(ClO)_2$) is one of the chemical disinfectants commnly used as(Buchholz and Matthews, 2010) demonstrared it .

2.1.1Gram positive bacteria

2.1.1.1 *Enterococcus faecalis*

E. faecalis is a Gram-positive obligate anaerobe bacterium(Valera *et al.*, 2009).It is also nonmotile, most cells are arranged in pairs or as short chains (Vidana, 2015).

E. faecalis is commonly present in the gastro-intestinal microbiota of warm-blooded animals and insect guts. These bacteria can also cause infections and diseases in immunocompromised individuals(Martino *et al.*, 2018).

2.1.1.2 *Listeria monocytogenes*

L.monocytogenes is a Gram-positive facultativeanaerobicbacterium that can cause meningitis and encephalitis(Tecellioglu *et al.*, 2019).

It found in soil and the intestines of a wide range of animals, including mammals, birds, fish, also in contaminated food, including pasteurized dairy products, undercooked meat, and drinking water, can be a source of infection for human beings (Rabinowitz and Conti, 2010).

It is the only one of six *Listeria*species pathogenic for humans (Weinberg, 2007); it can cause a rare but serious disease called listeriosis, especially among pregnant women, the elderly or individuals with a weakened immune (Ramaswamy *et al.*, 2007).

2.1.1.3*Staphylococcus aureus*

S.aureus is a pathogenic Gram-positive, facultative anaerobic, non spore-forming bacterium growing in grapelike clusters (Wirtanen and Salo, 2016).

S.aureus can proliferate and produce toxins that lead to strong inflammation of the mammary tissue(Rodríguez and Fernández, 2017). It is the second most common bacterium isolated from blood cultures and is by far the most common hospital-acquired infection (Ricke et al., 2012). It can cause a wide range of diseases, including skin infections, infections in internal organs, and intoxications (Langsrud, 2009).

2.1.1.4 *Bacillus cereus*

B.cereus is Gram-positive; rod shaped; spore-forming bacterium which have been recognized as one of the major foodborne pathogens; its pathogenicity associated with its toxin-producing ability varies among strains(Prakitchaiwattana and Det-udom, 2017); it has the ability to grow at a variety of temperatures and pH(El-Arabi and Griffiths, 2013).

B. cereus causes two distinct forms of foodborne disease: a diarrheal syndrome and an emetic syndrome; both through the production of distinct toxins;the emetic illness is associated with the production of a preformed heat stable toxin in foods while the diarrheal disease is caused by toxin production in the gut (Griffiths and Schraft, 2017).

2.1.2 Gram negative bacteria

2.1.2.1 *Escherichia coli*

E.coli is a non-spore-forming; Gram-negative bacterium, usually motile by peritrichous flagella ,Theodor Escherich was the first scientist to isolate *E. coli* in 1885 and it was initially known as *Bacterium coli* ; later it was renamed *Escherichia coli*(Bester, 2015).

It is the most common cause of acute urinary tractinfections.; *it* may also cause acute enteritis in humans as well as animals(Percival and Williams, 2014). *E. coli* are normal flora in the body of human beings and they can be non-pathogenic, commensal or pathogenic(Kaper *et al.*, 2004).

2.1.2.2 *Pseudomonas aeruginosa*

P. aeruginosa is a gram-negative bacillus bacterium found widely in nature; soil and water. It has a pearlescent appearance and grape-like; it grows well at 25℃ to 37℃ and its ability to grow at 42°C helps to distinguish it from many other *Pseudomonas*species.

P.aeruginosa is a ubiquitous microorganism which has the ability to survive under a variety of environmental conditions.

It not only causes disease in plants and animals, but also in humans, causing serious infections in immunocompromised patients with cancer and patients suffering from severe burns and cystic fibrosis(Golemi-Kotra, 2008; Wu and Li, 2015).

2.1.2.3 *Vibrio cholerae*

V.cholerae is a curved; motile ; gram-negative; bacillus; non-invasive ; intestinal pathogen bacterium; it is also a free-living aquatic bacterium(Bennett *et al.*, 2015)

In humans it causes cholera, the deadly diarrhoea that was responsible for millions of deaths during seven pandemics since 1817, and still thousands every year (Cava, 2017). Cholera results from secretory diarrhea caused by the actions of cholera toxin (CT) on intestinal epithelial cells(Bennett *et al.*, 2015).

2.1.2.*4 Klebsiella pneumoniae*

K.pneumoniae is a Gram-negative bacterium, nonmotile, encapsulated, rod-shaped bacillus the genus*Klebsiella* and family *Enterobacteriaceae*that is frequently found in the flora of the mouth, skin and intestines as well as in natural environments (Brabb *et al.*, 2012; Fahey and Westmoreland, 2012; Wu and Li, 2015).

K.pneumoniae is facultatively anaerobic bacterium, it produces acid and gas from lactose(Brabb *et al.*, 2012).*K.pneumoniae* is typically occurs in patients who are immunocompromised due to age, ethanol abuse, or diabetes mellitus. Infection produces hemorrhagic necrosis, microabscesses (Wu and Li, 2015).

2.1.2.5 *Klebsiella oxytoca*

K. oxytoca is a rod-shaped, nonmotile, Gram-negative bacterium *K.oxytoca* species are normally associated with the community and hospital-acquired infections particularly in immunocompromised patients (Trivedi *et al.*, 2015). It is distinguished from *K.pneumoniae* based on its ability to produce indole from tryptophan.It may also be resistant to multiple antibiotics.

There is evidence that *K.oxytoca* can cause hemorrhagic colitis associated with antibiotic use (Donnenberg, 2015). The incidence of meningitis caused by *K. pneumoniae*and *K. oxytoca* is unknown but particularly severe neurological complications such as hydrocephaly; empyema; and brain abscesses have been described in these cases (Carrie *et al.*, 2019).

2.1.2.6 *Salmonella spp.*

*Salmonella spp.*are Gram-negative bacteria capable of infecting a wide range of host species, including humans, domesticated and wild mammals, reptiles, birds and insects (Roy and Malo, 2002). One species of *Salmonella* may affect a wide variety of animal species (Shomer *et al.*, 2015).

It represents a major health risk to humans, capable of causing gastroenteritis (Ricke *et al.*, 2015). They multiply in the small intestine, colonising and producing an enterotoxin and causing an inflammatory reaction and diarrhoea (Bell and Kyriakides, 2009).

2.2 Antifungal activity

Disinfectants are defined as chemical agents capable of removing infectious microbes other than bacterial spores (Gupta *et al.*, 2002). A number of commonly used disinfectants are known to be relatively ineffective against fungi, moreover, not all fungal species are equally sensitive to a given product, and even different strains of the same fungal species may vary in resistance(Jeffrey, 1995).

Chlorine was the only agent capable of killing within 15 min all five fungal strains tested which oriented to study of the fungicidal activities of commonly used disinfectants and antifungal pharmaceutical spray preparations against clinical strains of *Aspergillus* and *Candida species*(Gupta *et al.*, 2002).

2.2.1 *Candida albicans*

Candida albicans is classified as an opportunistic fungus because it usually only causes disease in those who are immunocompromised. *Candida* species are yeast-type fungi. It is the most common pathogen among the *Candida* species Garbe (Dowd, 2007).

It is a particularly common fungal skin infection in both humans and other mammals (Issa and Hamblin, 2015).With stress, disease, immunosuppression, and long-term antimicrobial therapy, yeast overgrowth may occur and lead to morbidity and mortality if left untreated (Griner and Walch, 1978;Pollock, 2003;Werther et al., 2011).

Experimental part

- Chapter 3 -
Materiels and method

3.1 Materials

3.1.1. Laboratoryequipments

The reagents and apparatus used in this study are quoted in (Appendix I and II).

3.1.2 The chemical product tested

Calcium hypochlorite is a chemical substance used primarily for drinking water treatment. It is powder, readily dissolved in water and contains about 65 % available chlorine.

The calcium hypochlorite that we used in our study was provided by the Algerian directorate of water - Biskra unit, where it was kept away from moisture and light without exposing it to contact with any metal (Figure 2).

Figure 2. Calcium hypochlorite (original photo).

3.1.3. Biologicalmaterial

3.1.3.1. Pathological bacterial strains

Pathogenic bacterial strains used in this work, were obtained from the American Type Culture Collection (ATCC), except*Staphylococcus aureus* was obtained from the National Collection of Type Cultures (NCTC), those strains were provided by research laboratories from Biskra, Jijel, Mila, Algiers and TlemcenTable 3 demonstrates the deffrent ATCC codes and Gram type of each pathogenic bacterial strains used in this study.

Tableau 3. Pathological bacterial strains used

	Straine	ATCC code
Gram positive bacteria	*Listeria monocytogenes*	ATCC 35152
	Bacillus cereus	ATCC 14579
	Staphylococcus aureus	NCTC 8325
	Enterococcus faecalis	ATCC 10541
Gram negative bacteria	*Escherichia coli*	ATCC 25922
	Pseudomonas aeruginosa	ATCC 27853
	Klebsiella oxytoca	ATCC 13182
	Klebsiella pneumonia	ATCC 13883
	Salmonella spp.	ATCC 14028
	Vibrio cholerae	ATCC 14035

3.1.3.2 The fungus strain

To evaluate the antifungal activity of Calcium hypochlorite in this study we used *Candidaalbicans* strain was obtained from the American Type Culture Collection (ATCC), itsATCC code 10231, it was providedby a research laboratory from Tlemcen.

3.2. Method

3.2.1. The study of antimicrobial activity

3.2.1.1. The study of antibacterial activity (Agar well diffusion assay)

Antibiogram provides qualitative results by categorizing bacteria susceptible, intermediate or resistant (Reller *et al.*, 2009).

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts (Magaldi *et al.*, 2004; Valgas *et al.*, 2007).

In order to evaluate the antibacterial activity of calcium hypochlorite in vitro against ten pathological bacterial strains quoted in (Table 3).Agar well diffusion assay was employed with simple modifications as described by Irshad et *al*. (2012).

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured (Balouiri *et al.*, 2016).

Antibacterial agent diffusionleads to inhibition of bacterial growth in the vicinity of the source and to the formation of clear 'zones' without bacterial lawn. The diameter of these zones increases with antibacterial agent concentration.(Bonev *et al.*, 2008).

Antimicrobial activity of calcium hypochlorite is observed by the presence of an inhibition zone around the well plus the zone of inhibition is large plus the microbial strain is sensitive.

a) Agar well diffusion method steps

In order to achieve the protocol of agar well diffusion method we follow the next steps:

- **Sterilization of materiels**

In an alminium paper coated distilled water, physiological saline, cork-borer and Muller hinton agar culture medium that’s to sterilize all in the autoclave(Appendix I)at 121°C for 15 minutes (Harrar, 2018).

- **Preparation of culture medium**

Mueller Hinton agar, a medium widely used for the propagation of some fastidious pathogenic bacteria (Tanaka *et al.*, 2014) . We prepare this Mueller Hinton agar mediumfrom the components listed in (Annex II)(Lourens *et al.*, 2004).

In the sterile zone ofthe laboratorybench, we pour the Hünther Muller agar medium into the petri dishes to become more cohesive ,then it will be sterilized in the autoclave (Appendix I) at 121°C for 15 minutes (Harrar, 2018), with the various equipment that will be used in this experiment to become ready to use (Figure 3).

Figure 3. Preparing the culture medium and pouring petri dishes.

- **Preparation of bacterial suspensions (inoculum)**

The bacterial strains are seeded in nutrient agar and incubated at 37 ° C for 24 hr to optimize their growth.Platinum handle is used to remove some well-isolated and identical colonies of each bacterial strain to be tested. Discharge the platinum handle of each bacterian strain into 5 ml of sterile physiological water, the bacterial suspension is well homoginized, the sterile swab was vigorously mixed with a vortex (Appendix I) to ensure complete dissolution of the bacterial cells, the opacity of the susponion should be equivalent to 0.5 Mc Farland where a spectrophotometer (Appendix I) was used to measure an absorbance should be between 0.08 to 0.1 at 625 nm, the inoculum can be adjusted by adding either bacterial culture if it is low concentration or sterile physiological water if it is too concentrated(Harrar, 2018).

- **Preparation of the defferent concentrations of calcium hypochlorite**

In this step, we prepare various concentrations of calcium hypochlorite. Initially, we prepare a mother solution by mixing 1 gram of calcium hypochlorite powder in 100 mL of distilled water and placing this solution over a stirring plate (Appendix I) until calcium hypochlorite powder is fully dissolved in distilled water.

Based on this mother solution, its concentration is 10 g / L.We prepare three dilute son solutions, which are 7,5 g / L; 5g / L and 2,5g / L. We evaluate antibacterial activity and antifungal activity with these concentrations.

- **Seeding**

Seeding is performed by swabbing on petri dishes, a sterile swab is dipped in the bacterial suspension and then wringing it by pressing firmly on the inner tube wall, the swab is rubbed on the whole of the agar surface in the dish of petri up and down in tight streaks The operation repeated three times by turning the petri dish of 60°, each time the sowing is finished by passing the swab a last time on the border of the petri dish(Allouache *et al.*, 2018).

We do sowingofthe ten suspensions which contain the pathogenic bacterial strain used in this study in petri dishes.Theseeding of each bacterial suspenion is repeated 3 times by the same method (Figure 4).

Figure 4. Seeding the inoculum.

- **Formation and filling the wells**

Test plates were previously labeled into five parts around the center of petri dish wich is considred for the positive control :the first part for the negative control ,the second , the third the fourth and the fifth part specialised for the different concentrations of calcium hypochlorite 2,5g / L; 5g / L; 7,5 g / L and 10 g / L respectively.

We make the inoculation of the agar plate surface by spreading a volume of the microbial inoculum over the entire agar surface.In the sterile zonebetween tow bunsen burnerswells of about 6mm diameter were aseptically cut out from the inoculated plates using asterile cork-borer allowing 30mm between adjacent wells and the edge of the petri dishes.

We put the gentamicin disc in the appropriate part wich is the center of petri dish, this served as the positive control.50 µL of sterile distilled water added into well served as the negative control.50 µL of the different concentration of calcium hypochlorite was pipetted into its appropriate wells.The method was repeated three timesof each type of bacterian strain (Figure 5).

Figure 5. Filling the wells.

- **Incubation**

The petri dishes were incubated for 24 hours at 37ºC and observed for zone of inhibition millimeter(mm) around the wells(Nayeema, 2017).The antibacterial agent diffuses in the agar

medium and inhibits the growth of the microbial strain tested(Balouiri *et al.*, 2016). In our study we use the calcium hypochlorite as an antibacterial agent.

b) Tree parameters to evaluate antibacterian activity

- **measurement of zone of inhibition**

The growth plates were evaluated after 24 hours of incubation,the zones of inhibition on Mueller-Hinton *(*MH) plates of each bacterial strain were read from the back of the agar dish, against a dark background.

A pair of calipers were used to make the measurements while holding the plates approximately 30 cm from the eye (Matuschek *et al.*, 2014) (Figure 6).The zones of inhibition were then statistically analyzed.

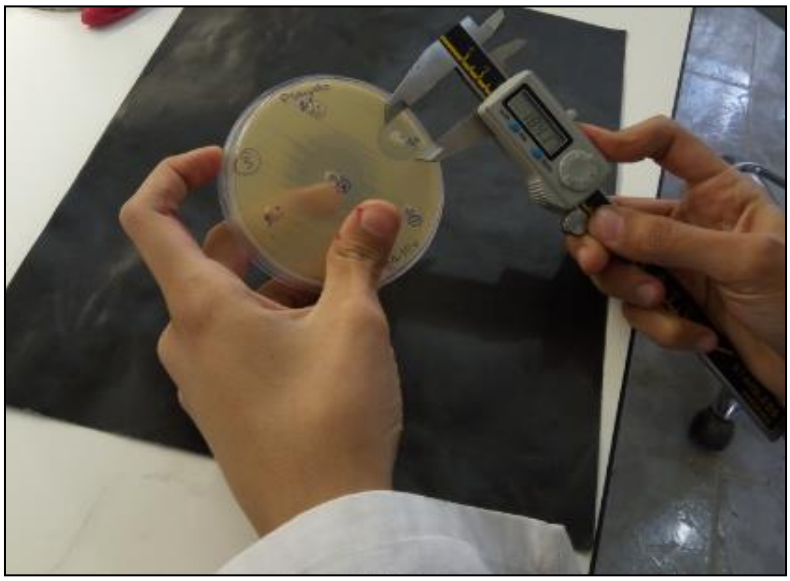

Figure 6. Reading inhibition zones.

- **Determination of the minimum inhibitory concentration (MIC) andthe minimum bactericidal concentration (MBC).**

The bacterial growth inhibition does not mean the bacterial death, agar well diffusion method can not distinguish bactericidal and bacteriostatic effects (Balouiri *et al.*, 2016).

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial ingredient or agent that is bacteriostatic (prevents the visible growth of bacteria). MICs are used to evaluate the antimicrobial efficacy of various compounds by

measuring the effect of decreasing concentrations of antibiotic or antiseptic(Vassallo *et al.*, 2018).

After the determination of the minimum inhibitory concentration we determine the minimum bactericidal concentration (MBC), by following the protocol of Vaquero *et al.*(2007), with simple modifications.

The minimum bactericidal concentration (MBC), also known as the minimum lethal concentration (MLC), is the most common estimation of bactericidal activity.

The MBC is defined as the lowest concentration of antimicrobial agent needed to kill 99.9% of the final inoculum after incubation for 24 h under a standardized set of conditions.

The minimum bactericidal concentration is determined by subculturing the preparations that would have shown no evidence of growth in the minimum inhibitory concentration (MIC) determination.These subcultures are made either in broth or in agar plates.On agar the lowestconcentration showing lack of growth represents the minimum bactericidal concentration (MBC) (Barry *et al.*, 1999; Ncube *et al.*, 2008).

We prepared another petri dishes containing Muller Hinton agar and labeled into five parts each part we put in it samples removed from the inhibition zones around each well of different concentrations of calcium hypochlorite by sterile platinum handle and plant them randomly in the corresponding parts in newly prepared Petri dishes. This later were incubated for 24 hours at 37ºC (Nayeema, 2017).

- **Measurement of activity index**

Activity Index represents inhibition zone of test sample divided by inhibition zone of a standard (M *et al.*, 2007).The activity index of the sample gives a quantifiable data for the identification of the level of antibacterial activity possessed by the sample (Nayeema, 2017).

$$Activity\ Index = \frac{\text{Zone of Inhibition of extract}}{\text{Zone of Inhibition of Antibiotics}}\ X\ 100$$

3.2.1.2 The evaluation of antifungal activity

In order to evalute the antifungal activity of calcium hypochlorite against *Candida albicans* its ATCC code 10231, we felow the same protocole that we used to evaluate antibacterial activity ,agar well diffusion assay was employed with simple modifications as

described by Irshad *et al.*(2012),by using the same concentration of calcium hypochlorite 2,5g / L; 5g / L; 7,5 g / L and 10 g / L .

We fellow the same steps only we change Muller hinton agar medium by Sabourau agar medium. We prepare it from the components quoted in (Annex II). because Sabourau agar medium used to cultivate fungi (Sandven and Lassen, 1999; Guinea et al., 2005).

The inhibition zones were determined after incubation at 27 °C for 48 hours(Sofiane, 2018).All experiments were repeated thrice

3.2.2 Statistical analysis

In the first case to evaluate antibacterial activity, we realized our statistical analysis of the effect of concentration and type of strain on the average diameter formed, that's why we apply ANOVA tow-way method.

In the second case to evaluate antifungal activity ,weto do our statistical analysis of the effect of Calcium hypochlorite's concentrations factor on the average diameter formed of *Candida albicans* fungal strain,we apply ANOVA one way method.

ANOVA tow-way and ANOVA one way methods are the most suitable for the statistical analyses of those tow problems; also to reach our objectivewe used Tukey's statistical method.

We release those statistical analyses by using SPSS IBM statistics program.

- Chapter 4 -
Results and discussion

4.1. Antibacterial activity

4.1.1 Zones of inhibition measurment

Antibacterial activity tested for four 4 concentrations of Calcium hypochlorite 2,5g / L; 5g / L; 7,5 g / L and 10 g / L were performed at ten 10 pathological bacteria by using agar well diffusion method. The zones of inhibition formed by deffrent concentrations of Calcium hypochlorite (Appendix III) were measured in milimeters and compared with the zones of inhibition of antibiotic which were used as positive control (Gentamicin), the means and standard deviations (±SD) of the diameters of zones of growthinhibitionshas been shown in Table 4 ,allmeasurements obtained from triplicate.

Table 4. The diameter of the zones of inhibition of bacterial growth formed by deffrent concentration of calcium hypochlorite and the positive control (mm).

	Strain	**Positive control (GN)**	**10 g/L**	**7,5g/L**	**5g/L**	**2,5g/L**
Gram positive bacteria	***E. faecalis***	28,00±2,00	11,67±0,57	10,66±0,57	9,00 ±0,00	8,66± 1,15
	L.monocytogenes	43,66±0,48	-	-	-	-
	S.aureus	39,66±0,47	-	-	-	-
	B. cereus	43,33±2,88	10,66±1,15	9,00 ± 1,73	7,33±0,57	6,33 ±0,57
Gram negative bacter	***E.coli***	29,66±0,57	11,00±1,00	9,00± 1,00	7,66 ± 1,15	6,66±2,08
	P.aeruginosa	28,66±0,57	10,66±1,15	9,00 ± 1,00	7,33 ±0,57	6,30±1,00
	V.cholerae	30,66 ± 1,15	16,66±0,57	13,00±1,00	11,33±1,15	9,66±1,52
	K.pneumoniae	30,33±0,57	15,33±0,57	12,66±0,57	11,00±1,00	7,33±1,15
	K. oxytoca	32,33±1,52	11,00±1,00	9,66 ± 1,15	8,66 ± 1,15	7,66±1,15
	S. spp.	28,33±1,527	14,66±1,15	9,66 ±0,57	8,33±0,57	6,33±0,57

The different bacterial strains tested gram positive (*E. faecalis, L.monocytogenes, S.aureus, B. cereus)* or gram negative(*E.coli,P.aeruginosa, V.cholerae, K.pneumoniae, K. oxytoca, S. spp.)*showed a sensitivity to antibiotic used as a positive control which is the (GN) gentamicin as it described byDean (2012) .

The results showed thatCalcium hypochlorite by his different concentrations from 2,5g / L to 10 g / L has an effect on bacterial growth by stopping their activity by varying degrees whether gram-positive or negative, exceptof two types of Gram-positive bacteria *Staphylococcus aureus* and *Listeria monocytogenes*,that did not show no sensitivity to defferent Calcium hypochlorite concentrations tested; as Virto *et al*.(2005)demonstrate that *L. monocytogenes*was more resistant to chlorine in distilled water. Only gentamicin exhibitsantimicrobial activityagainst *Staphylococcus aureus*and *Listeria monocytogenes*.

At each concentration of calcium hypochlorite from 2,5g / L to 10 g / L we note that the diameters ofthe inhibition zone in both gram-positive and gram-negative bacteria are different:

Where the diameter of the inhibition zone is often greater in gram-negative bacteria compared to the diameter of the inhibition zone of gram-positive bacteria in the same concentration; because generally, gram negative microorganisms are more fragile towards C hemical disinfectants than gram positive microorganisms (*L.monocytogenes, S.aureus)* due to the intracellular space between the two peptidoglycan layers in gram positive organisms providing more resistance to inactivation (Mir *et al.*, 1997; Bester, 2015).

The results of this study showed that There is a positive relationship between the length of the diameter of inhibition zone and the concentration of calcium hypochlorite in all the bacterial strains that have been sensitive to it where ,thesmalllest diameter of the inhibition zone at:

- Gram positive bacteria is *B. cereu*with an inhibition diameter zone of 6.33 ± 0.57 in 2,5g / L concentration of Calcium hypochlorite
- Gram-negative bacteria are P.aeruginosa and *S. spp.*with an inhibition diameter zone of 6.30 ± 1.00 and 6.33 ± 0.57 respectively in the same concentration of 2,5g / L.

The largest clear zone was seen in *V.cholerae* and *K.pneumoniae*with an inhibition diameter zone16,66±0,57 abd 15,33±0,57respectively for 10g/L concentration of Calcium hypochlorite

4.1.2 The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).

On the basis of the results obtainedby measuring the diameter of the zone of inhibition, it is found that there are zones of inhibitionare formed by all bacteria that show sensitivity to Calcium hypochlorite from the concentration 2.5g / L, we can therefore by the way deduce the minimum inhibitory concentration MIC of Calcium hypochlorite which prevents the visible growth of bacteria MIC from those concentrations used in this case MIC is less than or equal to 2.5 g / L for all bacteria tested, with the exception of bacteria that have not shown sensitivity to calcium hypochlorite which are *S.aureus* and *L. monocytogenes.*

The minimum bactericidal concentration (MBC)of Calcium hypochlorite against eight pathological bacteria is shown in (Figure7).

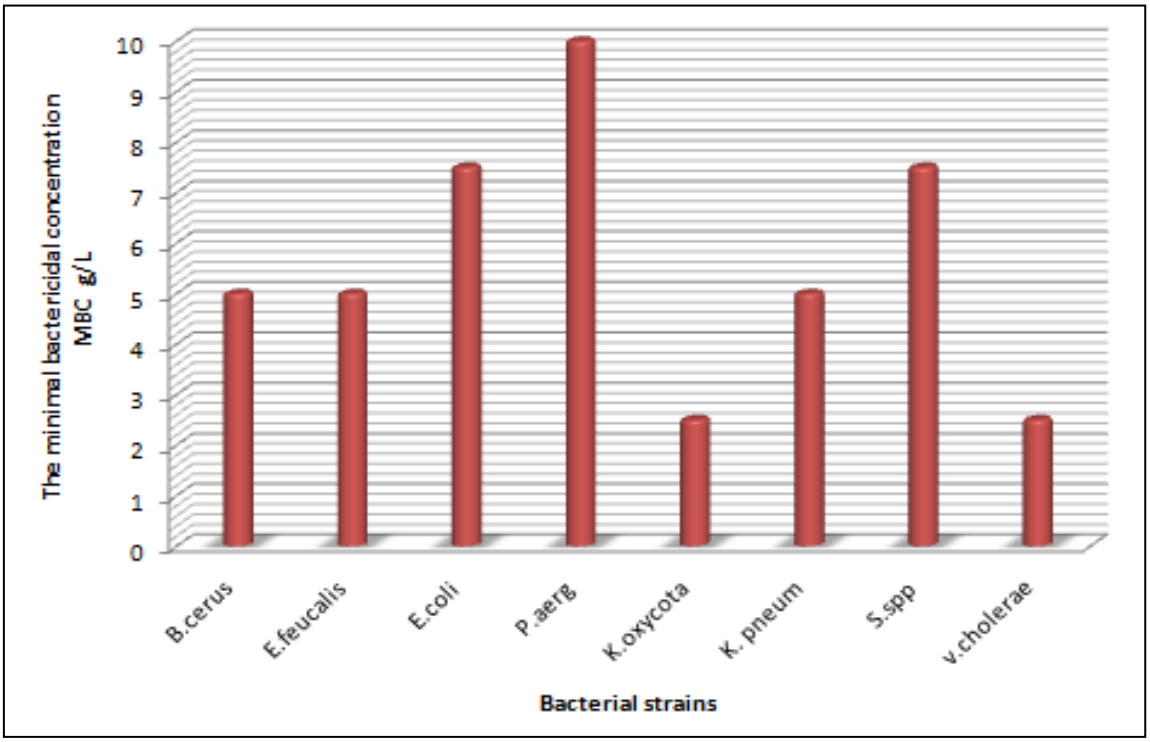

Figure 7. The minimum bactericidal concentration (MBC) of Calcium hypochlorite against eight pathological bacteria.

After the measurement of the diameter of each wellthe minimal bactericidal concentration (MBC) determined by cultivating samples from deffrent inhibition zones incubated. if we don't observe bacterial growth in the new culture that means this

concentration is bactericidal;if we observe bacterial growth in the new culture that means this concentration is bacteriostatistic concentration.

Based on the results shown in Figure 8585 we conclude the following:

- The least minimum bactericidal concentration (MBC) of calcium hypochloritevalue is 2,5g/L that it cans kill completely each of the two gram-negativebacteria (*V.cholerae, K. oxytoca)*because they are more sensitive towards chemical disinfectants .
- The medium minimum bactericidal concentration (MBC) of calcium hypochlorite value is 5g/L that it cans kill completely each of the two gram-positive bacteria (*E. faecalis, B. cereus).*This concentration can also completely eliminate one gram negative bacterium wich is (*K.pneumoniae).*
- The value of 7,5g/L of minimum bactericidal concentration (MBC) of calcium can also completely eliminate both of negativebacteria (*E.coli,S.spp.)*
- We found also that the highst minimum bactericidal concentration value is 10g/L that is the sufficient concentration capable of completely eliminating (*P.aeruginosa)*

4.1.3 Activity index

The bar chart below shows the comparison between the clear zones between the different concentrations of Calcium hypochlorite and the antibiotics used (gentamicin). Different colored bars are used to indicate the concentrations of Calcium hypochlorite shown in figure 8.

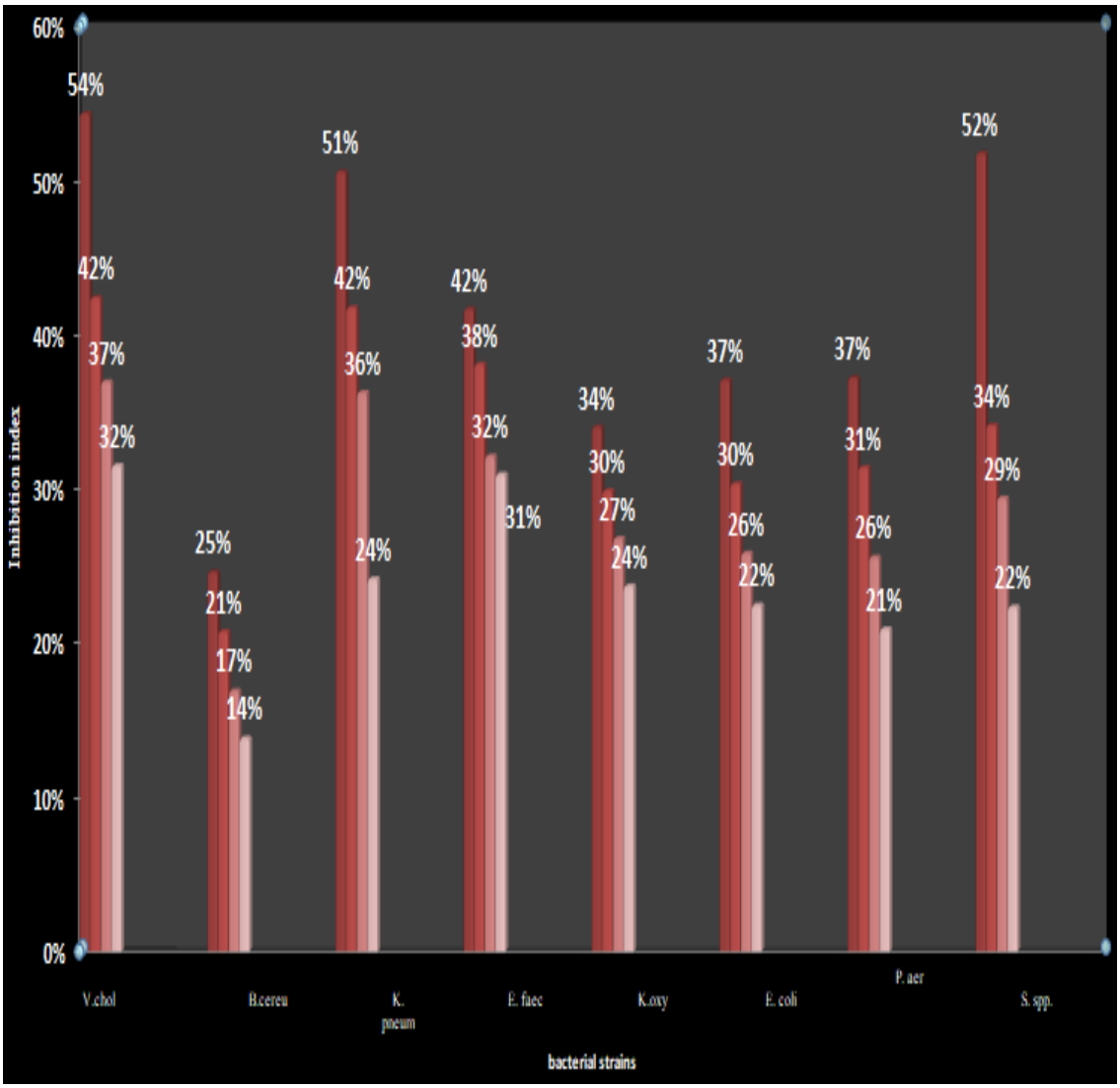

Figure 8. Graphical representation of the activity index of deffrent concentrations of Calcium hypochlorite.

In all four concentration of calcium hypochlorite we observe from the last results that:

- Generaly gram negative bacterian strains represent the bigest activity index than positive strains*(V.cholerae, K.pneumoniae, S.spp.),54%;51%;52%* at 10 g/L of Calcium hypochlorite respectively ouing to their sensitivity to chlorine disinfectant also the positive control gentamicin oriented basicly against negative strains bacteria that's why its represent a big activity index tested
- The and lowest activity index is fo*r (B. cereus) (25%,21%,17%,14%)*equivqlent to 10g/L,7,5g/L,5g/L,2,5g/L respectively, becaus*e (B. cereus)* representsgram positve bacterium wich resists chlorine and the diameter formed by gentamicin against*(B.*

cereus) is low when it compare it by gram negative bacteria lenth diameter ,*(B. cereus)*it has sporulated form that make it resistant as that demonstrated by Hamouda and Baker Jr (2000),vegetative bacteria are more sensitive to chemical agents than bacterial spores.

4.2 Antifungal activity

After treating *Candida albicans* with four concentrations of calcium hypochlorite 2,5g/L, /5g/L, /7,5g/L, /10 g/L.Diameters ofthe inhibition zones are measured around each concentration ,the results obtained have been shown in the figure 9,all measurements obtained from triplicate.

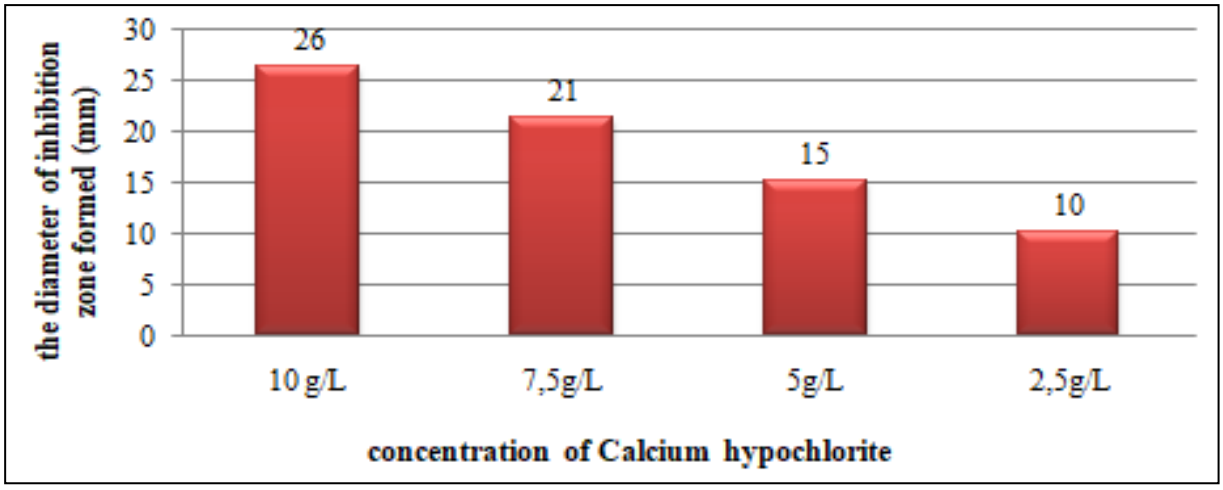

Figure 9. Graphical representation the diameters of inhibition Candida albicans growth at deffrent concentration of Calcium hypochlorite.

Through the results we conclude the following

- The diameters of the inhibition zone begin to form from the lowest concentration of calcium hypochlorite(2,5g/L),that is correspondant to the lowest diameters of the inhibition 10 (mm).
- This diameter is increased by an increase in the concentration of Calciumhypochlorite, 26 (mm) represents is the highest inhibition diameter which corresponds to thehighest concentration of Calcium hypochlorite 10 g/L.
- *Candida albicans* was more sensitive and had considerably larger zones of growth inhibition formed when it compared with inhibition zones diameters of deffrent bacterial strains treated by the same product (Calcium hypochlorite) and at the same concentrations.The diameters of the inhibitory zones resulting from the

treatment of various bacterial strains that showed a sensitivity against Calcium hypochlorite, in it hight concentration 10g/L the length of the inhibition diameter did not exceed (16.66mm), while the diameter of the inhibition zone of the *Candidaalbicans* when it treat by the same concentation of Calcium hypochlorite 10 g/L , the length of the inhibition diameter was (26 mm).

Differentiated results obtained from the diameters of inhibition zones of *Candida albicans* and various bacterial strains, after subjecting them to the same chemical product(calcium hypochlorite) and the same concentrations is logical because, sometimeseven different strains of the same species may show variations in their response to the chlorine disinfectant (Taylor *et al.*, 2000).

4.3 Statistical analysis

4.3.1 Statistical analysis of antibacterial activity

The statistical analysis ofthe effect of concentrationand the strain type on the average diameter formed indicates that these two factors as well as their interaction have a significant effect table 5)..

Table5. Table of tow-factor varience analysis with repetitions.

Tests of Between-Subjects Effects

DependentVariable:Diametre

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Souches	198,725	7	28,389	21,426	,000
Concentration	9348,333	4	2337,083	1763,836	,000
Souches * Concentration	598,067	28	21,360	16,120	,000
Error	106,000	80	1,325		
Total	10251,125	119			

Indeed, the use of the ANOVA tow way method, which is the most adequate for the statistical analysis of the problem addressed, shows that:

- The effect of the strain is highly significant on the average diameter (alpha> 0.000)
- The effect of the concentration is highly significant on the average diameter (alpha> 0.000)
- The effect of the interaction of the strain with the concentration is also highly significant on the average diameter (alpha> 0.000).

These last results only allow us to predict that there is at least one strain whose average diameter is different from the others, which remains true for the case of the concentrations. For further information we propose to make a multiple comparison (two by two) of the effect of strains and concentrations. In order to meet our objective, we used Tukey's statistical method. The results provided by the latter technique are summarized in Tables 6 and 7 for the case of comparison of the effect of the strains and Tables 8 and 9 for the case of comparison of the effect of the concentrations.

Table6. Multiple comparison of strain effect

Multiple Comparisons

Dependent Variable: Diametre
Tukey HSD

(I) Souches		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
V.cholerae	B.cereus	,9333	,42032	,351	-,3749	2,2416
	K.pneumoniae	,9333	,42032	,351	-,3749	2,2416
	E. faecalis	2,6667*	,42032	,000	1,3584	3,9749
	k.oxycota	2,4000*	,42032	,000	1,0917	3,7083
	E.coli	3,4667*	,42032	,000	2,1584	4,7749
	P. aeruginosa	3,9333*	,42032	,000	2,6251	5,2416
	S.spp	2,8000*	,42032	,000	1,4917	4,1083
B.cereus	K.pneumoniae	,0000	,42032	1,000	-1,3083	1,3083
	E. faecalis	1,7333*	,42032	,002	,4251	3,0416
	k.oxycota	1,4667*	,42032	,017	,1584	2,7749
	E.coli	2,5333*	,42032	,000	1,2251	3,8416
	P. aeruginosa	3,0000*	,42032	,000	1,6917	4,3083
	S.spp	1,8667*	,42032	,001	,5584	3,1749
K.pneumoniae	E. faecalis	1,7333*	,42032	,002	,4251	3,0416
	k.oxycota	1,4667*	,42032	,017	,1584	2,7749
	E.coli	2,5333*	,42032	,000	1,2251	3,8416
	P. aeruginosa	3,0000*	,42032	,000	1,6917	4,3083
	S.spp	1,8667*	,42032	,001	,5584	3,1749
E. faecalis	k.oxycota	-,2667	,42032	,998	-1,5749	1,0416
	E.coli	,8000	,42032	,553	-,5083	2,1083
	P. aeruginosa	1,2667*	,42032	,065	-,0416	2,5749
	S.spp	,1333	,42032	1,000	-1,1749	1,4416
k.oxycota	E.coli	1,0667*	,42032	,195	-,2416	2,3749
	P. aeruginosa	1,5333*	,42032	,011	,2251	2,8416
	S.spp	,4000	,42032	,980	-,9083	1,7083
E.coli	P. aeruginosa	,4667	,42032	,953	-,8416	1,7749
	S.spp	-,6667	,42032	,757	-1,9749	,6416
P. aeruginosa	S.spp	-1,1333*	,42032	,139	-2,4416	,1749

Table7. Homogeneous classes of strain effect

Diametre

Tukey HSD

Souches	N	Subset		
		1	2	3
P. aeruginosa	15	12,3333		
E.coli	15	12,8000	12,8000	
S.spp	15	13,4667	13,4667	
E. faecalis	15	13,6000	13,6000	
k.oxycota	15		13,8667	
B.cereus	15			15,3333
K.pneumoniae	15			15,3333
V.cholerae	15			16,2667
Sig.		,065	,195	,351

According to the multiple comparison using Tukey test, it is found that, for any alpha risk threshold $\geq$ 1.7% (Table 6), the strains can be classified according to their average diameter in three homogeneous sets (each set contains strains whose difference in diameter is (significantly zero). Indeed, the first set contains the strain *P. aeruginosa* (smaller diameter), the second set continent *K.oxycota* strain (mean diameter) and the third and last set contains

the three strains *B.cereus*, *K.pneumoniae* and *V.cholerae*. While the *E. coli*, *S.spp* and *E. faecalis* strains belong to the intersection of the first two sets. The latter can be expressed by: in average the diameter of these three strains is significantly close to the right side to the diameter of the *P.aeruginosa* strain and it is close to the left side of the diameter of the *K.oxycota* strain.

Table 8. Multiple comparisonof the effect of concentrations

Multiple Comparisons

Dependent Variable: Diametre
Tukey HSD

(I) Concentration		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
10	7.5	2,3750*	,33229	,000	1,4476	3,3024
	5	3,8750*	,33229	,000	2,9476	4,8024
	2.5	5,3750*	,33229	,000	4,4476	6,3024
	T+	-18,7083*	,33229	,000	-19,6357	-17,7809
7.5	5	1,5000*	,33229	,000	,5726	2,4274
	2.5	3,0000*	,33229	,000	2,0726	3,9274
	T+	-21,0833*	,33229	,000	-22,0107	-20,1559
5	2.5	1,5000*	,33229	,000	,5726	2,4274
	T+	-22,5833*	,33229	,000	-23,5107	-21,6559
2.5	T+	-24,0833*	,33229	,000	-25,0107	-23,1559

Table 9. Homogeneous classes of the effect of concentrations

Diametre

Tukey HSD

Concentration	N	Subset				
		1	2	3	4	5
2.5	24	7,3333				
5	24		8,8333			
7.5	24			10,3333		
10	24				12,7083	
T+	24					31,4167
Sig.		1,000	1,000	1,000	1,000	1,000

For comparison of the effect of calcium hypochlorite concentration on the average diameter of strains considered in our experiments, the Tukey test indicates that each concentration belongs to a separate set. This means that on average each effect concentration,

on the diameter of the strains, significantly (strongly) different from other concentrations (alpha> sig = 0.000). In addition, the diameter of the strains is proportional to the concentration of the product where the minimum diameter is measured for the lowest concentration (2.5 g/L) while the maximum diameter is measured in the case of the positive control.

4.3 Statistical analysis

4.3.2 Statistical analysis of antifungal activity

The statistical analysis ofthe effect of concentrationon the average diameter formed indicates that this one factor has significant effect (Table10).

Table. 10. Table of one-factor variance analysis with repetition

ANOVA one way

Diametre : Alpha = ,05.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1941,667	3	647,222	298,718	,000
Within Groups	17,333	8	2,167		
Total	1959,000	11			

Indeed, the use of the ANOVA one way method, which is the most adequate for the statistical analysis of the problem addressed, shows that:

- The effect of the concentration is highly significant on the average diameter (alpha> 0.000).

Table11. Multiple comparison of effect of concentrations

Multiple Comparisons

Dependent Variable: Diametre

Tukey HSD

(I) Concentration		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
10	7.5	21,66667*	1,20185	,000	17,8179	25,5154
	5	28,33333*	1,20185	,000	24,4846	32,1821
	2.5	33,33333*	1,20185	,000	29,4846	37,1821
7.5	10	-21,66667*	1,20185	,000	-25,5154	-17,8179
	5	6,66667*	1,20185	,002	2,8179	10,5154
	2.5	11,66667*	1,20185	,000	7,8179	15,5154
5	10	-28,33333*	1,20185	,000	-32,1821	-24,4846
	7.5	-6,66667*	1,20185	,002	-10,5154	-2,8179
	2.5	5,00000*	1,20185	,013	1,1513	8,8487
2.5	10	-33,33333*	1,20185	,000	-37,1821	-29,4846
	7.5	-11,66667*	1,20185	,000	-15,5154	-7,8179
	5	-5,00000*	1,20185	,013	-8,8487	-1,1513

Table12. Homogeneous classes of the effect of concentrations

Diametre

Tukey HSD= Alpha = 0,05.

Concentration	N	Subset for alpha = 0.05			
		1	2	3	4
2.5	3	10,0000			
5	3		15,0000		
7.5	3			21,6667	
10	3				43,3333
Sig.		1,000	1,000	1,000	1,000

The table 11represents the effect of the concentration which is strongly significant on the average diameter (alpha> 0,013) , for comparison of the effect of calcium hypochlorite concentration on the average diameter of the fungus considered in our experiments read table 12, the Tukey test indicates that each concentration belongs to a separate set. This means that on average each effect concentration, on the measured diameter, is significantly different from the effect of other concentrations (alpha> sig = 0.013).

Conclusion

The method of cleaning chlorination is one of the greatest revolutions in history, where it addressed the problem of pollution that was killing the lives of thousands of people and chlorine cleaning techniques are still the most important topics of modern science where scientists are working on developing protocols to increase its effectiveness and more detailed in the work of chlorine sterilized at the level molecular understanding in a deep way

Our interest in this study was to evaluate*in vitro*antibacterial and antifungal activitis of the Calcium hypochlorite which represents a form of chlorine, by using ten ATCC bacteian strains are : four gram positive bacteria (*E. faecalis, L.monocytogenes, S.aureus, B. cereus)*and sixgram negative bacteria *E.coli,P.aeruginosa, V.cholerae, K.pneumoniae, K. oxytoca, S. spp.)*and only one fungus strain wich is *Candida albicans;* by applying agar well diffusion method and depending on the statistical analysis program SPSS that's leads us to get many informations.

At these concentrations of Calcium hypochlorite 10g/L; are 7,5 g / L; 5g / L and 2,5g / L, all bacterial strains used in this study are sensitive to it except for two types of gram positive bacteria are *Staphylococcus aureus* and *Listeria monocytogenes.*

The smalllest diameter of the inhibition zone showed in :gram positive bacteria wich is *B. cereu* with an inhibition diameter zone of 6.33 ± 0.57 in 2,5g / L concentration of Calcium hypochloriteand in gram-negative bacteria are P.aeruginosa and *S. spp.*with an inhibition diameter zone of 6.30 ± 1.00 and 6.33 ± 0.57 respectively in the same concentration of 2,5g L.The largest clear zone was seen in *V.cholerae* and *K.pneumoniae*with an inhibition diameter zone 16,66±0,57 abd 15,33±0,57respectively for 10g/L concentration of Calcium hypochlorite.

The minimum inhibitory concentration (MIC)of Calcium hypochlorite is less than or equal to 2.5 g / L for all bacteria tested, with the exception of bacteria that have not shown sensitivity to Calcium hypochlorite, the highst minimum bactericidal concentrationvalue is 10g/L that is the sufficient concentration capable of completely eliminating (*P.aeruginosa).*

Generally gram negative bacterial strains represent the biggest activity index than positive strains *(V.cholerae, K.pneumoniae, S.spp.),54%;51%;52%* at 10 g/L of Calcium hypochlorite respectively, and lowest activity index is fo*r (B. cereus*) (25%,21%,17%,14%)equivalent to 10g/L,7,5g/L,5g/L,2,5g/L respectively

Candida albicans was more sensitive and had considerably larger zones of growth inhibition formed when it compared with inhibition zones diameters of different bacterial strains at the same concentrations .

In antibacterial activitythe statistical analysis which is the most adequate is ANOVA tow way method showsthat: there is a highly significant effect of the strain and the concentration and both of them (strain and concentration) on the average diameter formed where(alpha> 0.000).

In antigungal activitythe statistical analysis which is the most adequate is ANOVA one way method showes that the effect of the concentration of calcium hypochlorite which is strongly significant on the average diameter formed in petri dishes wich contai,s Candida albicans strain

As perspectifs, in ordre to improve the study around this subject much more deep we propose to :

- Determinate the cytotoxicity of Calcium hypochlorite.
- Study the synergistic activity of Calcium hypochlorite and antibiotics.
- Use ANCOVA biostatisc analysis for better understunding the effect of concentration at the deffrent strain

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Appendixes

Appendix I. Materials and products used

1. Equipment used

- Magnetic agitator
- Autoclave
- Precision balance (KERN, ABT 220-5DM)
- Normal balance (Adventurer Pro)
- Calipers
- Autoclave (MEMMERT)
- pH meter
- Stirring plate (VELP)
- Vortex (IKA)
- Cork borer
- Stirring hot plate
- platinum handle

2. Products used

Reactants

- *Gentamicin solution for injection 80 mg*

Solvents

- Physiological water (1 g NaCl in one liter of distilled water).
- Distilled water.

Appendix II . Compositions of the culture mediums used in one Litre

Sabouraud agar:

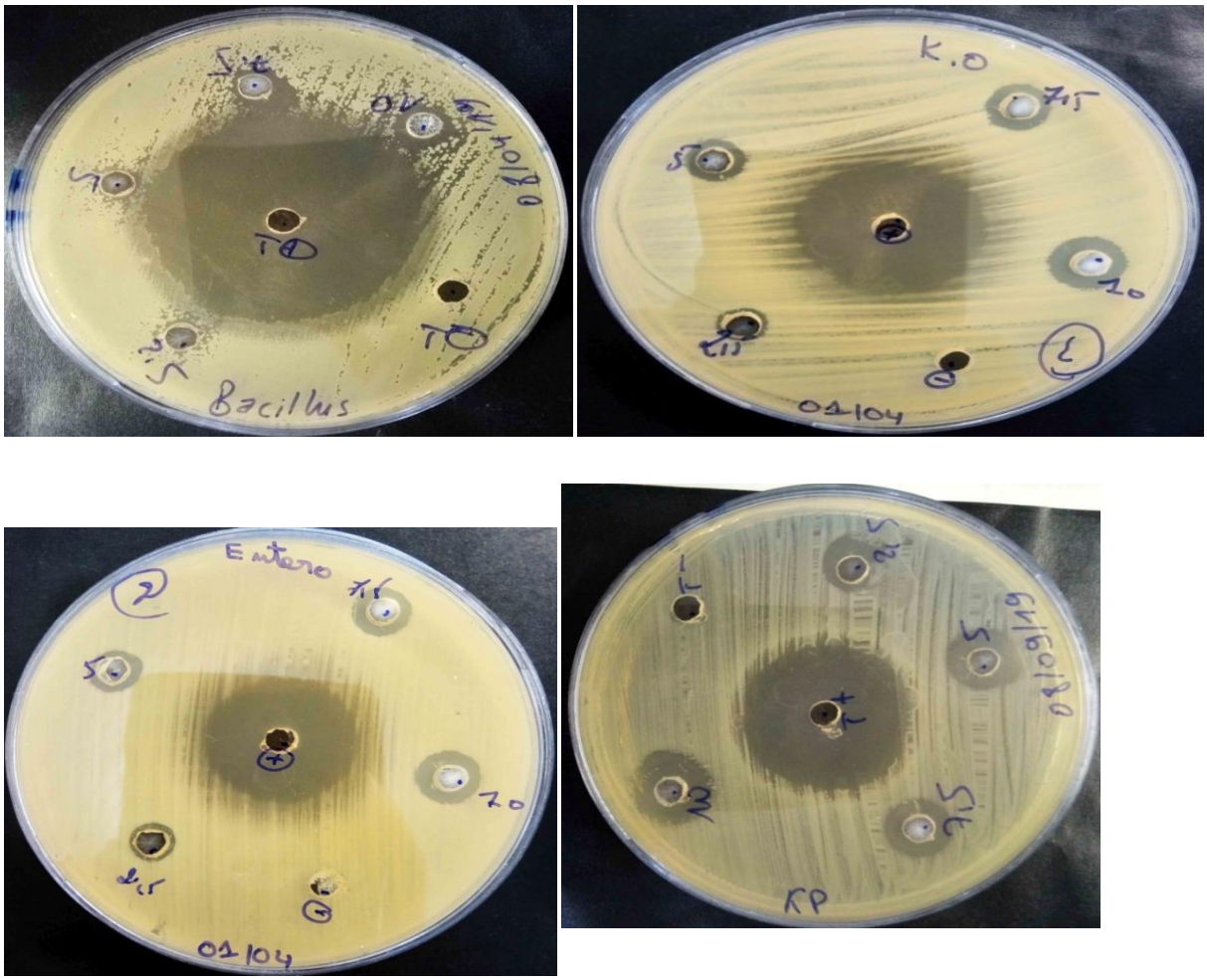
- 40 g/L dextrose
- 10 g/L peptone

- 20 g/L agar
- pH 5.6Agar

Mueller-Hinton Agar:

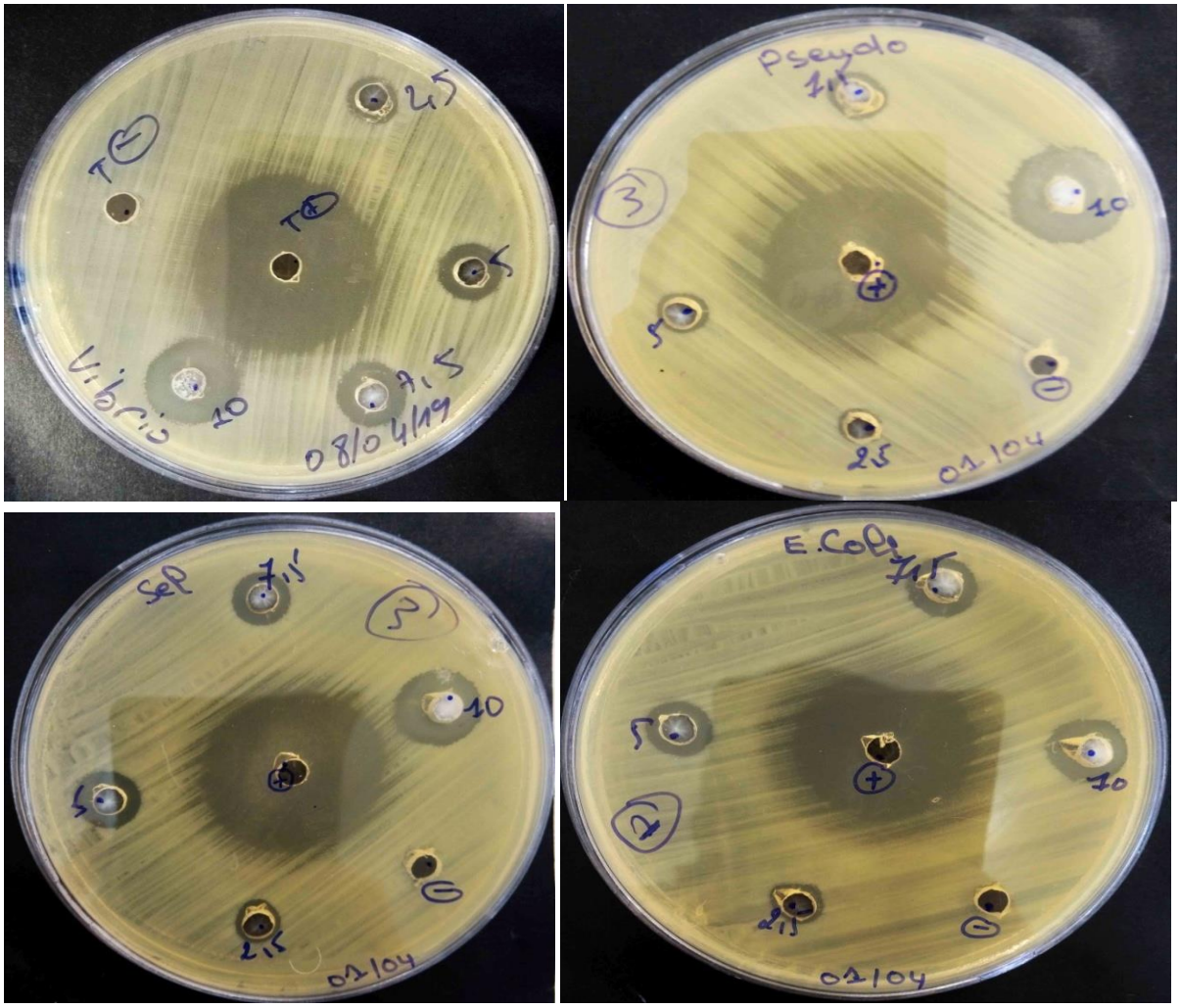
- infusion of beef: 300 ml
- casein peptone: 17.5 g
- corn starch: 1.5 g
- agar: 17.0 g
- pH = 7.4

Appendix III.Antibacterial results



Antibacterial Activities of different concentration of calcium hypochlorite

* All tests were performed in triplicate



Antibacterial Activities of different concentration of calcium hypochlorite

* All tests were performed in triplicate

ملخص

الهدف من هذه الدراسة هو تقييم نشاط مضاد الميكروبي لمادة هيبوكلوريت الكالسيوم في التركيزات التالية (2.5 غ / ل ، 5 غ / ل ، 7.5 غ / ل ، 10 غ / ل) ، ضد عشرة سلالات بكتيرية .موجبة و سالبة الغرام وسلالة فطرية واحدة هي كانديدا ألبيكانس ، وفقًا لمنهجية الإنتشار في الثقوب في وسط جيلوزي استنادًا إلى ثلاثة معايير أولاً ، مقياس قطر مناطق التثبيط التي تظهر أن البكتيريا سالبة الجرام هي الأكثر حساسية ، وثانياً ، تحديدالتركيز الأدنى المثبط أقل أو يساوي 2.5 غ / ل من هيبوكلوريت الكالسيوم أيضًا تحديدالتركيز الأدنى المبيد ، ثالثًا تحديد مؤشر النشاط من خلال استخدام الاختبارات الإحصائيةالمناسبة.

الكلمات المفتاحية : هيبوكلوريت الكالسيوم ، منهجية الإنتشار ، نشاط مضادات الميكروبات ، التركيز الأدنى المثبط ،التركيز الأدنى المبيد.

Abstract

The objective of this study is to evaluate the antimicrobial activity of Calcium hypochlorite at the following concentrations (2.5g / L, 5g / L, 7.5g / L, 10g / L), against ten bacterial strains. of positive and negative gram bacteria and a single fungal strain is *Candida albicans*, according to the method of wells in an agar medium based on three criteria first, the measure of diameter of the zones of inhibition which shows that gram negative bacteria are the most sensitive than gram posives, secondly it is the determination of MIC that is less than or equal to 2.5 g / L Calcium hypochlorite also the determination of the MBC, third it is the determination of the indexe of activity through the use of adequate statistical tests.

Key words: Calcium hypochlorite, agar well diffusion method, antimicrobial activity, MIC, MBC.

Résumé

L’objectif de cette étude c’est l’évaluation de l’activité antimicrobienne de Calcium hypochlorite à des concentrations suivants (2,5g/L ;5g/L ;7,5g/L ;10g/L), contre dix souches bactériennes de gram positives et négatives et une seul souche fongique c’est *Candida albicans* ,selon la méthode des puits dans un milieu gélosé on basant sur trois critéres premiérement, la mésure de diamétre des zones d’inhibition quit montre que les bactéries de gram négative sont les plus sensible que des gram posives, deuxiément c’est la détermination de CMI qui est inferieur ou égal à 2,5 g/L de Calcium hypochlorite aussi la détermination de la CMB, troisièmement c’est la determination de l’indexe d’activité par l’utilisation des tests statistiques adéquat .

Mots clés : Calcium hypochlorite, méthode des puits, activité antimicrobienne, CMI,CMB.