DEDICATION

I dedicate this mini-dissertation to Bhagawan Sri Sathya Sai Baba. I thank Him for guiding me towards Homoeopathy and for assisting me during the years of my studies.

I also dedicate this mini-dissertation to my parents, Ronny and Poppy, my wife Rajeshwari and my brother Sudeish. Thank you for believing in me.

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ABSTRACT

The aim of this *in vitro* study was to determine the effect of *Commiphora molmol* tincture prepared in 86% v/v ethanol, *Hydrastis canadensis* tincture prepared in 62% v/v ethanol and *Warburgia salutaris* tincture prepared in 62% v/v ethanol against *Candida albicans* (*C. albicans*) with 62% v/v ethanol, 86% v/v ethanol and fluconazole as the control agents, and to determine the Minimum Inhibitory Concentration/s (MIC/s) of the effective tincture/s using the disc diffusion assay.

This study was divided into two parts.

In Part 1 (Experiment 1), Sabouraud's dextrose agar was inoculated with *C. albicans* and poured onto 15 plates. Six filter paper discs representing the three tinctures, the two negative control substances (62% and 86% ethanol) and the positive control fluconazole disc were placed equidistant from each other on the agar of each plate using aseptic methods. All 15 plates were incubated at 37°C for 24 hours after which the zones of inhibition produced by the test tinctures and control substances were measured and recorded. *Hydrastis*

canadensis 62% v/v tincture was the only tincture that produced a zone of inhibition.

Part 2 (Experiments 2-6) of this study involved determining the MIC/s of the tincture/s that was/were effective in inhibiting the growth of *C. albicans*. Since it was established in Part 1 that only *Hydrastis canadensis* 62% v/v tincture was effective against *C. albicans,* 1:2, 1:5, 1:10, 1:50 and 1:100 dilutions of this tincture and it's 62% v/v ethanol control were prepared with distilled water. Sabouraud's dextrose agar was inoculated with *C. albicans* and poured onto 75 plates. Each dilution test was replicated 15 times. Due to the prohibitive cost of fluconazole discs, only three plates per dilution test contained fluconazole discs. All 75 plates were incubated at 37°C for 24 hours after which the zones of inhibition produced by the *Hydrastis canadensis* 62% v/v tincture dilutions, their relative 62% v/v ethanol dilutions and fluconazole were measured and recorded.

The results were analyzed with the SPSS statistical package. The Kruskal-Wallis test was used to compare the means of the zones of inhibition produced by all the test tinctures and control samples. The Mann-Whitney U test was used to compare the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and it's dilutions (1:2, 1:5, 1:10, 1:50 and 1:100) to fluconazole. The Friedman's test was used to compare all the zones of inhibition produced by fluconazole in all Experiments.

The results showed that *Commiphora molmol* 86% v/v tincture and *Warburgia salutaris* 62% v/v tincture were ineffective in inhibiting the *in vitro* growth of *C. albicans*, whilst *Hydrastis canadensis* 62% v/v tincture, *Hydrastis canadensis* 62% v/v 1:2 dilution, *Hydrastis canadensis* 62% v/v 1:5 dilution, *Hydrastis canadensis* 62% v/v 1:10 dilution and *Hydrastis canadensis* 62% v/v 1:50 dilution were effective in inhibiting the *in vitro* growth of *C. albicans*. The Minimum Inhibitory Concentration of *Hydrastis canadensis* 62 % v/v tincture was *Hydrastis canadensis* 62% v/v 1:50 dilution.

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DEFINITION OF TERMS

Bactericidal: to destroy bacteria (Mims, *et al.*, 1999:560).

Bacteriostatic: to inhibit bacterial growth (Mims, *et al.*, 1999:560).

Tincture: A tincture is a herbal preparation that is based in alcohol and water (Kircher, 2001: 167).

CHAPTER ONE

Introduction

1.1 Overview

The yeast, *C. albicans,* is part of the normal micro-flora of both the gastrointestinal tract and mucocutaneous areas (Howard, *et al.*, 1980:616). It is an opportunistic pathogen and occurs most frequently in infants, the elderly and in severely immunocompromised patients, including those with acquired immune deficiency syndrome (AIDS) (Greenwood, *et al*., 1994:682).

In the past, orthodox medicine relied mainly on “gentian violet” for the treatment of candidal infections. The advent of polyene anti-fungal agents such as nystatin and azole anti-fungal agents like fluconazole has revolutionized the treatment of candidal infections. (McFadden, 1995:47-49). Present day anti-fungal agents can effectively treat candidal infections, but their use can also lead to colonization with less susceptible species and to resistance among normally susceptible strains (Sobel, *et al*., 2001:286-294). The AIDS pandemic and the constant threat of drug-resistant diseases have stimulated great interest in alternative and complementary medicine (Sumner, 2000:9).

The potent healing qualities of plants have been used in different therapeutic philosophies throughout history (Hoffmann, 1997:17). Orthodox medicine has its roots in the use of herbs (Hoffmann, 1997:17) but with the advent of synthetic

chemistry, much of the past century has seen a decreased reliance on botanicals as sources of original therapeutic compounds (Sumner, 2000:9).

The screening of herbal/traditional medicinal plants is necessary because of the increasing acceptance of traditional medicine as an alternative form of health care (Rabe and van Staden, 1997:81-87). The relative value of a herb for human consumption depends on the competent evaluation of the strength of the data supporting its use, hence the necessity for good scientific research (Mowrey, 1986:XIV-XV).

In this *in vitro* study, the anti-candidal activities of *Commiphora molmol*, *Hydrastis canadensis* and *Warburgia salutaris* tinctures were tested using the disc diffusion technique (Cheesbrough, 1993:198), also known as the agar overlay method (Rios, *et al.*, 1988). The effectiveness of each herbal tincture as an anti-candidal agent was determined by the diameters of the zones of inhibition that each tincture produced.

1.2 **Research Objectives**

The objectives of this study were:

1.2.1 to determine the effect of *Commiphora molmol* 86% v/v tincture against *C. albicans* (with 86% v/v ethanol and fluconazole as the negative and positive controls respectively) using the disc diffusion assay.

1.2.2 to determine the effect of *Hydrastis canadensis* 62% v/v tincture against *C. albicans* (with 62% v/v ethanol and fluconazole as the negative and positive controls respectively) using the disc diffusion assay.

1.2.3 to determine the effect of *Warburgia salutaris* 62% v/v tincture against *C. albicans* (with 62% v/v ethanol and fluconazole as the negative and positive controls respectively) using the disc diffusion assay.

1.2.4 to determine the Minimum Inhibitory Concentration/s of the effective herbal tincture/s.

CHAPTER TWO

Literature Review

2.1 ***Candida albicans (C. albicans)***

2.1.1 **Classification**

C. albicans belongs to the Class Three Fungi, which are Fungi Imperfecti or Deuteromycetes (Anderson and Sobieski, 1980:470-471).

2.1.2 **Morphology and Identification**

C. albicans is a yeast, whose cells are spherical, oval or elongated and reproduce by budding from one or more points (Duguid, *et al*., 1978:544). These buds, which are 2 - 4 µm in diameter, are called blastospores (Nolte, 1973:198). The blastospores usually separate, but one to two generations of buds remain attached to each other. In cases when they remain attached, the yeast may appear filamentous because the cells are elongated and the terminal buds are long. This form is called a pseudomycelium or a pseudohyphae (Dugiud, *et al*., 1978:544.) Pseudomyceliums of *C. albicans* appear under semi-aerobic conditions (Nolte, 1973:198). When placed in nutritionally poor media such as corn meal agar, at temperatures lower than 26°C, it produces large thick walled spores (7 – 17 µm in diameter) called chlamydospores (Duguid, *et al*., 1978:544). Chlamydospores are important morphologic characters in the identification of *C. albicans* (Nolte, 1973:198).

C. albicans grows well on Sabouraud's dextrose agar or blood agar, with colonies reaching 0,5 mm in diameter after 18 hours, which develop into high convex, off-white colonies, 1,5 mm in diameter after two days (Duguid, *et al*., 1978:544). A distinct yeasty odour develops after four to five days (Nolte, 1973:199). The behaviour of *C. albicans* in other media, it's capacity to assimilate and ferment carbohydrates and it's failure to split urea, are used in differentiating it from other yeasts and other *Candida* species (Duguid, *et al*., 1978:544).

2.2 **Candidiasis**

C. albicans is part of the normal microflora of both the gastrointestinal tract and mucocutaneous areas (Howard, *et al*., 1980:616). It is also an opportunistic pathogen, giving rise to local inflammation with a variety of symptoms, and can infect virtually all organ systems (Duguid, *et al*., 1978:545).

Candidiasis refers to any infection caused by a member of the genus *Candida* (Howard, *et al*., 1980:616) and occurs in the mouth, gastrointestinal tract and perianal region, nails, endocardium, lungs, urinary tract, skin and vagina (Burnett and Schuster, 1978:349). *C. albicans* changes to the pseudohyphal form to establish infection. The mechanisms involved in *C. albicans* adherence to epithelial surfaces are not well known but transformation to the pseudohyphal form seems to increase adherence (De Almeid and Scully, 2002:19-26).

C. albicans causes infection when the normal host defenses are compromised (Howard, *et al*., 1980:617). Oral candidiasis is seen in virtually 100% of patients

with Acquired Immunodeficiency Syndrome (AIDS) and often may be the initial sign (Allen, *et al*., 1997:1046).

Opportunistic candida infections may occur in neonatal debility, senility, minor trauma, continued exposure of the skin to moisture and alcoholism (Duguid, *et al*., 1978:545).

Other predisposing conditions include:

- Skin that has been damaged by maceration of tissue, wounds and abrasions, thermal or chemical burns, and intra-vascular catheters.
- Mucosal barriers that have been altered by diabetes, anti-microbial agents, irradiations, smoking, cytotoxic drugs, corticosteriods, cimetidene, vagotomy resulting in an increased gastric pH, and foreign bodies, such as dentures, nasogastric tubes, and diaphragms.
- Hormonal or nutritional imbalances induced by diabetes, oral contraceptive use, pregnancy, menses, malnutrition and uremia.
- Leukemia, irradiation, cancer chemotherapy, and agranulocytosis resulting in decreased numbers of phagocytic cells.
- Intrinsic defects in the function of phagocytic cells resulting from chronic granulomatous diseases and myeloperoxidase deficiency.
- Phagocytic cell functions that are altered by uremia, viral infections, and the use of corticosteriods and anti-microbial agents such as aminoglycosides and sulfonamides.

- Cell-mediated immunity problems arising from defects such as chronic mucocutaneous candidiasis and DiGeorge syndrome, corticosteriod use, irradiation, cancer chemotherapy, immunosuppression for transplantation, and collagen vascular diseases. (Howard, *et al*., 1980:617.)

Localized lesions either remain benign or become acute and disseminated. Organisms may disseminate directly or haematogenously from localized lesions. (Burnett and Schuster, 1978:349.)

The initial infection is more likely to involve the skin or mucosa of the mouth, gastrointestinal tract, vagina and lungs. Depending on the location of the initial infection, when disseminated directly into the tissues, it can involve the urinary and gastrointestinal tracts, middle ear and mastoids, vagina, pleura or diaphragm. (Burnett and Schuster, 1978:349.)

2.2.1 **Different forms of candidiasis**

2.2.1.1 Oral

Candidal infection of the lips, called perlèche, is a symmetrical erosion of the labial commisures. The upper layers of the epidermis are lost, while those beneath are gray in appearance. Deep cracks covered with a gray or white membrane develop in the folds at the corner of the mouth. (Burnett and Schuster, 1978:349.)

Candidal infection of the mouth is called “thrush”. It may occur in newborn infants or in immunocompromised persons. (Jensen, *et al*., 1997:489.) Oral thrush in infants occurs in those that are debilitated or bottle-fed (Duguid, *et al*., 1978:545). Lesions develop within eight to nine days after contact with an infected birth canal, or from infected nipples or pacifiers. Infections appear as whitish, flaky, loosely adherent membranes covering part or all of the tongue, lips, gums or buccal mucosa. Beneath the membrane, the mucosa appears bright red and moist. (Burnett and Schuster, 1978:349.) Oral candidiasis in the adult is either sub-acute or chronic. The thrush membrane is thicker and less friable than it is in the infant (Nolte, 1973:202). The sub-acute adult type is characterized by white, cream coloured, or grayish plaques, which are scattered over all or part of the oral mucous membrane. These can be removed, but with some difficulty, thereby exposing the remaining base, which appears brightly inflamed. (Burnett and Schuster, 1978:351.) In the chronic adult type, the buccal mucosa is dry and red, with little or no covering membrane at all. The tongue has a “raw-beet”, shiny appearance and is dry, fissured, cracked and swollen. Mastication of anything but bland food is difficult, since the entire mucous membrane is dry and burning. (Burnett and Schuster, 1978:351.)

2.2.1.2 Oropharyngeal and oesophageal

Oesophageal involvement is the most frequent type of invasive mucosal disease (Tiernay, *et al*., 2000:1463). Infections are characterized by the development of a whitish pseudomembrane, sometimes large enough to occlude the oesophagus.

Ulcerations may develop which sometimes can cause fatal bleeding. (Burnett and Schuster, 1978:349-350.) Clinically, oesophageal candidiasis presentation may range from being asymptomatic to a complete inability to swallow with consequent dehydration (Wilcox and Mönkemüller, 1998:1002-1008). Individuals usually present with substernal odynaphagia, gastroeosophageal reflux, or nausea without substernal pain (Tierney, *et al*., 2000:1463).

2.2.1.3 Gastrointestinal

This form is seen in patients who have undergone major gastric or abdominal surgery and in those with neoplastic disease. Infection occurs when the organism passes through the intestinal wall and spreads from a gastrointestinal focus. (Mims, *et al*., 1999:399.) Gastrointestinal candidiasis presents as pruritis ani or diarrhoea (Duguid, *et al*., 1978:545).

2.2.1.4 Respiratory

Respiratory candidiasis occurs mainly in infants. The disease resembles diphtheria when the tonsils and larynx are involved. When the pharynx is involved, the infant may also present with an ear infection, because infection sometimes spreads through the eustachian tube to involve the ear or mastoids. (Burnett and Schuster, 1978:351.) Bronchopulmonary candidiasis is common and presents clinically as bronchitis. Chronic oral candidiasis can spread to the lungs resulting in bronchial and pulmonary candidiasis. (Nolte, 1973:204.)

Sputum samples contain many yeast cells, but are often mucoid and gelatinous, rather than purulent (Duguid, *et al*., 1978:545).

2.2.1.5 Vaginal

Vaginal candidiasis commonly presents as a sore, itchy vulva, with white curd-like plaques, inflamed mucous membranes and a white discharge (Kant, 2000:24). This may be accompanied by urethritis and dysuria and may present as a urinary tract infection (Mims, *et al*., 1999:240). The vaginal discharge has a pH below 5,2 and contains pus and yeast cells (Duguid, *et al*., 1978:545). Predisposing factors may be excessive glucose in body fluids and blood, and the accumulation of polysaccharides in the vagina (Burnett and Schuster, 1978:351). Vaginal candidiasis occurs in an estimated 75% of women during their lifetime (Tierney, *et al*., 2000:1463). In non-AIDS females, it is often associated with diabetes, pregnancy and the use of oral contraceptives (Allen, *et al*., 1997:1046).

2.2.1.6 Urinary

Manifesting as cystitis and pyelonephritis, candidiasis of the urinary tract occurs either from a bladder infection or from hematogenous spread from a distant primary site of infection. Urinary candidiasis is relatively rare. (Allen, *et al*., 1997: 1046.)

2.2.1.7 Cutaneous

Cutaneous candidiasis of infants is common, and usually starts in the peri-anal region and extends to the thigh or abdomen (Burnett and Schuster, 1978:351) and may involve the entire napkin area in infants (Duguid, *et al*., 1978:545). Diaper rash infection of neonates is a common manifestation (Allen, *et al*., 1997:1046). Weeping lesions also occur at the neck or axillary region, and almost the entire cutaneous surface may be involved (Burnett and Schuster, 1978:351).

In adults, lesions consist of superficially denuded, beefy-red areas in the depths of the body's folds (Tierney, *et al*., 2000:158) such as in the webs of the fingers and toes, beneath the female breasts, in the armpits and in the folds of the groin (Allen, *et al*., 1997:1046). Lesions are characterized by erythema, exudation and desquamation. Cutaneous candidal infections are common in diabetics whose skin may be mildly traumatized. (Duguid, *et al*., 1978:545.)

2.2.1.8 Ungual

Candidal infection of the nail proper is called onychomycosis, and paronychia, if the folds of skin encasing the nails are involved (Allen, *et al*., 1997:1046). In onychomycosis, initial changes occur at the free edge of the nail, which then becomes yellow and crumbly (Kant, 2000:24).

2.2.1.9 Endocarditis

Candidal endocarditis results from direct inoculation at the time of valvular heart surgery (Tierney, *et* al., 2000:1464) or in persons with pre-existing valvular disease, particularly following episodes of septicemia associated with the use of indwelling catheters and prolonged intravenous infusions, or through repeated inoculation with intravenous drug use (Allen, *et al*., 1997:1046). Splenomegaly and petechiae are common and there's a predilection for large vessel embolization (Tierney, *et al*., 2000:1464).

2.2.1.10 Others

Candidal meningitis is a rare condition, which results from secondary dissemination from sites of infection in the gastrointestinal or respiratory tracts, or from septic emboli released from infected heart valves (Allen, *et al*., 1997:1046).

2.2.2 **Candidiasis and Acquired Immunodeficiency Syndrome (AIDS)**

People with AIDS are concomitantly infected with multiple pathogens, which are difficult to eradicate despite prolonged, appropriate and aggressive treatment. Immunodeficiency deepens as the patient progresses from human immunodeficiency virus (HIV) seropositivity to full-blown AIDS, and organisms that are usually controlled by cell-mediated immunity are able to reactivate and cause disseminated infections which are not seen in the immunologically normal individual (Mims, *et al*., 1999:399).

C. albicans is the most frequently diagnosed opportunistic agent in immunocompromised patients (Fong, *et al*., 1997:85-93). Candidiasis suggests that some immunological alteration is present in the host, and can be recognized as a true opportunistic infection (Lachman, 1995:213). Oral candidiasis may occur at any stage of the HIV infection, but is most commonly associated with low CD4 and lymphocyte counts (Fong, *et al*., 1997:85-93.) Oropharyngeal candidiasis is the most frequently diagnosed fungal infection in AIDS patients (Tierney, *et al*., 2000:1463) and may occur at CD4 counts as high as 300 cells/mm^3 while oesophageal candidiasis occurs most commonly when the CD4 count is <100 cells/mm^3 (Hage, *et al*., 2002:236-241). Candidiasis of the vagina is usually the first and most frequent opportunistic infection in women with AIDS (Tierney, *et al*., 2000:1463). Oral colonization with *C. albicans* becomes more common as the HIV infection progresses, and clinical infection heralds the development of full-blown AIDS (Lachman, 1995:213).

Anti-candidal drugs used in early AIDS treatment are oral amphotericin B, nystatin or clotrimazole. These are often inadequate in advanced AIDS treatment. (Lachman, 1995:214.) Fluconazole has gained wide acceptance as an effective continuous treatment of candidiasis (Lachman, 1995:214) and is the recommended treatment for severe or recurrent oropharyngeal disease and oesophageal candidiasis (Hage, *et al*., 2002:236-241).

2.3 **Diagnosis**

2.3.1 **Signs and Symptoms**

- Severe itching of vulva, anus, or body folds.
- Superficial denuded, beefy-red areas of skin.
- Whitish, curd-like concretions on the oral and vaginal mucous membranes. (Tierney, *et al*., 2000:158.)

2.3.2 **Laboratory findings**

2.3.2.1 Culture

Sabouraud's dextrose or blood agar is inoculated with scrapings or swabs from infected areas (Greenwood, *et al*., 1994:683). Cream coloured, pasty colonies with a yeasty odour appear after 24 - 48 hours of incubation at 37°C. *C. albicans* can be identified by the formation of pseudomycelia and chlamydospores, if the yeast is grown on corn meal agar (Cheesbrough, 1993:390).

2.3.2.2 Direct microscopy

Gram stained preparations of skin, urine, vaginal discharge or other exudates from mucosal surfaces (Cheesbrough, 1993:389) will reveal Gram-positive yeast cells and pseudomycelia (Greenwood, *et al*., 1994:683).

C. albicans isolates can be identified by performing the germ tube test (Greenwood, *et* al.,1994:683), where sprouting yeast cells (tube-like outgrowths from the cells) known as "germ tubes" are visible on microscopic examination

(Cheesbrough, 1993:389). This test involves suspending a small portion of an isolated yeast colony in a test tube containing 0,5 ml of rabbit, human plasma or serum, then incubating it at 35°C for no longer than two hours. A drop of the yeast-serum suspension is examined. (Allen, *et al*., 1997:1043.)

Yeast cannot be identified as *C. albicans*, if this test is performed and germ tubes are not visible (Cheesbrough, 1993:390).

2.3.2.3 Serological tests

Candidiasis serologic test results are controversial (Jawetz, *et al*., 1989:309). A positive finding does not necessarily indicate infection, since the antigens that are employed are unable to differentiate antibodies produced during mucosal involvement, from those formed during deep infection. Similarly, a negative antibody test does not rule out the possibility of deep-seated candidiasis in immunocompromised patients. The ELISA (Enzyme-Linked Immuno-Sorbent Assay) test, which is a more sensitive test, would give better results (Greenwood, *et al*., 1994:696).

2.4 **Conventional treatment of candidiasis**

2.4.1 **Nystatin**

Nystatin is the most commonly prescribed agent for the treatment of candidiasis of the skin and mucous membranes, but is not absorbed when administered orally and cannot be administered parentally because of its low solubility and toxicity (Edwards, *et al*., 1995:81-82).

2.4.2 **Clotrimazole and econazole**

Clotrimazole and econazole are used for the topical treatment of cutaneous candidiasis (Edwards, *et al*., 1995:82,144).

2.4.3 **Itraconazole**

Oral itraconazole is indicated for oropharyngeal, genital and ungual candidiasis (Edwards, *et al*., 1995:82,144).

2.4.4 **Ketoconazole**

Intravenous ketoconazole is used for systemic candidiasis while oral ketoconazole is indicated for ungual candidiasis (Edwards, *et al*., 1995:82,144).

2.4.5 **Miconazole**

Intravenous miconazole is used for systemic candidiasis (Edwards, *et al*., 1995:144).

2.4.6 **Flucytosine**

Flucytosine is used for the treatment of systemic candidiasis, sometimes in combination with amphotericin B. *C. albicans* can develop resistance to flucytosine. (Edwards, *et al*., 1995:82,144.)

2.4.7 **Amphotericin B**

Intravenous amphotericin B is used for systemic candidiasis (Edwards, *et al*., 1995:82,144).

2.4.8 Fluconazole

Oral administration of fluconazole is indicated for mucocutaneous and systemic candidiasis (Edwards, *et al*., 1995:82).

A more detailed description of fluconazole will follow because it forms part of this study.

2.4.8.1 Uses

Fluconazole is used in the treatment of oropharyngeal, oesophageal, or vulvovaginal candidiasis, and in the treatment of other serious systemic candidal infections (e.g., urinary tract infections, peritonitis, disseminated candidiasis, meningitis and pneumonia) (Medscape Druginfo, 2001). It is also used in the treatment of cryptococcal meningitis (Goodman Gillman, 1991:1172). It is used for prophylaxis of serious fungal infections (e.g., candidiasis, cryptococcosis,

histoplasmosis and coccidioidomycosis) in HIV infected patients (Medscape Druginfo, 2001) or for anti-fungal prophylaxis in certain immunocompromised individuals (e.g., cancer patients, bone marrow and solid organ transplant patients) (Sypula and Kale-Pradhan, 2002:155-159).

2.4.8.2 Chemistry

Fluconazole, a synthetic azole anti-fungal agent, is a triazole derivative. It is structurally related to imidazole-derivative azole anti-fungal agents (e.g., clotrimazole, econazole, ketoconazole, miconazole) since it contains a 5-membered azole ring attached by a carbon-nitrogen bond to other aromatic rings. While imidazoles have 2 nitrogens in their imidazole rings, fluconazole and other triazoles (e.g., itraconazole and terconazole) have 3 nitrogens in their triazole rings. Replacing the imidazole ring with a triazole ring greatly increases an agent's antifungal activity. (Medscape Druginfo, 2001.)

2.4.8.3 Mechanism of action

Fluconazole exerts its anti-fungal activity by altering cellular membranes, resulting in increased membrane permeability, leakage of essential elements (e.g., amino acids, potassium) and impaired uptake of precursor molecules (e.g., purine and pyrimidine precursors to DNA) (Medscape Druginfo, 2001). Fluconazole selectively inhibits the yeast cytochrome P-450 and sterol C-14-alpha-demethylation (Chan-Tack, 2002:14).

2.4.8.4 Dosage

The usual dosage of fluconazole in paediatric patients ranges from 3-12 mg/kg daily. Doses exceeding 600 mg daily are not recommended (Medscape Druginfo, 2001).

For adults, fluconazole dosage varies according to the type and severity of the infection, identity of the causative organism, and the patient's renal function and response to therapy (Medscape Druginfo, 2001). Seth (1999:610) suggests the following protocol: for mucosal infections, 50 - 100 mg daily for 14-30 days; for systemic infections, 400 mg on the first day followed by 200 - 400 mg once daily. Maintenance therapy with fluconazole is usually necessary to prevent relapse in patients with AIDS, cryptococcal meningitis or recurrent oropharyngeal candidiasis (Medscape Druginfo, 2001). Seth (1999:610) suggests 100 - 200 mg daily as maintenance to prevent cryptococcal meningitis and 50 - 100 mg as prophylaxis of fungal infections.

2.4.8.5 Pharmacokinetics

2.4.8.5.1 Absorption

Fluconazole is rapidly and completely absorbed through the gastrointestinal tract and its bioavailability is not altered by food or gastric acidity (Goodman Gilman, *et al*., 1991:1172). There is no evidence of first-pass metabolism (Medscape Druginfo, 2001). Fluconazole diffuses readily into body fluids, including sputum and saliva (Goodman Gilman, 1991:1172).

2.4.8.5.2 Distribution

Fluconazole is distributed widely into body tissues and fluids following oral or intravenous administration (Medscape Druginfo, 2001). Plasma concentrations are essentially the same when the drug is administered orally or intravenously (Goodman Gilman, 1991:1172). While it is not known whether fluconazole crosses the placenta, there is evidence that human milk concentrations of fluconazole are similar to those of plasma (Medscape Druginfo, 2001).

2.4.8.5.3 Elimination

In healthy adults, the drug is eliminated principally by renal excretion, with the plasma elimination half-life of fluconazole ranging from 20 to 50 hours. The mean plasma half-life of fluconazole in children 9 months to 15 years of age ranges from about 15 to 25 hours. (Medscape Druginfo, 2001.)

2.4.8.6 Adverse effects

Mild to moderate nausea, vomiting, abdominal pain and diarrhoea have been reported in 1,5 to 8,5% of patients (Medscape Druginfo, 2001). Diffuse rash accompanied by eosinophilia and pruritus, have been reported in up to 5% of patients. Stevens-Johnson syndrome, which can be fatal, has been reported in patients receiving fluconazole. Hepatotoxicity fatalities have occurred mainly in patients with underlying disease (e.g., AIDS, cancer). Eosinophilia, anaemia, leukopenia, thrombocytopenia (Medscape Druginfo, 2001), hypokalemia and

headache (Chan-Tack, 2002) have also been reported. Alopecia can occur in patients receiving long-term fluconazole treatment. (Medscape Druginfo, 2001.)

2.4.9 Fluconazole resistance

Anti-fungal agents can effectively treat mucosal candidiasis, but their use can lead to colonization with less susceptible species and to resistance among normally susceptible strains (Sobel, *et al*., 2001:286-294). Imidazole-resistant *C. albicans* has increased in frequency in immunocompromised patients, particularly in patients with late stage AIDS receiving chronic suppressive fluconazole (Tierney, *et al*., 2000:1464). Morschhäuser (2002:240-248) says that the emergence of fluconazole-resistant *C. albicans* strains is a significant problem after long term treatment of recurrent oropharyngeal candidiasis in AIDS patients. However, Sobel and associates (2001:286-294) state that azole resistance among *C. albicans* may be less widespread than previously thought, and rare among HIV sero-positive females at the stages that precede advanced immunodeficiency. Progressively increased colonization with non-*C. albicans* strains with declining susceptibility to fluconazole is a new observation that has emerged from their study.

There is controversy concerning fluconazole's effectiveness in the treatment of systemic candidiasis, especially in critically ill patients. Clinical trials do not support the prophylactic or empirical use of fluconazole in the intensive care unit. (Kam and Lin, 2002:33-41.) Retrospective and surveillance studies of nosocomial

fungal infections suggest that the use of fluconazole may be contributing to the shift in fungal flora causing these infections, and that the isolates are more fluconazole resistant (Sypula and Kale-Pradhan, 2002:155-159).

After widespread reports of fluconazole-resistant oropharyngeal candidiasis in patients with AIDS, several retrospective studies with different study designs attempted to identify risk factors for the development of azole resistance. The dominant risk factor that emerged was patient exposure to fluconazole. (Sobel, *et al*., 2001:286-294.)

2.5 Complementary and Alternative therapies

Diet plays an important role in the treatment of candidiasis in complementary medicine. The Anti-Yeast diet is a diet that is low in carbohydrates and high in protein (De Schepper, 1989:33). Alternative therapies also use natural anti-fungal supplements like:

- Vitamins C and E, and selenium, which are anti-inflammatory (Anderson, *et al*., 2000:16).
- Anti-inflammatory essential fatty acids like omega-3 and omega-6 (Anderson, *et al*., 2000:16).
- Biotin, which inhibits a form of candida that is the most irritating to membranes (Anderson, *et al*., 2000:16).
- *Lactobacillus acidophilus*, which assists in restoring the normal balance of bowel and mucous membranes (Anderson, *et al*., 2000:16).
- FOS (fructo-oligosaccharides), which promote bowel bacterial growth (Bilanow and Calvo, 1999:227).
- Caprylic acid, which is an antifungal fatty acid (Anderson, *et al*., 2000:16).
- Homoeopathic isotherapeutic treatment with the candida nosode 30CH (Jouanny, 1993:181).

Herbal and Traditional Medicine

Herbs that are often used to treat candidiasis are goldenseal (*Hydrastis canadensis*), oregon grape root (*Mabonia nervosa*), barberry (*Berberis vulgaris*), chamomile (*Matricaria recicuta*), liquorice (*Glycyrrhiza glabra*), tea tree oil

(*Melaleuca alternifolia*) (Anderson, *et al*., 2000:16), marigold (*Calendula officinalis*) (McFadden, 1995:47-59), myrrh (*Commiphora molmol*) (McFadden, 1995:47-59), rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*) (Reid, 2001:22, 29-30). The pepper-bark tree (*Warburgia salutaris*) and african ginger (*Siphonochilus aethiopicus*) are used in traditional African medicine for the treatment of candidal infections (Gericke, 2001:3-15).

Commiphora molmol, *Hydrastis canadensis* and *Warburgia salutaris* will be described in detail because they form part of this study.

2.5.1 ***Commiphora molmol***

2.5.1.1 Family

Commiphora molmol belongs to the *Burseraceae* family (Iwu, 1993:160).

2.5.1.2. Common names

Commiphora molmol is commonly known as myrrh or molmol (Iwu, 1993:160).

2.5.1.3 African names:

- Arabic: morrh.
- Hausa: dashi, biskiti.
- Yoruba: turari.
- Swahili: mbebe, mbele. (Iwu, 1993:160.)

2.5.1.4 Botanical description

Commiphora molmol is a shrubby desert tree that grows to a height of 2,7 m, and has a light gray, thick trunk. The main branches are knotted, with smaller branches protruding at a right angle and ending in sharp spines. Leaves are hairless and roughly toothed, and are divided into one pair of small, oval leaflets with a larger terminal leaflet. Yellow-red flowers grow on stalks in an elongated and branching cluster. The tree produces small brown fruit that are oval in shape and which taper to a point. (Gale Encyclopedia of Alternative Medicine, 2001.)

2.5.1.5 Habitat and distribution

Commiphora molmol is native to the Arabian peninsula (Peirce, 1999:449), Ethiopia, Somalia, and is also cultivated in Kenya and Tanzania (Iwu, 1993:160).

2.5.1.6 Constituents

Commiphora molmol consists of about 60% of gums, which are usually composed of arabinose, galactose, 4-0-methylglucuronic acid and xylose. Eugenol, cadinene, furanodiene, furansesquiterpenes, heerabolene, cuminaldehyde and elemol were isolated in its volatile components. The resinous fraction is about 30% of the total weight and contains commiferin, α-, β- and γ-commiphoric acids, α- and β- heerabomyrrhols, commophorinic acid and heeraboresene. It also contains sterols and proteins. (Iwu, 1993:161.)

2.5.1.7 Parts used

The oleo-gum/resin from the bark of *Commiphora molmol* is used. The resin is collected as exudates from fissures or incisions in the bark, which dry in collecting vessels forming irregular shapes. Once completely dry, the resin is brown in colour with thin translucent splinters. It has a bitter, but pleasant taste and an aromatic odour. (Iwu, 1993:160.)

2.5.1.8 Preparations

Commiphora molmol is available in tincture, capsule and powder form. It is also an ingredient in lip balm, tooth powder and toothpaste. (Peirce, 1999:450.)

2.5.1.9 Actions

Commiphora molmol is an anti-microbial, astringent, carminative, anti-catarrhal, expectorant, vulnerary (Hoffmann, 1997:2180), stimulant, emmenagogue, purgative, anti-septic, anti-inflammatory, anti-spasmodic (Iwu, 1993:160) and a menstruation promoter (Peirce, 1999:449).

2.5.1.10 Medicinal uses

- Infections.

  Commiphora molmol is claimed to be an effective anti-microbial agent whereby it assists the body in combating infections (Hoffmann, 1997:218). It is specifically used for infections in the mouth such as mouth ulcers and gingivitis (Hoffmann, 1997:218), and for a sore throat and hoarseness of

the voice (Peirce, 1999:449). Iwu (1993:161) says that it is an ingredient in mouthwashes because of its anti-septic and stimulant properties.

It also assists in catarrhal problems like pharyngitis and sinusitis.

Systemically, it is of value in the treatment of boils, glandular fever and brucellosis. (Hoffmann, 1997:218.) As an expectorant, it assists with congestion caused by cough and asthma (Peirce, 1999:449). In West Africa, vapour from the resin in boiling water is used for the treatment of eye inflammation (Iwu, 1993:160).

- External uses.

  Commiphora molmol is rated highly as a topical anti-septic for wounds and abrasions (Hoffman, 1997:218), haemorrhoids and bedsores (Peirce, 1999:449). Iwu (1993:160) says that a decoction of the stem bark, is used by the Nyamwezi as a snake bite remedy, while in West Africa, it is used as a remedy for scorpion bite. Traditional Chinese healers valued it for treating haemorrhages, pain, swelling and wounds (Peirce, 1999:449).

- Other uses.

  Commiphora molmol is used as an incense and a perfume, and as flavouring in foods (Peirce, 1999:449-450). It was also used for embalming in ancient Egypt (Dolara, *et al*., 2000:356-358).

2.5.1.11 Research studies

Dolara and associates (2000:356-358), isolated 8 sesquiterpenes fractions from *Commiphora molmo*l and demonstrated with the disc diffusion technique that a mixture of furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one was anti-bacterial and anti-fungal against standard pathogenic strains of *Escherichia coli*, *Staphylococus aureaus*, *Pseudamonas aeruginosa* and *C. albicans* with minimum inhibitory concentrations ranging from 0,18 to 2,8 µg/ml.

McFadden (1995:47-59) compared the inhibitory characteristics of twelve plant extracts (including *Commiphora molmol*) using the agar dilution technique and concluded that *Commiphora molmol* exhibited a slight inhibitory effect on *C. albicans* with the mean *C. albicans* colony size being 17,38 mm^2.

2.5.2 ***Hydrastis canadensis***

2.5.2.1 Family

Hydrastis canadensis belongs to the *Ranunculaceae* family (Hoffmann, 1997:204).

2.5.2 2. Common name

The common name for *Hydrastis canadensis* is goldenseal (Hoffmann, 1997:204).

2.5.2.3 Botanical description

Hydrastis canadensis is a perennial herb with a knotty yellow rhizome, from which arises a single leaf and an erect hairy stem. In early spring, it bears two 5-9 lobed rounded leaves near the top, which are terminated by a single greenish-white flower. (Murray, 1995:162.)

2.5.2.4 Habitat and distribution

Hydrastis canadensis is native to eastern North America (Peirce, 1999:311) and is cultivated in Oregon and Washington (Murray, 1995:162).

2.5.2.5 Constituents

5% of the root consists of the alkaloids hydrastine, berberine, canadine (Hoffmann, 1997:204), berberastine, candaline, hydrastinine and other related

alkaloids. Other constituents include meconin, chlorogenic acid, phytosterins and resins. (Murray, 1995:163.)

2.5.2.6 Parts used

The dried rhizome and roots of *Hydrastis canadensis* are used (Hoffmann, 1997:204).

2.5.2.7 Preparations

Hydrastis canadensis is available in capsule, powder and tincture form (Peirce, 1999:313).

2.5.2.8 Actions

Hydrastis canadensis acts as a tonic, an astringent, an anti-catarrhal, an anti-microbial, a laxative, a muscular stimulant, an oxytocic and a bitter (Hoffmann, 1997:204).

2.5.2.9 Medicinal uses

The broad anti-microbial effects of berberine combined with its anti-infective and immune-stimulating actions, support the historical use of *Hydrastis canadensis* in infections of the mucous membranes of the oral cavity, throat, sinuses, bronchi, genitourinary tract and gastrointestinal tract (Murray, 1995:167).

- Infections.

  Berberine, one of the alkaloids discovered in *Hydrastis canadensis*, has been found to be effective against traveler's diarrhoea, shigellosis, food poisoning, giardiasis and cholera. It appears that it is effective in treating the majority of common gastrointestinal infections (Murray, 1995:167) such as gastritis, septic ulceration and colitis (Hoffmann, 1995:204).

  All catarrhal states benefit from this herb, especially upper respiratory tract catarrh (Hoffmann, 1997:204).

  In the past, the American Indians used *Hydrastis canadensis* for a variety of eye complaints. In recent times, berberine has shown remarkable effect in the treatment of trachoma, which is an infectious eye disease that is responsible for the high incidence of blindness and impaired vision in under-developed countries. (Murray, 1995:169.)

- Liver disorders.

  Berberine has been shown in several clinical studies, to stimulate the secretion of bile and bilirubin. It also has been shown to correct metabolic abnormalities in patients with liver cirrhosis. (Murray, 1995:169.)

- Gynaecological.

  Hydrastis canadensis helps in uterine conditions like menorrhagia (excessive menstruation) and haemorrhage, because of its tonic and astringent actions. It is also an excellent aid during childbirth. (Hoffmann, 1997:204.)

- External uses.

  It is used for the treatment of eczema and ringworm (Hoffmann, 1997:204).

2.5.2.10 Precautions

As *Hydrastis canadensis* stimulates the involuntary muscles of the uterus, it should be avoided during pregnancy, but can be used safely under supervision during childbirth (Hoffmann, 1997:204).

2.5.2.11 Research studies

Scazzocchio and associates (2001:561-564) evaluated the bactericidal (using the contact test whereby they measured the “killing time” on a low density bacterial inoculum) and bacteriostatic (in liquid medium by means of M.I.C. values) activities of the extract and major alkaloids of *Hydrastis canadensis* (berberine, β-hydrastine, canadine and canadaline) against *Staphylococcus aureus, Streptococcus sanguis, Escherichia coli and Pseudomonas aeruginosa*. They demonstrated that berberine was bactericidal against all tested strains

except *Pseudomonas aeruginosa*, while canadaline was more active than berberine at the same concentration on the Gram-positive tested strains. Canadaline's bactericidal activity was the same as berberine for the Gram-negative tested strains. Both canadaline and canadine were active against *Pseudomonas aeruginosa* while canadine was inactive on *Escherichia coli*. β-hydrastine was found to be inactive against all tested microorganisms. M.I.C.s of the tested alkaloids showed bacteriostatic activity between 0,12 to 0,5 mg/ml against Gram-positive strains while all tested substances did not show bactericidal activity against Gram-negative strains. (Scazzocchio, *et al*., 2001:561-564.)

McFadden (1995:47-59) compared the inhibitory characteristics of twelve plant extracts (including *Hydrastis canadensis*) and demonstrated using the agar dilution technique that *Hydrastis canadensis* exhibited a slight inhibitory effect on *C. albicans* with the mean *C. albicans* colony size being 19,50 mm^2.

Berberine has shown anti-microbial activity against bacteria, protozoa and fungi, including *C. albicans* (Murray, 1995:166).

2.5.3 ***Warburgia salutaris***

2.5.3.1 Family

Warburgia salutaris belongs to the *Canellaceae* family (Hutchings, 1996:204).

2.5.3.2 Synonyms

Warburgia salutaris is also known as *Chibaca salutaris*, *Warburgia breyeri*, and *Warburgia ugandensis* (Hutchings, 1996:204).

2.5.3.3 Common names

Warburgia salutaris is commonly known as the pepper-bark tree, fever tree, koorsboom and peperbasboom (Hutchings, 1996:204).

2.5.3.4 African names:

- Kikiyu: maseka.
- Masai: olsokoni, isibaha esimbomvu (root bark), isibaya esimhlope (stem bark).
- Meru: musuni.
- Kisii: omenyakige.
- Shangana: xibaha.
- Sotho: sebaha.
- Venda: isibaha (Iwu, 1993:258), mulanga and manaka.
- Zulu: isibaha (Van Wyk, *et al*., 1997:272).

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2.5.3.5 Botanical description

Warburgia salutaris is a medium size, spreading, evergreen tree of about 10 to 25 m in height (Iwu, 1993:258), with a rough, mottled bark, which is reddish on the inner side (Van Wyk, *et al*., 1997:272). Its leaves are oblong, about 60 mm long (Van Wyk, *et al*., 1997:272), simple, glossy, dark green above and pale green below (Iwu, 1993:258). Small, greenish-yellow flowers are produced between the leaves on the stem (Van Wyk, *et al*., 1997:272). It produces small green fruit (approximately 5 cm in diameter are ellipsoidal at first and later become subspherical), which become purple with time. The seeds (1 to 2 cm long) are yellow-brown and compressed. (Iwu, 1993:258.)

2.5.3.6 Habitat and distribution

Warburgia salutaris is found in lowland rainforest, upland dry evergreen forests and secondary bushland and grassland (Iwu, 1993:258). It is restricted primarily to the southern and eastern regions of Africa, and is found in Kenya, Uganda, Ethiopia, Tanzania, Zaire, (Iwu, 1993:258) and in the north-eastern parts of South Africa (Van Wyk, *et al*., 1997:272). It is being severely over-harvested because of its medicinal value, and wild trees are on the brink of extinction in South Africa (Gericke, 2001:3-13).

2.5.3.7 Constituents

The bark contains numerous sesquiterpenoids such as warburganal, polygodial (Van Wyk, *et al*., 1997:272), drimenol, warburgin, warburgiadione,

ugandensoline, ugandensidial, cinnamoide, bemadienolide and muzigadial (Iwu, 1993: 258). It is also said to contain mannitol (Van Wyk, *et al*., 1997:272).

2.5.3.8 Parts used

The bark or root bark of *Warburgia salutaris* is used (Van Wyk, *et al*., 1997:272). The inner bark, root bark and leaves have a pungent ginger or pepper-like taste (Hutchings, 1996:204).

2.5.3.9 Preparations

Powdered bark of *Warburgia salutaris* is mixed with cold water and taken as an infusion or it is smoked as a cough and cold remedy (Van Wyk, *et al*., 1997:272). It is also available in tablet form (Phyto Nova, 2002).

2.5.3.10 Actions

Warburgia salutaris is an emetic, a purgative, an anti-inflammatory (Hutchings, 1996:204) and an anti-pyretic (Iwu, 1993:258).

2.5.3.11 Medicinal uses

Warburgia salutaris is considered a general tonic and remedy for a variety of illnesses (Iwu, 1993:258).

- Infections.

  It's bark and leaves have been used to treat yeast, fungal, bacterial and protozoal infections for centuries (Gericke, 2001:3-13). The root and stem bark are used as expectorants or smoked for coughs and colds (Hutchings, 1996:204), and chest complaints (Van Wyk, *et al*., 1997:272). It is particularly sought after for a serious cough that produces purulent sputum (Van Wyk, *et al*., 1997:204). It is a natural antibiotic, particularly for bronchitis (Gericke, 2001:3-13). Powdered bark is used as a snuff to clear sinuses (Iwu, 1993:258).

  It is indicated for candidiasis syndrome, vaginal thrush, and for oral and oesophageal thrush in AIDS patients (Gericke, 2001:3-13).

  Powdered bark is mixed with fat and used for irritation of the penis, while ointment made from pounded leaves and stalks mixed with bark and fat is applied to the penis for inflammation and sores (Felhaber and Mayeng, 1997:219).

- Rheumatism.

  Warburgia salutaris is used for muscle pains, weak joints and general body pains (Iwu, 1993:258).

- Other.

  It is also a remedy for toothache, fever (Iwu, 1993:258), headache, and gastric ulcers (Van Wyk, *et al*., 1997:272), skin complaints, stomach pain and constipation (Felhaber and Mayeng, 1997:219) and for ailments known as *isibhobo* or *amanxeba*, which are traditionally thought to be caused by sorcery (Hutchings, 1996:204). It is used as an aphrodisiac (Hutchings, 1996:204).

2.5.3.12 Research studies

Rabe and van Staden (1997:81-87) tested 21 South African medicinal plants (including *Warburgia* salutaris) against *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* using the agar diffusion and dilution methods. *Warburgia salutaris* aqueous preparation was effective against *Staphylococcus aureus*, *Staphylococcus epidermis* and *Bacillus subtilis. Warburgia salutaris* methanol extract was effective against *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilis* and *Klebsiella pneumoniae* (Rabe and van Staden, 1997:81-87.)

Taniguchi and associates (1983:149-154) demonstrated using a two-fold dilution method that warburganal exhibited a broad anti-microbial activity against all yeasts and filamentous fungi tested and that it was highly active against *Saccharomyces*. *cerevisiae*, *Candida utilis* and *Sclerotinia libertiana*.

Drewes and associates (2001:383-386) used flash column chromatography, thin-layer chromatography and a quantitative ^{1}H NMR technique to determine ratios of polygodial to warburganal in both leaf and bark samples of *Warburgia salutaris.* The Mann-Whitney U test showed that differences in constituent ratios between the leaves and the bark were statistically non-significant and thus indicating that the leaves of *Warburgia salutaris* represent a good source of its two active components (Drewes, *et al*., 2001:383-386).

CHAPTER THREE

Methodology

3.1 Data

The research consists of two forms of data: primary and secondary.

3.1.1 Primary data

1. Results of the experiment determining the effects of *Commiphora molmol* 86% v/v ethanol tincture on *C. albicans*.
2. Results of the experiment determining the effects of *Hydrastis canadensis* 62% v/v ethanol tincture on *C. albicans*.
3. Results of the experiment determining the effects of *Warburgia salutaris* 62% v/v ethanol tincture on *C. albicans*.
4. Results of the experiment determining the effects of 86% v/v ethanol on *C. albicans*.
5. Results of the experiment determining the effects of 62% v/v ethanol on *C. albicans*.
6. Results of the experiment determining the effects of fluconazole on *C. albicans*.
7. Results of the experiment determining the effects of *Hydrastis canadensis* 62% v/v ethanol tincture 1:2 distilled water dilution on *C. albicans*.
8. Results of the experiment determining the effects of *Hydrastis canadensis* 62% v/v ethanol tincture 1:5 distilled water dilution on *C. albicans*.
9. Results of the experiment determining the effects of *Hydrastis canadensis* 62% v/v ethanol tincture 1:10 distilled water dilution on *C. albicans*.

10. Results of the experiment determining the effects of *Hydrastis canadensis* 62% v/v ethanol tincture 1:50 distilled water dilution on *C. albicans*.
11. Results of the experiment determining the effects of *Hydrastis canadensis* 62% v/v ethanol tincture 1:100 distilled water dilution on *C. albicans*.
12. Results of the experiment determining the effects of 62% v/v ethanol 1:2 distilled water dilution on *C. albicans*.
13. Results of the experiment determining the effects of 62% v/v ethanol 1:5 distilled water dilution on *C. albicans*.
14. Results of the experiment determining the effects of 62% v/v ethanol 1:10 distilled water dilution on *C. albicans*.
15. Results of the experiment determining the effects of 62% v/v ethanol 1:50 distilled water dilution on *C. albicans*.
16. Results of the experiment determining the effects of 62% v/v ethanol 1:100 distilled water dilution on *C. albicans*.

3.1.2 Secondary data

Articles from books and journal publications.

3.2 Criteria governing the admissibility of data.

Only data gathered from experiments conducted by the researcher in the research microbiology laboratory of the Department of Biotechnology, M.L. Sultan campus, Durban Institute of Technology were used.

3.3 Materials and Methods

3.3.1 Preparation of the tinctures

3.3.1.1 Preparation of *Commiphora molmol* tincture

Commiphora molmol tincture (batch no. 032043, expiry date: 02/2007) was prepared in 86% ethanol (*Commiphora molmol* is stable in 86% ethanol) by Parceval Pharmaceuticals (Pty) Ltd according to method HAB 4a of the German Homoeopathic Pharmacopoeia (Lilje, 2002).

3.3.1.2 Preparation of *Hydrastis canadensis* tincture

Hydrastis canadensis tincture (batch no. 10143, expiry date 09/2006) was prepared in 62% ethanol (*Hydrastis canadensis* is stable in 62% ethanol) by Parceval Pharmaceuticals (Pty) Ltd according to method HAB 4a of the German Homoeopathic Pharmacopoeia (Lilje, 2002).

3.3.1.3 Preparation of *Warburgia salutaris* tincture

Warburgia salutaris tincture (batch no. 04919 , expiry date 03/2004) was prepared in 62% ethanol by Parceval Pharmaceuticals (Pty) Ltd according to method HAB 4a of the German Homoeopathic Pharmacopoeia (Lilje, 2002).

3.3.2 Preparation of the controls

3.3.2.1 Preparation of 62% v/v ethanol (negative control)

The 62% v/v ethanol used in this study was prepared by Parceval Pharmaceuticals (Pty) Ltd in the following way: 65,68 parts of 94,4% ethanol was mixed with 34,32 parts of distilled water (by weight) (Lilje, 2002).

3.3.2.2 Preparation of 86% v/v ethanol (negative control)

The 86% v/v ethanol used in this study was prepared by Parceval Pharmaceuticals (Pty) Ltd in the following manner: 91,1 parts of 94,4% ethanol was mixed with 8,9 parts of distilled water (by weight) (Lilje, 2002).

3.3.2.3 Preparation of fluconazole (positive control)

The fluconazole discs (lot no. 117073, expiry date 01/10/2002) used in this study were manufactured by Mast Diagnostics (Mast Group Ltd). Each disc contained 25 µg of fluconazole.

3.3.3 Preparation of *Hydrastis canadensis* dilutions

3.3.3.1 Preparation of *Hydrastis canadensis* 1:2 dilution

1 ml of *Hydrastis canadensis* 62% v/v tincture was transferred using a sterile micropipette to a sterile 50 ml screw-top amber bottle containing 1 ml of distilled water. The lid was closed and the bottle was swirled, so as to evenly mix the dilution. (Naudé, 2002.)

3.3.3.2 Preparation of *Hydrastis canadensis* 1:5 dilution

1 ml of *Hydrastis canadensis* 62% v/v tincture was transferred using a sterile micropipette to a sterile 50 ml screw-top amber bottle containing 4 ml of distilled water. The lid was closed and the bottle was swirled, so as to evenly mix the dilution. (Naudé, 2002.)

3.3.3.3 Preparation of *Hydrastis canadensis* 1:10 dilution

1 ml of *Hydrastis canadensis* 62% v/v tincture was transferred using a sterile micropipette to a sterile 50 ml screw-top amber bottle containing 9 ml of distilled water. The lid was closed and the bottle was swirled, so as to evenly mix the dilution. (Naudé, 2002.)

3.3.3.4 Preparation of *Hydrastis canadensis* 1:50 dilution

0,1 ml of *Hydrastis canadensis* 62% v/v tincture was transferred using a sterile micropipette to a sterile 50 ml screw-top amber bottle containing 4,9 ml of distilled water. The lid was closed and the bottle was swirled, so as to evenly mix the dilution. (Naudé, 2002.)

3.3.3.5 Preparation of *Hydrastis canadensis* 1:100 dilution

0,1 ml of *Hydrastis canadensis* 62% v/v tincture was transferred using a sterile micropipette to a sterile 50 ml screw-top amber bottle containing 9,9 ml of distilled water. The lid was closed and the bottle was swirled, so as to evenly mix the dilution. (Naudé, 2002.)

3.3.4 Preparation of 62% v/v ethanol dilutions

3.3.4.1 Preparation of 62% v/v ethanol 1:2 dilution

1 ml of 62% v/v ethanol was transferred with a sterile micropipette to a plastic screw-top 15 ml test tube containing 1 ml of distilled water. The lid was closed and the test tube was swirled, so as to evenly mix the dilution. (Naudé, 2002.)

3.3.4.2 Preparation of 62% v/v ethanol 1:5 dilution

1 ml of 62% v/v ethanol was transferred with a sterile micropipette to a plastic screw-top 15 ml test tube containing 4 ml of distilled water. The lid was closed and the test tube was swirled, so as to evenly mix the dilution. (Naudé, 2002.)

3.3.4.3 Preparation of 62% v/v ethanol 1:10 dilution

1 ml of 62% v/v ethanol was transferred with a sterile micropipette to a plastic screw-top 15 ml test tube containing 9 ml of distilled water. The lid was closed and the test tube was swirled, so as to evenly mix the dilution. (Naudé, 2002.)

3.3.4.4 Preparation of 62% v/v ethanol 1:50 dilution

0,1 ml of 62% v/v ethanol was transferred with a sterile micropipette to a plastic screw-top 15 ml test tube containing 4,9 ml of distilled water. The lid was closed and the test tube was swirled, so as to evenly mix the dilution. (Naudé, 2002.)

3.3.4.5 Preparation of 62% v/v ethanol 1:100 dilution

0,1 ml of 62% v/v ethanol was transferred with a sterile micropipette to a plastic screw-top 15 ml test tube containing 9,9 ml of distilled water. The lid was closed and the test tube was swirled, so as to evenly mix the dilution. (Naudé, 2002.)

3.3.5 Preparation of the filter paper discs

The 0,6 mm filter paper discs (lot no. CQ 0999-1; ref. no. 10321260) used in this study were manufactured by Schleicher & Schuell and were obtained from the Department of Biotechnology, M.L. Sultan campus, Durban Institute of Technology.

3.3.6 Preparation of the inoculum

C. albicans (ATCC 10231) used in this study was obtained from the freeze dried culture collection of the microbiological laboratory of the Department of Biotechnology at the ML Sultan campus of the Durban Institute of Technology.

3.3.6.1 Preparation of the inoculum for Part 1 (to determine the effect of *Commiphora molmol*, *Hydrastis Canadensis, Warburgia salutaris,* 86% v/v ethanol, 62% v/v ethanol and fluconazole against *C. albicans*) of the Experiment

C. albicans was transferred using a sterile needle under aseptic conditions to a plate containing Sabouraud's dextrose agar. This culture containing plate was incubated for 48 hours at 37°C and transferred into four sterile test tubes, which were labeled 1 to 4. A loop of the *C. albicans* was then transferred into a sterile

“stock” test tube containing 10 ml distilled water. 1 ml of the stock was then pipetted into a sterile test tube test tube 1 containing 9 ml of distilled water and shaken, so as to evenly mix the mixture. Next, 1 ml of test tube 1 was pipetted into a test tube 2 containing 9 ml of distilled water. This constituted the 1:10 dilution. This was further serially diluted by ten fold dilution to the fourth serial dilution with the result being that test tube 4 contained a dilution of 1:10 000 with the dilution factor being 10 000. The Dry-Slide technique (Ross, 1986:170-175) was then used to determine the cell count of the fourth serial dilution. A loop of the inoculum from test tube 4 was transferred to a special slide with counting grids called a hemocytometer. The number of cells in 10 blocks were counted and averaged.

The cells/ml were determined using the following equation:

Cells/ml = (no. of cells counted / no. of squares counted) X dilution factor

(Pelzar, *et al.*, 1993:189).

No. of cells counted = 50

No. of blocks counted = 4

Dilution factor = 10 000

Cells/ml = $\frac{50}{10}$ X 10 000

= 50 000 cells/ml of *C. albicans*.

Hence, concentration of *C. albicans* used in this experiment was 50 000 cells/ml.

3.3.6.2 Preparation of the inoculum for Part 2 (to determine the minimum inhibitory concentration of *Hydrastis canadensis* tincture against *C. albicans*) of the Experiment

The procedure in 3.3.6.1 was repeated 4 times resulting in 4 test tubes being prepared, each containing 10 ml of the inoculum.

3.3.7 Preparation of the media

The media that was used in the experiments was Sabouraud's dextrose agar (Cheesbrough, 1993:423), and was manufactured by Biolab Diagnostics (Pty) Ltd (batch no. 1016559).

The Disc Diffusion /Agar Overlay Method (Rios, *et al*, 1988) was used in this study. In this method, each plate contained 2 layers of agar; an initial layer of Sabouraud's dextrose covered by a second layer of Sabouraud's dextrose agar, which had been inoculated, with the standardized inoculum of *C. albicans*.

3.3.7.1 Preparation of the media for Experiment 1:

Sabouraud's dextrose agar was prepared according to the directions on the bottle leaflet (Biolab Diagnostics (Pty) Ltd [batch no. 1016559]).

1. 15 g of Sabouraud's dextrose agar powder was weighed out using a trial balance.
2. 0,25 litre of distilled water was poured into a 1 litre sterile Erlen Meyer flask.

3. The 15 g of Sabouraud's dextrose agar was then added to the distilled water in the Erlen Meyer flask and the mixture swirled and shaken so as to evenly mix it.
4. The mouth of the flask was closed with cotton wool, which was then covered with foil.
5. The mixture was autoclaved at 121°C for 15 min.
6. The flask was left aside to cool slightly, before being put onto a magnetic stirring machine, which prevented the mixture from solidifying.

While the media was cooling, the under surfaces of 15 plates were divided into six equal parts using a permanent marker and a ruler. A particular letter was chosen for each of the test tinctures and control substances and written in each space.

A. *Commiphora molmol* 86% v/v ethanol tincture
B. *Hydrastis canadensis* 62% v/v ethanol tincture
C. *Warburgia salutaris* 62% v/v ethanol tincture
D. 86% v/v ethanol
E. 62% v/v ethanol
F. fluconazole

Once the media had sufficiently cooled, it was poured into the 15 plates at a depth of approximately 5 ml per plate. The 15 plates were then covered with their lids and stored in a refrigerator for 24 hours.

After 24 hours, the above procedure 1-6 was then repeated and the new agar that was prepared was inoculated with the 10 ml standardized inoculum of *C. albicans* and poured into the 15 plates.

3.3.7.2 Preparation of the media for Experiments 2, 3, 4, 5 and 6:

Sabouraud's dextrose agar was prepared according to the directions on the bottle leaflet (Biolab Diagnostics (Pty) Ltd [batch no. 1016559]).

1. 60 g of Sabouraud's dextrose agar powder were weighed out using a trial balance.
2. 1 litre of distilled water was poured into a 2 litre sterile Erlen Meyer flask.
3. The Sabouraud's dextrose agar was then added to the distilled water in the Erlen Meyer flask and the mixture swirled and shaken so as to evenly mix it.
4. The mouth of the flask was closed with cotton wool, which was then covered with foil.
5. The mixture was autoclaved at 121°C for 15 min.
6. The flask was left aside to cool slightly, before being put onto a magnetic stirring machine, which prevented the mixture from solidifying.

While the media was cooling, the under surfaces of 75 plates were divided into three equal parts using a permanent marker and a ruler. The letters that were chosen in Part 1 for *Hydrastis canadensis* 62% v/v tincture (B), 62% v/v ethanol (E) and fluconazole (F) were retained and written on each of the three parts. The

75 plates were then divided into 5 groups of 15 plates, and each group was marked with1:2 (Experiment 2), 1:5 (Experiment 3), 1:10 (Experiment 4), 1:50 (Experiment 5) and 1:100 (Experiment 6) these being the 5 dilutions that were investigated in this study.

Once the media had cooled to 45°C to 55°C, it was poured into 75 plates at a depth of approximately 5 ml per plate. The 75 plates were then covered with their lids and stored in a refrigerator for 24 hours.

After 24 hours, the above procedure 1-6 was then repeated and the new agar that was prepared was inoculated with a standardized inoculum of *C. albicans* and poured into the 75 plates.

3.3.8 Inoculation of the media

Inoculation of the media in Part 1 of the study:

The 10 ml standardized *C. albicans* inoculum was poured into the 1 litre Erlen Meyer flask containing the Sabouraud's dextrose agar when the agar was at a temperature of 37°C. After 24 hours the 15 plates, which contained the initial layer of Sabouraud's dextrose agar, were removed from the refrigerator and 5 ml of the inoculated media was poured into each plate. The plates were then left to cool and solidify.

Inoculation of the media in Part 2 of the study:

The 40 ml (from the 4 test tubes containing 10ml each) standardized *C. albicans* inoculum was poured into the 2 litre Erlen Meyer flask containing the Sabouraud's dextrose agar when the agar was at a temperature of 37°C. After 24 hours, the 75 plates, which contained the initial layer of Sabouraud's dextrose agar, were removed from the refrigerator and 5 ml of the inoculated media was poured into each plate. The plates were then left to cool and solidify.

3.3.9 Impregnation, placement of the discs and incubation of the plates

Part 1 of the study (Experiment 1).

Five sterile filter paper discs were dipped into the 3 herbal tinctures and the two negative control substances (62% and 86% ethanol) using sterile forceps and placed together with the fluconazole disc using aseptic methods at equal distances on each plate. All 15 plates were incubated at 37°C for 24 hours.

Part 2 of the study (Experiment 2-6)

Two sterile filter paper discs were dipped into their respective *Hydrastis canadensis* 62% v/v tincture and 62% v/v ethanol dilutions (1:2, 1:5, 1:10, 1:50 and 1:100) and placed on the agar in their premarked spaces on each plate. Due to the prohibitive cost of fluconazole discs, only 3 of the 15 plates of each dilution contained fluconazole discs (Odhav, 2002). All 75 plates were incubated at 37°C for 24 hours.

3.3.10 Measuring and recording of the results

After 24 hours, the plates were removed from the incubator, and the zones of inhibition were measured in quadruplicate using a ruler and the results were recorded in millimetres. Photographs of the plates were then taken.

3.4. **Analysis of the data**

3.4.1 Sample size

The sample size of the study was 15 hence making this study statistically viable, since each test was conducted 15 times.

3.4.2 Statistical methods for the processing of the data

3.4.2.1 The Kruskal-Wallis non-parametric analysis of variance by ranks test

The Kruskal-Wallis non-parametric analysis of variance by ranks test is a simple non-parametric test that compares the medians of three or more independent samples (Fowler and Cohen, 1992:168). This test was used to compare the following:

3.4.2.1.1 Inter-group comparison of *Commiphora molmol* 86% v/v tincture, *Hydrastis canadensis* 62% v/v tincture, *Warburgia salutaris* 62% v/v tincture, 86% v/v ethanol, 62 % v/v ethanol and fluconazole.

(a) Hypothesis

The null hypothesis (H_0) states that there is no difference between the means of the zones of inhibitions produced by the substances.

The alternative hypothesis (H_1) states that there is a difference between the means of the zones of inhibitions produced by the test tinctures and the negative and positive controls.

$H_0 : \mu_1 = \mu_2 = \mu_3$

$H_1 : \mu_1 \neq \mu_2 \neq \mu_3$

(b) The Decision Rule

Reject the null hypothesis if $P < \alpha$ or accept the alternative hypothesis at the same level of significance.

If $P < \alpha$ then reject H_0

If $P \geq \alpha$ then accept H_0

Where:

- P = observed level of significance or probability value.
- α = the level of significance (0,05)

If the null hypothesis is rejected then the Dunn Procedure for the Kruskal-Wallis test must be used to determine which of the test substances are significantly different.

<u>The Dunn Procedure for use with the Kruskal-Wallis test</u>

Let,

$\overline{R}_i$ and $\overline{R}_j$ be the means of the ranks of the *i*th and *j*th samples respectively.

$\overline{R_i}$ α: be the experiment-wise error rate. The values of α are usually 0,15, 0,20 and 0,25 depending upon the value of *k* (as *k* increases, α increases).

If $|\overline{R_i} - \overline{R_j}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}(\frac{1}{n_i}+\frac{1}{n_j})}$

then the difference $|\overline{R_i} - \overline{R_j}|$ is declared significant at the α level.

In the above formula:

- *k* = number of samples
- *n* = number of observations
- *N* = number of observations in all samples combined.
- *Z* = the value in the inverse normal distribution corresponding to (1-α/*k*(*k*-1)])

If *k* = 3, α = 0,15, *z* = 1,96

If *k* = 4, α = 0,20, *z* = 2,12

If *k* = 5, α = 0,25, *z* = 2,326

If *k* = 6, α = 0,30, *z* = 2,533

(Fisher and van Belle, 1993:430).

3.4.2.1.2 Inter-group comparison of *Hydrastis canadensis* 62% v/v tincture, 62% v/v ethanol and fluconazole.

(a) Hypothesis

As per 3.4.2.1.1 (a)

(b) The Decision Rule

As per 3.4.2.1.1 (b)

3.4.2.1.3 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:2 dilution, 62% v/v ethanol 1:2 dilution and fluconazole.

(a) Hypothesis

As per 3.4.2.1.1 (a)

(b) The Decision Rule

As per 3.4.2.1.1 (b)

3.4.2.1.4 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:5 dilution, 62% v/v ethanol 1:5 dilution and fluconazole.

(a) Hypothesis

As per 3.4.2.1.1 (a)

(b) The Decision Rule

As per 3.4.2.1.1 (b)

3.4.2.1.5 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:10 dilution, 62% v/v ethanol 1:10 dilution and fluconazole.

(a) Hypothesis

As per 3.4.2.1.1 (a)

(b) The Decision Rule

As per 3.4.2.1.1 (b)

3.4.2.1.6 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:50 dilution, 62% v/v ethanol 1:50 dilution and fluconazole.

(a) Hypothesis

As per 3.4.2.1.1 (a)

(b) The Decision Rule

As per 3.4.2.1.1 (b)

3.4.2.1.7 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:100 dilution, 62% v/v ethanol 1:100 dilution and fluconazole.

(a) Hypothesis

As per 3.4.2.1.1 (a)

(b) The Decision Rule

As per 3.4.2.1.1 (b)

3.4.2.1.8 Inter-group comparison of 62% v/v ethanol, 62% v/v ethanol 1:2 dilution, 62% v/v ethanol 1:5 dilution, 62% v/v ethanol 1:10 dilution, 62% v/v ethanol 1:50 dilution and 62% v/v ethanol 1:100 dilution.

(a) Hypothesis

As per 3.4.2.1.1 (a)

(b) The Decision Rule

As per 3.4.2.1.1 (b)

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3.4.2.1.9 Inter-group comparison of *Hydrastis canadensis* 62% v/v tincture, *Hydrastis canadensis* 62% v/v 1:2 dilution, *Hydrastis canadensis* 62% v/v 1:5 dilution, *Hydrastis canadensis* 62% v/v 1:10 dilution, *Hydrastis canadensis* 62% v/v 1:50 dilution and *Hydrastis canadensis* 62% v/v 1:00 dilution.

(a) Hypothesis

As per 3.4.2.1.1 (a)

(b) The Decision Rule

As per 3.4.2.1.1 (b)

3.4.2.2 The Mann-Whitney U Test

The Mann-Whitney U test is a simple non-parametric test that compares the medians of two independent samples (Daniel, 1999:678).

This test was used for the following comparisons:

3.4.2.2.1 Inter-group comparison of *Hydrastis canadensis* 62% v/v tincture and fluconazole

(a) Hypothesis

The null hypothesis (H_0) states that there is no difference between the means of the zones of inhibition produced by the two substances.

The alternative hypothesis (H_1) states that there is a difference between the means of the zones of inhibition produced by the two substances.

(H_0) : $M_1 = M_2$

(H_1) : $M_1 \neq M_2$

(b) The Decision Rule

Reject the null hypothesis if $P < \alpha$ or accept the alternative hypothesis at the same level of significance.

If $P < \alpha$ then reject H_0

If $P \geq \alpha$ then accept H_0

Where:

- P = observed level of significance or probability value.
- α = the level of significance (0,05)

(Fisher and van Belle, 1993:315).

3.4.2.2.2 Inter-group comparison of *Hydrastis canadensis* 62 % v/v 1:2 dilution and fluconazole

(a) Hypothesis

As per 3.4.2.2.1 (a)

(b) The Decision Rule

As per 3.4.2.2.1 (b)

3.4.2.2.3 Inter-group comparison of *Hydrastis canadensis* 62 % v/v 1:5 dilution and fluconazole

(a) Hypothesis

As per 3.4.2.2.1 (a)

(b) The Decision Rule

As per 3.4.2.2.1 (b)

3.4.2.2.4 Inter-group comparison of *Hydrastis canadensis* 62 % v/v 1:10 dilution and fluconazole

(a) Hypothesis

As per 3.4.2.2.1 (a)

(b) The Decision Rule

As per 3.4.2.2.1 (b)

3.4.2.2.5 Inter-group comparison of *Hydrastis canadensis* 62 % v/v 1:50 dilution and fluconazole

(a) Hypothesis

As per 3.4.2.2.1 (a)

(b) The Decision Rule

As per 3.4.2.2.5 (b)

3.4.2.2.6 Inter-group comparison of *Hydrastis canadensis* 62 % v/v 1:100 dilution and fluconazole

(a) Hypothesis

As per 3.4.2.2.1 (a)

(b) The Decision Rule

As per 3.4.2.2.5 (b)

3.4.2.3 Friedman's Test

Friedman's test is a simple non-parametric test that compares the medians of three or more dependant/related samples (Daniel, 1999:701).

This test was used for the following comparison:

3.4.2.3.1 Intra-group comparison of fluconazole for all experiments

(a) Hypothesis

The null hypothesis (H_0) states that there is no difference between the means of the zones of inhibition produced by fluconazole in all experiments.

The alternative hypothesis (H_1) states that there is a difference between the means of the zones of inhibitions produced by fluconazole in all experiments.

The Decision Rule

Reject the null hypothesis if P < α or accept the alternative hypothesis at the same level of significance.

If P < α then reject H_0

If P ≥ then accept H_0

Where:

- P = observed level of significance or probability value
- α: The level of significance.

If the null hypothesis is rejected then the Dunn Procedure for the Friedman's test must be used to determine which of the means of the zones of fluconazole in all the experiments are different.

Dunn Procedure for use with Friedman's T-test

Let,

- R_j and $R_{j'}$: be the j^{th} and j'^{th} fluconazole rank totals.
- α: be the experimentwise error rate,. Usually α = 0,10

If $| R_j - R_{j'} | \geq z \frac{\sqrt{bk(k+1)}}{\sqrt{6}}$, then R_j and $R_{j'}$ are declared significant.

In the above formula:

- *b* = the number of blocks.
- *k* = the number of experiments.

- z = the value in the inverse normal distribution corresponding to $(1-[\alpha/k(k-1)])$.

To compute the treatment rank totals, rank values in each block and then compute the sum of the ranks for each treatment.

When $k = 3$, $\alpha = 0{,}10$, $z = 2{,}12$

$k = 4$, $\alpha = 0{,}10$, $z = 2{,}409$

(Fisher and van Belle, 1993:430).

CHAPTER FOUR

Results

4.1 Photographs

4.1.1 The effect of *Commiphora molmol* 86% v/v tincture, *Hydrastis canadensis* 62% v/v tincture, *Warburgia salutaris* 62% v/v tincture, 86% v/v ethanol, 62% v/v ethanol and fluconazole against *C. albicans.* No clearing growth was visible around *Commiphora molmol* 86% v/v tincture, *Warburgia salutaris* 62% v/v tincture, 86% v/v ethanol and 62% v/v ethanol. Clearing growth was visible around *Hydrastis canadensis* 62% v/v tincture and fluconazole.

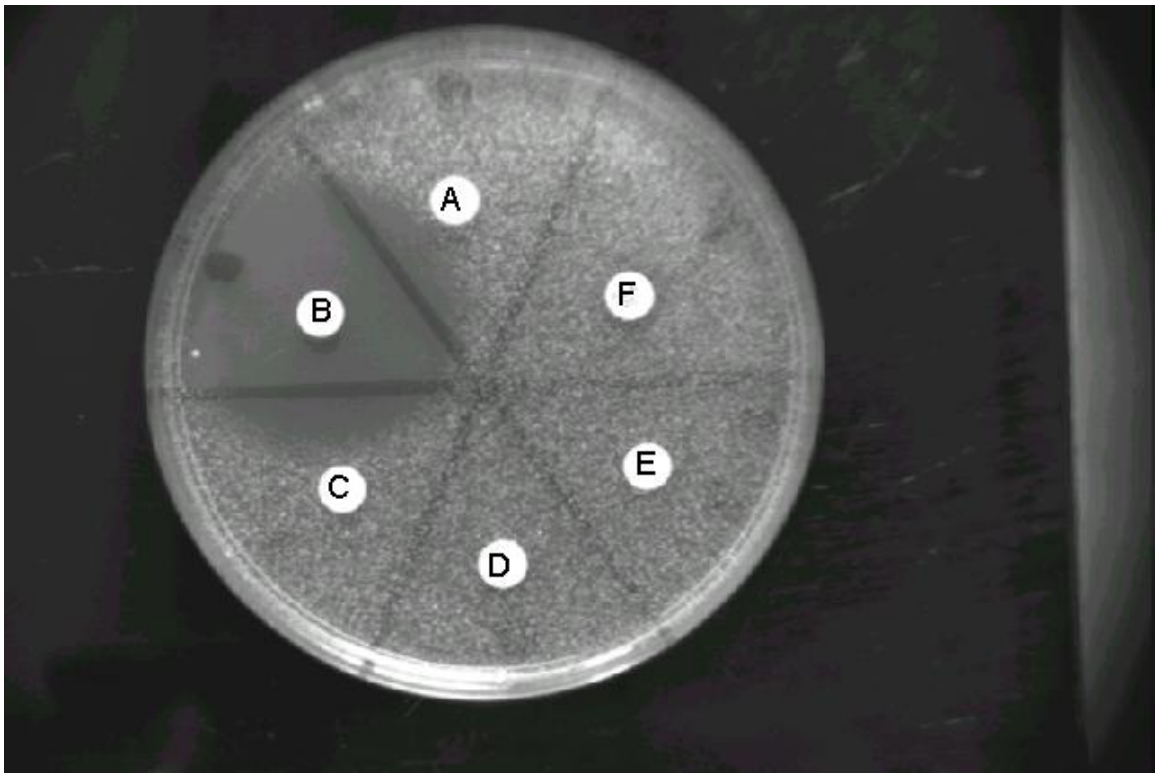

Plate 4.1.1 Photograph of plate 5 of experiment 1.

A – *Commiphora molmol* 86% v/v tincture.

B – *Hydrastis canadensis* 62% v/v tincture.

C – *Warburgia salutaris* 62% *v/v tincture.*

D – 86% v/v ethanol.

E – 62% v/v ethanol.

F – fluconazole

4.1.2 The effect of *Hydrastis Canadensis* 62% v/v 1:2 dilution, 62% v/v ethanol 1:2 dilution and fluconazole against *C. albicans.* No clearing growth was visible around 62% v/v ethanol 1:2 dilution. Clearing growth was visible around *Hydrastis Canadensis* 62% v/v 1:2 dilution and fluconazole.

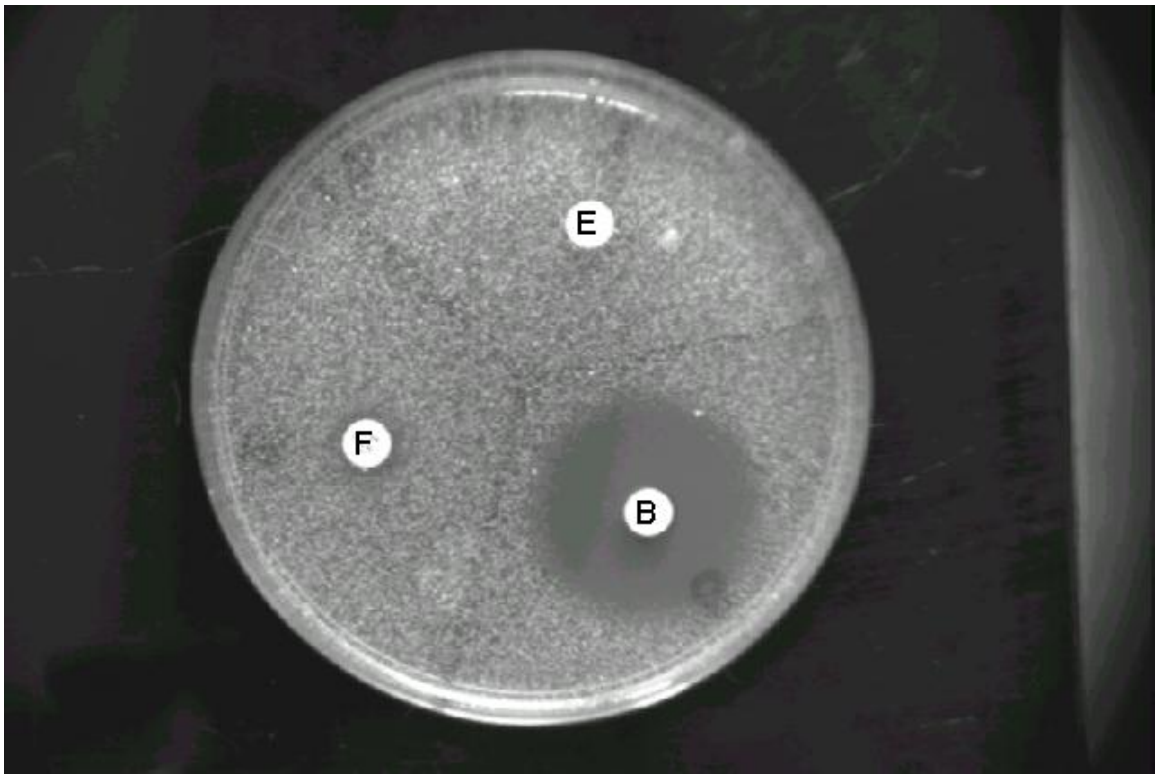

Plate 4.1.2 Photograph of plate 7 of experiment 2.

B – *Hydrastis canadensis* 62% v/v 1:2 dilution.

E – 62 % v/v ethanol 1:2 dilution.

F – Fluconazole

4.2 Statistical Analysis of Data

4.2.1. Inter-group comparison of *Commiphora molmol* 86% v/v tincture, *Hydrastis canadensis* 62% v/v tincture, *Warburgia salutaris* 62% v/v tincture, 86% v/v ethanol, 62 % v/v ethanol and fluconazole in Experiment 1

The sample size (N), means, standard deviations, minimum and maximum zone diameters are shown in Table 4.1.

The Kruskal-Wallis test showed that there was a significant difference (P=.000) between the means of the zones of inhibition produced by *Commiphora molmol* 86% v/v tincture, *Hydrastis canadensis* 62% v/v tincture, *Warburgia salutaris* 62% v/v tincture, 86% v/v ethanol, 62 % v/v ethanol and fluconazole in Experiment 1 (see Table 4.2).

The Dunn Procedure used the Mean Ranks in Table 4.3 to identify which of the substances were significantly different. A difference was found between *Hydrastis canadensis* 62% v/v tincture and *Commiphora molmol* 86% v/v tincture, *Hydrastis canadensis* 62% v/v tincture and *Warburgia salutaris* 62% v/v tincture, fluconazole and 62 % v/v ethanol, fluconazole and *Commiphora molmol* 86% v/v tincture, *Hydrastis canadensis* 62% v/v tincture and 86% v/v ethanol, *Hydrastis canadensis* 62% v/v tincture and 62% v/v ethanol, and fluconazole and *Warburgia salutaris* 62% v/v tincture.

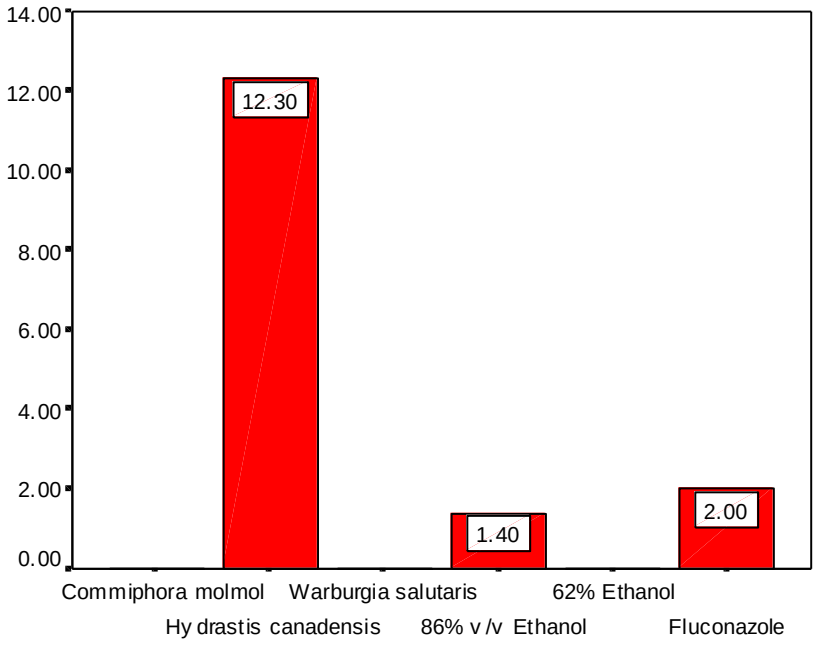

Figure 4.1 Bar graph comparing the mean zones of inhibition of Commiphora molmol 86% v/v tincture, Hydrastis canadensis 62% v/v tincture, Warburgia salutaris 62% v/v tincture, 86% v/v ethanol, 62% v/v ethanol and fluconazole in Experiment 1.

Table 4.1 Descriptive statistics for the test tinctures, negative and positive controls in Experiment 1.

	N	Mean	Std. Deviation	Minimum	Maximum
Test & Controls	90	2.6167	4.5294	.00	14.00
Experiment 1	90	3.5000	1.7174	1.00	6.00

Table 4.2 Kruskal-Wallis test statistics for the test tinctures, negative and positive controls in Experiment 1. [a,b]

	Test & Controls
Chi-Square	73.413
df	5
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Experiment

Conclusion: P=.000 (<.001), therefore P<α, hence the null hypothesis was rejected and the Dunn Procedure was used to identify which of the substances were significantly different.

Table 4.3 Mean ranks for the test tinctures, negative and positive controls in Experiment 1.

	Group	N	Mean Rank
Tests & Controls	A	15	28.50
	B	15	83.00
	C	15	28.50
	D	15	43.13
	E	15	28.50
	F	15	61.37
	Total	90	

If $|\overline{R_i} - \overline{R_j}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}(\frac{1}{n_i} + \frac{1}{n_j})}$

$\overline{R_1} = 28,50$

$\overline{R_2} = 83,00$

$\overline{R_3} = 28,50$

$\overline{R_4} = 43,13$

$\overline{R_5} = 28,50$

$\overline{R_6} = 61,37$

$k = 6$

$\alpha = 0,30$

$z = 2,41$

$N = 90$

$n = 15$

$$|\overline{R_2} - \overline{R_1}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|83{,}00 - 28{,}50| > 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|54{,}50| > 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|54{,}50| > 2{,}41.\sqrt{91{,}00}$$

$$|54{,}50| > (2{,}41)(9{,}54)$$

$$|54{,}50| > 22{,}99$$

The difference between $\overline{R_2}$ and $\overline{R_1}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and *Commiphora molmol* 86% v/v tincture.

$$\overline{R_2} - \overline{R_3} > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|83{,}00 - 28{,}50| > 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|54{,}50| > 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|54{,}50| > 2{,}41.\sqrt{91{,}00}$$

$$|54{,}50| > (2{,}41)(9{,}54)$$

$$|54{,}50| > 22{,}99$$

The difference between $\overline{R_2}$ and $\overline{R_3}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and *Warburgia salutaris* 62% v/v tincture.

$\overline{R_4}$ - $\overline{R_3}$ > $Z_{(1-[\alpha/k(k-1)])}\sqrt{\frac{N(N+1)}{12}(\frac{1}{n_i}+\frac{1}{n_j})}$

$I43,13 - 28,50I > 2,41\sqrt{\frac{90(90+1)}{12}(\frac{1}{15}+\frac{1}{15})}$

$I14,63I < 2,41.\sqrt{(682,50)(0,13)}$

$I14,63I < 2,41.\sqrt{91,00}$

I14,63I < (2,41)(9,54)

I14,63I < 22,99

The difference between $\overline{R_4}$ and $\overline{R_3}$ was not significant. Hence there was no difference in the zones of inhibition produced by 86% v/v ethanol and *Warburgia salutaris* 62% v/v tincture.

$\overline{R_4}$ - $\overline{R_5}$ > $Z_{(1-[\alpha/k(k-1)])}\sqrt{\frac{N(N+1)}{12}(\frac{1}{n_i}+\frac{1}{n_j})}$

$I43,13 - 28,50I > 2,41\sqrt{\frac{90(90+1)}{12}(\frac{1}{15}+\frac{1}{15})}$

$I14,63I < 2,41.\sqrt{(682,50)(0,13)}$

$I14,63I < 2,41.\sqrt{91,00}$

I14,63I < (2,41)(9,54)

I14,63I < 22,99

The difference between $\overline{R_4}$ and $\overline{R_5}$ was not significant. Hence there was no difference in the zones of inhibition produced by 86% v/v ethanol and 62% v/v ethanol.

$$|\overline{R_6} - \overline{R_5}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|61{,}37 - 28{,}50| > 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|32{,}87| > 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|32{,}87| > 2{,}41.\sqrt{91{,}00}$$

$$|32{,}87| > (2{,}41)(9{,}54)$$

$$|32{,}87| > 22{,}99$$

The difference between $\overline{R_6}$ and $\overline{R_5}$ was significant. Hence there was a difference in the zones of inhibition produced by fluconazole and 62% v/v ethanol.

$$|\overline{R_1} - \overline{R_3}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|28{,}50 - 28{,}50| < 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|0{,}00| < 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|0{,}00| < 2{,}41.\sqrt{91{,}00}$$

$$|0{,}00| < (2{,}41)(9{,}54)$$

$$|0{,}00| < 22{,}99$$

The difference between $\overline{R_1}$ and $\overline{R_3}$ was not significant. Hence there was no difference in the zones of inhibition produced by *Commiphora molmol* 86% v/v tincture and *Warburgia salutaris* 62% v/v tincture.

$$|\overline{R_4} - \overline{R_1}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|43{,}13 - 28{,}50| < 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|14{,}33| < 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|14{,}33| < 2{,}41.\sqrt{91{,}00}$$

$$|14{,}33| < (2{,}41)(9{,}54)$$

$$|14{,}33| < 22{,}99$$

The difference between $\overline{R_4}$ and $\overline{R_1}$ was not significant. Hence there was no difference in the zones of inhibition produced by 86% v/v ethanol and *Commiphora molmol* 86% v/v tincture.

$$|\overline{R_5} - \overline{R_1}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|28{,}50 - 28{,}50| < 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|0{,}00| < 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|0{,}00| < 2{,}41.\sqrt{91{,}00}$$

$$|0{,}00| < (2{,}41)(9{,}54)$$

$$|0{,}00| < 22{,}99$$

The difference between $\overline{R_5}$ and $\overline{R_1}$ was not significant. Hence there was no difference in the zones of inhibition produced by 62% v/v ethanol and *Commiphora molmol* 86% v/v tincture.

$$|\overline{R_6} - \overline{R_1}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|61,37 - 28,50| > 2,41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|32,87| > 2,41.\sqrt{(682,50)(0,13)}$$

$$|32,87| > 2,41.\sqrt{91,00}$$

$$|32,87| > (2,41)(9,54)$$

$$|32,87| > 22,99$$

The difference between $\overline{R_6}$ and $\overline{R_1}$ was significant. Hence there was a difference in the zones of inhibition produced by fluconazole and *Commiphora molmol* 86% v/v tincture.

$$|\overline{R_2} - \overline{R_4}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|83,00 - 43,13| > 2,41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|39,87| > 2,41.\sqrt{(682,50)(0,13)}$$

$$|39,87| > 2,41.\sqrt{91,00}$$

$$|39,87| > (2,41)(9,54)$$

$$|39,87| > 22,99$$

The difference between $\overline{R_2}$ and $\overline{R_4}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and 86% v/v ethanol.

$$|\overline{R_2} - \overline{R_5}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|83{,}00 - 28{,}50| > 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|54{,}50| > 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|54{,}50| > 2{,}41.\sqrt{91{,}00}$$

$$|54{,}50| > (2{,}41)(9{,}54)$$

$$|54{,}50| > 22{,}99$$

The difference between $\overline{R_2}$ and $\overline{R_5}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and 62% v/v ethanol.

$$|\overline{R_2} - \overline{R_6}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|83{,}00 - 61{,}37| > 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|21{,}63| < 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|21{,}63| < 2{,}41.\sqrt{91{,}00}$$

$$|21{,}63| < (2{,}41)(9{,}54)$$

$$|21{,}63| < 22{,}99$$

The difference between $\overline{R_2}$ and $\overline{R_6}$ was not significant. Hence there was no difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and fluconazole.

$$|\overline{R_5} - \overline{R_3}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|28{,}50 - 28{,}50| < 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|0{,}00| < 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|0{,}00| < 2{,}41.\sqrt{91{,}00}$$

$$|0{,}00| < (2{,}41)(9{,}54)$$

$$|0{,}00| < 22{,}99$$

The difference between $\overline{R_5}$ and $\overline{R_3}$ was not significant. Hence there was no difference in the zones of inhibition produced by 62% v/v ethanol and *Warburgia salutaris* 62% v/v tincture.

$$|\overline{R_6} - \overline{R_3}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|61{,}37 - 28{,}50| > 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|32{,}87| > 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|32{,}87| > 2{,}41.\sqrt{91{,}00}$$

$$|32{,}87| > (2{,}41)(9{,}54)$$

$$|32{,}87| > 22{,}99$$

The difference between $\overline{R_6}$ and $\overline{R_3}$ was significant. Hence there was a difference in the zones of inhibition produced by fluconazole and *Warburgia salutaris* 62% v/v tincture.

$$|\overline{R_6} - \overline{R_4}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|61{,}37 - 43{,}13| < 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|18{,}24| < 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|18{,}24| < 2{,}41\sqrt{91{,}00}$$

$$|18{,}24| < (2{,}41)(9{,}54)$$

$$|18{,}24| < 22{,}99$$

The difference between $\overline{R_6}$ and $\overline{R_4}$ was not significant. Hence there was no difference in the zones of inhibition produced by fluconazole and *Warburgia salutaris* 62% v/v tincture.

4.2.2 Inter-group comparison of *Hydrastis canadensis* 62% v/v tincture, 62 % v/v ethanol and fluconazole in Experiment 1

The sample size (N), means, standard deviations, minimum and maximum zone diameters are shown in Table 4.4.

The Kruskal-Wallis test showed that there was a significant difference (P=.000) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture, 62 % v/v ethanol and fluconazole (see Table 4.5).

The Dunn Procedure used the Mean Ranks in Table 4.6 to identify which of the substances were significantly different. A difference was found between

Hydrastis canadensis 62% v/v tincture and 62 % v/v ethanol, fluconazole and 62 % v/v ethanol, and *Hydrastis canadensis* 62% v/v tincture and fluconazole.

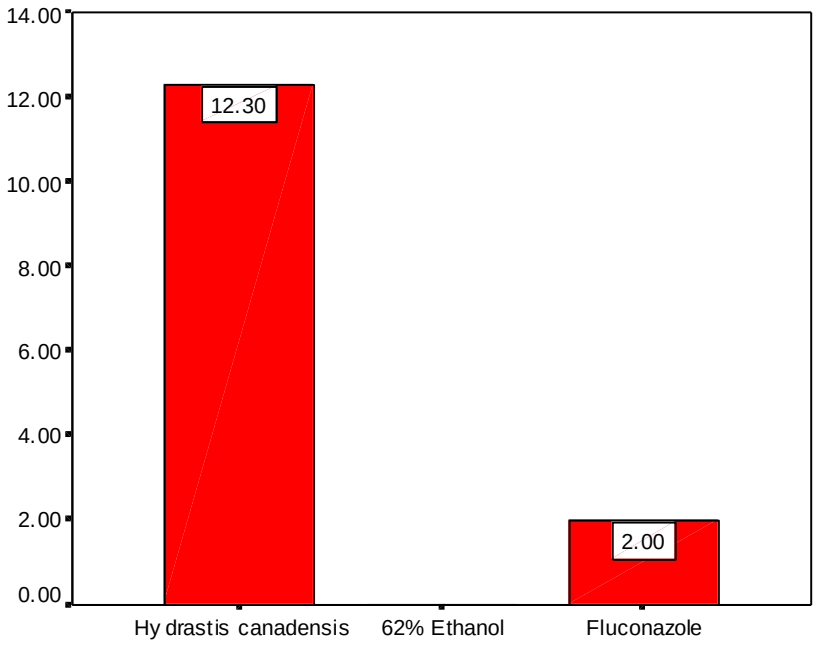

Figure 4.2 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v tincture, 62% v/v ethanol and fluconazole in Experiment 1.

Table 4.4 Descriptive statistics for Hydrastis canadensis 62% v/v tincture, 62% v/v ethanol and fluconazole in Experiment 1.

	N	Mean	Std. Deviation	Minimum	Maximum
Test & Controls	45	4.7667	5.4911	.00	14.00
Exp 1	45	2.00	.83	1	3

Table 4.5 Kruskal-Wallis test statistics for Hydrastis canadensis 62% v/v tincture, 62% v/v ethanol and fluconazole in Experiment 1[a,b]

	Exp 1
Chi-Square	40.144
df	2
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Experiment 1

Conclusion: P=.000 (<.001), therefore P<α, hence the null hypothesis was rejected and the Dunn Procedure was used to identify which of the substances were significantly different.

Table 4.6 Mean ranks for Hydrastis canadensis 62% v/v tincture, 62% v/v ethanol and fluconazole in Experiment 1.

	Exp 1	N	Mean Rank
Groups	B	15	38.00
	E	15	8.50
	F	15	22.50
	Total	45	

$$\text{If } |\overline{R_i} - \overline{R_j}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$\overline{R_1} = 38{,}00$

$\overline{R_2} = 8{,}50$

$\overline{R_3} = 22{,}50$

$k = 3$

$\alpha = 0{,}15$

$z = 1{,}96$

$N = 45$

$n = 15$

$$|\overline{R_1} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|38{,}00 - 8{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|29{,}50| > 1{,}96 . \sqrt{(172{,}50)(0{,}13)}$$

$$|29{,}50| > 1{,}96 \sqrt{23{,}00}$$

$$|29{,}50| > (1{,}96)(4{,}80)$$

$$|29{,}50| > 9{,}40$$

The difference between $\overline{R_1}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and 62% v/v ethanol.

$$|\overline{R_3} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|22{,}50 - 8{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|14| > 1{,}96 . \sqrt{(172{,}50)(0{,}13)}$$

$$|14| > 1{,}96 \sqrt{23{,}00}$$

$$|14| > (1{,}96)(4{,}80)$$

$$|14| > 9{,}40$$

The difference between $\overline{R_3}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by fluconazole and 62% v/v ethanol.

$$|\overline{R_1} - \overline{R_3}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|38{,}00 - 22{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|15{,}50| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|15{,}50| > (1{,}96)\sqrt{23{,}00}$$

$$|15{,}50| > (1{,}96)(4{,}80)$$

$$|15{,}50| > 9{,}40$$

The difference between $\overline{R_1}$ and $\overline{R_3}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and fluconazole.

4.2.3 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:2 dilution, 62 % v/v ethanol 1:2 dilution and fluconazole in Experiment 2

The sample size (N), means, standard deviations, minimum and maximum zone diameters are shown in Table 4.7.

The Kruskal-Wallis test showed that there was a significant difference (P=.000) (see Table 4.8) between the means of the zones of inhibition produced by

Hydrastis canadensis 62% v/v 1:2 dilution, 62 % v/v ethanol 1:2 dilution and fluconazole in Experiment 2.

The Dunn Procedure used the Mean Ranks in Table 4.9 to identify which substances were significantly different. A difference was found between *Hydrastis canadensis* 62% v/v 1:2 dilution and 62 % v/v ethanol 1:2 dilution, fluconazole and 62 % v/v ethanol 1:2 dilution, and *Hydrastis canadensis* 62% v/v 1:2 dilution and fluconazole.

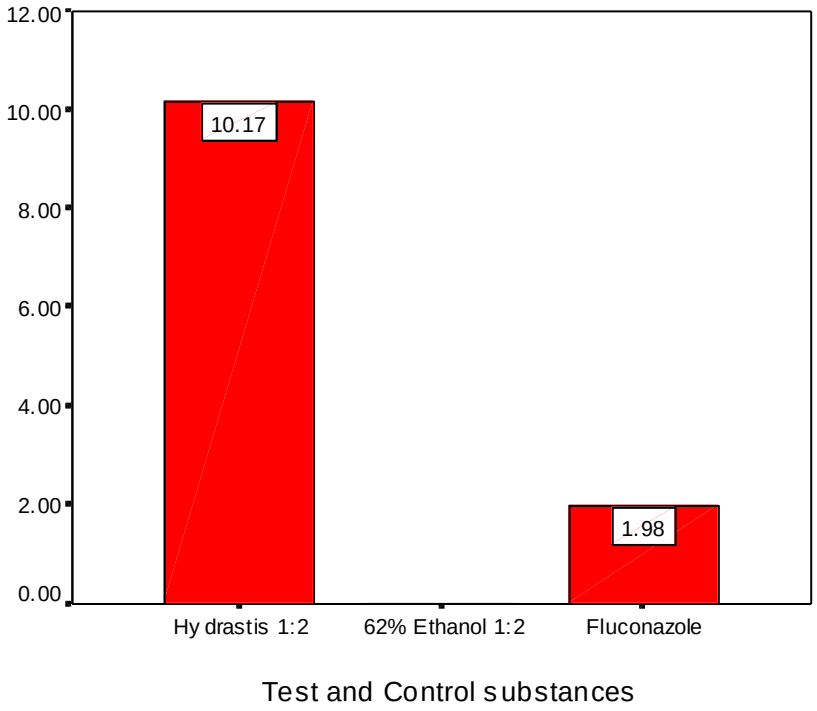

Figure 4.3 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v 1:2 dilution, 62% v/v ethanol 1:2 dilution and fluconazole in Experiment 2.

Table 4.7 Descriptive statistics for Hydrastis canadensis 62% v/v 1:2 dilution, ethanol 62% v/v 1:2 dilution and fluconazole in Experiment 2.

	N	Mean	Std. Deviation	Minimum	Maximum
Groups	45	4.0500	4.4986	.00	11.00
Exp 2	45	2.0000	.8257	1.00	3.00

Table 4.8 Kruskal-Wallis test statistics for Hydrastis canadensis 62% v/v 1:2 dilution, ethanol 62% v/v 1:2 dilution and fluconazole in Experiment 2.[a,b]

	Exp 2
Chi-Square	40.245
df	2
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Experiment 2

Conclusion: P=.000 (<.001), therefore P<α, hence the null hypothesis was rejected and the Dunn Procedure was used to identify which of the substances were significantly different.

Table 4.9 Mean ranks for Hydrastis canadensis 62% v/v 1:2 dilution, ethanol 62% v/v 1:2 dilution and fluconazole in Experiment 2.

	Experiment 2	N	Mean Rank
Test & Controls	B	15	38.00
	E	15	8.50
	F	15	22.50
	Total	45	

$$\text{If } |\overline{R_i} - \overline{R_j}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$\overline{R_1}$ = 38,00

$\overline{R_2}$ = 8,50

$\overline{R_3}$ = 22,50

k = 3

α = 0,15

z = 1,96

N = 45

n = 15

$$|\overline{R_1} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|38{,}00 - 8{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|29{,}50| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|29{,}50| > (1{,}96)\sqrt{23{,}00}$$

$$|29{,}50| > (1{,}96)(4{,}80)$$

$$|29{,}50| > 9{,}40$$

The difference between $\overline{R_1}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:2 dilution and 62% v/v ethanol 1:2 dilution.

$$|\overline{R_3} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|22{,}50 - 8{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|14| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|14| > (1{,}96)\sqrt{23{,}00}$$

$$|14| > (1{,}96)(4{,}80)$$

$$|14| > 9{,}40$$

The difference between $\overline{R_3}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by fluconazole and 62% v/v ethanol 1:2 dilution.

$$|\overline{R_1} - \overline{R_3}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|38{,}00 - 22{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|15{,}50| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|15{,}50| > (1{,}96)\sqrt{23{,}00}$$

$$|15{,}50| > (1{,}96)(4{,}80)$$

$$|15{,}50| > 9{,}40$$

The difference between $\overline{R_1}$ and $\overline{R_3}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:2 dilution and fluconazole.

4.2.4 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:5 dilution, 62 % v/v ethanol 1:5 dilution and fluconazole in Experiment 3

The sample size (N), means, standard deviations, minimum and maximum zone diameters are shown in Table 4.10.

The Kruskal-Wallis test showed that there was a significant difference (P=.000) (see Table 4.11) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:5 dilution, 62 % v/v ethanol 1:5 dilution and fluconazole in Experiment 3.

The Dunn Procedure used the Mean Ranks in Table 4.12 to identify which substances were significantly different. A difference was found between *Hydrastis canadensis* 62% v/v 1:5 dilution and 62 % v/v ethanol 1:5 dilution, fluconazole and 62 % v/v ethanol 1:5 dilution, and *Hydrastis canadensis* 62% v/v 1:5 dilution and fluconazole.

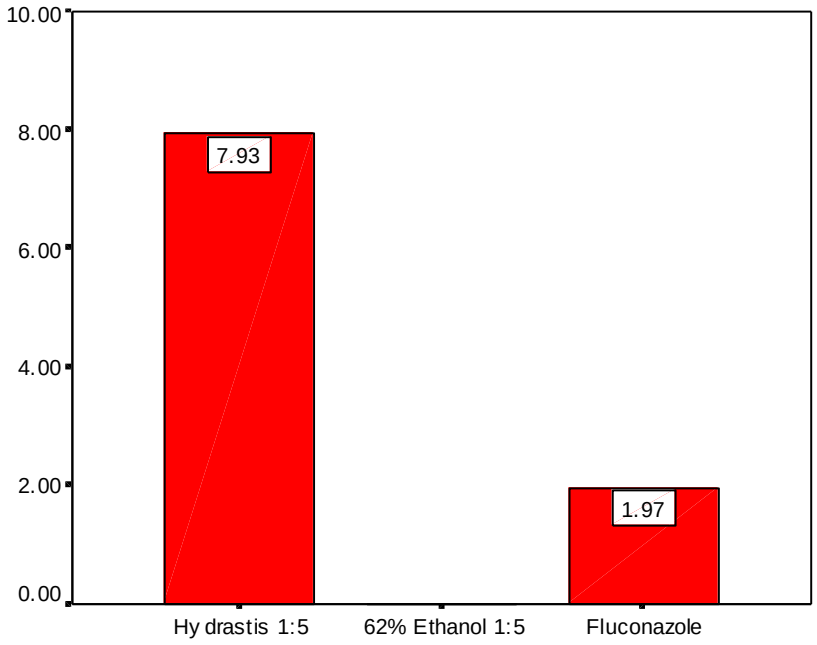

Figure 4.4 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v 1:5 dilution, 62% v/v ethanol 1:5 dilution and fluconazole in Experiment 3.

Table 4.10 Descriptive statistics for Hydrastis canadensis 62% v/v 1:5 dilution, 62% v/v ethanol 1:5 dilution and fluconazole in Experiment 3.

	N	Mean	Std. Deviation	Minimum	Maximum
Test & Controls	45	3.3000	3.4713	.00	9.00
Exp 3	45	2.0000	.8257	1.00	3.00

Table 4.11 Kruskal-Wallis test statistics for Hydrastis canadensis 62% v/v 1:5 dilution, 62% v/v ethanol 1:5 dilution and fluconazole in Experiment 3.[a,b]

	Exp 3
Chi-Square	40.279
df	2
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Experiment 3

Conclusion: P=.000 (<.001), therefore P<α, hence the null hypothesis was rejected and the Dunn Procedure was used to identify which of the substances were significantly different.

Table 4.12 Mean ranks for Hydrastis canadensis 62% v/v 1:5 dilution, 62% v/v ethanol 1:5 dilution and fluconazole in Experiment 3.

	Experiment 3	N	Mean Rank
Groups	B	15	38.00
	E	15	8.50
	F	15	22.50
	Total	45	

$$\text{If } |\overline{R_i} - \overline{R_j}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$\overline{R_1}$ = 38,00

$\overline{R_2}$ = 8,50

$\overline{R_3}$ = 22,50

k = 3

α = 0,15

z = 1,96

N = 45

n = 15

$$|\overline{R_1} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|38{,}00 - 8{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|29{,}50| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|29{,}50| > (1{,}96)\sqrt{23{,}00}$$

$$|29{,}50| > (1{,}96)(4{,}80)$$

$$|29{,}50| > 9{,}40$$

The difference between $\overline{R_1}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:5 dilution and 62% v/v ethanol 1:5 dilution.

$$|\overline{R_3} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|22{,}50 - 8{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|14| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|14| > (1{,}96)\sqrt{23{,}00}$$

$$|14| > (1{,}96)(4{,}80)$$

$$|14| > 9{,}40$$

The difference between $\overline{R_3}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by fluconazole and 62% v/v ethanol 1:5 dilution.

$$|\overline{R_1} - \overline{R_3}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|38{,}00 - 22{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|15{,}50| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|15{,}50| > (1{,}96)\sqrt{23{,}00}$$

$$|15{,}50| > (1{,}96)(4{,}80)$$

$$|15{,}50| > 9{,}40$$

The difference between $\overline{R_1}$ and $\overline{R_3}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:5 dilution and fluconazole

4.2.5 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:10 dilution, 62 % v/v ethanol 1:10 dilution and fluconazole in Experiment 4

The sample size (N), means, standard deviations, minimum and maximum zone diameters are shown in Table 4.13.

The Kruskal-Wallis test showed that there was a significant difference (P=.000) (see Table 4.14) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:10 dilution, 62 % v/v ethanol 1:10 dilution and fluconazole in Experiment 4.

The Dunn Procedure used the Mean Ranks in Table 4.15 to identify which substances were significantly different. A difference was found between *Hydrastis canadensis* 62% v/v 1:10 dilution and 62 % v/v ethanol 1:10 dilution, fluconazole and 62 % v/v ethanol 1:10 dilution, and *Hydrastis canadensis* 62% v/v 1:10 dilution and fluconazole.

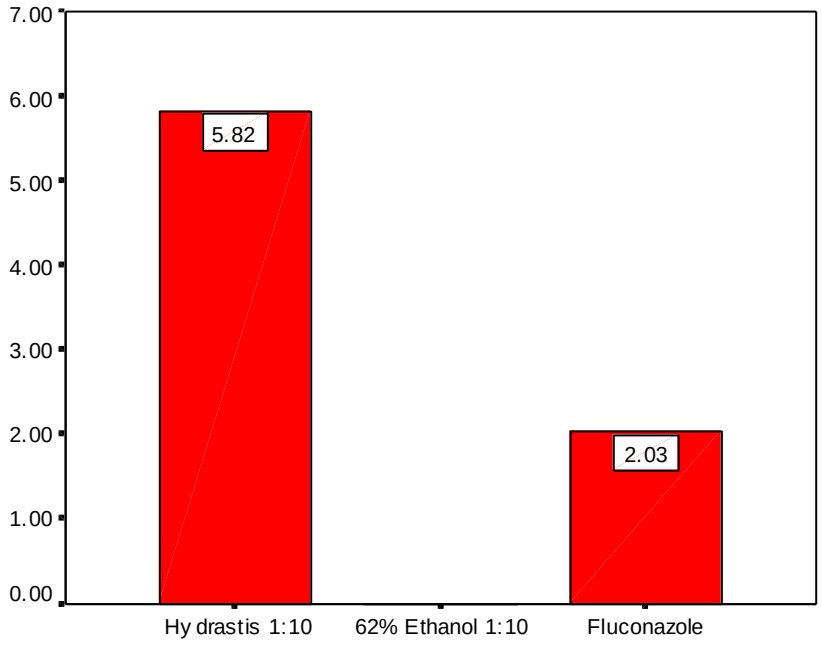

Figure 4.5 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v 1:10 dilution, 62% v/v ethanol 1:10 dilution and fluconazole in Experiment 4.

Table 4.13 Descriptive statistics for Hydrastis canadensis 62% v/v 1:10 dilution 62% v/v ethanol 1:10 dilution and fluconazole in Experiment 4.

	N	Mean	Std. Deviation	Minimum	Maximum
Test & Controls	45	2.6167	2.5515	.00	7.00
Exp 4	45	2.0000	.8257	1.00	3.00

Table 4.14 Kruskal-Wallis test statistics for Hydrastis canadensis 62% v/v 1:10 dilution, 62% v/v ethanol 1:10 dilution and fluconazole in Experiment 4.[a,b]

	Groups
Chi-Square	39.801
df	2
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Experiment 4

Conclusion: P=.000 (<.001), therefore P<α, hence the null hypothesis was rejected and the Dunn Procedure was used to identify which of the substances were significantly different.

Table 4.15 Mean ranks for Hydrastis canadensis 62% v/v 1:10 dilution, 62% v/v ethanol 1:10 dilution and fluconazole in Experiment 4.

	GROUP	N	Mean Rank
Exp 4	B	15	37.93
	E	15	8.50
	F	15	22.57
	Total	45	

If $|\overline{R_i} - \overline{R_j}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}(\frac{1}{n_i} + \frac{1}{n_j})}$

$\overline{R_1} = 37{,}93$

$\overline{R_2} = 8{,}50$

$\overline{R_3} = 22{,}57$

$k = 3$

$\alpha = 0{,}15$

$z = 1{,}96$

$N = 45$

$n = 15$

$$|\overline{R_1} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|37{,}93 - 8{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|29{,}43| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|29{,}43| > (1{,}96)\sqrt{23{,}00}$$

$$|29{,}43| > (1{,}96)(4{,}80)$$

$$|29{,}43| > 9{,}40$$

The difference between $\overline{R_1}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:10 dilution and 62% v/v ethanol 1:10 dilution.

$$|\overline{R_3} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|22{,}57 - 8{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|14{,}07| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|14{,}07| > (1{,}96)\sqrt{23{,}00}$$

$$|14{,}07| > (1{,}96)(4{,}80)$$

$$|14{,}07| > 9{,}40$$

The difference between $\overline{R_3}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by fluconazole and 62% v/v ethanol 1:10 dilution.

$$|\overline{R_1} - \overline{R_3}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|37{,}93 - 22{,}57| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|15{,}36| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|15{,}36| > (1{,}96)\sqrt{23{,}00}$$

$$|15{,}36| > (1{,}96)(4{,}80)$$

$$|15{,}36| > 9{,}40$$

The difference between $\overline{R_1}$ and $\overline{R_3}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:10 dilution and fluconazole.

4.2.6 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:50 dilution, ethanol 62 % v/v 1:50 dilution and fluconazole in Experiment 5

The sample size (N), means, standard deviations, minimum and maximum zone diameters are shown in Table 4.16.

The Kruskal-Wallis test showed that there was a significant difference (P=.000) (see Table 4.17) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:50 dilution, 62 % v/v ethanol 1:50 dilution and fluconazole in Experiment 5.

The Dunn Procedure used the Mean Ranks in Table 4.18 to identify which substances were significantly different. A difference was found between *Hydrastis canadensis* 62% v/v 1:50 dilution and 62 % v/v ethanol 1:50 dilution, and fluconazole and 62 % v/v ethanol 1:50 dilution.

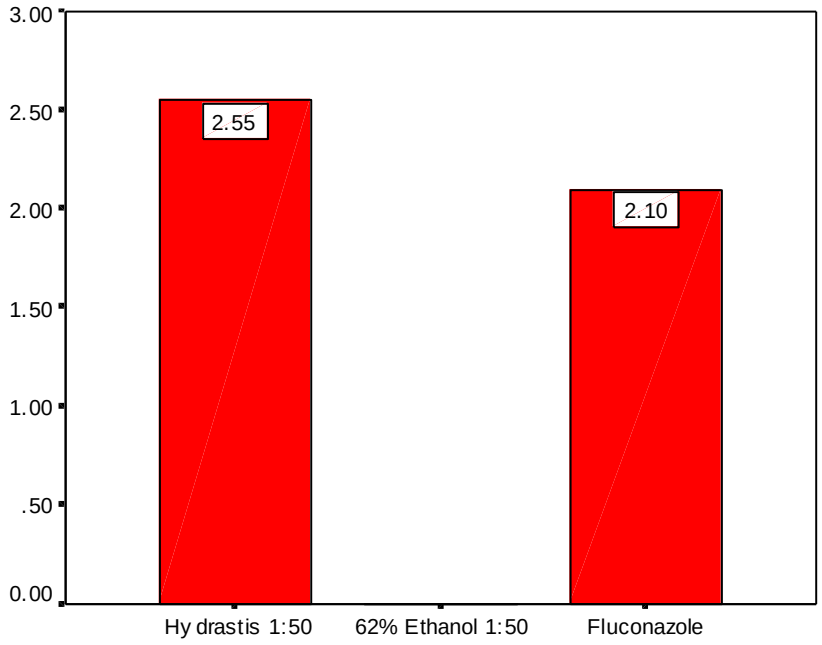

Figure 4.6 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v 1:50 dilution, 62% v/v ethanol 1:50 dilution and fluconazole in Experiment 5.

Table 4.16 Descriptive statistics for Hydrastis canadensis 62% v/v 1:50 dilution 62% v/v ethanol 1:50 dilution and fluconazole in Experiment 5

	N	Mean	Std. Deviation	Minimum	Maximum
Test & Controls	45	1.5500	1.3030	.00	4.50
Exp 5	45	2.0000	.8257	1.00	3.00

Table 4.17 Kruskal-Wallis test statistics for Hydrastis canadensis 62% v/v 1:50 dilution, 62% v/v ethanol 1:50 dilution and fluconazole in Experiment 5.[a,b]

	Exp 5
Chi-Square	31.708
df	2
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Experiment 5

Conclusion: P=.000 (<.001), therefore P<α, hence the null hypothesis was rejected and the Dunn Procedure was used to identify which of the substances were significantly different.

Table 4.18 Mean ranks for Hydrastis canadensis 62% v/v 1:50 dilution, 62% v/v ethanol 1:50 dilution and fluconazole in Experiment 5.

	Experiment 5	N	Mean Rank
Groups	B	15	33.03
	E	15	8.50
	F	15	27.47
	Total	45	

If $|\overline{R_i} - \overline{R_j}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}(\frac{1}{n_i} + \frac{1}{n_j})}$

$\overline{R_1} = 33{,}03$

$\overline{R_2} = 8{,}50$

$\overline{R_3} = 27{,}47$

$k = 3$

$\alpha = 0{,}15$

$z = 1{,}96$

$N = 45$

$n = 15$

$$|\overline{R_1} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|33,03 - 8,50| > 1,96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|24,53| > 1,96 \sqrt{(172,50)(0,13)}$$

$$|24,53| > (1,96)\sqrt{23,00}$$

$$|24,53| > (1,96)(4,80)$$

$$|24,53| > 9,40$$

The difference between $\overline{R_1}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:50 dilution and 62% v/v ethanol 1:50 dilution.

$$|\overline{R_3} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|27,47 - 8,50| > 1,96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|18,97| > 1,96 \sqrt{(172,50)(0,13)}$$

$$|18,97| > (1,96)\sqrt{23,00}$$

$$|18,97| > (1,96)(4,80)$$

$$|18,97| > 9,40$$

The difference between $\overline{R_3}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by fluconazole and 62% v/v ethanol 1:50 dilution.

$$|\overline{R_1} - \overline{R_3}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|33{,}03 - 27{,}47| < 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|5.56| < 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|5.56| < (1{,}96)\sqrt{23{,}00}$$

$$|5.56| < (1{,}96)(4{,}80)$$

$$|5.56| < 9{,}40$$

The difference between $\overline{R_1}$ and $\overline{R_3}$ was not significant. Hence there was no difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:50 dilution and fluconazole.

4.2.7.Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:100 dilution, 62 % v/v ethanol 1:100 dilution and fluconazole in Experiment 6

The sample size (N), means, standard deviations, minimum and maximum zone diameters are shown in Table 4.19.

The Kruskal-Wallis test showed that there was a significant difference (P=.000) (see Table 4.20) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:100 dilution, 62 % v/v ethanol 1:100 dilution and fluconazole in Experiment 6.

The Dunn Procedure used the Mean Ranks in Table 4.21 to identify which substances were significantly different. A difference was found between fluconazole and 62 % v/v ethanol 1:100 dilution, and fluconazole and *Hydrastis canadensis* 62% v/v 1:100 dilution.

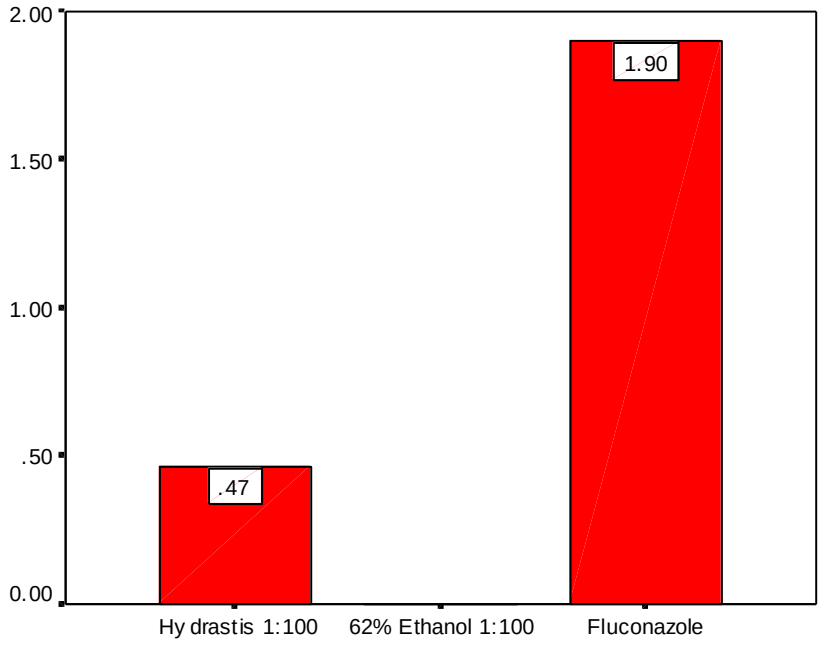

Figure 4.7 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v 1:100 dilution, 62% v/v ethanol 1:100 dilution and fluconazole in Experiment 6.

able 4.19 Descriptive statistics for Hydrastis canadensis 62% v/v 1:100 dilutio 62% v/v ethanol 1:100 dilution and fluconazole in Experiment 6.

	N	Mean	Std. Deviation	Minimum	Maximum
Groups	45	.79	1.04	0	5
Experiment 6	45	2.0000	.8257	1.00	3.00

able 4.20 Kruskal-Wallis test statistics for Hydrastis canadensis 62% v/v 1:100 dilution, 62% v/v ethanol 1:100 dilution and fluconazole in Experiment 6.[a,b]

	Groups
Chi-Square	30.139
df	2
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Experiment 6

Conclusion: P=.000 (<.001), therefore P<α, hence the null hypothesis was rejected and the Dunn Procedure was used to identify which of the substances were significantly different.

Table 4.21 Mean ranks for Hydrastis canadensis 62% v/v 1:100 dilution, 62% v/v ethanol 1:100 dilution and fluconazole in Experiment 6.

	Experiment 6	N	Mean Rank
Groups	1.00	15	20.43
	2.00	15	12.50
	3.00	15	36.07
	Total	45	

If $|\overline{R_i} - \overline{R_j}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}(\frac{1}{n_i} + \frac{1}{n_j})}$

$\overline{R_1} = 20{,}43$

$\overline{R_2} = 12{,}50$

$\overline{R_3} = 36{,}07$

$k = 3$

$\alpha = 0{,}15$

$z = 1{,}96$

$N = 45$

$n = 15$

$$|\overline{R_1} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|20{,}43 - 12{,}50| < 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|7{,}93| < 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|7{,}93| < (1{,}96) \sqrt{23{,}00}$$

$$|7{,}93| < (1{,}96)(4{,}80)$$

$$|7{,}93| < 9{,}40$$

The difference between $\overline{R_1}$ and $\overline{R_2}$ was not significant. Hence there was no difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:100 dilution and 62% v/v ethanol 1:100 dilution.

$$|\overline{R_3} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|36{,}07 - 12{,}50| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|23{,}57| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|23{,}57| > (1{,}96) \sqrt{23{,}00}$$

$$|23{,}57| > (1{,}96)(4{,}80)$$

$$|23{,}57| > 9{,}40$$

The difference between $\overline{R_3}$ and $\overline{R_2}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by fluconazole and 62% v/v ethanol 1:100 dilution.

$$|\overline{R_3} - \overline{R_1}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|36{,}07 - 20{,}43| > 1{,}96 \sqrt{\frac{45(45+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|15.64| > 1{,}96 \sqrt{(172{,}50)(0{,}13)}$$

$$|15.64| > (1{,}96)\sqrt{23{,}00}$$

$$|15.64| > (1{,}96)(4{,}80)$$

$$|15.64| > 9{,}40$$

The difference between $\overline{R_3}$ and $\overline{R_1}$ was significant. Hence there was a difference in the means of the zones of inhibition produced by fluconazole and *Hydrastis canadensis* 62% v/v 1:00 dilution.

4.2.8 Inter-group comparison of 62% v/v ethanol, 62% v/v ethanol 1:2 dilution, 62% v/v ethanol 1:5 dilution, 62% v/v ethanol 1:10 dilution, 62% v/v ethanol 1:50 dilution and 62% v/v ethanol 1:100 dilution

The sample size (N), means, standard deviations, minimum and maximum zone diameters are shown in Table 4.22.

The Kruskal-Wallis test showed that there was no difference (P=1.000) (see Table 4.23) in the means of the zones of inhibition produced by 62 % v/v ethanol, 62 % v/v ethanol 1:2 dilution, 62 % v/v ethanol 1:5 dilution, 62 % v/v ethanol 1:10 dilution, 62 % v/v ethanol 1:50 dilution and 62 % v/v ethanol 1:100 dilution in all the Experiments.

Table 4.22 Descriptive statistics for 62% v/v ethanol, 62% v/v ethanol 1:2 dilution, 62% v/v ethanol 1:5 dilution, 62% v/v ethanol 1:10 dilution, 62% v/v ethanol 1:50 dilution and 62% v/v ethanol 1:100 dilution.

	N	Mean	Std. Deviation	Minimum	Maximum
62 % ethanol & its dilutions	90	.0000	.0000	.00	.00
	90	3.5000	1.7174	1.00	6.00

Table 4.23 Kruskal-Wallis test statistics for 62% v/v ethanol, 62% v/v ethanol 1:2 dilution, 62% v/v ethanol 1:5 dilution, 62% v/v ethanol 1:10 dilution, 62% ethanol 1:50 dilution and 62% ethanol 1:100 dilution.[a,b]

	62% ethanol & its dilutions
Chi-Square	.000
df	5
Asymp. Sig.	1.000

a. Kruskal Wallis Test

b. Grouping Variable: Experiments

Conclusion: P=.1.000, therefore P>α, therefore the null hypothesis was accepted. Hence there was no difference in the zones of inhibition produced by 62% v/v ethanol, 62% v/v ethanol 1:2 dilution, 62% v/v ethanol 1:5 dilution, 62% v/v ethanol 1:10 dilution, 62% v/v ethanol 1:50 dilution and 62% v/v ethanol 1:100 dilution.

4.2.9 Inter-group comparison of *Hydrastis canadensis* 62% v/v tincture, *Hydrastis canadensis* 62% v/v 1:2 dilution, *Hydrastis canadensis* 62% v/v 1:5 dilution, *Hydrastis canadensis* 62% v/v 1:10 dilution, *Hydrastis canadensis* 62% v/v 1:50 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution

The sample size (N), means, standard deviations, minimum and maximum zone diameters are shown in Table 4.24.

The Kruskal-Wallis test showed that there was a significant difference (P=.000) (see Table 4.25) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:100 dilution, 62 % v/v ethanol 1:100 dilution and fluconazole in all the Experiments.

The Dunn Procedure used the Mean Ranks in Table 4.26 to identify which substances were significantly different. A difference was found between *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:5 dilution, *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:10 dilution, *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:50 dilution, *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:100 dilution, *Hydrastis canadensis* 62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:10 dilution, *Hydrastis canadensis* 62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:50 dilution, *Hydrastis canadensis* 62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution, *Hydrastis canadensis* 62% v/v 1:5 dilution and *Hydrastis canadensis*

62% v/v 1:50 dilution, and *Hydrastis canadensis* 62% v/v 1:5 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution.

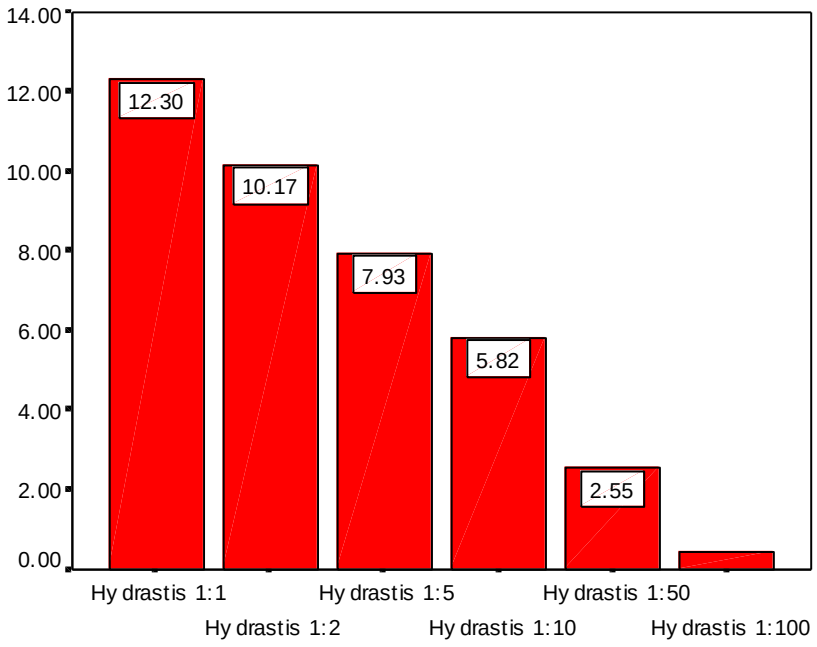

Figure 4.8 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v tincture, Hydrastis canadensis 62% v/v 1:2 dilution, Hydrastis canadensis 62% v/v 1:5 dilution, Hydrastis canadensis 62% v/v 1:10 dilution, Hydrastis canadensis 62% v/v 1:50 dilution and Hydrastis canadensis 62% v/v 1:100 dilution in all the experiments.

Table 4.24 Descriptive statistics for Hydrastis canadensis 62% v/v tincture, Hydrastis canadensis 62% v/v 1:2 dilution, Hydrastis canadensis 62% v/v 1:5 dilution, Hydrastis canadensis 62% v/v 1:10 dilution, Hydrastis canadensis 62% v/v 1:50 dilution and Hydrastis canadensis 62% v/v 1:100 dilution.

	N	Mean	Std. Deviation	Minimum	Maximum
Hydrastis canadensis and dilutions	90	6.5389	4.1835	.00	14.00
Experiments	90	3.5000	1.7174	1.00	6.00

Table 4.25 Kruskal-Wallis test statistics for Hydrastis canadensis 62% v/v tincture, Hydrastis canadensis 62% v/v 1:2 dilution, Hydrastis canadensis 62% v/v 1:5 dilution, Hydrastis canadensis 62% v/v 1:10 dilution, Hydrastis canadensis 62% v/v 1:50 dilution and Hydrastis canadensis 62% v/v 1:100 dilution.[a,b]

	Hydrastis canadensis & dilutions
Chi-Square	86.671
df	5
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Experiments

Conclusion: P=.000 (<.001), therefore P<α, hence the null hypothesis was rejected and the Dunn Procedure was used to identify which of the substances were significantly different.

Table 4.26 Mean ranks for Hydrastis canadensis 62% v/v tincture, Hydrastis canadensis 62% v/v 1:2 dilution, Hydrastis canadensis 62% v/v 1:5 dilution, Hydrastis canadensis 62% v/v 1:10 dilution, Hydrastis canadensis 62% v/v 1:50 dilution and Hydrastis canadensis 62% v/v 1:100 dilution.

	Experiments	N	Mean Rank
Hydrastis canadensis and dilutions	1 (1:1)	15	82.87
	2 (1:2)	15	68.00
	3 (1:5)	15	53.00
	4 (1:10)	15	38.13
	5 (1:50)	15	23.00
	6 (1:100)	15	8.00
	Total	90	

$$\text{If } |\overline{R_i} - \overline{R_j}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$\overline{R_1}$ = 82,87

$\overline{R_2}$ = 68,00

$\overline{R_3}$ = 53,00

$\overline{R_4}$ = 38,13

$\overline{R_5}$ = 23,00

$\overline{R_6}$ = 8,00

k = 6

α = 0,30

z = 2,41

N = 90

n = 15

$$|\overline{R_1} - \overline{R_2}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

$$|82,87 - 68,00| < 2,41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15} + \frac{1}{15}\right)}$$

$$|14,87| < 2,41.\sqrt{(682,50)(0,13)}$$

$$|14,87| < 2,41.\sqrt{91,00}$$

$$|14,87| < (2,41)(9,54)$$

$$|14,87| < 22,99$$

The difference between $\overline{R_1}$ and $\overline{R_2}$ was not significant. Hence there was no difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:2 dilution.

$|\overline{R_2} - \overline{R_3}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}(\frac{1}{n_i}+\frac{1}{n_j})}$

$|68{,}00 - 53{,}00| < 2{,}41 \sqrt{\frac{90(90+1)}{12}(\frac{1}{15}+\frac{1}{15})}$

$|15{,}00| < 2{,}41.\sqrt{(682{,}50)(0{,}13)}$

$|15{,}00| < 2{,}41.\sqrt{91{,}00}$

$|15{,}00| < (2{,}41)(9{,}54)$

$|15{,}00| < 22{,}99$

The difference between $\overline{R_2}$ and $\overline{R_3}$ was not significant. Hence there was no difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:5 dilution.

$|\overline{R_3} - \overline{R_4}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}(\frac{1}{n_i}+\frac{1}{n_j})}$

$|53{,}00 - 38{,}13| < 2{,}41 \sqrt{\frac{90(90+1)}{12}(\frac{1}{15}+\frac{1}{15})}$

$|14{,}87| < 2{,}41.\sqrt{(682{,}50)(0{,}13)}$

$|14{,}87| < 2{,}41.\sqrt{91{,}00}$

$|14{,}87| < (2{,}41)(9{,}54)$

$|14{,}87| < 22{,}99$

The difference between $\overline{R_3}$ and $\overline{R_4}$ was not significant. Hence there was no difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:5 dilution and *Hydrastis canadensis* 62% v/v 1:10 dilution.

$$|\overline{R_4} - \overline{R_5}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|38{,}13 - 23{,}00| < 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|15{,}13| < 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|15{,}13| < 2{,}41.\sqrt{91{,}00}$$

$$|15{,}13| < (2{,}41)(9{,}54)$$

$$|15{,}13| < 22{,}99$$

The difference between $\overline{R_4}$ and $\overline{R_5}$ was not significant. Hence there was no difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:10 dilution and *Hydrastis canadensis* 62% v/v 1:50 dilution.

$$|\overline{R_5} - \overline{R_6}| > Z_{(1-[\alpha/k(k-1)])} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|23{,}00 - 8{,}00| < 2{,}41 \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|15{,}00| < 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|15{,}00| < 2{,}41.\sqrt{91{,}00}$$

$$|15{,}00| < (2{,}41)(9{,}54)$$

I15,00I < 22,99

The difference between $\overline{R_5}$ and $\overline{R_6}$ was not significant. Hence there was no difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:50 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution.

$$I\overline{R_1} - \overline{R_3}I > Z_{(1-[\alpha/k(k-1)])}\sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$I82,87 - 53,00I > 2,41\sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$I29,87I > 2,41.\sqrt{(682,50)(0,13)}$$

$$I29,87I > 2,41.\sqrt{91,00}$$

I29,87I > (2,41)(9,54)

I29,87I > 22,99

The difference between $\overline{R_1}$ and $\overline{R_3}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:5 dilution.

$$I\overline{R_1} - \overline{R_4}I > Z_{(1-[\alpha/k(k-1)])}\sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$I82,87 - 38,13I > 2,41\sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$I44,74I > 2,41.\sqrt{(682,50)(0,13)}$$

$$I44,74I > 2,41.\sqrt{91,00}$$

I44,74I > (2,41)(9,54)

I44,74I > 22,99

The difference between $\overline{R_1}$ and $\overline{R_4}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:10 dilution.

$$I\overline{R_1} - \overline{R_5}I > Z_{(1-[\alpha/k(k-1)])}\sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$I82,87 - 23,00I > 2,41\sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$I59,87I > 2,41.\sqrt{(682,50)(0,13)}$$

$$I59,87I > 2,41.\sqrt{91,00}$$

I59,87I > (2,41)(9,54)

I59,87I > 22,99

The difference between $\overline{R_1}$ and $\overline{R_5}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:50 dilution.

$$I\overline{R_1} - \overline{R_6}I > Z_{(1-[\alpha/k(k-1)])}\sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$I82,87 - 8,00I > 2,41\sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$I74.87I > 2,41.\sqrt{(682,50)(0,13)}$$

$$I74,87I > 2,41.\sqrt{91,00}$$

I74,87I > (2,41)(9,54)

I74,87I > 22,99

The difference between $\overline{R_1}$ and $\overline{R_6}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:100 dilution.

$$I\overline{R_2} - \overline{R_4}I > Z_{(1-[\alpha/k(k-1)])}\sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$I68{,}00 - 38{,}13I > 2{,}41\sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$I29.87I > 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$I29{,}87I > 2{,}41.\sqrt{91{,}00}$$

I29,87I > (2,41)(9,54)

I29,87I > 22,99

The difference between $\overline{R_2}$ and $\overline{R_4}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:10 dilution.

$$I\overline{R_2} - \overline{R_5}I > Z_{(1-[\alpha/k(k-1)])}\sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|68{,}00 - 38{,}13| > 2{,}41\ \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|45{,}00| > 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|45{,}00| > 2{,}41.\sqrt{91{,}00}$$

$$|45{,}00| > (2{,}41)(9{,}54)$$

$$|45{,}00| > 22{,}99$$

The difference between $\overline{R_2}$ and $\overline{R_5}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:50 dilution.

$$|\overline{R_2} - \overline{R_6}| > Z_{(1-[\alpha/k(k-1)])}\ \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|68{,}00 - 8{,}00| > 2{,}41\ \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|60{,}00| > 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|60{,}00| > 2{,}41.\sqrt{91{,}00}$$

$$|60{,}00| > (2{,}41)(9{,}54)$$

$$|60{,}00| > 22{,}99$$

The difference between $\overline{R_2}$ and $\overline{R_6}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution.

$$|\overline{R_3} - \overline{R_5}| > Z_{(1-[\alpha/k(k-1)])}\ \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|53{,}00 - 23{,}00| > 2{,}41\ \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|30{,}00| > 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|30{,}00| > 2{,}41.\sqrt{91{,}00}$$

$$|30{,}00| > (2{,}41)(9{,}54)$$

$$|30{,}00| > 22{,}99$$

The difference between $\overline{R_3}$ and $\overline{R_5}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:5 dilution and *Hydrastis canadensis* 62% v/v 1:50 dilution.

$$|\overline{R_3} - \overline{R_6}| > Z_{(1-[\alpha/k(k-1)])}\ \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|53{,}00 - 8{,}00| > 2{,}41\ \sqrt{\frac{90(90+1)}{12}\left(\frac{1}{15}+\frac{1}{15}\right)}$$

$$|45{,}00| > 2{,}41.\sqrt{(682{,}50)(0{,}13)}$$

$$|45{,}00| > 2{,}41.\sqrt{91{,}00}$$

$$|45{,}00| > (2{,}41)(9{,}54)$$

$$|45{,}00| > 22{,}99$$

The difference between $\overline{R_3}$ and $\overline{R_6}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:5 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution.

$$|\overline{R_4} - \overline{R_6}| > Z_{(1-[\alpha/k(k-1)])}\ \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}$$

$$|38,13 - 8,00| > 2,41\sqrt{\frac{90(90+1)}{12}(\frac{1}{15}+\frac{1}{15})}$$

$$|30,13| > 2,41.\sqrt{(682,50)(0,13)}$$

$$|30,13| > 2,41.\sqrt{91,00}$$

$$|30,13| > (2,41)(9,54)$$

$$|30,13| > 22,99$$

The difference between $\overline{R_4}$ and $\overline{R_6}$ was significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:10 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution.

4.2.10. Inter-group comparison of *Hydrastis canadensis* 62% v/v tincture and fluconazole in Experiment 1 using the Mann-Whitney U test

The sample size (N), Mean Ranks and Sum of Ranks are shown in Table 4.27. The Mann-Whitney U test showed that there was a significant difference (P=.000) (see Table 4.28) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and fluconazole in Experiment 1.

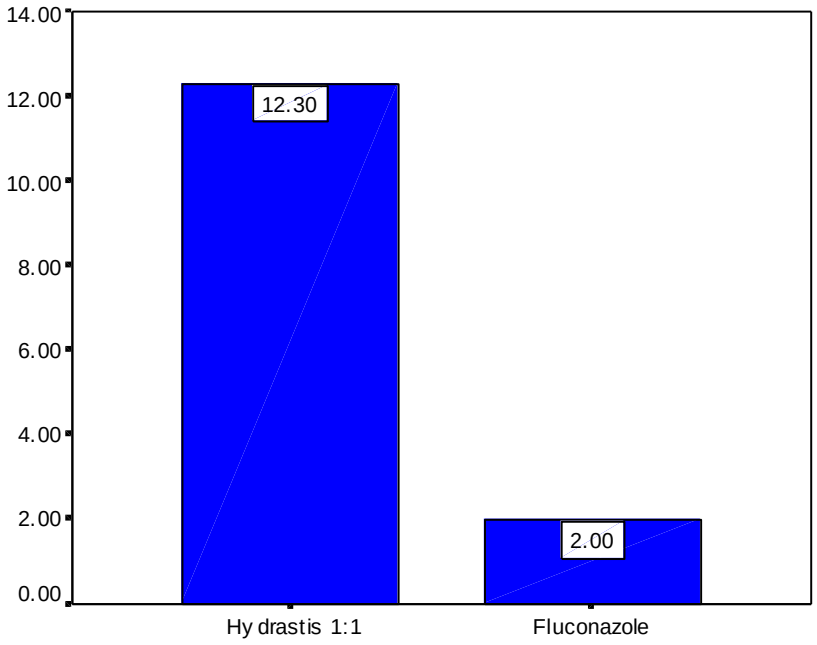

Figure 4.9 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v tincture and fluconazole in Experiment 1.

Table 4.27 Mean ranks for Hydrastis canadensis 62% v/v tincture and fluconazole in Experiment 1.

	Groups	N	Mean Rank	Sum of Ranks
Exp 1	B	15	23.00	345.00
	F	15	8.00	120.00
	Total	30		

Table 4.28 Mann-Whitney U test statistics for Hydrastis canadensis 62% v/v tincture and fluconazole in Experiment 1.[b]

	Exp 1
Mann-Whitney U	.000
Wilcoxon W	120.000
Z	-4.763
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

Conclusion: P=.000 (<.001), therefore P<α and is significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62 % v/v tincture and fluconazole in Experiment 1.

4.2.11 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:2 dilution and fluconazole in Experiment 2 using the Mann-Whitney U test

The sample size (N), Mean Ranks and Sum of Ranks are shown in Table 4.29. The Mann-Whitney U test showed that there was a significant difference (P=.000) (see Table 4.30) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:2 dilution and fluconazole in Experiment 2.

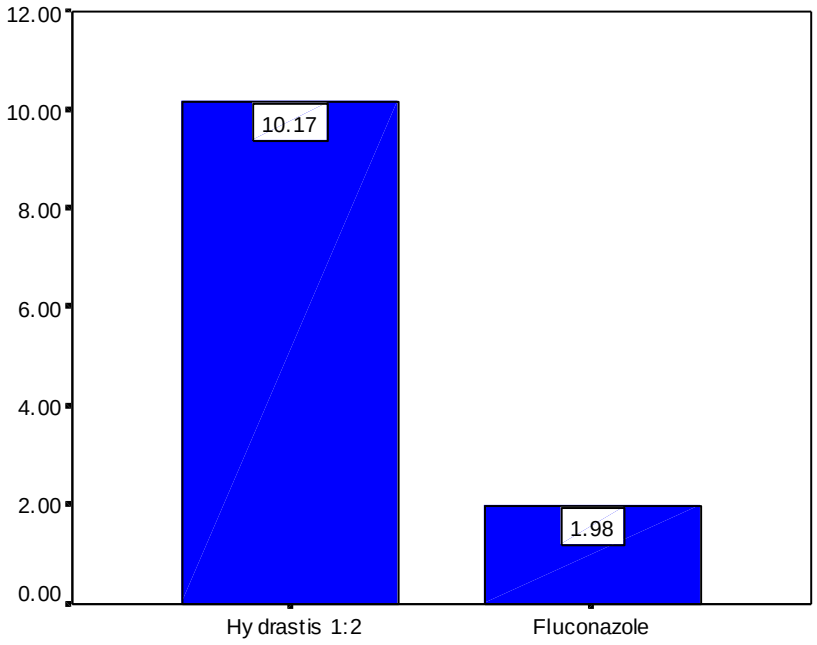

Figure 4.10 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v 1:2 dilution and fluconazole in Experiment 2.

Table 4.29 Mean ranks for Hydrastis canadensis 62% v/v 1:2 dilution and fluconazole in Experiment 2.

	Groups	N	Mean Rank	Sum of Ranks
Exp 2	B	15	23.00	345.00
	F	15	8.00	120.00
	Total	30		

Table 4.30 Mann-Whitney U test statistics for Hydrastis canadensis 62% v/v 1:2 dilution and fluconazole in Experiment 2.[b]

	Exp 2
Mann-Whitney U	.000
Wilcoxon W	120.000
Z	-4.783
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

Conclusion: P=.000 (<.001), therefore P<α and is significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62 % v/v 1:2 dilution and fluconazole in Experiment 2.

4.2.12 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:5 dilution and fluconazole in Experiment 3 using the Mann-Whitney U test

The sample size (N), Mean Ranks and Sum of Ranks are shown in Table 4.31. The Mann-Whitney U test showed that there was a significant difference (P=.000) (see Table 4.32) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:5 dilution and fluconazole in Experiment 3.

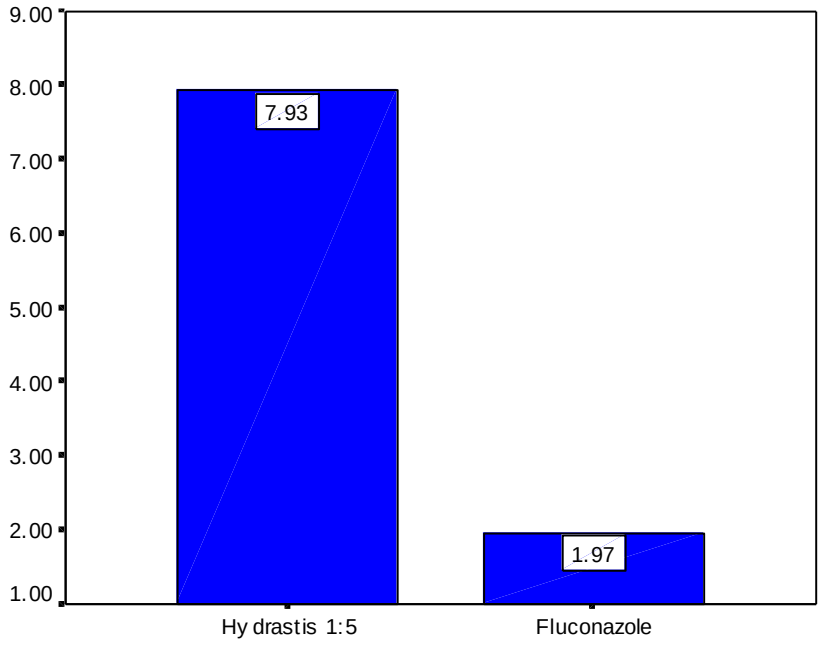

Figure 4.11 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v 1:5 dilution and fluconazole in Experiment 3.

Table 4.31 Mean ranks for Hydrastis canadensis 62% v/v 1:5 dilution and fluconazole in Experiment 3.

	Groups	N	Mean Rank	Sum of Ranks
Exp 3	B	15	23.00	345.00
	F	15	8.00	120.00
	Total	30		

Table 4.32 Mann-Whitney U test statistics for Hydrastis canadensis 62% v/v 1:5 dilution and fluconazole in Experiment 3.[b]

	Exp 3
Mann-Whitney U	.000
Wilcoxon W	120.000
Z	-4.789
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

Conclusion: P=.000 (<.001), therefore P<α and is significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62 % v/v 1:5 dilution and fluconazole in Experiment 3.

4.2.13 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:10 dilution and fluconazole in Experiment 4 using the Mann-Whitney U test

The sample size (N), Mean Ranks and Sum of Ranks are shown in Table 4.33. The Mann-Whitney U test showed that there was a significant difference (P=.000) (see Table 4.34) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:10 dilution and fluconazole in Experiment 4.

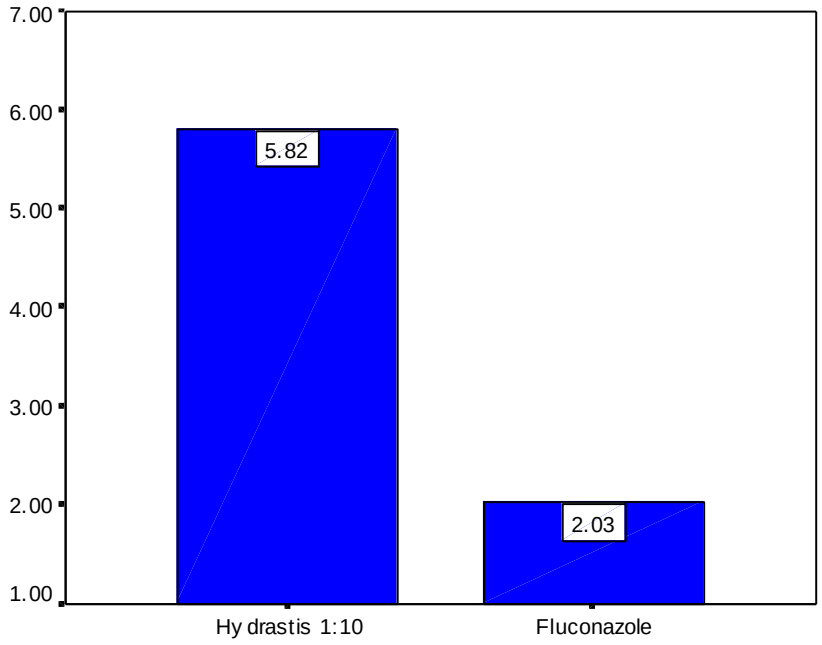

Figure 4.12 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v 1:10 dilution and fluconazole in Experiment 4.

Table 4.33 Mean ranks for Hydrastis canadensis 62% v/v 1:10 dilution and fluconazole in Experiment 4.

	Groups	N	Mean Rank	Sum of Ranks
Exp 4	B	15	22.93	344.00
	F	15	8.07	121.00
	Total	30		

Table 4.34 Mann-Whitney U test statistics for Hydrastis canadensis 62% v/v 1:10[b] dilution and fluconazole in Experiment 4.

	Exp 4
Mann-Whitney U	1.000
Wilcoxon W	121.000
Z	-4.690
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

Conclusion: P=.000 (<.001), therefore P<α and is significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62 % v/v 1:10 dilution and fluconazole in Experiment 4.

4.2.14 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:50 dilution and fluconazole in Experiment 5 using the Mann-Whitney U test

The sample size (N), Mean Ranks and Sum of Ranks are shown in Table 4.35. The Mann-Whitney U test showed that there was no difference (P=.116) (see Table 4.36) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:50 dilution and fluconazole in Experiment 5.

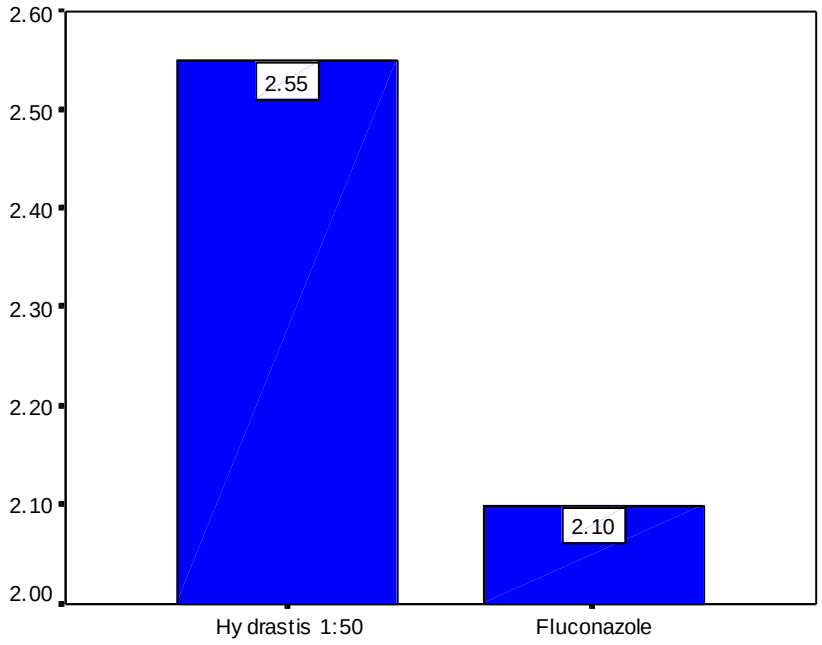

Figure 4.13 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v 1:50 dilution and fluconazole in Experiment 5.

Table 4.35 Mean ranks for Hydrastis canadensis 62% v/v 1:50 dilution and fluconazole in Experiment 5.

	Groups	N	Mean Rank	Sum of Ranks
Exp 5	B	15	18.03	270.50
	F	15	12.97	194.50
	Total	30		

Table 4.36 Mann-Whitney U test statistics for Hydrastis canadensis 62% v/v 1:50[b] dilution and fluconazole in Experiment 5.

	Exp 5
Mann-Whitney U	74.500
Wilcoxon W	194.500
Z	-1.721
Asymp. Sig. (2-tailed)	.085
Exact Sig. [2*(1-tailed Sig.)]	.116[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

Conclusion: P=.0,85 (>.001), therefore P>α and is not significant. Hence there was no difference in the zones of inhibition produced by *Hydrastis canadensis* 62 % v/v 1:50 dilution and fluconazole in Experiment 5.

4.2.15 Inter-group comparison of *Hydrastis canadensis* 62% v/v 1:100 dilution and fluconazole in Experiment 6 using the Mann-Whitney U test

The sample size (N), Mean Ranks and Sum of Ranks are shown in Table 4.37. The Mann-Whitney U test showed that there was a significant difference (P=.000) (see Table 4.38) between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:00 dilution and fluconazole in Experiment 6.

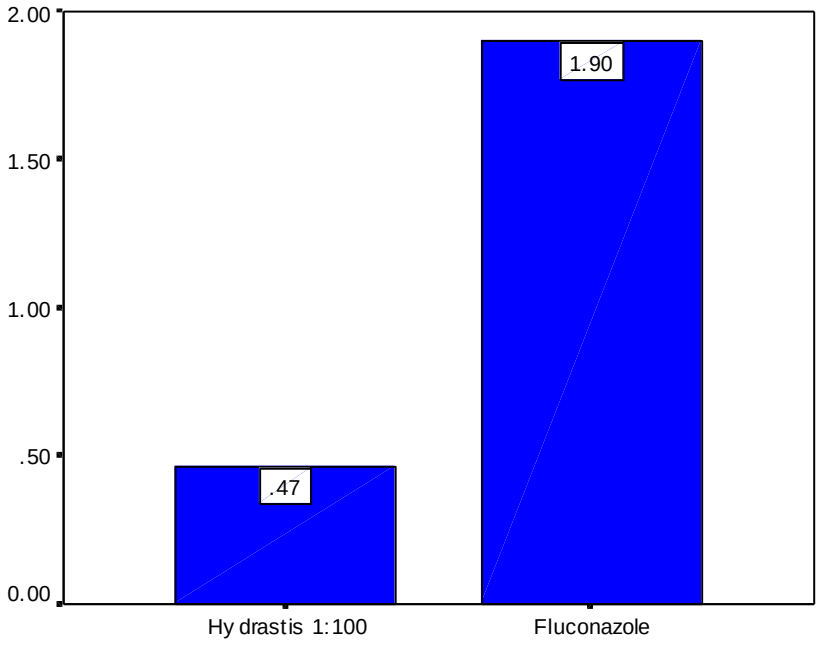

Figure 4.14 Bar graph comparing the mean zones of inhibition of Hydrastis canadensis 62% v/v 1:100 dilution and fluconazole in Experiment 6.

Table 4.37 Mean ranks for Hydrastis canadensis 62% v/v 1:100 dilution and fluconazole in Experiment 6.

	Groups	N	Mean Rank	Sum of Ranks
Exp 6	B	15	9.43	141.50
	F	15	21.57	323.50
	Total	30		

Table 4.38 Mann-Whitney U test statistics for Hydrastis canadensis 62% v/v 1:100 dilution and fluconazole in Experiment 6.[b]

	Exp 6
Mann-Whitney U	21.500
Wilcoxon W	141.500
Z	-3.957
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

Conclusion: P=.000 (<.001), therefore P<α and is significant. Hence there was a difference in the zones of inhibition produced by *Hydrastis canadensis* 62 % v/v 1:100 dilution and fluconazole in Experiment 6.

4.2.16 Intra-group comparison of fluconazole in all the Experiments using Friedman's Test

The sample size (N), means, standard deviations, minimum and maximum zone diameters are shown in Table 4.39. Friedman's Test results (P=.343) show no difference between the means of the zones of inhibition produced by fluconazole in all the Experiments (see Table 4.40).

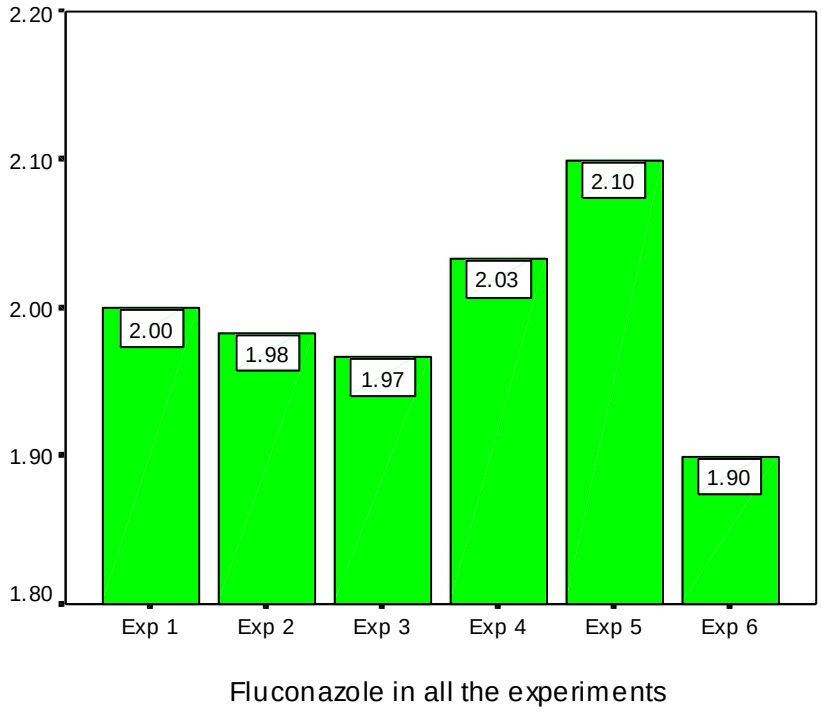

Figure 4.15 Bar graph comparing the mean zones of inhibition of fluconazole in all the experiments.

Table 4.39 Descriptive statistics for fluconazole in all the Experiments.

	N	Mean	Std. Deviation	Minimum	Maximum
Exp 1	15	2.0000	.9820	.00	4.50
Exp 2	15	1.9833	.9750	.00	4.50
Exp 3	15	1.9667	.9722	.00	4.50
Exp 4	15	2.0333	1.0768	.00	4.50
Exp 5	15	2.1000	1.0385	.00	4.50
Exp 6	15	1.9000	1.0036	.00	4.50

Table 4.40 Friedman's test statistics[a] for fluconazole in all the Experiments.

N	15
Chi-Square	5.640
df	5
Asymp. Sig.	.343

a. Friedman Test

Conclusion: P=.343, therefore P>α and the null hypothesis is accepted. Hence there was no difference in the zones of inhibition produced by fluconazole in all the experiments.

CHAPTER FIVE

5.1 **Discussion**

5.1.1 The effect of *Commiphora molmol* 86% v/v tincture against *C. albicans* as compared to 86% v/v ethanol (negative control) and fluconazole (positive control)

Commiphora molmol did not produce any zones of inhibition and thus had no effect on *C. albicans*. Although the mean rank of 86% v/v ethanol (48,13) was greater than the mean rank of *Commiphora molmol* 86% v/v tincture (28,50), the Dunn Procedure demonstrated that there was no difference between the means of the zones of inhibition produced by *Commiphora molmol* 86% v/v tincture and 86% v/v ethanol. The Kruskal-Wallis test and the Dunn Procedure showed that fluconazole (positive control) had an effect on *C. albicans* whereas *Commiphora molmol* 86% v/v tincture did not.

5.1.2 The effect of *Hydrastis canadensis* 62% v/v tincture against *C. albicans* as compared to 62% v/v ethanol (negative control) and fluconazole (positive control)

Hydrastis canadensis 62% v/v tincture had an effect on *C. albicans*. The Kruskal-Wallis test showed that *Hydrastis canadensis* 62% v/v tincture had an effect on *C. albicans* as whereas 62% v/v ethanol did not. The Dunn Procedure results showed a difference in the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and 62% v/v ethanol (negative control). The Kruskal-Wallis and Mann-Whitney U test results showed that *Hydrastis canadensis* 62% v/v tincture had a greater effect than fluconazole on *C.albicans*, while the Dunn Procedure demonstrated that there was a difference in the means

of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and fluconazole.

5.1.3 The effect of *Warburgia salutaris* 62% v/v tincture on *C.albicans* as compared to 62% v/v ethanol (negative control) and fluconazole (positive control)

Warburgia salutaris 62% v/v tincture did not produce any zones of inhibition and thus had no effect on *C. albicans*. The Kruskal-Wallis test and the Dunn Procedure showed that there was no difference in the means of the zones of inhibition produced by *Warburgia salutaris* 62% v/v tincture and 62% v/v ethanol. The Kruskal-Wallis test and the Dunn Procedure results showed that fluconazole did have an effect on *C. albicans* whereas *Warburgia salutaris* 62% v/v tincture did not.

5.1.4 The effect of *Commiphora molmol* 86% v/v tincture, *Hydrastis canadensis* 62% v/v tincture and *Warburgia salutaris* 62% v/v tincture on *C. albicans*

The Kruskal-Wallis test and the Dunn Procedure showed that *Hydrastis canadensis* 62% v/v tincture had an effect on *C. albicans*, while *Commiphora molmol* 86% v/v tincture and *Warburgia salutaris* 62% v/v tincture had no effect on *C. albicans*.

5.1.5 The effect of *Hydrastis canadensis* 62% v/v 1:2 dilution, 62% v/v ethanol 1:2 dilution (negative control) and fluconazole (positive control) on *C. albicans*

Hydrastis canadensis 62% v/v 1:2 dilution had an effect on *C. albicans*. The Kruskal-Wallis test showed that *Hydrastis canadensis* 62% v/v 1:2 dilution had an effect on *C. albicans* whereas 62% v/v ethanol 1:2 dilution did not. The Dunn Procedure demonstrated that the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:2 dilution and 62% v/v ethanol 1:2 dilution were significantly different.

The Kruskal-Wallis and Mann-Whitney U tests demonstrated that *Hydrastis canadensis* 62% v/v 1:2 dilution had a greater effect on *C. albicans* than fluconazole, while the Dunn Procedure showed that the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:2 dilution and fluconazole were significantly different.

5.1.6 The effect of *Hydrastis canadensis* 62% v/v 1:5 dilution, 62% v/v ethanol 1:5 dilution (negative control) and fluconazole (positive control) on *C. albicans*

Hydrastis canadensis 62% v/v 1:5 dilution had an effect on *C. albicans*. The Kruskal-Wallis test showed that *Hydrastis canadensis* 62% v/v 1:5 dilution had an effect on *C. albicans* whereas 62% v/v ethanol 1:5 dilution did not. The Dunn Procedure demonstrated that the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:5 dilution and 62% v/v ethanol 1:5 dilution were significantly different. The Kruskal-Wallis and Mann-Whitney U tests demonstrated that *Hydrastis canadensis* 62% v/v 1:5 dilution had a greater effect

on *C. albicans* than fluconazole, while the Dunn Procedure showed that the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:5 dilution and fluconazole were significantly different.

5.1.7 The effect of *Hydrastis canadensis* 62% v/v 1:10 dilution, 62% v/v ethanol 1:10 dilution (negative control) and fluconazole (positive control) on *C. albicans*

Hydrastis canadensis 62% v/v 1:10 dilution had an effect on *C. albicans*. The Kruskal-Wallis test showed that *Hydrastis canadensis* 62% v/v 1:10 dilution had an effect on *C. albicans* whereas 62% v/v ethanol 1:10 dilution did not. The Dunn Procedure demonstrated that the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:10 dilution and 62% v/v ethanol 1:10 dilution were significantly different.

The Kruskal-Wallis and Mann-Whitney U tests demonstrated that *Hydrastis canadensis* 62% v/v 1:10 dilution had a greater effect on *C. albicans* than fluconazole, while the Dunn Procedure showed that the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:10 dilution and fluconazole were significantly different.

5.1.8 The effect of *Hydrastis canadensis* 62% v/v 1:50 dilution, 62% v/v ethanol 1:50 dilution (negative control) and fluconazole (positive control) on *C. albicans*

Hydrastis canadensis 62% v/v 1:50 dilution had an effect on *C. albicans*. The Kruskal-Wallis test showed that *Hydrastis canadensis* 62% v/v 1:50 dilution had an effect on *C. albicans* whereas 62% v/v ethanol 1:50 dilution did not. The Dunn

Procedure demonstrated that the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v 1:50 dilution and 62% v/v ethanol 1:50 dilution were significantly different.

The Dunn Procedure and Mann-Whitney U test (P=.085) demonstrated that there was no difference between the means of the zones of inhibition produced *Hydrastis canadensis* 62% v/v 1:50 dilution and fluconazole.

5.1.9 The effect of *Hydrastis canadensis* 62% v/v 1:100 dilution, 62% v/v ethanol 1:100 dilution (negative control) and fluconazole (positive control) on *C. albicans*

Hydrastis canadensis 62% v/v 1:100 dilution had no effect on *C. albicans*. The Kruskal-Wallis test and the Dunn Procedure showed that there was no difference between *Hydrastis canadensis* 62% v/v 1:100 dilution and 62% v/v ethanol 1:100 dilution.

The Kruskal-Wallis and the Mann-Whitney U tests showed that fluconazole had an effect on *C. albicans* whereas *Hydrastis canadensis* 62% v/v 1:100 dilution did not. The Dunn Procedure showed that the means of the zones of inhibition produced by fluconazole and *Hydrastis canadensis* 62% v/v 1:100 dilution were significantly different.

5.1.10 The inter-group comparison of 62% v/v ethanol, 62% v/v ethanol 1:2 dilution, 62% v/v ethanol 1:5 dilution, 62% v/v ethanol 1:10 dilution, 62% v/v ethanol 1:50 dilution and 62% v/v ethanol 1:100 dilution on *C. albicans*

The Kruskal-Wallis test results (P=1.000) showed that 62% v/v ethanol,

62% v/v ethanol 1:2 dilution, 62% v/v ethanol 1:5 dilution, 62% v/v ethanol 1:10 dilution, 62% v/v ethanol 1:50 dilution and 62% v/v ethanol 1:100 dilution had no effect on *C. albicans*.

5.1.11 The inter-group comparison of *Hydrastis canadensis* 62% v/v tincture, *Hydrastis canadensis* 62% v/v 1:2 dilution, *Hydrastis canadensis* 62% v/v 1:5 dilution, *Hydrastis canadensis* 62% v/v 1:10 dilution, *Hydrastis canadensis* 62% v/v 1:50 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution on *C. albicans*

The Dunn Procedure showed that there was no difference between the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:2 dilution, *Hydrastis canadensis* 62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:5 dilution, *Hydrastis canadensis* 62% v/v 1:5 dilution and *Hydrastis canadensis* 62% v/v 1:10 dilution, *Hydrastis canadensis* 62% v/v 1:10 dilution and *Hydrastis canadensis* 62% v/v 1:50 dilution, *Hydrastis canadensis* 62% v/v 1:50 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution.

The Dunn Procedure demonstrated that the means of the zones of inhibition produced by *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:5 dilution, *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:10 dilution, *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:50 dilution, *Hydrastis canadensis* 62% v/v tincture and *Hydrastis canadensis* 62% v/v 1:100 dilution, *Hydrastis canadensis*

62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:10 dilution, *Hydrastis canadensis* 62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:50 dilution, *Hydrastis canadensis* 62% v/v 1:2 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution, *Hydrastis canadensis* 62% v/v 1:5 dilution and *Hydrastis canadensis* 62% v/v 1:50 dilution, *Hydrastis canadensis* 62% v/v 1:5 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution, *Hydrastis canadensis* 62% v/v 1:10 dilution and *Hydrastis canadensis* 62% v/v 1:100 dilution were significantly different.

5.1.12 The intra-group comparison of fluconazole in all the experiments

The Friedman's test results (P=.343) demonstrated that there were no differences in the means of the zones of inhibition produced by fluconazole in all the experiments.

5.2 Summary

Hydrastis canadensis 62% v/v tincture was the only tincture that demonstrated an inhibitory effect on the *in vitro* growth of *C. albicans* in this study, while the 1:50 aqueous dilution of *Hydrastis canadensis* 62% v/v tincture was shown to be the minimum inhibitory concentration (MIC).

5.3 General Discussion

Commiphora molmol tincture did not have an inhibitory effect on the *in vitro* growth of *C. albicans* in this study even though it did demonstrate an inhibitory effect on the *in vitro* growth of *C. albicans* in the studies conducted by Dolara *et al*., (2000:356-358) and McFadden (1995).

The results of *Warburgia salutaris* in this study do not correlate with those of Taniguchi and associates (1983:149-154) and other related literature. Clinical doses of 100 to 200 mg (Gericke, 2001:3-13) have been reported to have cured oral and vaginal candidiasis in patients and it is thus assumed that higher concentrations and frequent doses of *Warburgia salutaris* may inhibit the growth of *C. albicans* but this can only be established through future controlled *in vivo* studies.

Hydrastis canadensis does inhibit the *in vitro* growth of *C. albicans* and these results do correlate with the studies of Scazzocchio *et al*., (2001:561-564) and McFadden (1995), and other related literature.

86% v/v ethanol did have a slight inhibitory effect on *C. albicans* while 62% v/v ethanol had no effect on *C. albicans*. This result does not correlate with Reid's (2001:22) study in which 62% v/v ethanol did have an inhibitory effect on *C. albicans*.

Fluconazole did have an inhibitory effect on *C. albicans* in this study and this result does support all studies and related literature. Wilcox and Mönkemüller, (1998:1002-1008) state that 50 to 100 mg of fluconazole is an effective form of prophylaxis against candidiasis. However, the concentration of fluconazole in this study was quite low (25 µg) and this could be the reason why the zones of inhibition produced by *Hydrastis canadensis* tincture and its dilutions were much larger than those produced by fluconazole.

The above discussions cannot be related to experimental error since Friedman's test results of fluconazole in all experiments showed that there was no difference in the means of the zones of inhibition produced by fluconazole in all the experiments. This validifies the reproducibility of this methodology.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The purpose of this study was to determine the effect of *Commiphora molmol* 86% v/v tincture, *Hydrastis canadensis* 62% v/v tincture and *Warburgia salutaris* 62% v/v tincture on the *in vitro* growth *C. albicans* as compared to fluconazole using the disc diffusion assay.

Commiphora molmol 86% v/v tincture was ineffective in inhibiting the *in vitro* growth of *C. albicans.*

Warburgia salutaris 62% v/v tincture was ineffective in inhibiting the *in vitro* growth of *C. albicans*

Hydrastis canadensis 62% v/v tincture was effective in inhibiting the *in vitro* growth of *C. albicans*. The MIC of *Hydrastis canadensis* 62% v/v tincture was the 1:50 dilution.

Fluconazole was effective in inhibiting the *in vitro* growth of *C. albicans*, but was less effective than *Hydrastis canadensis* 62% v/v tincture and its 1:2, 1:5 1:10 and 1:50 dilutions.

In accordance with the aims set out in this study, *Hydrastis canadensis* 62% v/v tincture is a good alternative to fluconazole for treating *C. albicans* infections.

6.2 Recommendations

1. *Hydrastis canadensis* 62% v/v tincture should undergo a clinical trial to determine its efficacy in patients.
2. *Hydrastis canadensis* 62% v/v tincture and low potency *Hydrastis canadensis* homoeopathic preparations could be compared to each other in *in vitro* and *in vivo* studies.
3. *Hydrastis canadensis* 62% v/v tincture could be used in the treatment of *C. albicans* infections, which is one of the most serious complications in our current AIDS epidemic.

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APPENDIX A

EXPERIMENT: 1

	PLATE														
TEST & CONTROL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A. *Commiphora molmol* 86%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. *Hydrastis canadensis* 62%	12.25	12	12	11	13	12.25	12	12	12	12.5	12	13	12	14	12.5
C. *Warburgia salutaris* 62%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. 86% v/v ethanol	3	0	0	5	0	3	0	5	0	0	5	0	0	0	0
E. 62% v/v ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. fluconazole	2	2	2.5	2	2	1	0	1	2	2	2	4.5	3	2	2
	ZONE DIAMETER (MM)														

EXPERIMENT: 2

	PLATE														
TEST & CONTROL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B. *Hydrastis canadensis* 1:2	9	10	10	10	10.5	11	9	10	11	11	11	10	10	10	10
E. 62% v/v ethanol 1:2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. fluconazole	2	2.2	2	2	2	1	0	1	2	2	2	4.5	3	2	2
	ZONE DIAMETER (MM)														

EXPERIMENT: 3

	PLATE														
TEST & CONTROL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B. *Hydrastis canadensis* 1:5	7	8	7	8	7.5	9	8.5	8	7.5	9	7.5	8	8	8	8
E. 62% v/v ethanol 1:5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. fluconazole	2	2	2	2	2	1	0	1	2	2	2	4.5	3	2	2
	ZONE DIAMETER (MM)														

APPENDIX B

EXPERIMENT: 4	PLATE														
TEST & CONTROL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B. *Hydrastis canadensis* 1:10	6	5	6	7	6	4	5	6	6	6	5	6	6.5	5.75	7
E. 62% v/v ethanol 1:10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. fluconazole	3	1	3	2	2	1	0	1	2	2	2	4.5	3	2	2
	ZONE DIAMETER (MM)														

EXPERIMENT: 5	PLATE														
TEST & CONTROL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B. *Hydrastis canadensis* 1:50	3.25	3	2	3	3	3	2	2	2	3	2	2	2	3	3
E. 62% v/v ethanol 1:50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. fluconazole	2	3	3	2	2	1	0	1	2	2	2	4.5	3	2	2
	ZONE DIAMETER (MM)														

EXPERIMENT: 6	PLATE														
TEST & CONTROL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B. *Hydrastis canadensis* 1:100	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0
E. 62% v/v ethanol 1:100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. fluconazole	1	2	2	2	2	1	0	1	2	2	2	4.5	3	2	2
	ZONE DIAMETER (MM)														