MIDLANDS STATE UNIVERSITY

THE ANTIBIOTIC EFFECTS OF APPLE CIDER VINEGAR ON *CANDIDA ALBICANS* AND *CANDIDA TROPICALIS*.

BY

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ABSTRACT

Candida species are the most common cause of fungal infections in humans. *Candida* species produce infections that range from non life-threatening mucocutaneous illnesses to invasive processes that may involve virtually any organ and this broad range of infections. *Candida albicans* coexists with a highly diverse human bacterial microbiota. *Candida tropicalis* is a common pathogen in neutropenic hosts in whom it may spread through the bloodstream to peripheral organs. *Candida tropicalis* has been identified as the most prevalent pathogenic yeast species of the *Candida*-non *albicans* (CNA) group. Antibiotic affects of Apple Cider Vinegar (ACV) were investigated on the two species *C. albicans* and *C. tropicalis* using the disc diffusion method. The effect of four ACV concentrations on the *Candida* species was determined by measuring the zone of inhibition. A one-way analysis of variance (ANOVA) was carried out for the diameter of inhibition of ACV on the two yeast species. A multiple comparisons for the four concentrations of ACV used and the two controls clotrimazole (positive) and distilled water (negative) were done using Tukey post hoc test. The results revealed that the effect of apple cider vinegar on *Candida albicans* was significant ($p < 0.05$) and there was a significant differences between the means of both *C. albicans* and *C. tropicalis*. This study indicated that Apple cider Vinegar can be effectively used for the treatment of yeast infections associated with *Candida albicans* and *Candida tropicalis*.

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DEDICATION

M. M. Katu, D. Z. Katu, and Ms H. Zimbudzana, this is for you. For all the stress and sacrifice you went through so that you provide me with the best education I can get, I dedicate this project to your love.

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LIST OF ABBREVIATIONS

CNA …….Candida-non albicans

ACV …….Apple Cider Vinegar

SPSS …….Statistical Parkage for the Social Sciences

ANOVA…… Analysis of Variance

CHAPTER ONE: INTRODUCTION

1.1BACKGROUND

Candida species are the most common cause of fungal infections in humans (Smith, 2017). *Candida* species produce infections that range from non life-threatening mucocutaneous illnesses to invasive processes that may involve virtually any organ and this broad range of infections requires an equally broad range of diagnostic and therapeutic strategies (Smith, 2017). The human commensal fungus *Candida albicans* is part of the microbiota of healthy individuals and colonizes several niches, such as skin, gastrointestinal and urogentinal tracts. In immunosuppressed human hosts, however, *C. albicans* causes opportunistic mucosal and, often fatal, bloodstream infections (Bruno, Bartellia, Rodriguesa and Briones, 2018).

Candida albicans is an opportunistic pathogenic yeast that is a common member of the human gut flora. It does not proliferate outside the human body and it is detected in the gastrointestinal tract and mouth in 40-60% of healthy adults (Odds, 1998). Quite a number of people have some level of *Candida albicans* in their intestines and it usually coexists peacefully with the other bacteria and yeasts that are found in the intestines (Smith, 2017). However, other factors may lead to the *Candida albicans* populations getting out of control establishing fast growing colonies and biofilms (Smith, 2017). At the point of biofilm establishment, it begins to weaken the immune system and affect the digestion and damages the intestinal wall, thus, allowing its toxic by-products to escape into the bloodstream and spread throughout your body causing damage to your body tissues and organs (Smith, 2017).

Candida albicans is responsible for yeast infections of the vaginal and oral mucosa especially as an opportunistic infection in diseases like HIV/AIDS and uncontrolled diabetes (Smith, 2017). Vaginal infections, urinary tract infections, rectal itching or vaginal itching and severe seasonal allergies or itchy ears are some of the infections caused by the yeast. Vaginal yeast

infections are also called vaginal Candidiasis or vulvovaginal candidiasis. The species will normally exist on the human skin but weakening immune system or homeostatic control for the body may lead to their overgrowth and subsequent infection (Manyarara, Chifamba, and Tarugarira, 2016). Oropharyngeal candidiasis develops in the mouth and throat and can be invasive leading to ulceration of the upper gastrointestinal tract and systemic infection from the *Candida* species. Vaginal candidiasis is associated with a white discharge, irritation and burning sensation and it occurs as a result of the imbalance of vaginal bacterial flora due to a weakened immune system, imbalance between estrogen and progesterone hormones in the menstrual cycle and poor sugar dietary control. Antifungal drug resistance has become a major problem in late stages of AIDS patients (Manyarara *et al*., 2016).

Bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* and *Staphylococcus aureus* are commonly isolated from infections with *C. albicans* (Bruno, Bartellia, Rodriguesa, Marcelo, and Briones, 2018). These pathogens are able to colonize the host or medical devices together, forming mixed biofilms that are associated with increased antimicrobial resistance and virulence (Bruno *et al*., 2018).

Candida tropicalis has been identified as the most prevalent pathogenic yeast species of the *Candida*-non *albicans* (CNA) group (Kothavade, Kura, Valand, and Panthaki, 2010). *Candida tropicalis* is a common pathogen in neutropenic hosts in whom it may spread through the bloodstream to peripheral organs (Kothavade *et al*., 2010). *Candida tropicalis* is a diploid ascomycetous yeast commonly found on the skin and in digestive tracts of healthy human hosts worldwide (Desnos-Ollivier, Bretagne, Bernède, Robert, Raoux, Chachaty, Forget, Lacroix, and Dromer, 2008). *Candida albicans* has been the major species responsible for causing candidiasis in immunocompromised and immunocompetent patients, however, infections due to *Candida tropicalis* have increased dramatically on a global scale (Kothavade *et al*., 2010).

Candida tropicalis is among the *Candida* species that are mostly isolated from clinical specimens (Chai, Denning, and Warn, 2010). Candidiasis and candidemia are treated by amphotericin B and *Candida tropicalis* is usually susceptible to all antifungal agents. However, drug resistance reports were made (Law, Moore, Joseph, Keaney, and Denning, 1996). The antifungal resistance was related to mutations.

Apple cider vinegar known as cider vinegar (ACV), is a type of vinegar made from cider or apple must and has a pale medium colour. Unpasteurized or organic ACV contains some pectin, vitamin B1, vitamin B2, and vitamin B6, biotin, folic acid, niacin pantothenic acid and vitamin C (Mishra, 2018).

It is a rich source of enzymes, potassium and other minerals. They prevent the bacteria causing UTI from multiplying and growing (Pulipati, Babu, and Narasu, 2017). It acts as a natural antibiotic to treat infections. It possesses acetic acid which is one of the best natural disinfecting compounds that kills resistant bacteria (Pulipati *et at*., 2017).

1.2 PROBLEM STATEMENT

The opportunistic fungus *Candida* tends to affect people living with HIV/AIDS. Due to the weakening of the immune system by the HIV virus many infected people are vulnerable to *Candida* infections.

Studies show that *Candida tropicalis* is mutating and becoming resistant to antifungal drugs like fluconazole (Kothavade *et al*., 2010). The *in vitro* resistance to antifungals among *Candida tropicalis* isolates obtained from human sources shows an increased number with rates reaching 70% (Castelo-branco, Teixeira, Marques, Bittencourt, Carvalho, Bandeira, Brilhante, Moreira, Pereira-Neto, Sidrim and Rocha. 2015).

As the yeast is mutating and becoming antifungal resistant, for example *Candida tropicalis* which is becoming resistant to fluconazoles, there is need for the introduction of new and efficient antifungals that can help in treating the fungal infections (Law, 1996).

1.3 JUSTIFICATION

The diagnosis of candidiasis is common in people who live with HIV/AIDS and those who live with diabetes due to their compromised immune system. About 5-10% of oral candidiasis is now intractable with the antifungal drug fluconazole and up to 33% of oral *Candida* isolates from AIDS patients are resistant to fluconazole (Manyarara *et al*., 2016. Resistance appears to be correlated with the total cumulative dose, which is a reflection of long term prophylaxis or therapy (Kothavade *et al*., 2010).

New medical research (Mishra, 2018) suggests that apple cider vinegar use can help cure acid reflux, lower blood pressure and improves diabetes. The benefits of apple cider vinegar come from its powerful healing compounds which include acetic acid, potassium, magnesium, probiotics and enzymes. Acetic acid has the ability to kill bad bacteria (Mishra, 2018) and at the same time to foster the growth of beneficial bacteria. Acetic acid kills unwanted bacteria when it comes into contact with them, it essentially acts as a natural antibiotic. Apple cider vinegar naturally provides numerous benefits related to skin, digestion, and immunity health without any side effects (Mishra, 2018). The acids found in apple cider vinegar, malic and acetic acids are responsible for the sour taste of vinegar which lowers the blood's pH levels thereby creating an inhospitable intestinal environment for bacterial and fungal infections (Dees, 2006) as well as fighting fungal and bacterial infections.

However as *Candida* yeast infections are associated with various bacterial infections leading to a mixed biofilm that is antimicrobial resistant (Bruno *et al*., 2018), there is need for the use of antimicrobials that kill both the yeast and bacterial infections without giving room for any mutations that will lead to resistance. The properties that are found in apple cider vinegar

are known not to favour fungal and bacterial growth (Mishra, 2018). Therefore an investigation on its antibiotic effects on the *Candida* species was necessary.

The study, however, was carried to investigate whether apple cider vinegar has antifungal effects on *Candida albicans* and *Candida tropicalis*.

1.4 OBJECTIVES

1.4.1 MAIN OBJECTIVE:

- to investigate the antibiotic effects of apple cider vinegar on *Candida albicans* and *Candida tropicalis*.

1.4.2 SPECIFIC OBJECTIVES

- to determine the sensitivity of *Candida albicans* and *Candida tropicalis* to apple cider vinegar,
- to determine the most effective concentration of apple cider vinegar on *Candida albicans* and *Candida tropicalis.*

CHAPTER TWO: LITERATURE REVIEW

2.1 History of *Candida*

2.1.1 Candida

Candida species are among of the four most common causes of bloodstream and cardiovascular infections (Kabir, Hussain, and Ahmad, 2012). Bloodstream infections caused by *Candida* are responsible for as high as 50% mortality rate among the infected patients (Kabir *et al*., 2012).

There are over 200 known species of the genus *Candida*, but only a relatively small number of *Candida* are pathogenic to humans (Magalhães, Bomfim, Melônio, Ribeiro, Cosme, Rhoden,and Marques, 2015). A number of species have been isolated from humans, including *C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. krusei, C. lusitaniae, C. guilliermondii, C. dubliniensis, C. famata, C. utilis, C. lipolytica, C. norvegensis, C. inconspicua, C. kefyr, C. rugosa, C. haemulonii, C. viswanathii* and *C. albicans* (Magalhães *et al*., 2015).

The spectrum of diseases caused by *Candida* consists of superficial and invasive Candida infections (Ruhnke, Rickerts, Cornely, Buchheidt, Glockner, Heinz, Hohl, Horre´, Karthaus, Kujath, Willinger, Prester, Rath, Ritter, Glasmacher, Lass-Flo and Groll, 2011). *Candida* species produce infections that range from non life-threatening mucocutaneous illnesses to invasive conditions which may involve severe infection to destruction of vital organs (Hani, Shivakumar, Vaghela, Osmani and Shrivastava, 2015).

Candida research suffered a great deal due to its diploid nature in which genetic manipulation was not amenable (Kabir *et al*., 2012). However, in the last two decades, several important techniques have been developed and applied to the genetic manipulation and proteomic studies with respect to its interaction with the host, biofilm formation, drug resistance, morphogenetic states and phenotypic switching (Kabir *et al*., 2012). Current techniques like

DNA transformation, sequential rapid gene disruption, RNA isolation, RNA sequencing, epitope tagging, use of reporter genes and regulatory promoters, chromatin immunoprecipitation and DNA microarray which are used in molecular genetic studies are being used to study *Candida* species.

2.1.2 Candida albicans

Candida albicans is a dimorphic fungus that exists as a commensal of warm blooded animals including humans. *Candida albicans* does not have a sexual cycle and it is a diploid organism which has made it difficult to manipulate genetically. *Candida albicans* has the ability to grow in two different ways, reproduction by budding, that is, forming an ellipsoid bud and in hyphal form, which can periodically fragment and give rise to new mycelia or yeast like forms (Molero, Navarro-garcía, and Sánchez-pérez, 1998).

Candida albicans coexists with a highly diverse human bacterial microbiota, where fungi only represent a small part of this population and these communities often produce mixed species biofilms which have a significant impact in the survival and reproductive success of both bacteria and fungi (Bruno *et al*., 2018).

In the 1970s and 1980s some S*accharomyces cerevisiae* laboratories started working on *Candida albicans* and in the 1990s a large number of yeast laboratories switched to study different aspects of *Candida albicans* resulting in the initiation of genome sequencing of this pathogen in 1996 (Kabir *et al*., 2012). The genome sequence of *Candida albicans* was completed and was available in 2004, and this made it possible to initiate rigorous research activities and expanded our knowledge on the pathogen (Kabir *et al*., 2012).

The affinity of *Candida albicans* mycoses for human immunodeficiency virus positive patients has led to the search for improved epidemiological knowledge of the responsible strains (Tibayrenc, 1993).

2.1.3 Candida tropicalis

Candida tropicalis is prevalent in organically enriched soil and aquatic environments and the use of azole compounds in the environment may select organisms that exhibit reduced susceptibility to drugs, which may find their way to animals and humans (Candel, Pacheco, Ruiz-Camps, Maseda, Sánchez- Benito, Montero, Puig, Gilsanz, Aguilar and Matesanz, 2017).

The types of disease caused by *C. tropicalis* vary depending on the location the species colonizes (Chai *et al*., 2010). With an infection in the mucous membrane, the subject will experience oropharyngeal candidiasis, angular cheilitis (Chai *et al*., 2010). *C. tropicalis* is reported to secrete additional products that can preferably target T-cell deficient hosts. *C. tropicalis* is part of the normal flora which is found on the skin and nails on approximately 10% of the patients (Chai *et al*., 2010). *C. tropicalis* infections have been reported in immunocompromised patients who have had chronic mucocutaneous candidiasis (Kothavade *et al*., 2010).

C. tropicalis has been associated with invasive candidiasis, being the first or second most common non-*Candida albicans. Candida* species isolated in humans with candidemia and candiduria, as well as being frequently isolated from healthy animals (Castelo-branco *et al*., 2015). *C. tropicalis* is the frequent cause of fungemia and represents an important cause of fungemi in oncological and nononcological patients (Goldani and Mario, 2003). *C. tropicalis* also expresses many virulence factors, including hydrolytic enzymes such as aspartyl proteases, phospholipases and lipases.

C. tropicalis alone or in association with *C. parapsilosis* is the second most prevalent *Candida* species after *C. albicans*. *C. tropicalis* is an emerging pathogen and it is causing higher mortality rates than *C. albicans* (Kothavade *et al*., 2010). Infections caused by *C.*

tropicalis are reported in 4–24% of the patients with candidemia (Desnos-Ollivier, Bretagne, Bernède, Robert, Raoux, Chachaty, Forget, Lacroix and Dromer, 2008).

C. tropicalis has been fully genome sequenced. *C. tropicalis* genome sequences available from Gen-Bank databases and from the Broad Institute (Desnos-Ollivier *et al*., 2008).

2.1.4 Resistance to drugs

The incidence of fungal infections has increased significantly, so contributing to morbidity and mortality. This is caused by an increase in antimicrobial resistance and the restricted number of antifungal drugs, which retain many side effects (Giannini, 2018).

Increased virulence of *C. tropicalis* isolates was observed when given orally to compromised mice, which parallels clinical observations in immune compromised patients (Kothavade *et al*., 2010). Some studies showed that *C. tropicalis* is even more invasive than *C. albicans* in the human intestine, particularly in oncology patients (Kothavade *et al*., 2010).

C. tropicalis showed a moderate level of fluconazole resistance. This indicates that there may be a risk of fluconazole resistance through upregulation of efflux transporters upon exposure to increasing concentrations of the drug (Pfaller, Boyken, Hollis, Kroeger, Messer, Tendolkar, Diekema and ARTEMIS DISK Global Antifungal Surveillance Group, 2009). This indicates that there is a risk of fluconazole resistance through upregulation of efflux transporters upon exposure to increasing concentrations of the drug (Kothavade *et al*., 2010).

Biofilm production is also associated with a high level of antimicrobial resistance of the associated organisms. The ability of *Candida* species to form drug- resistant biofilms is an important factor in their contribution to human disease (Giannini, 2018).

2.2 Candidiasis

Candidiasis is an infection caused by any of several types of yeasts called *Candida*. The most common is called *Candida albicans*. This yeast is normally present on the skin, in the

intestines and in the vagina, but does not cause disease. However, sometimes it can develop into an infection usually of the mouth, vagina or skin that causes red or white patches, itching and irritation (States, 2014). These infections are broad spectrum, ranging from superficial oral thrush and vaginitis to deep cited systemic which are mostly life threatening (Hani *et al.*, 2015).

Oropharyngeal candidiasis is found in 6% to 93% of HIV-infected patients (Ruhnke *et al*., 2011). In people living with HIV/AIDS, oropharyngeal candidiasis is the most common fungal infection and it is predictive of the development of AIDS. Antifungal treatments are often initially successful but relapses are more common (Tibayrenc, 1993). Oropharyngeal candidiasis can spread to the larynx and the oesophagus. These manifestations may also occur in the absence of oral disease and are characterised by painful swallowing (Ruhnke *et al*., 2011).

Systemic infections are commonly referred to as candidemia and are one of the prominent co-infections in immune compromised patients such as those suffering from cancer, HIV (Hani *et al*., 2015). Candidaemia is the most frequent manifestation of deeply invasive candidiasis (Ruhnke *et al*., 2011). Candidiasis is not usually a dangerous infection, but in some people it can spread through the bloodstream to other parts of the body such as the heart valves, the spleen, the kidneys and the eyes. This invasive candidiasis is a much more serious condition and it is fatal (States, 2014).

The term acute disseminated candidosis is not widely used in Europe (Ruhnke *et al*., 2011). This entity is found in patients with malignancy and prolonged granulocytopenia. Patients present with sepsis, persisting candidaemia, haemodynamic instability and disseminated skin or organ involvement. It is associated with a high mortality (Ruhnke *et al*., 2011). Chronic

disseminated candidosis like hepatosplenic candidosis is also found in patients with malignancy typically after recovery from granulocy topenia (Ruhnke *et al*., 2011).

Cutaneous candidiasis and vaginal infections are more likely to be associated with a phagocytic response involving neutrophils and mononuclear phagocytes (Giannini, 2018).

2.3 Importance of *Candida*

Candida species are considered important pathogens due to their versatility and ability to survive in various anatomical sites (Giannini, 2018). When it is at its proper levels in the body, *Candida* is a fungus that aids with nutrient absorption and digestion.

2.4 History of apple cider vinegar

Apple cider vinegar is product of fermentation. It is made by crushing apples and squeezing out the liquid. Bacteria and yeast are added to the liquid to start the alcoholic fermentation process, and the sugars are turned into alcohol, the alcohol is converted into vinegar by acetic acid forming bacteria (Mishra, 2018). Apple cider vinegar is a very light vinegar that is sometimes used in Iranian foods (Chan, Nia and Nazari, 2012).

Apple cider vinegar production is encountered in more than 25 countries around the world in temperate regions where apple trees can flourish. The highest production is in Europe where the term cider refers strictly to fermented products (Nogueira and Wosiacki, 2012). Within Europe, the main cider producing countries are England, Spain, France, Germany and Ireland (Cousin, Guellec, Schlusselhuber, Dalmasso, Laplace and Cretenet, 2017).

Apple cider vinegar was used in the ancient civilizations of Egypt, Babylonia, Greece and the Roman Empire (Yu, 2001). It was used for every known medical condition from simple digestive problems, for endurance and stamina and for external wound care. In 400 B.C in Greece, Hippocrates treated his patients with apple cider vinegar and honey for all sorts of ailments (Yu, 2001).

Apple cider vinegar was particularly used during the American Civil War for disinfecting the wounds of soldiers. It has more valuable properties and ingredients that suggest their therapeutic effects (Chan *et al*., 2012).

2.5 Properties of apple cider vinegar

Apple cider vinegar is rich in potassium, enzymes and many organic acids. It also contains minerals like boron, iron, trace elements and pectin soluble fibre. Potassium is considered the mineral of youthfulness. It keeps the arteries flexible and resilient, and maintains youthful, healthy skin. Potassium deficiency can stunt growth. A shortened lifespan occurs for people living on foods from potassium deficient soil (Yu, 2001).

Apple cider vinegar comprises mainly of acetic acid, typically 4-18% acetic acid by mass (Ismael, 2013). The organic acids like acetic acid, lactic acid and propionic acid promote digestion, balance acid/alkaline levels of the blood, help detoxify the body, dissolve fats, and kill viruses, bacteria and fungus (Yu, 2001).

ACV ingredients that include calcium, iron, sodium, potassium, malic acid and pectin which are found in balanced amounts (Dees, 2006). Malic acid helps to fight infections from harmful bacteria and fungi and acetic acid accounts for the sour taste of vinegar which lowers the blood's pH levels thereby creating an inhospitable intestinal environment for bacterial and fungal infections (Dees, 2006).

Apple cider vinegar contains polyphenolic compounds that have beneficial health effects. Its antioxidant flavonoid content can reduce the harmful effects of high cholesterol diets (Chan *et al*., 2012).

2.6 Medicinal value

Natural apple cider vinegar is a wonderful natural cure for a number of ailments which usually require antibiotics and other medications that have a number of side effects (Main and Sylva 2012).

Apple cider vinegar prevents the bacteria causing urinary tract infections (UTI) from multiplying and growing, which are caused by pathogenic bacteria such as *Escherichia coli, Staphylococcus saprophyticus, Klebsiella pneumoniae, Proteus mirabilis* and fungi *Candida albicans* and *tropicalis*. It acts as a natural antibiotic to treat the infection. Drinking apple cider vinegar daily increases acidic environment in urinary tract and discourages the growth of UTI causing bacteria (Pulipati *et al*., 2017). Antimicrobial agents of apple cider vinegar act as affordable and safe alternative remedy to treat UTIs without increasing the risk of antibiotic resistance (Pulipati *et al*., 2017).

Apple cider vinegar stimulates production of digestive juices (Mishra, 2018). Apple cider vinegar restores alkaline acid balance. Proponents of the alkaline acid theory believe that a diet high in acid producing foods leads to lack of energy, excessive mucous production, infections, anxiety, irritability, headache, sore throat, a nasal and sinus congestion, allergic reactions, and increased risk of conditions such as arthritis and gout (Mishra, 2018). Apple cider vinegar into the diet adds to the alkaline acid balance thus lowering the risks of suffering from the diseases. Apple cider vinegar and honey treats arthritis and also application of the vinegar externally on painful joints (Mishra, 2018).

Apple cider vinegar has been traditionally used since many years ago to treat a certain number of diseases including hyperlipidemia which is known as a risk factor for atherosclerosis. Early prevention and treatment of atherosclerosis can prevent complications of cardiovascular diseases (Chan *et al*., 2012). Consumption of apple cider vinegar reduces

the lower density lipoprotein (LDL), triglyceride, and cholesterol levels in patients with hyperlipidemia (Mishra, 2018).

The acetic acid of apple cider vinegar decreases fatty acid oxidation, inhibits lipogenesis in the liver, and it decreases triglyceride and cholesterol concentrations (Chan *et al*., 2012). Apple cider vinegar promotes fat, reduces sugar cravings and improves detoxification (Mishra, 2018). Apple Cider Vinegar also breaks down fat and is widely used to lose weight. It has also been reported that a daily dose of apple cider vinegar in water has high blood pressure under control (Vinegar, 2005).

Apple Cider Vinegar is a fantastic natural remedy for diarrhoea since the high pectin concentration acts as a protective coating which soothes the irritated lining of the colon (Main and Sylva, 2012). ACV helps to speed up the metabolism and it burns calories. A number of nutritionists also believe that combining Vitamin B6 and Lecithin with ACV is highly effective for weight loss (Main and Sylva, 2012).

ACV has also been shown to be effective in the prevention and control of microbial contamination in intra canal treatment of apical periodontitis in teeth (Estrela, Holland, Bernabe, De Souza and Estrela, 2004). Due to its acidic properties, ACV makes a wonderful remedy for bad breath or halitosis (Main and Sylva, 2012) .

Apple Cider Vinegar is also wonderful for pets. It helps them with arthritic conditions, controls fleas and barn flies, and gives a beautiful shine to their coats (Vinegar, 2005).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study site

The study was carried out at the Midlands State University (MSU), Department of Applied Biosciences and Biotechnology laboratories.

3.2. Sources of samples

The pure cultures of *Candida albicans* and *Candida tropicalis* used were obtained from the University of Zimbabwe, Department of Medical Microbiology at Parirenyatwa group of hospitals and were transported to MSU in a Thermo bag. Apple cider vinegar was purchased from TM Pick n Pay supermarket Gweru. Clotrimazole cream was purchased over the counter at Greenwood pharmacy, Harare.

3.3. Experimental design

The study had three replicates for each ACV concentration and two controls, clotrimazole as the positive control and distilled water as the negative control

Table 1: Experimental design

	ACV Concentration %				**Controls**	
Dependent	25	50	75	100	Clotrimazole	Distilled water
Candida albicans	TR	TR	TR	TR	TR	TR
	R1	R1	R1	R1	R1	R1
	R2	R2	R2	R2	R2	R2
	R3	R3	R3	R3	R3	R3
Candida tropicalis	TR	TR	TR	TR	TR	TR
	R1	R1	R1	R1	R1	R1
	R2	R2	R2	R2	R2	R2
	R3	R3	R3	R3	R3	R3

KEY

TR Treatment

R1 Replicate 1

R2 Replicate 2

R3 Replicate 3

3.4. Media Preparation

Sabouraud dextrose agar media measuring to 1000 ml was prepared using the manufacturer instructions (Appendix 1). The agar was sterilized in the autoclave for 15 minutes. The agar was poured into sterile labelled petri dishes and was left to set before culturing the yeast species.

3.5. Culturing *Candida* species

3.5.1 Preparation of fungal cultures

In the lamina flow cabinet and over a flame *C. albicans* and *C. tropicalis* were sub-cultured from the obtained pure cultures and were suspended in 0.9% saline buffer and standardized to turbidity. The fungal species were sub-cultured into labelled test tubes.

3.5.2 Preparation of apple cider vinegar concentrations by serial dilutions

Concentrations of 75%, 50% and 25% apple cider vinegar were prepared by diluting the apple cider vinegar by parts of distilled water. Concentration of 100%, 75%, 50% and 25% of apple cider vinegar were used in the study.

Clotrimazole which is known to inhibit fungal growth was used as a positive control and distilled water was used as a negative control.

3.5.3 Preparation of antibiotic discs

Filter paper discs were dipped in the four apple cider concentrations, clotrimazole and distilled water in the lamina flow cabinet. The filter paper discs were allowed to suck in the liquids. The filter paper discs were air dried and kept under aseptic conditions.

3.5.4 Fungal species plating

A clean and sterile cotton swab was dipped in the standardized fungal suspensions for *Candida albicans* and *Candida tropicalis* and was swabbed uniformly and evenly on the labelled petri dishes, respectively to the labeling. The surface of the medium was left to dry under aseptic conditions before inoculation of the treatment.

3.5.5 Treatment by apple cider vinegar using disc diffusion assay

Using a sterile forceps in the lamina flow and over a flame the antibiotic discs were gently placed on the dried surface of the fungal inoculated dishes respectively to the labelling.

3.5.6 Incubation

The inoculated dishes were incubated at an inverted position in an incubator at 37^{o}C for 24 hours.

3.6 Determination of the antibiotic effects of apple cider vinegar

3.6.1 Measuring zone of inhibition

The diameter of the zones of inhibition in all observed dishes were measured using a transparent millimetre graduated rule and the obtained results were recorded.

3.7 Data analysis

The antimicrobial susceptibilities characteristics were compared using one way analysis of variance (ANOVA) and Tukey's multiple comparisons post test. The statistical analyses were performed with IBM Statistical Parkage for the Social Sciences (SPSS) software.

Dependent variable - diameter of the zone of inhibition

Independent variable - apple cider concentrations.

CHAPTER FOUR: RESULTS

All the apple cider vinegar concentrations inhibited the growth of both *Candida albicans* and *Candida tropicalis* that were under study. However, inhibition was in varying degrees between concentrations. *Candida tropicalis* generally performed better in all the ACV concentrations with the mean diameter of inhibition, 100% ACV on *C. tropicalis* (11.5±0.57mm) being higher than the standard clotrimazole mean diameter of inhibition (11±0.82mm) (Appendix 2b).

4.1 Effects of ACV on *Candida albicans*

Table 2: Effects of ACV on *Candida albicans*

	ACV Concentration %				**Controls**	
	25	50	75	100	Clotrimazole	Distilled water
Zone of inhibition in millimetres	6	7	9	11	11	0
	5	6	9	10	12	0
	5	8	8	11	12	0
	5	7	7	11	12	0
Mean value	5.25±0.5	7±0.82	8.25±0.96	10.75±0.5	11.75±0.5	0±0

The results in Table 2 and Figure 1 show the zone of inhibition diameters for the species *Candida albicans.* The mean diameter of the zone of inhibition measured for the species ranged from 0 mm to 11.75 mm including controls and 5.25 mm to 10.75 mm excluding controls (Appendix 2a).

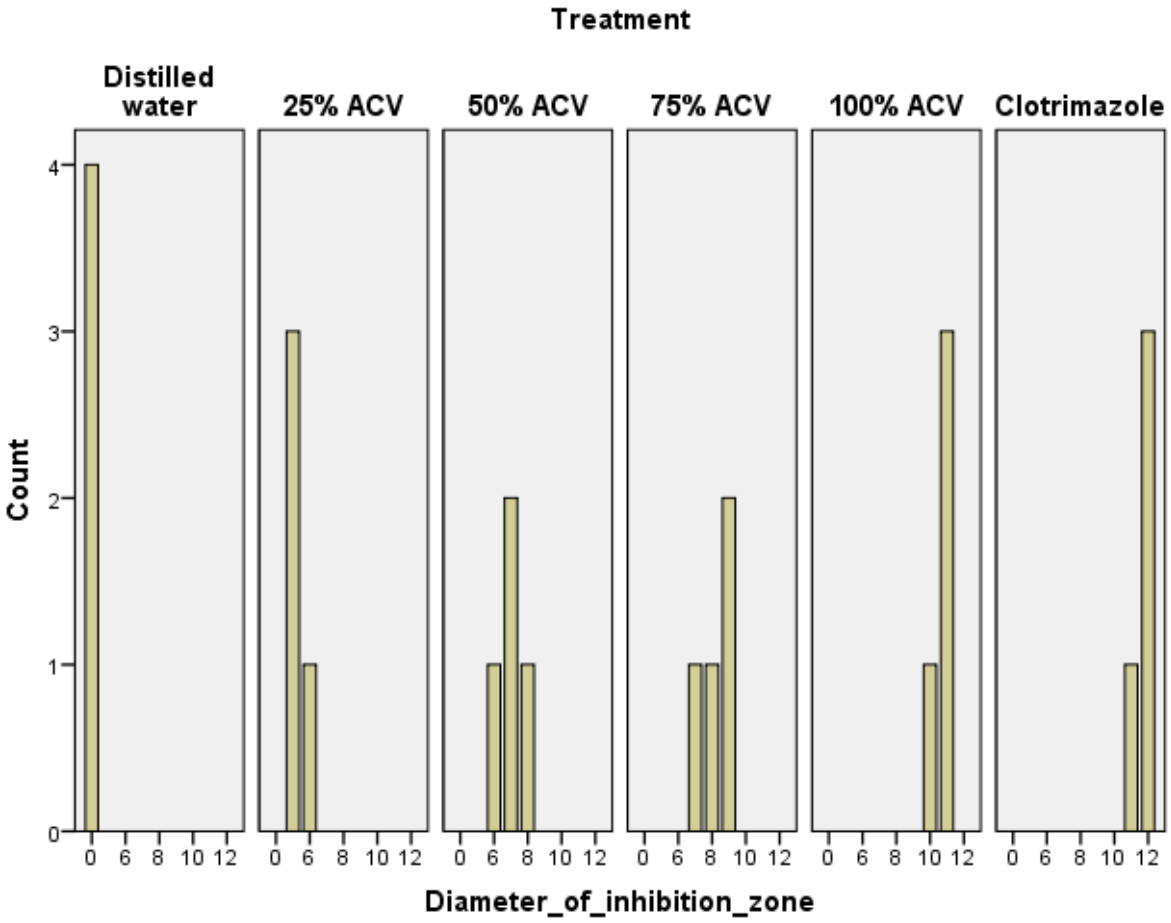

Figure 1: Effects of ACV on *Candida albicans*

Apple cider vinegar concentrations under study, the highest zone of inhibition was measured in the 100% ACV concentration (11 mm) with a mean diameter (10.75±0.5 mm). The 25% ACV concentration measured the lowest zone of inhibition (5mm) with a mean diameter (5.25±0.5 mm).

An analysis of variance showed that the effect of apple cider vinegar on *Candida albicans* was significant, $p < 0.05$ (ANOVA appendix 2a) therefore there is a significant differences between the means (ANOVA p .000). However, a Tukey post hoc, $p > 0.05$, revealed that there was no significant difference between the 50% ACV and 75% ACV ($p = .097$, Post Hoc Test appendix 2a) and also between 100% ACV and the control clotrimazole ($p = .257$, Post Hoc Test appendix 2a)

4.2 Effects of ACV on *Candida tropicalis*

Table 3: Effects of ACV on *Candida tropicalis*

	ACV Concentration %				Controls	
	25	50	75	100	Clotrimazole	Distilled water
Zone of	7	9	10	11	11	0
inhibition	5	7	8	12	12	0
in	5	7	9	12	11	0
millimetres	6	6	7	11	10	0
Mean Values	5.75±0.96	7.25±1.3	8.5±1.3	11.5±0.58	11±0.82	0±0

The Table 3 and Figure 2 show the zone of inhibition diameter for the species *Candida tropicalis.* The mean diameter of the zone of inhibition measured for the species ranged from 5.75 mm to 11.50 mm exclusive of controls and 0 mm to 11 mm inclusive of controls (see Descriptives in appendix 2b).

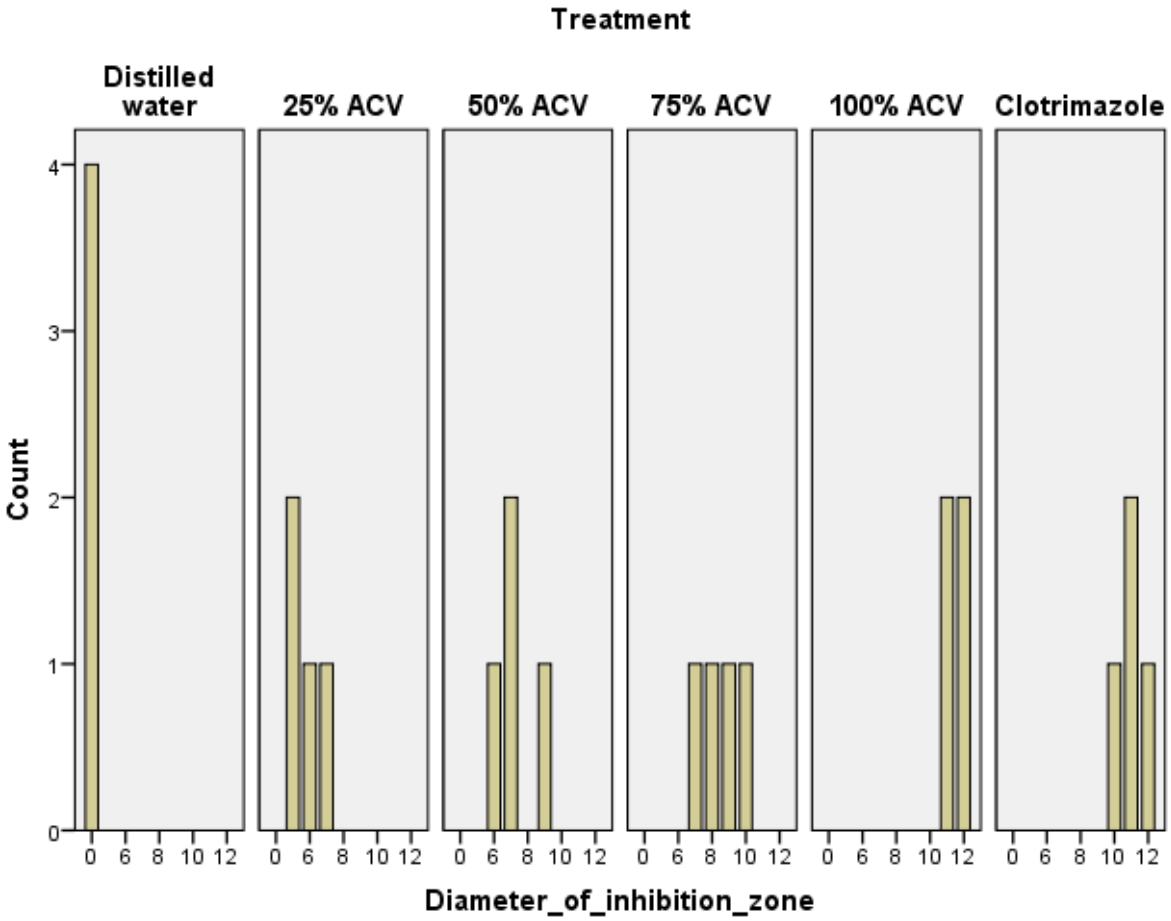

Figure 2: Effects of ACV on *Candida* tropicalis

Among the different apple cider vinegar concentrations under study, the highest zone of inhibition was measured in the 100% ACV concentration (12 mm) with a mean diameter (11.50±0.57 mm), followed by 75% ACV concentration with the highest diameter of inhibition (10 mm) with a mean diameter (8.50±1.3 mm). The control 0% ACV (distilled water) showed no inhibition.

An analysis of variance showed that the effect of apple cider vinegar on *Candida tropicalis* was significant, $p<0.05$ (ANOVA appendix 2b).

Tukey post hoc revealed that there was no significant difference, $p>0.05$, between the 25% ACV and 50% ACV ($p = .250$, Post Hoc Test appendix 2b), 50% ACV and 75% ACV ($p =$

.430, Post Hoc Test appendix 2b) and between 100% ACV and the control clotrimazole (p = .971, Post Hoc Test appendix 2b).

CHAPTER FIVE: DISCUSSION

5.0 Discussion

All the four apple cider concentrations inhibited the growth of both *Candida albicans* and *Candida tropicalis,* however, in varying degrees. The inhibition of growth on *Candida albicans* and *Candida tropicalis* by the apple cider vinegar shows that the vinegar can be used in the treatment of *Candida* infections associated with the species *C. albicans* and *C. tropicalis*. *C. tropicalis* was more susceptible to ACV treatment than clotrimazole treatment, with the 100% ACV concentration having a larger zone of inhibition mean diameter than the standard clotrimazole.

The diluted apple cider vinegar with the concentrations 75%, 50% and 25% showed lesser antibiotic activity as compared to the undiluted apple cider vinegar. The dilution of the apple cider vinegar with distilled water altered the pH of the vinegar. Vinegar is acidic as it contains acids like folic acid and acetic acid which are used in the antibiotic activities of the vinegar (Chan *et al*., 2012). The alteration by adding distilled water increases the vinegar's pH to alkalinity therefore decreasing the level of antibiotic activity of the cider vinegar.

The highest diameter of inhibition measured in the *Candida albicans* and *Candida tropicalis* can be attributed to the high acidity content of the apple cider vinegar that did not undergo dilution since the highest zone of inhibition was measured in the 100% concentrations and the lowest recorded in the heavily diluted 25% concentration. The action of inhibition could involve the acetic acid component of apple cider vinegar which is able to reduce the cell hydrogen potential hence could potentially facilitate diffusion across the plasma membrane of microbes (Yagnik, Vlad and Shah, 2018).

Present findings were close to those of Yagnik,Vlad and Shah (2018) who found out that apple cider vinegar has multiple antimicrobial properties on different microbial species,

affecting microbe growth, suppressing mononuclear cytokine and phagocytic responses (Yagnik *et al.* 2018). Yagnik *et al*. (2018) detected the absence of key enzymes which are fundamental in maintaining cell integrity and biosynthetic pathways on *Candida albicans* that had been treated with apple cider vinegar. Apple cider vinegar acts like other anti-pathogenic compounds in diverting monocyte responses through toll receptor signalling pathways (Yagnik *et al*., 2018).

The mechanism of apple cider vinegar activity could be attributed, in part, to the apple polyphenol content (Mishra, 2018). Yang, Li, Wang, and Wu. (2010) reported on the cellular protective effects of apple polyphenols on induced liver damage whereby histo-pathological tissue destruction was limited and liver activity maintained in mice that received the polyphenols. The mechanisms involved were free radical scavenger action, lipid peroxidation modulation and the antioxidant upregulation capacity of apple cider vinegar (Yagnik *et al.* 2018) .

Candida tropicalis which is the species that is becoming more resistant to antifungal drugs showed high susceptibility to the treatment (apple cider vinegar) in the present study. *Candida tropicalis* measured the highest zones of inhibition diameters as compared to *Candida albicans*. Having to be more susceptible to the apple cider vinegar, as *Candida tropicalis* growth can be inbited by apple cider vinegar.

The ability of the apple cider vinegar to inhibit the growth of the yeast species *C. tropicalis* can be attributed to its broad spectrum of antibody components like acetic acid, flavonoids such as gallic acid, tyrosol catechin, epicatechin, benzoic acid, vaninilin, caftaric acid, coutaric acid, caffeic acid, and ferrulic acid which affect the immune defence and oxidative responses.

Studies done by Yagnik *et al.* (2018) concluded that apple cider vinegar can have multiple antimicrobial effects directly on *E. coli, S. aureus* and *C. albicans*. Apple cider vinegar addition can also decrease induced inflammatory cytokine release during mononuclear leukocyte infection and increases monocyte phagocytic capacity (Yagni *et al.* 2018). Mechanisms include alteration of the microbial protein physiology destroying structural pathogenic proteins and metabolic enzymes.

The ability of apple cider vinegar to inhibit the growth of *E. coli, S. aureus* and other microbes as stated by Yagnik *et al*. (2018) can lead to the growth inhibition of biofilms which are inherently resistant to antimicrobial agents and drugs. Biofilms are partly due to the upregulation of several genes associated with drug resistance, including the multidrug resistance and *Candida* drug resistance. The ACV properties are able to inhibit the growth of biofilms, as ACV has the ability of inhibiting the bacteria, yeasts and fungi that are associated with biofilms.

5.1 Conclusion

Apple cider vinegar can successfully be used to cure yeast infections caused by *Candida albicans* and *Candida tropicalis* as it was shown in the study with its ability to inhibit the growth of the two yeast species. The results revealed that various concentrations of the apple cider vinegar can be used to inhibit the growth of the yeast, with the highest concentrations having the highest antibiotic properties. The results of this study could have clinical implications as apple cider vinegar can be used as an additive component of an antimicrobial therapeutic regimen especially in immune-compromised patients presenting with infections associated with *Candida albicans* and *Candida tropicalis*.

5.2 Recommendations

Further studies need to be carried out to find the antibiotic effects of apple cider vinegar on other *Candida* non *albicans* species that are isolated from people suffering from yeast

infections so as to have full knowledge on whether all the human isolated species are susceptible to the apple cider vinegar.

The studies are to be furthered to investigate on any potential side effects that the apple cider vinegar can cause to the patients receiving treatment.

Apple cider vinegar can however, be used as a preventative measure of the yeast infection by including it in the diet

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APPENDICES

Appendix 1: Sabouraud Dextrose Agar Manufacturer's instruction

1. Suspend 65g of the medium in one litre of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121^{o}C for 15 minutes.

Appendix 2a:SPSS output for *Candida albicans*

Oneway

ANOVA

diameter_of_inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	360.333	5	72.067	185.314	.000
Within Groups	7.000	18	.389		
Total	367.333	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: diameter_of_inhibition

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
					Lower Bound
distilled water	25% acv	-5.250*	.441	.000	-6.65
	50% acv	-7.000*	.441	.000	-8.40
	75% acv	-8.250*	.441	.000	-9.65
	100% acv	-10.750*	.441	.000	-12.15
	clotrimazole	-11.750*	.441	.000	-13.15

	distilled water	5.250*	.441	.000	3.85
	50% acv	-1.750*	.441	.010	-3.15
25% acv	75% acv	-3.000*	.441	.000	-4.40
	100% acv	-5.500*	.441	.000	-6.90
	clotrimazole	-6.500*	.441	.000	-7.90
	distilled water	7.000*	.441	.000	5.60
	25% acv	1.750*	.441	.010	.35
50% acv	75% acv	-1.250	.441	.097	-2.65
	100% acv	-3.750*	.441	.000	-5.15
	clotrimazole	-4.750*	.441	.000	-6.15
	distilled water	8.250*	.441	.000	6.85
	25% acv	3.000*	.441	.000	1.60
75% acv	50% acv	1.250	.441	.097	-.15
	100% acv	-2.500*	.441	.000	-3.90
	clotrimazole	-3.500*	.441	.000	-4.90
	distilled water	10.750*	.441	.000	9.35
	25% acv	5.500*	.441	.000	4.10
100% acv	50% acv	3.750*	.441	.000	2.35
	75% acv	2.500*	.441	.000	1.10
	clotrimazole	-1.000	.441	.257	-2.40
	distilled water	11.750*	.441	.000	10.35
	25% acv	6.500*	.441	.000	5.10
clotrimazole	50% acv	4.750*	.441	.000	3.35
	75% acv	3.500*	.441	.000	2.10
	100% acv	1.000	.441	.257	-.40

Multiple Comparisons

Dependent Variable: diameter_of_inhibition

Tukey HSD

(I) treatment	(J) treatment	95% Confidence Interval
		Upper Bound
distilled water	25% acv	-3.85*
	50% acv	-5.60*
	75% acv	-6.85*
	100% acv	-9.35*
	clotrimazole	-10.35*
25% acv	distilled water	6.65*
	50% acv	-.35*
	75% acv	-1.60*
	100% acv	-4.10*
	clotrimazole	-5.10*
50% acv	distilled water	8.40*
	25% acv	3.15*
	75% acv	.15
	100% acv	-2.35*
	clotrimazole	-3.35*
75% acv	distilled water	9.65*
	25% acv	4.40*
	50% acv	2.65
	100% acv	-1.10*
	clotrimazole	-2.10*
100% acv	distilled water	12.15*
	25% acv	6.90*
	50% acv	5.15*
	75% acv	3.90*
	clotrimazole	.40

	distilled water	13.15*
	25% acv	7.90*
clotrimazole	50% acv	6.15*
	75% acv	4.90*
	100% acv	2.40

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

diameter_of_inhibition

Tukey HSD[a]

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
distilled water	4	.00			
25% acv	4		5.25		
50% acv	4			7.00	
75% acv	4			8.25	
100% acv	4				10.75
clotrimazole	4				11.75
Sig.		1.000	1.000	.097	.257

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
treatment	24	1	6	3.50	1.745
diameter_of_inhibition	24	0	12	7.17	3.996
Valid N (listwise)	24				

Appendix 2b:SPSS output for *Candida tropicalis*

Oneway

ANOVA

diameter_of_inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	353.833	5	70.767	82.181	.000
Within Groups	15.500	18	.861		
Total	369.333	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: diameter_of_inhibition

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
					Lower Bound
distilled water	25% acv	-5.750*	.656	.000	-7.84
	50% acv	-7.250*	.656	.000	-9.34
	75% acv	-8.500*	.656	.000	-10.59
	100% acv	-11.500*	.656	.000	-13.59

	clotrimazole	-11.000*	.656	.000	-13.09
	distilled water	5.750*	.656	.000	3.66
	50% acv	-1.500	.656	.250	-3.59
25% acv	75% acv	-2.750*	.656	.006	-4.84
	100% acv	-5.750*	.656	.000	-7.84
	clotrimazole	-5.250*	.656	.000	-7.34
	distilled water	7.250*	.656	.000	5.16
	25% acv	1.500	.656	.250	-.59
50% acv	75% acv	-1.250	.656	.430	-3.34
	100% acv	-4.250*	.656	.000	-6.34
	clotrimazole	-3.750*	.656	.000	-5.84
	distilled water	8.500*	.656	.000	6.41
	25% acv	2.750*	.656	.006	.66
75% acv	50% acv	1.250	.656	.430	-.84
	100% acv	-3.000*	.656	.003	-5.09
	clotrimazole	-2.500*	.656	.014	-4.59
	distilled water	11.500*	.656	.000	9.41
	25% acv	5.750*	.656	.000	3.66
100% acv	50% acv	4.250*	.656	.000	2.16
	75% acv	3.000*	.656	.003	.91
	clotrimazole	.500	.656	.971	-1.59
	distilled water	11.000*	.656	.000	8.91
	25% acv	5.250*	.656	.000	3.16
clotrimazole	50% acv	3.750*	.656	.000	1.66
	75% acv	2.500*	.656	.014	.41
	100% acv	-.500	.656	.971	-2.59

Multiple Comparisons

Dependent Variable: diameter_of_inhibition

Tukey HSD

(I) treatment	(J) treatment	95% Confidence Interval
		Upper Bound
distilled water	25% acv	-3.66*
	50% acv	-5.16*
	75% acv	-6.41*
	100% acv	-9.41*
	clotrimazole	-8.91*
	distilled water	7.84*
25% acv	50% acv	.59
	75% acv	-.66*
	100% acv	-3.66*
	clotrimazole	-3.16*
	distilled water	9.34*
50% acv	25% acv	3.59
	75% acv	.84
	100% acv	-2.16*
	clotrimazole	-1.66*
	distilled water	10.59*
75% acv	25% acv	4.84*
	50% acv	3.34
	100% acv	-.91*
	clotrimazole	-.41*
	distilled water	13.59*
100% acv	25% acv	7.84*
	50% acv	6.34*
	75% acv	5.09*

	clotrimazole	2.59
	distilled water	13.09*
	25% acv	7.34*
clotrimazole	50% acv	5.84*
	75% acv	4.59*
	100% acv	1.59

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

diameter_of_inhibition

Tukey HSD[a]

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
distilled water	4	.00			
25% acv	4		5.75		
50% acv	4		7.25	7.25	
75% acv	4			8.50	
clotrimazole	4				11.00
100% acv	4				11.50
Sig.		1.000	.250	.430	.971

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation

treatment	24	1	6	3.50	1.745
diameter_of_inhibition	24	0	12	7.33	4.007
Valid N (listwise)	24				