**THE EFFICACY OF CERTAIN *COMPOSITAE* SPECIES *(ARCTIUM LAPPA, CALENDULA OFFICINALIS AND ECHINACEA PURPUREA)* HERBAL EXTRACTS AS COMPARED TO NYSTATIN, IN THE INHIBITION OF *IN VITRO* GROWTH OF *CANDIDA ALBICANS***

**BY**

**SHAVASHNI RAMLACHAN**

Mini-dissertation submitted in partial compliance with the requirements of the Master's Degree in Technology: Homoeopathy in the Faculty of Health Sciences at Durban Institute of Technology.

I, Shavashni Ramlachan do declare that this mini-dissertation represents my own work in both conception and execution.

_____
**Signature of Student**

22/07/2002
**Date of Signature**

APPROVED FOR FINAL SUBMISSION

_____
**Signature of Supervisor**
**Dr Richard Steele**
**B.A., H.D.E., M.Tech (Hom)**

27-09-02
**Date of Signature**

## Dedication

I dedicate this study to my Parents.

Thank you for all your love, support and encouragement throughout my life, and for the host of opportunities you have made available to me.

## Acknowledgements

The author wishes to express her sincere appreciation to the following persons for their assistance in preparing this dissertation.

| | |
|---|---|
| Dr. Richard Steele | Supervisor |
| Mrs. D. Macdonald | Co-Supervisor |
| Mrs. Reena Brijlal | Lab Technician (Dept. of Biotechnology) |
| Mr. Thomas Kavanel | Statistician |
| Mr. Ron Gauldie | Dept. of Microbiology |
| Dr. Kim Reid | |
| Miss Deepa Ramlachan | |
| Miss Thirashni Ramlachan | |

## Abstract

The purpose of this study was to determine the efficacy of certain *Compositae* species herbal extracts (*Arctium lappa, Calendula officinalis and Echinacea purpurea*) in the inhibition of *in vitro* growth of *Candida albicans* as compared to nystatin in terms of the disc diffusion test.

*Candida albicans* was obtained from the Department of Biotechnology (Technikon Natal). Three components were tested on *Candida albicans*: namely the herbal extracts which were the experimental group, nystatin which was the allopathic component serving as a positive control and the 62% ethanol which was the negative control. Commercially available herbal extracts of *Arctium lappa*, *Calendula officinalis* and *Echinacea purpurea* respectively, with an ethanol concentration of 62% v/v, were purchased from Parceval (Pty) Ltd. The herbal extracts were prepared according to the German Herbal Pharmacopoeia (1991) standards. Commercially available nystatin suspension was obtained from Bristol-Myers Squibb (Pty) Ltd, 62% (v/v) ethanol was prepared according to the German Homoeopathic Pharmacopoeia (1991) standards.

In carrying out the experiment, 5mm filter paper discs were placed on Sabouraud's dextrose agar plates which were streaked with *Candida albicans*. Thereafter 0.7 microlitres of the test and control substances were pipetted onto

the filter paper discs. The agar plates were incubated and observations were made at 18 hours, 24 hours and 36 hours for the presence of growth inhibition areas, which were indicated by a clear zone surrounding each disc.

The susceptibility of *Candida albicans* to a substance is determined by the size of the zone of inhibition. A small or no zone of inhibition indicates resistance and large zone indicates that the organism is susceptible to the test substance.

The zones were measured using a transparent ruler. The tests were carried out on 15 agar plates to ensure that the results were statistically viable. The Mann-Whitney U-Test, the Kruskal-Wallis non-parametric Analysis of Variance by Ranks method and Friedman's T test were used to statistically analyse the results obtained.

From the results obtained nystatin produced zones of inhibition that were larger than the zones of inhibition produced by the herbal extracts, indicating that nystatin has a greater antifungal effect than the herbal extracts. The size of the zones of inhibition produced by 62% ethanol (v/v) were similar to the zones of inhibition produced by the herbal extracts, indicating that the herbal extracts and the 62% ethanol (v/v) had the same antifungal effects.

The results obtained were in contrast to claims that the herbal extracts used in this study are antifungal. These *in vitro* results should serve as a motivation for

clinical trials that would prove whether these *in vitro* results extend *in vivo*. Clinical trials are a more appropriate method of testing the efficacy of these herbal extracts.

# Table of Contents

## List of Figures

**List of Tables**

## List of Plates – Appendix B

# CHAPTER ONE

## INTRODUCTION

The emergence of newly identified pathogens and the re-emergence of diseases that had previously been uncommon is a serious and growing public health problem (Dixon, et al.1996). In 1992, an Institute of Medicine report by Lederberg, et al. identified 3 fungi as the most important emerging pathogens, namely, *Candida albicans, Cryptococcus neoformans* and *Pneumocystis carinii* (reported in Dixon, et al.1996). Contributing factors to the emergence of fungal disease are:

- changing demographics and technology which results in an increase in the number of susceptible hosts;
- microbial adaptation that has resulted in the evolution of drug resistant fungi;
- land use and travel exposes previous immunocompetent persons to fungal infections;
- the breakdown of public health measures resulting in failing laboratory infrastructure. (Dixon, et al. 1996.)

Henderson (1996) (in Lipman and Saadia 1997) identified *Candida albicans* as the most frequently isolated organism in critically ill patients suffering from nosocomial fungal infections. A survey carried out by Banerjee (in Hedderwick and Kauffman, 1997) between 1980-1989, concluded that systemic *candida* infections are the 4$^{th}$ leading cause of bloodstream infections in the United

States and *Candida albicans* was isolated as the most common species responsible for infection.

*Candida albicans* has also been identified as the most common cause of superficial and deep forms of vaginal candidiasis, with the rate of isolation in swabs taken from women with vaginal infection being between 80% to 95% (Goorney and Roberts, 1995).

Treatment for *Candida albicans* infection is usually individualized and based on a comparison of the effectiveness, convenience, potential side-effects and cost. However, antifungal drug-resistance is crucial in treating common infections such as vulvovaginitis as well as life-threatening, invasive candidiasis. (Dixon, et al. 1996). Besides drug-resistance, Mann (1984) (in Robertson, 1995) states that the side-effects of drug therapies are increasing with estimates that over 15% of hospital beds are occupied by patients suffering from drug side-effects. Figures released by the United Kingdom government in 1977 indicate that 120 366 patients died as a result of harmful effects of drug treatment. (Robertson, 1995.)

The identification of *Candida albicans* in recent times as the most important emerging pathogen has lead to it being considered as a major iatrogenic disease of the 21st century. The disturbance of this harmoniously co-existing pathogen results in symptoms that have collectively earned the name "*Candida* Syndrome". Tiredness, lack of energy, extreme mood swings, depression, major digestive problems, highly allergic conditions and even

debilitating illness are the symptoms of this so-called syndrome. These symptoms are in contrast to what medical dogma holds, that *Candida albicans* is limited to thrush, onychomycosis, athletes foot and *candida* septicemia. The causes of this syndrome are related to the food we eat which is loaded with antibiotics and steroids. The increased intake of prescribed antibiotics, contraceptive pills and the increased consumption of artificial, junk food and foods containing excessive amounts of yeast and moulds are also contributing factors to this syndrome. Treatment of this modern age syndrome lies in changing of the diet and a naturopathic approach. (Moore, 1998.) It is evident that drug-resistance, the side-effects of conventional therapies and the "*Candida* Syndrome" has driven people to seek an alternative to conventional therapies, the most common alternative being herbal medicines.

In the United States, the market for herbal supplements is approaching $4 billion a year (Ernst, 2000). In the United Kingdom spending on herbal products is over £4 million a year, mainly from self-prescription over the counter products (Vickers and Zollman, 1999).

The use of plants for healing purposes predates human history and forms the origins of modern medicine. Many conventional drugs originate from plant sources. A century ago most of the effective drugs were plant based, e.g. aspirin from willow bark, digoxin from foxglove, quinine from cinchona bark and morphine from opium (Vickers and Zollman, 1999).

The rebirth of herbal medicine is, however, largely based on renewed interest of scientific researchers (Murray, 1995: 4). According to Angel and Kassir (in Vickers and Zollman, 1999) it is the public's interest in herbal medicine, as a result of their disillusionment with the impersonal care delivered by conventional medicine, that has contributed largely to the rebirth of herbal medicine. The aim of herbal treatment is to produce persisting improvement in well-being, to treat the underlying cause of disease and to correct the patterns of dysfunction (Vickers and Zollman, 1999). Herbal medicine can be adopted as an aspect of self-empowerment (Hoads, 1996: 3). It is less toxic and offers less risk of side-effects (Murray, 1995: 5).

Herbal medicine is likely to play a major role in future medicine as it illustrates a paradigm shift in medicine and appreciation is growing for the harmonious healing properties that herbal medicines possess (Murray, 1995: 5). A limitation however, with using herbal medicines is that we do not fully understand how they work (Ernst, 2000). While clinical evidence on the effectiveness of herbalism is sparse, laboratory work has been able to show a variety of pharmacological effects of plant extracts (Vickers and Zollman, 1999). Recent studies evaluating the effects of plant extracts have emerged. One such study evaluated the antibacterial effects of *Thymus vulgaris* tincture (Vosloo, 2002). The statistical results of this study indicate that the average P value was 0.005. As a result the null hypothesis was rejected and therefore there was a significant difference between the test and control substances indicating that *Thymus vulgaris* tincture has antibacterial effects (Vosloo, 2002). A second study evaluated the efficacy of certain *Labiatiae* species

herbal extracts (*Rosmarinus officinalis, Salvia officinalis and Thymus vulgaris)* in the inhibition of the *in vitro* growth of *Candida albicans* as compared to nystatin (Reid, 2002). The disc diffusion test was used and the statistical results indicated that the P values of *Rosmarinus officinalis,* for observations at 18 and 24 hours were, significantly different to the control. The results demonstrate the antifungal effect of *Rosmarinus officinalis* in 43% ethanol (v/v), compared to 43% ethanol (v/v). (Reid, 2002.)

This present study is therefore significant in expanding further our knowledge of the antifungal properties of other herbal extracts. The purpose of this study was to determine the efficacy of herbal extracts of certain *Compositae* species (*Arctium lappa, Calendula officinalis* and *Echinacea purpurea*) respectively in the inhibition of *in vitro* growth of *Candida albicans* as compared to nystatin in terms of the disc diffusion test. The antimicrobial activities of these herbs were evaluated by determining the diameter of the zone of inhibition of the pathogen (Williams and Home, 1995). The diameters of the zones of inhibition produced by the herbal extracts are compared to 62% ethanol (v/v) (negative control) and nystatin (positive control) in order to evaluate the efficacy of the herbal extracts.

# CHAPTER TWO

## REVIEW OF RELATED LITERATURE

*Candida albicans* naturally colonises the human gastrointestinal and genitourinary tracts. For these normally commensal yeasts to become pathogenic, interruption of the normal defences is necessary (Hedderwick and Kauffman, 1997).

The growing numbers of immunocompromised and other susceptible individuals in the population mean that fungal infections cause tremendous costs financially and in terms of human life (Dixon, et al. 1996). Data from the National Nosocomial Infections Surveillance System reveals that the incidence of candidal infection increased by 487 percent between 1980 and 1989. The reported mortality directly attributed to such infections varies between 38-50%. *Candida albicans* has been identified as the most common pathogen responsible for candidal infections and it is therefore a major public health problem (Hedderwick and Kauffman, 1997).

### 2.1 Classification, Morphology and Indentification

*Candida albicans* belongs to the genus *Candida* of the family Sacharomyceteae.

This genus is commonly known as a 'yeast-like fungi' because it produces a pseudomycelium (Dugaid, et al. 1978: 544).

*Candida* is a gram-postive, oval budding yeast, measuring 4-6μm. *Candida* grows on Sabouraud agar, cream-coloured colonies, with a yeasty odour. The surface growth consists of oval-budding cells and the submergered growth consists of pseudomycelium. The pseudohyphae form blastospores at the nodes. *Candida albicans* ferments glucose and maltose producing both acid and gas (Jawetz, et al. 1989: 307). The ability of *Candida albicans* to ferment carbohydrates and its failure to split urea is used in differentiating it from other yeast and other *Candida* species (Dugaid, et al. 1989: 544).

## 2.2 Pathology

Candidal infection is usually a superficial infection of the skin, nails or the mucus membranes, causing inflammation. A congenital immune deficiency of T lymphocytes predisposes to the syndrome of chronic mucocutaneous candidal infection. More severe iatrogenic forms of immune suppression may permit systemic infection in which the yeast invades tissues and forms micro-abscesses. (Edwards, et al. 1995: 144.)

## 2.3 Predisposing Factors

*Candida albicans* causes infection when the normal host defences are compromised.

Predisposing conditions include the following:

- skin barriers that have been damaged by maceration of tissue, wounds and abrasions, thermal or chemical burns and intravascular catheters;

- mucosal barriers that have been altered by diabetes, antimicrobial agents, irradiation, smoking, cytotoxic drugs, corticosteroids, cimetidine, vagotomy resulting in an increased gastric pH, and foreign bodies, such as dentures, nasogastric tubes and diaphragms; hormonal or nutritional imbalances resulting from diabetes, oral contraceptives, pregnancy, menses, malnutrition and uremia; decreased numbers of phagocytic cells as a result of leukemia, irradiation, cancer chemotherapy and agranulocytosis;
  - intrinsic defects in the function of phagocytic cells as the result of chronic granulomatous diseases and myeloperoxidase deficiency;
  - alterations in phagocyte functions caused by uremia, viral infections and the use of corticosteroids and antimicrobial agents such as the aminoglycosides and sulfonamides;
  - cell-mediated immunity problems arising from defects such as chronic mucocutaneous candidiasis and DiGeorge syndrome, from using corticosteroids, irradiation, cancer chemotherapy and immunosuppresion for transplantation, and from collagen vascular diseases (Howard, 1994: 617).

## 2.4 Types of Candidiasis (Candidal Infections)

### 2.4.1 Superficial

Superficial candidal infections are very common throughout the world. They involve the skin, nails and mucous membranes of the mouth and the vagina. Infection of the mucous membranes is commonly referred to as thrush (Greenwood, et al. 1992: 682). Superficial candidal infections can cause

significant morbidity in older adults, where increased medication, poor self-care and decreased salivary flow, can contribute to increased severity of infection (Hedderwick and Kauffman, 1997).

#### 2.4.1.1 Mucosal

These are the commonest forms of superficial candidiasis, and are characterized by the development of discrete white patches on the mucosal surface which may become confluent and form a curd-like pseudomembrane (Greenwood, et al. 1995: 683).

##### 2.4.1.1.1 Oral

This occurs mainly in debilitated or bottle fed infants and in old age (Dugaid, et al. 1978: 544). *Candida albicans* infections occur at a disproportionately high rate in persons age 65 and older (Hedderwick and Kauffman, 1997). Oral candidiasis manifests as white flecks on the buccal mucosa and the hard palate. These are adherant and can spread to the tongue (Greenwood, et al. 1995: 683). In addition some patients develop painful cracks at the corners of the mouth (Hedderwick and Kauffman, 1997), as a result of infection at the angles which occurs mainly in those with chronic oral candidiasis (Greenwood, et al. 1995: 683). Factors that predispose patients to the development of oral candidiasis include the use of broadspectrum antibiotics, inhaled corticosteriods and diminished cell-mediated immunity (Hedderwick and Kauffman 1997).

People who wear dentures are also prone to a variant of candidiasis, denture stomatitis, which presents as a chronic mucosal erythema beneath a denture. Patients who do not remove their dentures at night and who have poor oral hygiene are likely to be affected (Hedderwick and Kauffman, 1997).

*Candida albicans* is the most frequently diagnosed opportunistic infecting agent of the oral cavity, pharynx and the oesophagus in immunocompromised patients. Clinically the infection is a marker of moderate or severe immunosuppression and in patients who present with unexplained oral candidiasis, there is a high index of suspicion that this could be due to acquired immunodeficiency syndrome (AIDS) (Fong, et al. 1997). Most AIDS patients have oropharyngeal candidiasis during their illness. The typical presentation of white flecks on the buccal mucosa, hard palate and tongue are followed by increased sensitivity to spicy foods or they may have diminished sense of taste. Oesophageal candidiasis is commonly seen with oropharyngeal candidiasis. (Reisacher, et al. 1999.) Dysphagia is the commonest complaint in these patients and can be accompanied by odynophagia, heartburn and less commonly a retrosternal pain (Wilcox and Mankemullar 1998).

#### 2.4.1.1.2 Vaginal

The commonest *Candida* infection is vaginitis or vaginal thrush (Dugaid, et al. 1978: 544). According to Kant (2000) vaginal thrush is so common in today's society that at least 75% of all women will suffer at least one infection of vaginal thrush at some time. *Candida albicans* accounts for about 90% of

these infections (Goorney and Roberts, 1995). Vaginal thrush manifests as typical white lesions on the epithelial surfaces of the vulva, vagina and cervix and is accompanied by itching, soreness and a non-homogenous white discharge (Greenwood, et al. 1995: 683), with a pH below 5 (Dugaid, et al. 1978: 544). The mucosa appears inflamed and friable. The perivulval skin may become sore and small satellite pustules may appear around the perineum and the natal cleft (Greenwood, et al. 1995: 684). Dysuria and dyspareunia are common (Goorney and Roberts, 1995). An acid pH is normally maintained by the bacterial flora in the vagina, therefore loss of an acid pH predisposes to candidal vulvovaginitis (Jawetz, et al. 1989: 307). Vaginal candidiasis is most common in pregnant women, and a large proportion of women first present with infection during pregnancy. Broad spectrum antibiotic treatment also predisposes women to vaginal candidiasis (Goorney and Roberts, 1995). Reed (1992) (in Ringdahl, 2000) reports that antibiotics decrease the protective vaginal flora and allow colonization by *Candida albicans*. Vaginal colonization and infection is also common among women with diabetes mellitus because hyperglycemia enhances the ability of *Candida albicans* to bind to vaginal epithelial cells. (Ringdahl, 2000.)

Vulvovaginal candidiasis is considered recurrent when at least four specific episodes occur in one year or at least three episodes unrelated to antibiotic therapy occur within a year (Ringdahl, 2000). According to Geiger (in Ringdahl, 2000), more than 50% of all women older than 25 years have one episode of vulvovaginal candidiasis, but fewer than 5% of these women experience recurrent infection. Recurrent vulvovaginal candidiasis is

distinguished from persistent infection by the presence of a symptom-free interval (Ringdahl, 2000). Contraceptives such as spermicidal jellies and creams promote recurrent vulvovaginal candidiasis because they increase the susceptibility to infection by altering the vaginal flora (Ringdahl, 2000).

Women who have deficient cell-mediated immunity are prone to vulvovaginal candidiasis, and this condition is therefore common in AIDS patients (Ringdahl, 2000).

#### 2.4.1.2 Skin

*Candida* infections of the skin occur at the moist sites of the skin, such as the axillae, groin, perineum, submammary folds and occasionally at the toe cleft (Greenwood, et al. 1995: 684). Mildly traumatized skin is also prone to candida infection (Dugaid, et al. 1989: 544). In infants *Candida* species are frequently involved in napkin dermatitis (Greenwood, et al. 1995: 684). *Candida albicans* has been implicated as the most common pathogen responsible for invasive fungal dermatitis in low birth weight-neonates. Congenital candidiasis is characterised by an extensive skin rash within 12 hours of birth. This often resolves spontaneously, but may proceed to invasive disease and death in very low birth weight infants (Rowen, et al, 1995).

#### 2.4.1.3 Nails

The term onchomycosis refers to fungal infection of the nails. About 30% of all superficial fungal infections affect the nail. *Candida* infections affect the fingernails and the fingernail folds (Denning, et al, 1995). Infection of the

finger webs, nail folds, and nails are associated with frequent immersion of the hands in water and is an occupational disease among housewives, nurses and barmaids (Greenwood, et al. 1995: 684).

The nail is painful to touch with reddened swelling of the nail folds, resembling a pyogenic paronychia which leads to thickening and transverse grooving of the nail and eventually loss of the nail (Jawetz, et al. 1989: 307).

#### 2.4.1.4 Chronic Mucocutaneous

This is a rare form of candidiasis, which is usually apparent in childhood (Greenwood, et al. 1995: 684). This disorder is a sign of deficiency of cellular immunity in children (Jawetz, et al. 1989: 307). The disease takes the form of a persistent granulomatous infection of the mouth, skin and nails (Greenwood, et al. 1995: 684).

### 2.4.2 Systemic Candidiasis

Systemic forms of candidiasis may be localized in the urinary system, liver, heart valves, meninges or the peritoneal cavity, or the infection may be disseminated and associated with septicaemia (candidaemia) (Greenwood, et al. 1995: 695). *Candida* species are the most important bloodstream pathogens that are being isolated with increasing frequency. A Canadian study concluded that candidemia is predominantly caused by *Candida albicans* and the mortality rate attributable to *Candida albicans* during the period of the study (1992-1994) was significant at 38% (Yamamura, et al. 1999).

### 2.4.3 *Candidia albicans* and Intensive Care Units

*Candida albicans* has been identified as the most frequently isolated organism in intensive care units. Treatment with broad spectrum antibiotics is the single most important factor in promoting the overgrowth of *Candida.* However, prolonged ventilation, the presence of intravascular or urinary catheters, total parenteral nutrition and immunosuppression induced by major surgery, trauma, burns, cancer, bacterial sepsis, diabetes, steroids, chemotherapy, and immunosuppressive treatment after transplantation heighten the risk of contracting candidal infection (Lipman and Saadia, 1997). According to Solomkin (1993) (in Lipman and Saadia, 1997), *Candida albicans* lives as a commensal in the gut lumen and on the mucocutaneous surfaces. Therefore, the susceptible host is infected either endogenously by organisms from a persons own gastrointestinal tract or exogenously through hand contact as a result of a breach in hospital policies for infection control.

### 2.4.4 Other

Candida arteritis is very rare (less than 1% of all reported cases). However *Candida albicans* has been identified in aortic and common femoral arterial cultures postoperatively and this invasive candida infection is potentially fatal (Sailors, et al. 1996).

Pulmonary infection by *Candida albicans* infection is also very rare. The yeast may persistently be present in the sputum because they have colonised pockets of secretion in the bronchial tree damaged by bronchiectasis,

tuberculosis or carcinoma. The sputum is often mucoid and gelatinous rather than purulent (Dugaid, et al. 1978:544).

According to Nassoura (1993) (in D'Amelio, et al. 1995), candiduria is common in patients with a bladder catheterisation and defects in neutrophil function. Candiduria is an early marker of systemic infection in patients with ongoing sepsis and organ failure.

## 2.5 Diagnosis

Specimens consist of swabs and scrapings from the surface lesions, sputum, exudates and material from removed intravenous catheters (Jawetz, et al. 1989: 307).

### 2.5.1 Microscopic Examination

Sputum, exudates and thrombi are examined in Gram stained smears for pseudohyphae and budding cells. Skin and nail scrapings are collected and placed in potassium hydroxide mounts for microscopic examination. (Jawetz, et al. 1989: 307.)

### 2.5.2 Culture

*Candida* species grow well on Sabouraud's medium or on bacteriological media such as blood agar at 25-37° C. *Candida albicans* can be identified by the germ tube test. This test involves incubating the yeast at 37° C in serum for one-and-a-half hours to two hours and under these conditions *Candida albicans* produces hyphae known as germ tubes. (Greenwood, et al. 1995: 695.)

### 2.5.3 Serology

Serological tests lack specificity and sensitivity and the results are interpreted with care (Greenwood, et al. 1995: 696).

### 2.5.4 Skin Tests

A candida test is almost universally positive in normal adults, and is therefore used only as an indicator of competent cellular immunity (Greenwood, et al. 1995: 696).

## 2.6 Treatment

### 2.6.1 Conventional Treatment

Cutaneous candidiasis is treated with a topical ointment. Mucosal infection reponds well to lozenges or a suspension of nystatin or amphotericin. Persistent infection and nail infection require oral ketoconazole or itraconazole 100mg daily. Systemic infections should be treated with intravenous miconazole or amphotericin and maintained on oral ketoconazole. (Edwards, et al. 1995: 144.)

The above antifungal agents are very effective. However it was only after 1951, when nystatin was extracted from Actinomyces species in soil and then developed into an antifungal agent, that multiple topical antifungal agents with several different mechanisms of action were developed (Diehl, 1996).

### 2.6.1.1 Nystatin

#### 2.6.1.1.1 Source

Nystatin is an antifungal substance produced by the growth of certain strains of *Streptomyces nourseii.* It mainly contains tetraenes and the principle component is nystatin $A_1$. Nystatin is a yellow to light brown hydroscopic powder with a characteristic odour suggestive of cereals. (Reynolds, 1989: 432.)

#### 2.6.1.1.2 Chemistry

Nystatin is a polyene antibiotic, which has a very large ring system linked to mycosamine, an amino sugar. The empirical formula for nystatin is $C_{46}$ $H_{77}$ $NO_{19}$ and it quickly decomposes in the presence of water or plasma (Meyers, et al. 1974: 558). It is stable in the dry form (Meyers, et al. 1974: 558) and should be stored at a temperature between 2° C and 8°C in an airtight container protected from light (Reynolds, 1989: 432).

#### 2.6.1.1.3 Antifungal Activity

Nystatin is both fungistatic and fungicidal (Diehl, 1996) and it has no effect on bacteria or protozoa but *in vitro* it inhibits many fungi (Meyers, et al. 1974: 588). It's main action is against *Candida* species (Reynolds, 1989: 432). *Candida* is sensitive *in vitro* to concentrations ranging from 1.5 to 6.5 μg/ml (Goodman and Gilman, 1980: 1222). A recent study conducted in Chile by Valcic, et al. (1999), compared the antifungal effects of propolis and nystatin, using the disc diffusion test. The results of this study revealed that propolis in comparison to nystatin has moderate inhibitory activity. The average zone of

inhibition for propolis was 12mm and nystatin produced an average zone of 16mm. This study reinforces the antifungal effects of nystatin. (Valcic, 1999.)

#### 2.6.1.1.4 Mechanism of Action

Nystatin interferes with the permeability of the cell membrane of sensitive fungi by binding to sterols, chiefly ergosterol (Reynolds, 1989: 432). The binding results in a change of the permeability of the cell membrane. The change is due to the appearance of polyenes which form pores and channels (Goodman and Gilman, 1980: 1222), which result in the loss of cations (Meyers, et al. 1974: 588).

#### 2.6.1.1.5 Absorption and Excretion

Nystatin is poorly absorbed from the gastrointestinal tract (Reynolds, 1989: 432). Virtually all the nystatin taken orally is excreted in the faeces (Meyers, et al. 1974: 588). It is not absorbed through the skin or mucous membranes when it is applied topically (Reynolds, 1989: 432).

#### 2.6.1.1.6 Effectivity

Research to determine the drug resistance of 93 *Candida* species strains against nystatin by means of the disc diffusion method proved that the drug is highly effective against 81.72% of the strains (Lisiak, et al. 2000).

#### 2.6.1.1.7 Preparations

Preparations of nystatin include ointments, suspensions and tablets for oral and vaginal use. Creams, powders, ointments and suspensions contain 100

000 units of nystatin per milliliter. Many topical preparations also contain antibiotics such as neomycin, gramicidin and triamcinolone acetonide (Goodman and Gilman, 1980:1222).

### 2.6.1.2 Uses of Administration

Nystatin is used for the prophylaxis and treatment of candidiasis of the skin and mucous membranes, especially that caused by *Candida albicans*. In the treatment of intestinal or oesophageal candidiasis, nystatin is given in doses of 500 000 or 1000 000 units by mouth 3 or 4 times a day. In infants and children a dosage of 10 000 units or more may be given 4 times daily. For the prophylaxis of intestinal candidiasis in adults nystatin may be given in a dosage of 1000 000 units daily. Nystatin is administered with non-absorbable antibiotics in various regimens to suppress the overgrowth of gastrointestinal flora especially in immunocompromised patients (Reynolds, 1989: 433).

For treatment of lesions in the mouth, pastilles or suspension may be given in a dosage of 100 000 units 4 times daily. Nystatin 100 000 units daily may be given to neonates for the prophylaxis of oral candidiasis (Reynolds, 1989: 433). For vaginal infections nystatin is administered in a dosage of 100 000 to 200 000 units daily as pessaries or vaginal cream. For cutaneous lesions, ointment, gel, cream, dusting powder containing 100 000 units per gram may be applied 2 to 4 times daily (Reynolds, 1989: 433).

### 2.6.2 Herbal Treatment

#### 2.6.2.1 Compositae

The Compositae (plants of the daisy family) form one of the largest families of flowering plants, with some 25000 species in over 1500 genera. The Compositae are also the largest family of the muscular plants and they are distributed in almost all habitats (Lawrence, 1963: 727). Members of this family occur from the polar regions to the tropics and may range over all habitats from dry desert to swamp, and from rainforests to mountain peaks. In many regions of the world they compromise up to a tenth of the flora (Hind, 1996).

The general characteristics of this family are as follows:

- plants of this family are mainly herbaceous and they usually have a taproot system;
- leaves are alternate or opposite and they are either simple or compound, frequently in basal rosettes;
- flowers exhibit inflorescence racemosa, the flower (florets) are arranged in heads, the heads (capitula) are grouped into large inflorescences. Each head consists of a group of florets, borne on a receptacle, surrounded by green bracts;
- the corolla consists of 5 united petals, with a short or long tube and a limb;
- five stamens with short filaments alternate with the corolla and the anthers forming a tube around the style;

- the ovary is inferior and unilocular containing one erect, anatropous ovule with basal placentation which consists of a simple style and 2 branching stigmas (Hickey and King, 1997: 147).

In general, many Compositae are used as herbal medicines all over the world. In Europe, *Arnica* has been used for centuries in wound healing. In Mexico, 200 species of herbs are used in folk medicine (Hind, 1996). However, research to understand the medicinal properties of these species is minimal. The significance of the present study is to further our understanding of *Compositae*, in particular *Arctium lappa, Calendula officinalis* and *Echinacea purpurea*.

#### 2.6.2.1.1 *Arctium Lappa*

Common name: Burdock.

##### 2.6.2.1.1.1 Botanical Description

*Arctium lappa* is a large biennial with stems that grow up to 1.5m in height (Chevalliar, 1996: 62). The leaves are broad, blunt and cordate, up to about 40cm long. The purple flowers are globular with hooked bracts forming burrs. The taproot is longitudinally furrowed externally and it is whitish or buff-coloured internally. The fruits are brownish-grey, wrinkled and about 6 mm long by 4mm broad. (Wren, 1988: 49.)

##### 2.6.2.1.1.2 Habitat and Cultivation

*Arctium lappa* is native to Europe and Asia, but mainly grows in temperate regions throughout the world (Chevalliar, 1996: 62). It is found in waste

places, ditches, by hedges and by waysides, mainly in deep, rich but neglected soils from lowland to submontane elevations (Stary, 1998: 50). *Arctium lappa* is cultivated in Europe and China and is propagated from the seed in spring. The seeds are harvested in summer and the whole plant is dug up in midsummer. (Chevalliar, 1996: 62.)

#### 2.6.2.1.1.3 Parts Used

Roots, seeds and leaves (Hoffman, 1996: 61).

#### 2.6.2.1.1.4 History and Traditional Uses

In traditional herbal texts, *Arctium lappa* root is described as a "blood purifier" or an "alterative", it is believed to clear the bloodstream of toxins. It was used both internally and externally for eczema and psoriasis, as well as to treat painful joints and as a diuretic (Hobbs, 1990). *Arctium lappa* is a traditional remedy for gout, fevers and kidney stones. In the17$^{th}$ century, Culpeper wrote that the seed was commended to break kidney stones and cause them to be expelled by urine (Chevalliar, 1996: 62).

#### 2.6.2.1.1.5 Constituents

- Lignans, including arctigenin, its glycoside arctiin and matairesinol (Hoffmann, 2000a).
- Polyacetylenes, in the root, mainly tridecadienetetraynes and tridecatrienetriynes, with sulphur containing arctic acid (Wren, 1988: 49).
- Sequiterpenes, in the leaves including arctiol, β-eudesmol, fukinone, fukinanolide and derivatives, petasitolone and eremophillene (Wren,

1988: 49).

- Amino acid such as γ-guanidino-n-butyric acid (Hoffmann, 2000a).
- Inulin (up to 50%) in the roots (Wren, 1988: 62).
- Miscellaneous organic acids, fatty acids and phenolic acids; including acetic, isovaleric, lauric, myristic, caffeic and chlorogenic acid (Hoffman, 2000a).

#### 2.6.2.1.1.6 Medicinal Uses

The traditional uses of *Arctium lappa* have not changed in modern times. *Arctium lappa* is still used as a detoxifying herb in both Western and Chinese herbal medicine. The seeds are used to remove toxins in fevers and infections such as mumps and measles (Chevalliar, 1996: 62). The tonifying action of *Arctium lappa* increases it's ability to breakdown toxins and eliminate them from the blood, as a result it is known as a blood purifier (Hobbs, 2000).

A recent study strongly suggests that *Arctium lappa* has anti-inflammatory and free radical scavenger effects (Chuang, et al. 1996). In order to investigate the anti-inflammatory and liver protective effects of *Arctium lappa,* carrageenan-induced rat paw edema and $CCl_4$-induced hepatotoxicity models were used. Subcutaneous administration of *Arctium lappa* extract significantly decreased carrageenan-induced rat paw edema and when simultaneously treated with $CCl_4$, it produced pronounced activities against CCl4-induced acute liver damage. The free-radical scavenging activity *Arctium lappa* extract was examined by means of an electron spin resonance (ESR) spectrometer. The readings obtained on superoxide and hydroxyl scavenger activity was 2.06

mg/m and 11.8 mg/ml respectively which indicates that *Arctium lappa* has free radical scavenger effects as well. (Chuang, et al. 1996.)

These properties of *Arctium lappa* make it most valuable for the treatment of skin conditions which result in dry and scaly skin. It is most effective for psoriasis, as well as for rheumatic complaints associated with psoriasis (Hoffmann, 1996: 61). The diuretic, antibiotic and mildly bitter actions of *Arctium lappa* account for its action on the skin and they are also the key factors involved in treating acne, boils, abscesses, local skin infections and eczema (Chevalliar, 1996: 62). The high amount of inulin and mucilage in the root is responsible for soothing effects in the gastrointestinal tract. The bitter constituents of the root stimulate the secretion of the digestive juices and bile which increase the absorption of fat-soluble nutrients and this aids digestion (Hobbs, 2000). The unstable polyacetylenic compounds are reputed to have antiseptic and fungicidal properties (Stary, 1988: 50).

Research indicates that *Arctium lappa* has hypoglycemic effects as well as antitumour action (Chevalliar, 1996: 62). The antitumour action is due to an uncharacteristic desmutagenic factor of high molecular weight, that reduces the effect of a number of mutagens, including those that do not require metabolic action (Wren, 1988: 50). *Arctium lappa* in combination with *Rheum palmatum, Rumex acetosella* and *Ulmus fulva* has been used for more than 70 years in Canada as an alternative to conventional cancer therapy (Kaegi, 1998). The herbs in combination, are marketed as a product known as Essiac. Essiac reputedly strengthens the immune system, improves appetite, relieves

pain and improves the overall quality of life. Claims have also been made that Essiac reduces tumour size and prolongs the lives of people with cancer. An unpublished Canadian study of the effectiveness of orally administered Essiac, revealed no clinical benefits in terms of survival and tumour regression but some subjective improvements in control and well-being were documented (Kaegi, 1998).

*Arctium lappa* has also been screened for anti-HIV activity (Chang and Yeung, 1988). Researchers assayed 27 Chinese herbs by adding HIV to cell cultures in the presence of the largest non-toxic concentration of the extract. Every assay was repeated three times. To be considered as having anti-HIV activity, every one of the three assays had to reduce the percentage of infected cells by at least three standard deviations from the average value in control assays. The five best herbs were *Arctium lappa*, *Viola yedoensis*, *Andrographis paniculata*, *Lithospermum erythrorhizon* and *Alternanthera philoxeroides*. Although these herbs showed significant activity in the laboratory, this does not mean that it will have the same effect on humans. Human trials however cannot be ethically carried out therefore it is difficult to clinically test the effect of these herbs in patients (Chang and Yeung, 1988).

#### 2.6.2.1.1.7 Preparations

- A decoction of the root is made by putting 1 teaspoon of the root into a cup of water bringing it to the boil and letting it simmer for 10-15 minutes. This should be drunk 3 times a day (Hoffmann, 1996: 61).

- Tincture should be taken as 20 drops 2-3 times daily (Hoffmann, 1996: 61).

#### 2.6.2.1.2 *Calendula officinalis*

Common name: Marigold.

##### 2.6.2.1.2.1 Botanical Description

*Calendula* is an annual garden plant with an anular, branched, hairy stem that is 60cm high. The leaves are alternate, sessile, spatulate or oblanceolate, dentate with widely spaced teeth (Lord, 2001). The flowerheads of *Calendula* are bright yellow or orange. Ligulate, florets usually detach from the ovary, these are 15-25mm long and about 3mm broad. They have four or five veins and an entire margin with a short corolla tube containing the bifid stigma and style (Wren, 1988: 184).

##### 2.6.2.1.2.2 Habitat and Cultivation

*Calendula* is native to southern Europe and is cultivated in temperate regions around the world. It is easily propagated from the seed and it flourishes in almost all soils (Chevalliar, 1996: 69). Either the whole flower tops or just the petals are collected between early summer and early autumn. They are dried with great care to ensure that there is no discolouration (Hoffmann, 1996: 71).

##### 2.6.2.1.2.3 Parts Used

Petals and flowerheads (Wren, 1988: 184).

#### 2.6.2.1.2.4 History and Traditional Uses

Historically, *Calendula* is found to be antispasmodic, aperient, cholagogue, diaphoretic and vulnerary (Lord, 2001). *Calendula* flowers were historically considered beneficial for reducing inflammation, for wound healing and as an antiseptic. *Calendula* has also been used to treat skin conditions ranging from skin ulcerations to eczema (Hoffmann, 2000b).

#### 2.6.2.1.2.5 Chemical Constituents

- Triterpenes, pentacyclic alcohols such as faradol, brein, arnidiol, erythrodiol, calenduladiol, heliantriol Cand F, ursatriol, longispinogenine.
- Calendudulosides A-D.
- α- and β-amyrin, taraxasterol, τ-taraxasterol and lupeol.
- Flavonoids; isohamnetin glycosides including narcissin and quertin glycosides including rutin.
- Volatile oil.
- Chlorogenic acid.

  (Wren, 1988: 184).
- Bitter glycosides.
- Sterols.
- Mucilage.
- Carotenes.

  (Chevalliar, 1996: 69).

#### 2.6.2.1.2.6 Medicinal Uses

Internally *Calendula* infusions or tinctures are used for inflammatory problems of the digestive system such as gastritis, peptic ulcers, regional ileitis and colitis (Chevalliar, 1996: 69). As a cholagogue it helps to relieve gall-bladder problems and indigestion (Hoffmann, 1996: 71). *Calendula* is above all a remedy for the skin (Chevalliar, 1996: 71). The anti-inflammatory effects of *Calendula* are seen in the treatment of dermatological disorders. The constituents responsible for these effects are the saponins and the polysaccharides. A study to evaluate the anti-inflammatory effects of *Calendula* was conducted using $CO_2$ extracts of *Calendula* flowers (Della, et al. 1994). The *Calendula* extracts were tested on induced dermatitis of the mouse ear, using bioassay-orientated fractionation principles to isolate the active principles. The effectiveness of the extracts were measured by the degree to which they reduced edema in the test animal. The fraction which caused the greatest inhibition was found to be the triterpenic compounds. These compounds consist mainly of farodial monoester and it was concluded that these constituents are the main anti-inflammatory principles of *Calendula*. (Della, et al. 1994.)

*Calendula* is the ideal remedy for first-aid treatment of minor burns and scalds. The astringent action of *Calendula* on the capillaries enables it to be used externally for bleeding, wounds, bruising and strains. It is safely used wherever there is inflammation of the skin due to physical damage or infection (Hoffmann, 1996: 71). The wound healing effects of *Calendula* were researched in a controlled study involving standard wound models of male

albino rats (Gurumadhva, 1991). Mother tinctures of *Hypericum* and *Calendula* was used in this investigation. The *Calendula* tincture was applied topically to incision and excision wounds. The *Hypericum* was diluted a further 10 times and was given orally in a dose of 1ml for incision, excision and dead space wounds. *Hypericum* and *Calendula* were found to have had significant wound healing capacity. Both reduced the time of epithelisation and they also improved wound contraction (Gurumadhva, 1991).

*Calendula* is used for fungal infections such as ringworm, athlete's foot and thrush. It is reputed to be helpful for diaper rash and cradle cap, and it soothes nipples that are sore from breast-feeding (Chevalliar, 1996: 69).

Gynaecologically, *Calendula* has a mild estrogenic action and is used to reduce menstrual pain and regulate menstrual bleeding. It can also be used effectively as a douche for yeast infections (Chevalliar, 1996: 69). However, an *in vitro* study comparing the inhibitory effects of various plant extracts on the growth of *Candida albicans,* found *Calendula officinalis* to have no inhibitory characteristics (McFadden, 1995). The agar diffusion method was used to evaluate the depth of inhibition of the herbal extracts. The *Calendula* tincture used in the McFadden study was diluted at a ratio of 1: 8 with distilled water because he considered this dilution an average for topical applications e.g. mouth washes and vaginal douches. (McFadden, 1995.) The present study used mother tincture.

*Calendula* has a detoxifying effect and helps to treat the toxicity that underlies fevers and infections. The herb is also considered cleansing for the liver and gallbladder and is used to treat problems affecting these organs (Chevalliar, 1996: 69).

#### 2.6.2.1.2.7 Preparations

- An infusion is prepared by pouring a cup of boiling water onto 1-2 teaspoons of the petals, leaving it to infuse for 10-15 minutes. This should be drunk 3 times daily (Hoffmann, 1996: 71).
- The tincture should be taken 1-4ml, 3 times daily (Hoffmann, 1996: 71).
- Ointments and infused oils can be used externally to the affected area (Chevalliar, 1996: 69).

### 2.6.2.1.3 *Echinacea purpurea*

Common name: purple coneflower.

#### 2.6.2.1.3.1 Botanical Description

*E. purpurea* belongs to the **Echinacea** genus. Plants of this genus are perennials with vertical or horizontal roots. The stems are erect with rough, coarse hairs. The leaves are ovate to lanceolate or elliptical, entire, pubescent or smooth. A solitary flower is produced atop a long peduncle. *E. purpurea* differs from others of this genus in that it has a fibrous root in comparison to others which have a taproot system (McLaughlin, 1992). Another distinguishing feature of *E. purpurea* is a distinct purple coneflower (Ness, et al. 1999).

#### 2.6.2.1.3.2 Habitat and Cultivation

*E. purpurea* is native to the central parts of the United States, but it is also commercially cultivated in Europe (Chevalliar, 1996: 90). The natural range of *E. purpurea* is quite broad. In the wild it is a plant of the ecotone, preferring the shaded edges of the savannas and glades and open woodlands with partial sun exposure. *E. purpurea* is the most widely used and the most widely cultivated medicinal species of the genus. *E. purpurea* grows easily from the seeds and is drought tolerant. It will grow in full sun or partial shade and it thrives on neglect. (McLaughlin, 1992.)

#### 2.6.2.1.3.3 Parts Used

Cone flowers and the roots (Hoffmann, 1996: 87).

#### 2.6.2.1.3.4 History and Traditional Uses

Native Americans, the Commanche, used *E. purpurea* as a remedy for toothache and sore throats, and the Sioux used it for rabies and snakebites (Chevalliar, 1996: 90). *E. purpurea* was adopted by the early settlers for use as an antiseptic for abscesses and boils. The eclectic practitioners contended that the main action of *E. purpurea* was to balance changes in the body fluids produced by either internal or external causes. It was most successful in treating cases of blood poisoning and it was useful against cancerous growth especially of the mucous membranes. It was also used to lessen the pain and inflammation of gonorrhea and syphilis, tonsillitis and skin disorders i.e. psoriasis, acne and poison ivy irritation. (McLaughlin, 1992.)

#### 2.6.2.1.3.5 Chemical Constituents

- Polysaccharides – *E. purpurea* contains high molecular weight polysaccharides in the aerial parts of the plant. These polysaccharides are water soluble, acidic and have branched-chain heteroglycans which are composed of many types of sugars (Murray, 1995: 93).
- Flavanoids – The stems and the leaves of *E. purpurea* contain numerous flavanoids, with rutoside being the most abundant. The total flavanoid content is 0.38% (Murray, 1995: 93).
- Caffeic acid derivatives – Echinoside, cichoric acid, chlorogenic acid and cynarin are the caffeic acid derivatives present in *E. purpurea.* Cichoric acid is found in high concentrations in *E. purpurea,* and is responsible for stimulatory phagocytosis of granulocytes *in vitro.* (Murray, 1995: 93.)
- Essential oil – These contain humulene, caryophyllene and it's epoxide, germacrene D, and methyl-*p*-hydroxycinnamate (Wren, 1988: 105).
- Alkamides – The most commonly isolated alkamides are isobutylamides with olefinic and acetylenic bonds (Chevalliar, 1996: 90). They cause a tingling sensation on the tongue which is representative of their mild anaesthetic effect (Murray, 1995: 95).
- Miscellaneous chemicals – Vanillin, linolenic acid derivatives, a labdane derivative, alkanes and flavonoids, and the alkaloids tussilagine and isotussilagine (Wren, 1988: 95). Other compounds isolated include resins, glycoproteins, sterols, minerals, fatty acids (Murray, 1995: 95) as well as echinolone and betaine (Chevalliar, 1996: 90).

#### 2.6.2.1.3.6 Medicinal Uses

The main action of the constituents of *E. purpurea* is to stimulate the immune system (Iwu, 1993: 175) and to rid the body of both bacterial and viral infection (Hoffmann, 1996: 87). It does this by stimulating the production of white blood cells, thereafter boosting their activity once they exist (Schar, 1999: 107). The polysaccharides of *E. purpurea* have an anti-hyaluronidase action. The enzyme hyaluronidase increases connective tissue permeability and allows the organism to become more invasive, therefore inhibition of this enzyme will inhibit the ability of viruses to enter and take over the cells (Murray, 1995: 99).

According to Murray, the polysaccharides of *E. purpurea* have shown the ability to enhance macrophage phagocytosis and stimulate the macrophages to produce a number of immunpotentiating compounds. Research indicates that the macrophages can destroy tumour cells and inhibit *Candida albicans* infection. A study carried out over a six month period demonstrates that fresh-pressed juice of *E. purpurea* greatly accentuates the efficacy of a topical antimycotic agent, decreasing the recurrence of Candida *albicans* (Coeugniet and Kuhnast, 1986).

(See Table 2.1).

**Table 2.1** Treatment of recurrent candidiasis with fresh–pressed juice of *Echinacea purpurea.*

| THERAPEUTIC SCHEME | NO. OF PATIENTS | RECURRENCE RATE (%) |
|---|---|---|
| Topical antimycotic alone | 43 | 60.5 |
| Topical antimycotic + sub-cutaneous injection of fresh-pressed juice of *E. purpurea* | 20 | 15.0 |
| Topical antimycotic + intra-muscular injection of fresh-pressed juice of *E. purpurea* | 60 | 5.0 |
| Topical antimycotic + intra-venous injection of fresh-pressed juice of *E. purpurea* | 20 | 15.0 |
| Topical antimycotic + oral fresh-pressed juice of *E. purpurea* | 60 | 16.7 |

An *in vitro* study further reinforces the antifungal effects of *E. purpurea* (Roesler, et al. 1991). Purified polysaccharide cell cultures of *E. purpurea* were investigated for their ability to enhance phagocytotic activity. Macrophages from different organ origins were activated by *E. purpurea* to produce Interleukin-1, Tumour Necrosis Factor $\alpha$ and Interleukin-6, which in

turn elevated the amount of reactive carbon dioxide intermediates and inhibited the growth of *Candida albicans in vitro* (Roesler, et al. 1991).

*E. purpurea* is effectively used in the treatment of boils, septicemia and other infections. Chronic infections such as postviral fatigue syndrome, as well as chilblains, colds, influenza, skin disorders and respiratory problems can also be treated with *E. purpurea* (Chevalliar, 1996: 90). *E. purpurea* is most useful for upper respiratory infections, such as laryngitis, tonsillitis and for catarrhal infections of the nose. The tincture may be used as a mouthwash in the treatment of pyorrhoea and gingivitis (Hoffmann, 1996: 87). Intensive research into the use of *E. purpurea* for colds and upper respiratory infections has been carried out over the past few years. One such study was carried out on 108 patients who had reported more than three attacks of respiratory infections or the common cold in the preceeding year. These patients randomly received either 4 ml of fluid extract of *E. purpurea* or placebo juice (20% alcohol) for eight weeks. The outcome of this study showed that there were no significant differences in the incidence, duration or severity of colds or respiratory infections between the two groups. During the treatment period, 35 patients in the treatment group and 40 patients in the placebo group had at least one cold or respiratory infection. The colds and respiratory infections lasted a median of 4.5 days in the treatment group and 6.5 days in the placebo group (Grimm and Muller, 1999).

#### 2.6.2.1.3.7 Preparations

- Decoction - Put 1-2 teaspoonfuls of the root in one cup of water brought slowly to the boil and simmered for 10-15 minutes. This should be drunk 3 times daily (Hoffmann, 1996: 87).
- Tincture – 1-4ml of the tincture should be taken 3 times daily (Hoffmann, 1996: 87).

# CHAPTER THREE

## Methodology

### 3.1 The Data

This research involved two types of data: primary and secondary.

#### 3.1.1 The Primary Data

1. Results of the experiment determining the effects of *Arctium lappa* in 62% ethanol (v/v) on *Candida albicans*.
2. Results of the experiment determining the effects of *Calendula officinalis in 62%* ethanol(v/v) on *Candida albicans*.
3. Results of the experiment determining the effects of *Echinacea purpurea* in 62% ethanol (v/v) on *Candida albicans*.
4. Results of the effects of 62% ethanol (v/v) on *Candida albicans*.
5. Results of the effects of nystatin on *Candida albicans*.

#### 3.1.2 The Secondary Data

Research articles from journal publications, books and manuals.

### 3.2 Materials and Methods

#### 3.2.1 Preparation of the Media

The media was prepared according to the manufacturers directions (Oxoid Manual, 1979: 238) as follows:

1. 32.5 grams of Sabouraud dextrose agar was weighed and added to 0.5 litres of distilled water in a screw top flask.
2. A magnetic stirrer was added to the bottom of the flask to aid the dissolving process. The mixture was then shaken vigorously until the agar was dissolved.
3. The mixture was autoclaved for 15 minutes at 121° C.
4. The flask was removed from the autoclave and allowed to cool in a beaker of cold water.
5. Once the flask was cool enough to touch, the plates were poured as follows:

5.1 The top of the flask was flamed with a Bunsen burner to prevent contamination, before the agar was poured into the plates.

5.2 Each plate was poured to a depth of +/- 5mm.

5.3 The plates were stacked and allowed to cool and solidify. A total of 15 plates were obtained from 0.5 litres of liquid agar.

5.4 The plates were visually examined for contamination.

### 3.2.2 Preparation of Inoculum

1. A specimen of *Candida albicans* was transferred from a Sabouraud dextrose agar plate into 10ml of Sabouraud dextrose liquid medium using sterile techniques.
2. The culture was swirled to allow even distribution of the culture.
3. 1ml of the liquid medium was aseptically pipetted into a test tube containing 9ml of saline solution.
4. The culture was then vortexed to enable adequate mixing.

5. A spectrophotometer was used to measure the optical density of the *Candida albicans* suspension as follows:

5.1 The Spectronic 20 was switched on 20 minutes before use.

5.2 The wavelength was set at 100nm and the % Transmittance was set at 0 using the right hand dial.

5.3 10ml of saline was poured into a sample test, this being the blank. The outside of the test tube was wiped and was placed into the spectrophotometer.

5.4 % Transmittance was set at 100 using the right hand dial, this is inversely proportional to the optical density, which was 0 for the blank.

5.5 The blank was removed from the holder and the test tube containing the *Candida albicans* was given a swirl. The test tube was thereafter wiped and inserted into the holder of the spectrophotometer.

5.6 The optical density reading obtained was 0.05 % Transmittance (Cappuccino and Sherman, 1992: 77-89).

The purpose of finding the optical density of a culture is to standardise the amount of inoculum used, to allow for replication of the test.

### 3.2.3 Preparation of the Filter Paper Discs

Whatman No.3 filter paper was punched into discs 5mm in diameter. The discs were placed in a flask and were autoclaved at 121° C for 15 minutes to ensure that they were sterile.

### 3.2.4 Preparation of Test Substances

Commercially available herbal extracts (*Arctium lappa*, *Calendula officinalis* and *Echinacea purpurea*) respectively, with an ethanol concentration of 62% v/v were used as the test substances. The herbal extracts were purchased from Parceval (Pty) Ltd. The herbal extracts were prepared according to the German Herbal Pharmacopoeia standards (1991).

*Calendula officinalis* extract (Batch No. 09928) and *Echinacea purpurea* extract (Batch No. 02904) was prepared according to method HAB 3a : Extract from fresh plant with 33,3 parts of plant to 66,7 parts of alcohol. Final alcohol concentration was 62% w/w (Smidt, 2001). *Arctium lappa* extract (Batch No. 03003), was prepared according to method HAB 4a : Extract from fresh plant with 10 parts dried plant to 100 parts of alcohol. Final alcohol concentration was 62% w/w (Smidt, 2001).

### 3.2.5 Preparation of the Control Substances

- Nystatin, the allopathic component of the experiment served as the positive control. Commercially available nystatin suspension (Batch No. B006391) manufactured by Bristol-Myers Squibb (Pty) Ltd, was used.
- 62% (v/v) ethanol (negative control) was prepared according to the German Homoeopathic Pharmacopoeia standards (British Homeopathic Association, 1985: 5): 65.90ml 65.90ml of 96% (v/v) ethanol is diluted with sufficient distilled water to produce 100ml of 62% ethanol. The weight per ml as per hydrometer reading was between 0.8885 to 0.8864 grams.

### 3.2.6 Determination of the Antifungal Activity

The *in vitro* antifungal activity of *Arctium lappa* tincture, *Calendula officinalis* tincture and *Echinacea purpurea* tincture respectively, were determined using the disc diffusion method. The methodology below follows Cappuccino and Sherman (1992: 247-254).

#### 3.2.6.1 Inoculation of Agar Plates

A. Using sterile techniques the prepared Sabouraud dextrose agar plates were inoculated with *Candida albicans*.

B. A sterile cotton swab was dipped into a test tube containing *Candida albicans* culture, which had a premeasured optical density of 0.05 % Transmittance.

C. The swab was removed from the test tube ensuring that the excess inoculum was removed by pressing the saturated swab against the inner wall of the test tube.

D. The agar surface was then streaked using the swab, the entire surface was streaked in a vertical and then horizontal direction in order to ensure a confluent lawn of growth over the entire surface.

### 3.2.6.2 Placement and Impregnation of discs

A. The under surface of the plates were marked with a marker to represent the disc positions of the test and control substances.

B. Five sterile filter paper discs of uniform size (5mm), were placed onto the premarked agar plates by means of sterile forceps.

C. The five discs were placed 10mm apart from each other. They were gently placed onto the agar and not pressed into the agar.

D. A premeasured amount of 7 microlitres of each of the test substances and the controls were pipetted onto seperate filter discs.

E. The experiment was repeated 15 times to ensure that the results were statistically viable.

### 3.2.6.3 Incubation and Recording of Results

A. The agar plates were therefore covered and incubated for 36 hours at 37°C.

B. The plates were examined at 18, 24 and 36 hours for the presence of growth inhibition areas, which were indicated by a clear zone surrounding each disc. The susceptibility of *Candida albicans* to a substance is determined by the size of the zone of inhibition. A small or no zone of inhibition indicates resistance, a large zone indicates that the organism is susceptible to the test substance.

C. The diameters of the zones of inhibition were measured in millimeters using a transparent ruler. Three readings of the diameter around each disc were obtained and an average of the three was recorded on a table (Appendix A).

D. Photographs for observations at 18 hours were taken as a visual record (Appendix B).

### 3.2.7 Statistical Procedures

#### 3.2.7.1 Procedure 1.1

**Intergroup comparison between *Arctium lappa* in 62% ethanol (v/v) and 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs.**

The Mann-Whitney U-test was used to compare *Arctium lappa* in 62% ethanol (v/v) and 62% ethanol (v/v) with regard to observations at 18 hrs, 24hrs and 36 hrs.

- Hypothesis testing

  The null hypothesis $H_0$, states that there is no difference in diameter of the zone of inhibition between the herbal extract and 62% ethanol (v/v) with respect to the variable of comparison at the $\alpha = 0.05$ level of significance. The alternative hypothesis $H_1$, states that there is a difference at the same level of significance.

  $H_0$: $M_1 = M_2$

  $H_1$: $M_1 \neq M_2$

- Decision rule

  At $\alpha = 0.05$ level of significance, the null hypothesis is rejected if $P < \alpha$ where P is the observed significance level or probability value. Otherwise the null hypothesis is accepted at the same level of significance.

  Reject $H_0$ if $P < \alpha$

  Accept $H_0$ if $P \geq \alpha$

  P is the observed significance level or probability value (Fisher and van Belle, 1993: 315).

### 3.2.7.2 Procedure 1.2

**Intergroup comparison between *Calendula officinalis* in 62% ethanol (v/v) and 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs.**

The Mann-Whitney U-test was used to compare *Calendula officinalis* in 62% ethanol (v/v) and 62% ethanol (v/v) with regard to observations at 18 hrs, 24hrs and 36 hrs.

- Hypothesis testing

  As per Procedure 1.1.

- Decision rule

  As per Procedure 1.1.

### 3.2.7.3 Procedure 1.3

**Intergroup comparison between *Echinacea purpurea* in 62% ethanol (v/v) and 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs.**

The Mann-Whitney U-test was used to compare *Echinacea purpurea* in 62% ethanol (v/v) and 62% ethanol (v/v) with regard to observations at 18 hrs, 24hrs and 36 hrs.

- Hypothesis testing

  As per Procedure 1.1.

- Decision rule

  As per Procedure 1.1.

### 3.2.7.4 Procedure 1.4

**Intergroup comparison between *Arctium lappa* in 62% ethanol (v/v) and nystatin with regard observations at 18hrs, 24hrs and 36hrs.**

The Mann-Whitney U-test was used to compare *Arctium lappa* in 62% ethanol (v/v) and nystatin with regard to observations at 18 hrs, 24hrs and 36 hrs.

- Hypothesis testing

  The null hypothesis $H_0$, states that there is no difference in diameter of the zone of inhibition between the herb and nystatin, with respect to the variable of comparison at the $\alpha = 0.05$ level of significance. The alternative hypothesis $H_1$, states that there is a difference at the same level of significance.

$H_0$: $M_1 = M_2$

$H_1$: $M_1 \neq M_2$

- Decision rule

  As per Procedure 1.1.

### 3.2.7.5 Procedure 1.5

**Intergroup comparison between *Calendula officinalis* in 62% ethanol (v/v) and nystatin with regard to observations at 18hrs, 24hrs and 36hrs.**

The Mann-Whitney U-test was used to compare *Calendula officinalis* in 62% ethanol (v/v) and nystatin with regard to observations at 18 hrs, 24hrs and 36 hrs.

- Hypothesis testing

  As per Procedure 1.4.

- Decision rule

  As per Procedure 1.1.

### 3.2.7.6 Procedure 1.6

**Intergroup comparison between *Echinacea purpurea* in 62% ethanol (v/v) and nystatin with regard to observations at 18hrs, 24hrs and 36hrs.**

The Mann-Whitney U-test was used to compare *Echinacea purpurea* in 62% ethanol (v/v) and nystatin with regard to observations at 18 hrs, 24hrs and 36 hrs.

- Hypothesis testing

  As per Procedure 1.4.

- Decision rule

  As per Procedure 1.1.

### 3.2.7.7 Procedure 1.7

**Intergroup comparison between *Arctium lappa* in 62% ethanol (v/v) and *Echinacea purpurea* in 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs.**

The Mann-Whitney U-test was used to compare *Arctium lappa* in 62% ethanol (v/v) and *Echinacea purpurea* in 62% ethanol (v/v) with regard to observations at 18 hrs, 24hrs and 36hrs.

- Hypothesis testing

  As per Procedure 1.7

- Decision rule

  As per Procedure 1.1.

### 3.2.7.8 Procedure 1.8

**Intergroup comparison between *Arctium lappa* in 62% ethanol (v/v) and *Calendula officinalis* in 62% ethanol (v/v) with regard to observations at 18 hrs, 24hrs and 36hrs.**

The Mann-Whitney U-test was used to compare *Arctium lappa* in 62% ethanol (v/v) and *Calendula officinalis* in 62% ethanol (v/v) with regard to observations at 18 hrs, 24hrs and 36 hrs.

- Hypothesis testing

  As per Procedure 1.7

- Decision rule

  As per Procedure 1.1.

### 3.2.7.9 Procedure 1.9

**Intergroup comparison between *Echinacea purpurea* in 62% ethanol (v/v) and *Calendula officinalis* in 62% ethanol (v/v) with regard to observations at 18 hrs, 24hrs and 36hrs**

The Mann-Whitney U-test was used to compare *Echinacea purpurea* in 62% ethanol (v/v) and *Calendula officinalis* in 62% ethanol (v/v) with regard to observations at 18 hrs, 24hrs and 36hrs.

- Hypothesis testing

  As per Procedure 1.7

- Decision rule

  As per Procedure 1.1.

### 3.2.7.10 Procedure 1.10

**Intergroup comparison between *Arctium lappa* in 62% ethanol (v/v), *Calendula offficinalis* in 62% ethanol (v/v) *and Echinacea purpurea* in 62% ethanol (v/v) respectively with regard to observations at 18hrs, 24hrs and 36hrs.**

The Kruskal-Wallis non-parametric Analysis of Variance by Ranks method was used to compare the diameter of the zone of inhibition of the herbal

extracts to each other, with regard to observations at 18hrs, 24hrs and 36hrs. In each test, the null hypothesis states that there is no difference in diameter among the means of the herbal extracts. The alternative hypothesis states that there is a difference among the means.

$H_0$: $M_1 = M_2 = M_3$

$H_1$: All three means are not equal (at least one mean differs from the rest)

- Decision rule

At $\alpha = 0.05$ level of significance, the null hypothesis is rejected if $P < \alpha$ where P is the observed significance level or probability value. Otherwise the null hypothesis is accepted at the same level of significance.

Reject $H_0$ if $P < \alpha$

Accept $H_0$ if $P \geq \alpha$

P is the observed significance level or probability value

(Fisher and Van Belle, 1993: 315).

### 3.2.7.11 Procedure 1.11

**Intragroup comparison of *Arctium lappa* in 62% ethanol with regard to observations at 18hrs, 24hrs and 36hrs.**

The Friedman's T test was used to compare the diameter of the zones of inhibition at the different time intervals. In each test the null hypothesis states that there is no difference in the diameter of the zones of inhibition of *Arctium lappa* in 62% ethanol (v/v) during the different time intervals, at $\alpha$ level of significance. The alternative hypothesis states that at least two time intervals will differ significantly.

- Decision rule

At $\alpha = 0.05$ level of significance, the null hypothesis is rejected if $P < \alpha$, where P is the observed significance level or probability value. Otherwise the null hypothesis is accepted at the same level of significance.

Reject $H_0$ if $P < \alpha$

Accept $H_0$ if $P \geq \alpha$

P is the observed significance level or probability value

(Fisher and Van Belle, 1993: 430).

If $H_0$ is rejected, then the Dunn procedure will be applied to establish which two time intervals are significantly different.

Dunn procedure for use with Friedman's T test:

If the null hypothesis $H_0$ is rejected for Friedman's T test, then a multiple comparison procedure will have to be applied to determine which of the treatments are significantly different. Let $R_j$ and $R_j^1$ be the $j^{th}$ and $j^{1th}$ treatment ranks. Let $\alpha$ be the experiment wise error rate. Usually $\alpha = 0.10$

If $| R_j - R_j^1 | \geq z \sqrt{bk(k+1)/6}$, then $R_j$ and $R_j^1$ are declared significant

In the above formula:

b = the number of blocks

k = the number of agar plates

z = 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 (Fisher and van Belle, 1993: 432).

### 3.2.7.12 Procedure 1.12

**Intragroup comparison of *Calendula officinalis* in 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs.**

The Friedman's T test was used to compare the diameter of the zones of inhibition at the different time intervals. In each test the null hypothesis states that there is no difference in the diameter of the zones of inhibition of *Calendula officinalis* in 62% ethanol (v/v) during the different time intervals, at $\alpha$ level of significance. The alternative hypothesis states that at least two time intervals will differ significantly.

- Decision rule

  As per Procedure 1.11.

### 3.2.7.13 Procedure 1.13

**Intragroup comparison of *Echinacea purpurea* in 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs.**

The Friedman's T test was used to compare the diameter of the zones of inhibition at the different time intervals. In each test the null hypothesis states that there is no difference in the diameter of the zones of inhibition of *Echinacea purpurea* in 62% ethanol (v/v) during the different time intervals, at $\alpha$ level of significance. The alternative hypothesis states that at least two time intervals will differ significantly.

- Decision rule

  As per Procedure 1.11.

### 3.2.7.14 Procedure 1.14

**Intragroup comparison of 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs.**

The Friedman's T test was used to compare the diameter of the zones of inhibition at the different time intervals. In each test the null hypothesis states that there is no difference in the diameter of the zones of inhibition of 62% ethanol (v/v) during the different time intervals, at $\alpha$ level of significance. The alternative hypothesis states that at least two time intervals will differ significantly.

- Decision rule

  As per Procedure 1.11.

### 3.2.7.15 Procedure 1.15

**Intragroup comparison of nystatin with regard to observations at 18hrs, 24hrs and 36hrs.**

The Friedman's T test was used to compare the diameter of the zones of inhibition at the different time intervals. In each test the null hypothesis states that there is no difference in the diameter of the zones of inhibition of nystatin during the different time intervals, at $\alpha$ level of significance. The alternative hypothesis states that at least two time intervals will differ significantly.

- Decision rule

  As per Procedure 1.11.

### 3.2.7.16 Procedure 2

**Comparison using barcharts**

Visual summaries of the analytical findings are given by means of barcharts. Average readings were used to construct the barcharts represented.

### 3.2.7.17 Statistical Package

The statistical package Statistical Package for Social Sciences (SPSS) version 9 was used for data entry and analysis.

# CHAPTER FOUR

## Results

### 4.1 Criteria Governing the Admissibility of Data

Only data obtained from the laboratory experiments carried out by the researcher at the Department of Biotechnology, Technikon Natal was used.

## 4.2 Statistical Analysis of Results

### 4.2.1 Procedure 1.1 - 1.3

**See Table 4.1 and Figure 4.1**

**Table 4.1** Intergroup comparison between *Arctium lappa, Calendula officinalis* and *Echinacea purpurea* in 62% ethanol (v/v) respectively and 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs. (Mann-Whitney U-Test)

| **HERBS vs 62% ETHANOL (v/v)** | **TIME** | **P-VALUE** |
|---|---|---|
| Procedure 1.1<br>*Arctium lappa*<br>vs<br>62% ethanol (v/v) | 18hrs<br>24hrs<br>36hrs | 0.05<br>1.000<br>1.000 |
| Procedure 1.2<br>*Calendula officinalis*<br>vs<br>62% ethanol (v/v) | 18hrs<br>24hrs<br>36hrs | 0.10<br>1.000<br>1.000 |
| Procedure 1.3<br>*Echinacea purpurea*<br>vs<br>62% ethanol (v/v) | 18hrs<br>24hrs<br>36hrs | 0.12<br>1.000<br>1.000 |

- Interpretation of results for Procedure 1.1

  P = 0.05 at 18 hours

  The null hypothesis is accepted since $P \geq \alpha = 0.05$, indicating there is a trend towards a difference between the size of the zones of inhibition produced by *Arctium lappa* in 62% ethanol (v/v) and the 62% ethanol (v/v). The P values obtained at 24 hours and 36 hours are $> \alpha = 0.05$, therefore the null hypothesis is accepted. Thus, there is no difference between the zones of inhibition produced by the test and control substances at these time intervals.

- Interpretation of results for Procedure 1.2 and 1.3.

  The P values obtained are $> \alpha = 0.05$, therefore the null hypotheses are accepted. Thus, all three herbal extracts have no statistical difference in comparison to 62% ethanol (v/v).

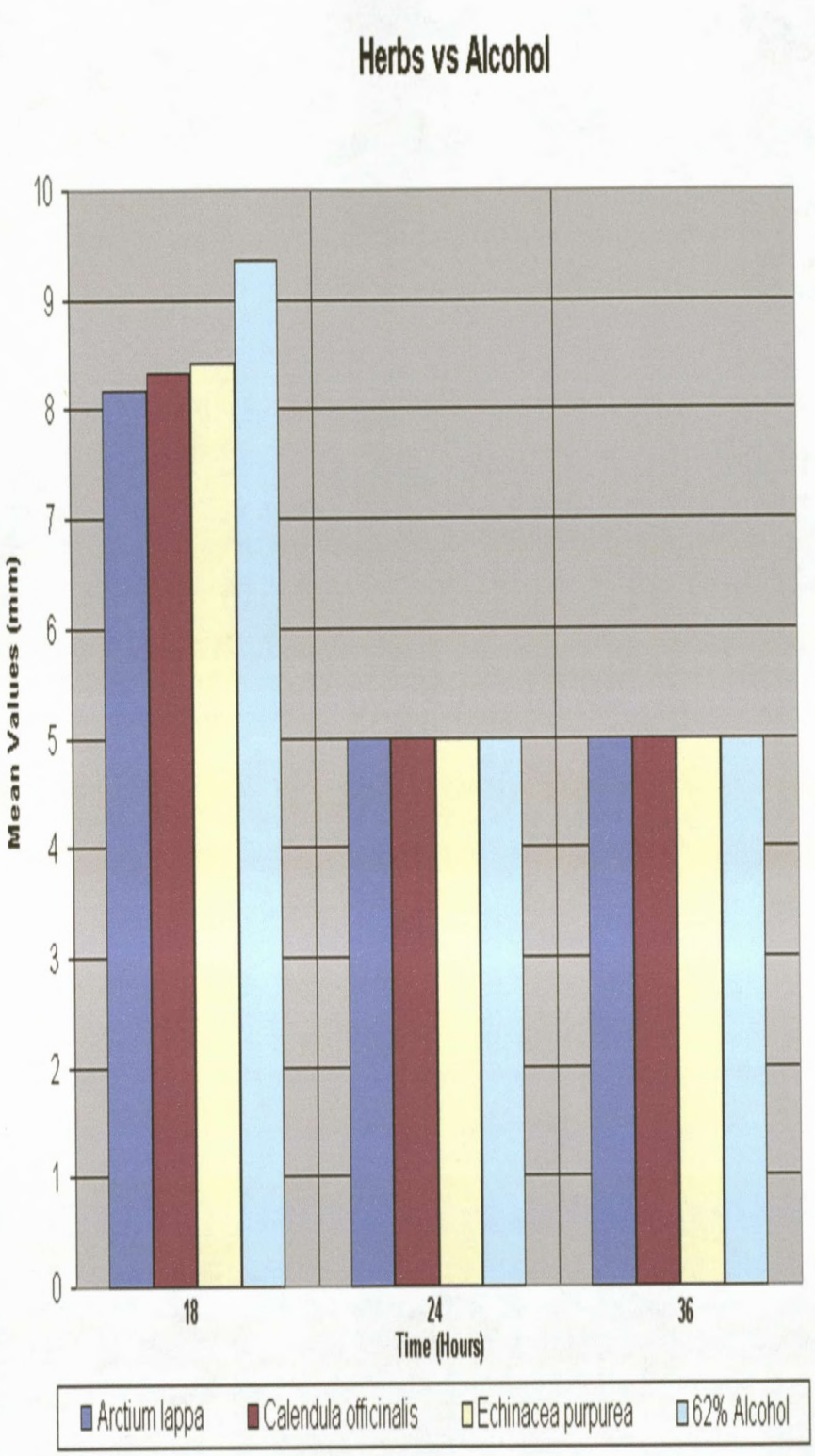


**Figure 4.1 Barchart showing the comparison between the herbal extracts and 62% ethanol (v/v)**

### 4.2.2 Procedure 1.4 - 1.6

**See Table 4.2 and Figure 4.2**

**Table 4.2** Intergroup comparison between *Arctium lappa* in 62% ethanol (v/v), *Calendula officinalis* in 62% ethanol (v/v) and *Echinacea purpurea* in 62% ethanol (v/v) respectively and nystatin with regard to observations at 18hrs, 24hrs and 36hrs. (Mann-Whitney U-Test)

| HERBS vs NYSTATIN | TIME | P-VALUE |
|---|---|---|
| Procedure 1.4<br>*Arctium lappa*<br>vs<br>Nystatin | 18hrs<br>24hrs<br>36hrs | 0.00<br>0.00<br>0.00 |
| Procedure 1.5<br>*Calendula officinalis*<br>vs<br>Nystatin | 18hrs<br>24hrs<br>36hrs | 0.00<br>0.00<br>0.00 |
| Procedure 1.6<br>*Echinacea purpurea*<br>vs<br>Nystatin | 18hrs<br>24hrs<br>36hrs | 0.00<br>0.00<br>0.00 |

Interpretation of results for Procedure 1.4 – 1.6

P = 0.00 for all the tests.

Since the P value is $< \alpha = 0.05$, the null hypotheses are rejected. Thus, there is a significant difference between all the herbal extracts and nystatin in terms of the diameter of the zones of inhibition.

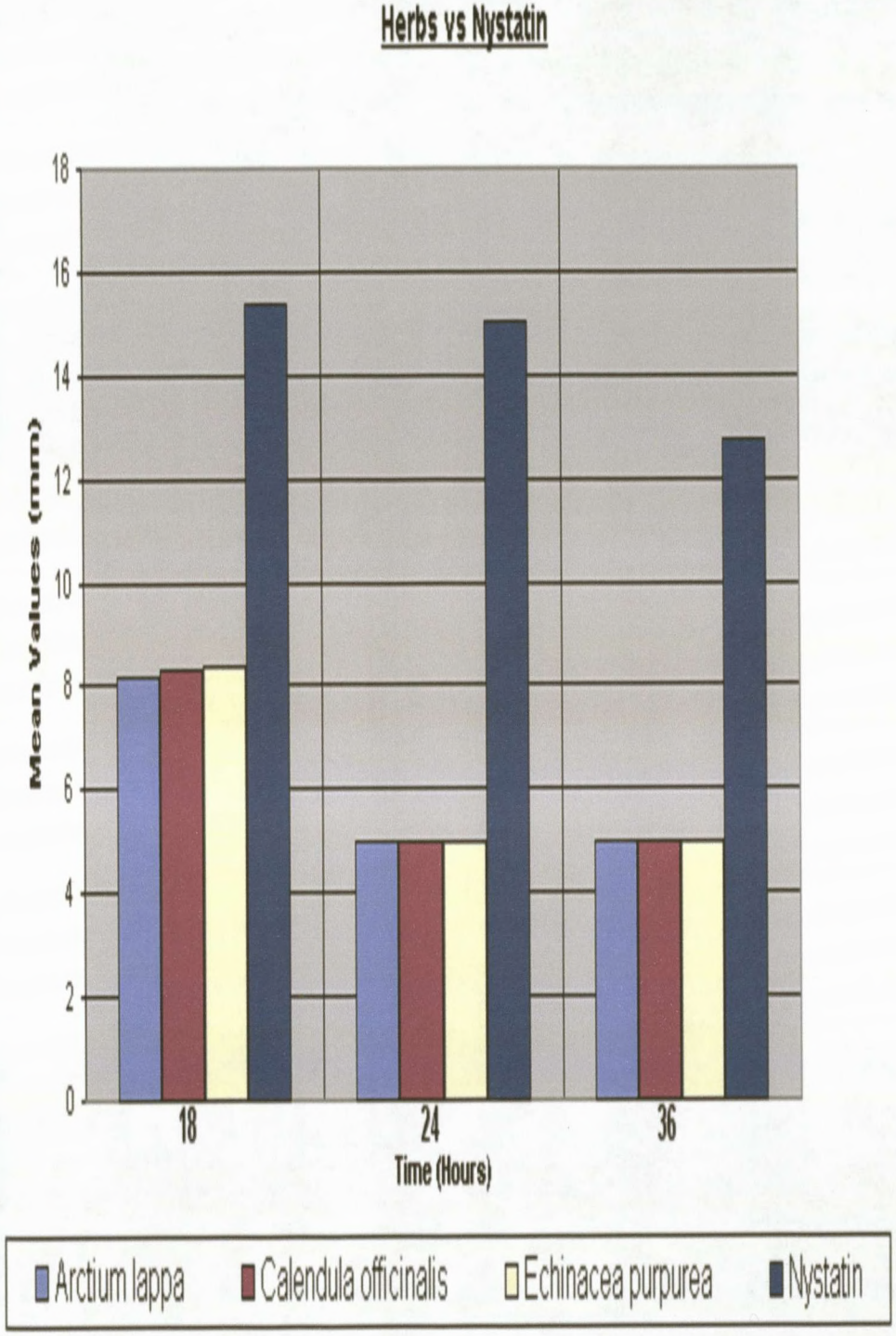


**Figure 4.2 Barchart showing the comparison between the herbal extracts and nystatin**

### 4.2.3 Procedure 1.7 - 1.9

**See Table 4.3 and Figure 4.3**

**Table 4.3** Intergroup comparison between *Arctium lappa* in 62% ethanol (v/v), *Calendula officinalis* In 62% ethanol (v/v) and *Echinacea purpurea* in 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs. (Mann-Whitney U-Test)

| HERBS | TIME | P-VALUE |
|---|---|---|
| Procedure 1.7 | | |
| *Arctium lappa* | 18hrs | 0.884 |
| vs | 24hrs | 1.000 |
| *Echinacea purpurea* | 36hrs | 1.000 |
| Procedure 1.8 | | |
| *Arctium lappa* | 18hrs | 0.589 |
| vs | 24hrs | 1.000 |
| *Calendula officinalis* | 36hrs | 1.000 |
| Procedure 1.9 | | |
| *Calendula officinalis* | 18hrs | 0.901 |
| vs | 24hrs | 1.000 |
| *Echinacea purpura* | 26hrs | 1.000 |

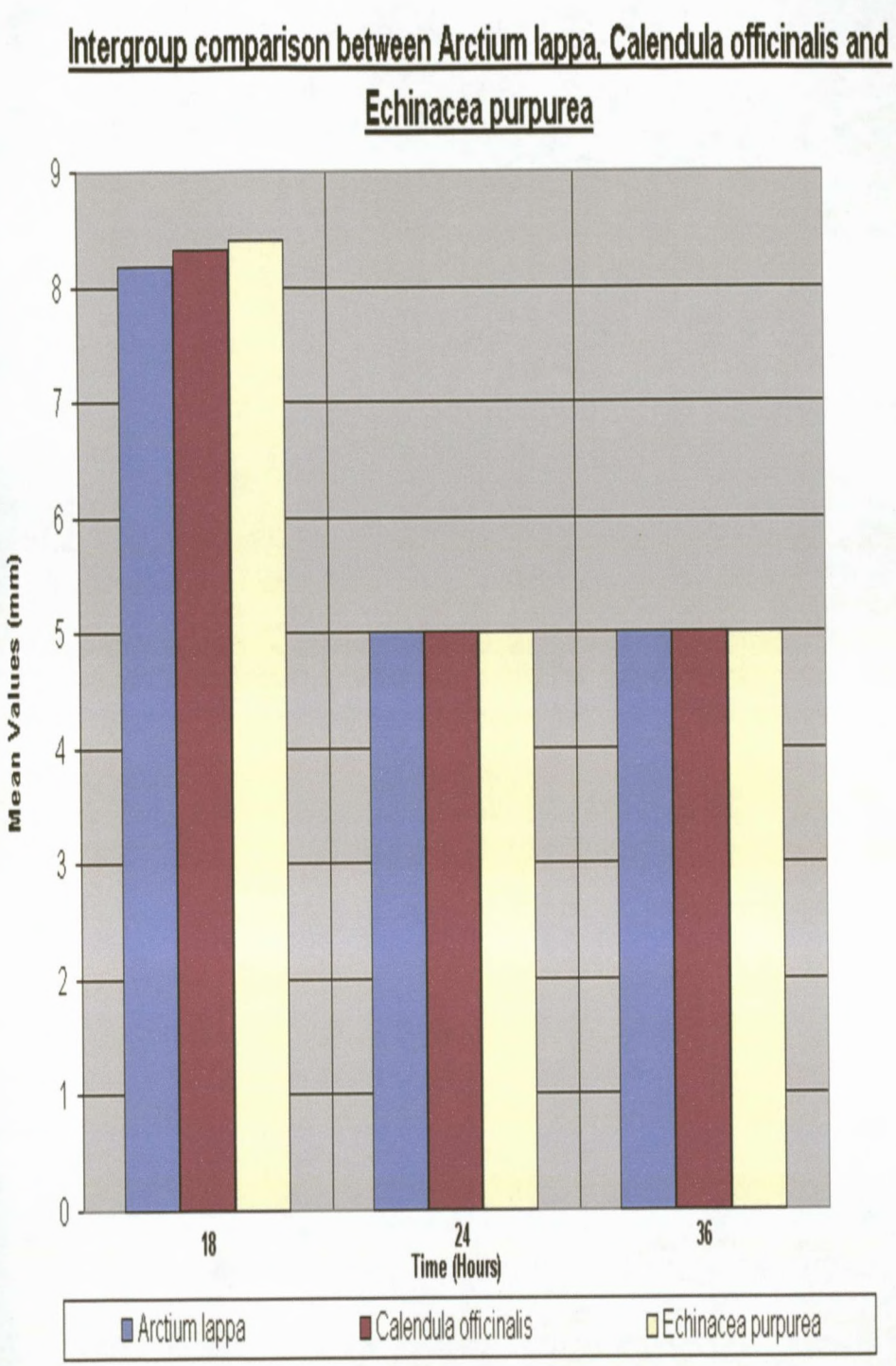


**Figure 4.3 Barchart showing the comparison between the herbal extracts**

Interpretation of results for Procedure 1.10

The P values for all these herbal extracts are > $\alpha$ = 0.05, therefore the null hypotheses are rejected for all of them. Thus, there are no significant differences between the herbal extracts in terms of the diameter of the zones of inhibition of the test substances.

### 4.2.4 Procedure 1.10

**See Tables 4.4.1 -2**

**Table 4.4.1** Intergroup comparison between *Arctium lappa* in 62% ethanol v/v), *Calendula officinalis* In 62% ethanol (v/v) and *Echinacea purpurea* in 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs. (Kruskal-Wallis Test)

| HERBS | NO. OF SAMPLES | MEAN | STD.DEVIATION | MINIMUM | MAX |
|---|---|---|---|---|---|
| 18hours | 75 | 9.9227 | 3.0352 | 5.00 | 20.30 |
| 24hours | 75 | 6.9987 | 4.0988 | 5.00 | 18.30 |
| 36hours | 75 | 6.5507 | 3.2498 | 5.00 | 16.70 |
| GROUP | 75 | 3.0000 | 1.4237 | 1.00 | 5.00 |

**Table 4.4.2** Kruskal-Wallis Test

| | **18 hours** | **24 hours** | **36 hours** |
|---|---|---|---|
| Chi-square | .205 | .000 | .000 |
| df | 2 | 2 | 2 |
| Asymptomatic significance (P value) | .902 | 1.000 | 1.000 |

The P value at 18 hours = .902, at 24 hours = 1.00 and at 36 hours = 1.00.

Since the P value at the different time intervals is $> \alpha = 0.05$, the null hypothesis is accepted for all the time periods. Thus there is no significant difference between the herbal extracts in terms of the diameters of the zones of inhibition.

### 4.2.5 Procedure 1.11

**See Tables 4.5.1 -3**

**Table 4.5.1** Intragroup comparison of *Arctium lappa* in 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs.

| ***ARCTIUM LAPPA*** | **NO. OF SAMPLES** | **MEAN** | **STD.DEVIATION** | **MINIMUM** | **MAXIMUM** |
|---|---|---|---|---|---|
| 18hours | 15 | 8.1733 | 1.0674 | 5.00 | 9.70 |
| 24hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |
| 36hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |

**Table 4.5.2** Friedman's T test statistics

| | |
|---|---|
| **NO. OF SAMPLES** | 15 |
| **CHI- SQUARE** | 28.000 |
| **df** | 2 |
| **ASYMPTOMATIC SIGNIFICANCE (P)** | .000 |

P (Level of significance) = .000

Since the level of significance is less than $\alpha$, the null hypothesis ($H_0$) is rejected. As a result of the rejection of $H_0$, the Dunn Procedure for Friedman's Test is applied.

The Dunn Procedure for *Arctium lappa* in 62% ethanol (v/v)

CALCULATION:

k = 3 z = 2.12 b = 15

$| R_j - R_j^1 | \geq z \sqrt{bk(k+1)/6}$

$| R_j - R_j^1 | \geq 2.12 \sqrt{15*3*4/6}$

$| R_j - R_j^1 | \geq 2.12\ (5.477)$

$| R_j - R_j^1 | \geq 11.61$

In the experiment $R_j$ and $R_j^1$ are the ranks for observations of the diameter of the zone of inhibition at the different time intervals, for the test and control substances respectively.

$R_1$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for *Arctium lappa* in 62% ethanol (v/v) at 18 hours.

$R_2$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for *Arctium lappa* in 62% ethanol (v/v) at 24 hours.

$R_3$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for *Arctium lappa* in 62% ethanol (v/v) at 36 hours.

**Table 4.5.3** The sum of ranks for *Arctium lappa* in 62% ethanol (v/v)

| **No. of *Arctium lappa* samples** | **$R_1$** | **$R_2$** | **$R_3$** |
|---|---|---|---|
| 1 | 7.6 (3) | 5 (1.5) | 5 (1.5) |
| 2 | 8.8 (3) | 5 (1.5) | 5 (1.5) |
| 3 | 8.8 (3) | 5 (1.5) | 5 (1.5) |
| 4 | 7.8 (3) | 5 (1.5) | 5 (1.5) |
| 5 | 7.6 (3) | 5 (1.5) | 5 (1.5) |
| 6 | 7.8 (3) | 5 (1.5) | 5 (1.5) |
| 7 | 9.7 (3) | 5 (1.5) | 5 (1.5) |
| 8 | 8.6 (3) | 5 (1.5) | 5 (1.5) |
| 9 | 7.7 (3) | 5 (1.5) | 5 (1.5) |
| 10 | 9 (3) | 5 (1.5) | 5 (1.5) |
| 11 | 8.7 (3) | 5 (1.5) | 5 (1.5) |
| 12 | 5 (3) | 5 (1.5) | 5 (1.5) |
| 13 | 8.2 (3) | 5 (1.5) | 5 (1.5) |
| 14 | 8.6 (3) | 5 (1.5) | 5 (1.5) |
| 15 | 8.7 (3) | 5 (1.5) | 5 (1.5) |
| **Sum of ranks** | **45** | **22.5** | **22.5** |

A comparison of *Arctium lappa* in 62% ethanol (v/v) at 18 hours and 24 hours:

$| R_1 - R_2 |$ = 45 - 22.5

= 22.5

Since $| R_1 - R_2 |$ > 11.61 there is a significant difference in the diameter of the

zones of inhibition between 18 hours and 24 hours.

A comparison of *Arctium lappa* in 62% ethanol (v/v) at 18 hours and 36 hours:

$| R_1 - R_3 |$ = 45 - 22.5

= 22.5

Since $| R_1 - R_3 |$ > 11.61 there is a significant difference in the diameter of the

zones of inhibition between 18 hours and 36 hours.

A comparison of *Arctium lappa* in 62% ethanol (v/v) at 24 hours and 36 hours:

$| R_2 - R_3 |$ = 22.5 - 22.5

= 0

Since $| R_2 - R_3 | < 11.61$ there is no significant difference in the diameter of the zones of inhibition between 24 hours and 36 hours.

### 4.2.6 Procedure 1.12

**See Tables 4.6.1 - 3**

**Table 4.6.1** Intragroup comparison of *Calendula officinalis* in 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs

| *CALENDULA OFFICINALIS* | NO. OF SAMPLES | MEAN | STD.DEVIATION | MINIMUM | MAX |
|---|---|---|---|---|---|
| 18hours | 15 | 8.3200 | 1.0483 | 5.00 | 9.70 |
| 24hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |
| 36hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |

**Table 4.6.2** Friedman's T test statistics

| | |
|---|---|
| **NO. OF SAMPLES** | 15 |
| **CHI- SQUARE** | 28.000 |
| **df** | 2 |
| **ASYMPTOMATIC SIGNIFICANCE (P)** | .000 |

P (Level of significance) = .000

Since the level of significance is less than $\alpha$, we reject the null hypothesis ($H_0$). As a result of the rejection of $H_0$, the Dunn Procedure for Friedman's Test is applied.

The Dunn Procedure for *Calendula officinalis* in 62% ethanol (v/v)

CALCULATION:

k = 3 z = 2.12 b = 15

$$| R_j - R_j^1 | \geq z \sqrt{bk(k+1)}/6$$

$$| R_j - R_j^1 | \geq 2.12 \sqrt{15*3*4}/6$$

$$| R_j - R_j^1 | \geq 2.12\ (5.477)$$

$$| R_j - R_j^1 | \geq 11.61$$

In the experiment $R_j$ and $R_j^1$ are the ranks for observations of the diameter of the zone of inhibition at the different time intervals, for the test and control substances respectively.

$R_1$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for *Calendula officinalis* in 62% ethanol (v/v) at 18 hours.

$R_2$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for *Calendula officinalis* in 62% ethanol (v/v) at 24 hours.

$R_3$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for *Calendula officinalis* in 62% ethanol (v/v) at 36 hours

**Table 4.6.3** The sum of ranks for *Calendula officinalis* in 62% ethanol (v/v)

| **No. of *Calendula officianalis* samples** | **$R_1$** | **$R_2$** | **$R_3$** |
|---|---|---|---|
| 1 | 8.5 (3) | 5 (1.5) | 5 (1.5) |
| 2 | 8 (3) | 5 (1.5) | 5 (1.5) |
| 3 | 8.3 (3) | 5 (1.5) | 5 (1.5) |
| 4 | 9.7 (3) | 5 (1.5) | 5 (1.5) |
| 5 | 7.8 (3) | 5 (1.5) | 5 (1.5) |
| 6 | 9.3 (3) | 5 (1.5) | 5 (1.5) |
| 7 | 8 (3) | 5 (1.5) | 5 (1.5) |
| 8 | 8.5 (3) | 5 (1.5) | 5 (1.5) |
| 9 | 8.5 (3) | 5 (1.5) | 5 (1.5) |
| 10 | 8.8 (3) | 5 (1.5) | 5 (1.5) |
| 11 | 8.3 (3) | 5 (1.5) | 5 (1.5) |
| 12 | 9 (3) | 5 (1.5) | 5 (1.5) |
| 13 | 8.8 (3) | 5 (1.5) | 5 (1.5) |
| 14 | 8.3 (3) | 5 (1.5) | 5 (1.5) |
| 15 | 5 (3) | 5 (1.5) | 5 (1.5) |
| **Sum of ranks** | **45** | **22.5** | **22.5** |

A comparison of *Calendula officinalis* in 62% ethanol (v/v) at 18 hours and 24 hours:

$| R_1 - R_2 | = 45 - 22.5$

$= 22.5$

Since $| R_1 - R_2 | > 11.61$ there is a significant difference in the diameter of the zones of inhibition between 18 hours and 24 hours.

A comparison of *Calendula officinalis* in 62% ethanol (v/v) at 18 hours and 36 hours :

$| R_1 - R_3 | = 45 - 22.5$

$= 22.5$

Since $| R_1 - R_3 | > 11.61$ there is a significant difference in the diameter of the zones of inhibition between 18 hours and 36 hours.

A comparison of *Calendula officinalis* in 62% ethanol (v/v) at 24 hours and 36 hours:

$| R_2 - R_3 |$ = 22.5 - 22.5

= 0

Since $| R_2 - R_3 | < 11.61$ there is no significant difference in the diameter of the zones of inhibition between 24 hours and 36 hours.

### 4.2.7 Procedure 1.13

**See Tables 4.7.1 – 3**

**Table 4.7.1** Intragroup comparison of *Echinacea purpurea* in 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs

| *ECHINACEA PURPUREA* | NO. OF SAMPLES | MEAN | STD.DEVIATION | MINIMUM | MAX |
|---|---|---|---|---|---|
| 18hours | 15 | 8.4133 | .9471 | 5.00 | 10.70 |
| 24hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |
| 36hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |

**Table 4.7.2** Friedman's T test statistics

| | |
|---|---|
| **NO. OF SAMPLES** | 15 |
| **CHI- SQUARE** | 30.000 |
| **Df** | 2 |
| **ASYMPTOMATIC SIGNIFICANCE (P)** | .000 |

P (Level of significance) = .000

Since the level of significance is less than $\alpha$, we reject the null hypothesis ($H_0$). As a result of the rejection of $H_0$, the Dunn Procedure for Friedman's Test is applied.

The Dunn Procedure for *Echinacea purpurea* in 62% ethanol (v/v)

CALCULATION:

k = 3 z = 2.12 b = 15

$| R_j - R_j^1 | \geq z \sqrt{bk(k+1)}/6$

$| R_j - R_j^1 | \geq 2.12 \sqrt{15*3*4}/6$

$| R_j - R_j^1 | \geq 2.12\ (5.477)$

$| R_j - R_j^1 | \geq 11.61$

In the experiment $R_j$ and $R_j^1$ are the ranks for observations of the diameter of the zone of inhibition at the different time intervals, for the test and control substances respectively.

$R_1$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for *Echinacea purpurea* in 62% ethanol (v/v) at 18 hours.

$R_2$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for *Echinacea purpurea* in 62% ethanol (v/v) at 24 hours.

$R_3$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for *Echinacea purpurea* in 62% ethanol (v/v) at 36 hours.

**Table 4.7.3** The sum of ranks for *Echinacea purpurea* in 62% ethanol (v/v)

| **No. of *Echinacea purpurea* samples** | **$R_1$** | **$R_2$** | **$R_3$** |
|---|---|---|---|
| 1 | 6.6 (3) | 5 (1.5) | 5 (1.5) |
| 2 | 7.3 (3) | 5 (1.5) | 5 (1.5) |
| 3 | 8.3 (3) | 5 (1.5) | 5 (1.5) |
| 4 | 8.2 (3) | 5 (1.5) | 5 (1.5) |
| 5 | 7.3 (3) | 5 (1.5) | 5 (1.5) |
| 6 | 8.3 (3) | 5 (1.5) | 5 (1.5) |
| 7 | 8.2 (3) | 5 (1.5) | 5 (1.5) |
| 8 | 8.8 (3) | 5 (1.5) | 5 (1.5) |
| 9 | 8.5 (3) | 5 (1.5) | 5 (1.5) |
| 10 | 8.5 (3) | 5 (1.5) | 5 (1.5) |
| 11 | 9.3 (3) | 5 (1.5) | 5 (1.5) |
| 12 | 8.6 (3) | 5 (1.5) | 5 (1.5) |
| 13 | 9 (3) | 5 (1.5) | 5 (1.5) |
| 14 | 8.7 (3) | 5 (1.5) | 5 (1.5) |
| 15 | 10.7 (3) | 5 (1.5) | 5 (1.5) |
| **Sum of ranks** | **45** | **22.5** | **22.5** |

A comparison of *Echinacea purpurea* in 62% ethanol (v/v) at 18 hours and 24 hours:

$| R_1 - R_2 | = 45 - 22.5$

$= 22.5$

Since $| R_1 - R_2 | > 11.61$ there is a significant difference in the diameter of the zones of inhibition between 18 hours and 24 hours.

A comparison of *Echinacea purpurea* in 62% ethanol (v/v) at 18 hours and 36 hours :

$| R_1 - R_3 | = 45 - 22.5$

$= 22.5$

Since $| R_1 - R_3 | > 11.61$ there is a significant difference in the diameter of the zones of inhibition between 18 hours and 36 hours.

A comparison of *Echinacea purpurea* in 62% ethanol (v/v) at 24 hours and 36 hours:

$$| R_2 - R_3 | = 22.5 - 22.5$$

$$= 0$$

Since $| R_2 - R_3 | < 11.61$ there is no significant difference in the diameter of the zones of inhibition between 24 hours and 36 hours.

### 4.2.8 Procedure 1.14

**See Tables 4.8.1 – 3**

**Table 4.8.1** Intragroup comparison of 62% ethanol (v/v) with regard to observations at 18hrs, 24hrs and 36hrs

| 62% ETHANOL (v/v) | NO. OF SAMPLES | MEAN | STD.DEVIATION | MINIMUM | MAX |
|---|---|---|---|---|---|
| 18hours | 15 | 9.3533 | .9349 | 8.20 | 11.00 |
| 24hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |
| 36hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |

**Table 4.8.2** Friedman's T test statistics

| | |
|---|---|
| **NO. OF SAMPLES** | 15 |
| **CHI- SQUARE** | 30.000 |
| **Df** | 2 |
| **ASYMPTOMATIC SIGNIFICANCE (P)** | .000 |

P (Level of significance) = 0.00

Since the level of significance is less than $\alpha$, we reject the null hypothesis ($H_0$). As a result of the rejection of $H_0$, the Dunn Procedure for Friedman's Test is applied.

The Dunn Procedure for 62% alcohol (v/v)

CALCULATION:

k = 3 z = 2.12 b = 15

$$| R_j - R_j^1 | \geq z \sqrt{bk(k+1)}/6$$

$$| R_j - R_j^1 | \geq 2.12 \sqrt{15*3*4}/6$$

$$| R_j - R_j^1 | \geq 2.12\ (5.477)$$

$$| R_j - R_j^1 | \geq 11.61$$

In the experiment $R_j$ and $R_j^1$ are the ranks for observations of the diameter of the zone of inhibition at the different time intervals, for the test and control substances respectively.

$R_1$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for 62% ethanol (v/v) at 18 hours.

$R_2$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for 62% ethanol (v/v) at 24 hours.

$R_3$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for 62% ethanol (v/v) at 36 hours.

**Table 4.8.3** The sum of ranks for 62% ethanol (v/v)

| No. of 62% ethanol samples | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 1 | 8.8 (3) | 5 (1.5) | 5 (1.5) |
| 2 | 8.2 (3) | 5 (1.5) | 5 (1.5) |
| 3 | 10.3 (3) | 5 (1.5) | 5 (1.5) |
| 4 | 8.2 (3) | 5 (1.5) | 5 (1.5) |
| 5 | 9 (3) | 5 (1.5) | 5 (1.5) |
| 6 | 8.5 (3) | 5 (1.5) | 5 (1.5) |
| 7 | 10.3 (3) | 5 (1.5) | 5 (1.5) |
| 8 | 8.8 (3) | 5 (1.5) | 5 (1.5) |
| 9 | 8.6 (3) | 5 (1.5) | 5 (1.5) |
| 10 | 9.5 (3) | 5 (1.5) | 5 (1.5) |
| 11 | 11 (3) | 5 (1.5) | 5 (1.5) |
| 12 | 8.8 (3) | 5 (1.5) | 5 (1.5) |
| 13 | 10.7 (3) | 5 (1.5) | 5 (1.5) |
| 14 | 9.3 (3) | 5 (1.5) | 5 (1.5) |
| 15 | 10.3 (3) | 5 (1.5) | 5 (1.5) |
| **Sum of ranks** | **45** | **22.5** | **22.5** |

A comparison of 62% ethanol (v/v) at 18 hours and 24 hours:

$| R_1 - R_2 |$ = 45 - 22.5

= 22.5

Since $| R_1 - R_2 |$ > 11.61 there is a significant difference in the diameter of the zones of inhibition between 18 hours and 24 hours.

A comparison of 62% ethanol (v/v) at 18 hours and 36 hours :

$| R_1 - R_3 |$ = 45 - 22.5

= 22.5

Since $| R_1 - R_3 |$ > 11.61 there is a significant difference in the diameter of the zones of inhibition between 18 hours and 36 hours.

A comparison of 62% ethanol (v/v) at 24 hours and 36 hours:

$| R_2 - R_3 |$ = 22.5 - 22.5

= 0

Since $| R_2 - R_3 | < 11.61$ there is no significant difference in the diameter of the zones of inhibition between 24 hours and 36 hours.

### 4.2.9 Procedure 1.15

See Tables 4.9.1 – 3

**Table 4.9.1** Intragroup comparison of nystatin with regard to observations at 18hrs, 24hrs and 36hrs

| NYSTATIN | NO. OF SAMPLES | MEAN | STD.DEVIATION | MINIMUM | MAX |
|---|---|---|---|---|---|
| 18hours | 15 | 15.3533 | 2.0657 | 11.60 | 20.30 |
| 24hours | 15 | 14.9933 | 1.7886 | 11.50 | 18.30 |
| 36hours | 15 | 12.7533 | 2.0729 | 8.60 | 16.70 |

**Table 4.9.2** Friedman's T test statistics

| | |
|---|---|
| **NO. OF SAMPLES** | 15 |
| **CHI- SQUARE** | 26.815 |
| **df** | 2 |
| **ASYMPTOMATIC SIGNIFICANCE (P)** | .000 |

P (Level of significance) = .000

Since the level of significance is less than $\alpha$, we reject the null hypothesis ($H_0$). As a result of the rejection of $H_0$, the Dunn Procedure for Friedman's Test is applied.

The Dunn Procedure for nystatin

CALCULATION:

k = 3 z = 2.12 b = 15

$| R_j - R_j^1 | \geq z \sqrt{bk(k+1)}/6$

$| R_j - R_j^1 | \geq 2.12 \sqrt{15*3*4}/6$

$| R_j - R_j^1 | \geq 2.12\ (5.477)$

$| R_j - R_j^1 | \geq 11.61$

In the experiment $R_j$ and $R_j^1$ are the ranks for observations of the diameter of the zone of inhibition at the different time intervals, for the test and control substances respectively.

$R_1$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for nystatin at 18 hours.

$R_2$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for nystatin at 24 hours.

$R_3$ = sum of the ranks of the observations of the diameter of the zone of inhibition, for nystatin at 36 hours.

**Table 4.9.3** The sum of ranks for nystatin

| No. of nystatin samples | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 1 | 14.3 (2.5) | 14.3 (2.5) | 11.2 (1) |
| 2 | 13.3 (3) | 12.8 (2) | 10.7 (1) |
| 3 | 11.6 (3) | 11.5 (2) | 8.6 (1) |
| 4 | 16.3 (3) | 15.3 (2) | 14.3 (1) |
| 5 | 14.7 (3) | 14.3 (2) | 12.7 (1) |
| 6 | 13 (3) | 12.2 (2) | 10 (1) |
| 7 | 15.3 (2.5) | 15.3 (2.5) | 12 (1) |
| 8 | 15 (2.5) | 15 (2.5) | 12.3 (1) |
| 9 | 15.3 (3) | 14.7 (2) | 14.2 (1) |
| 10 | 16 (2.5) | 16 (2.5) | 13.3 (1) |
| 11 | 16.7 (2.5) | 16.7 (2.5) | 14.3 (1) |
| 12 | 17.3 (2.5) | 17.3 (2.5) | 15 (1) |
| 13 | 20.3 (3) | 18.3 (2) | 16.7 (1) |
| 14 | 15.7 (3) | 15 (2) | 13 (1) |
| 15 | 16 (3) | 15.7 (2) | 13 (1) |
| **Sum of ranks** | **42** | **33** | **15** |

A comparison of nystatin at 18 hours and 24 hours:

$| R_1 - R_2 |$ = 42 - 33

= 11

Since $| R_1 - R_2 |$ < 11.61 there is no significant difference in the diameter of the zones of inhibition between 18 hours and 24 hours.

A comparison of nystatin at 18 hours and 36 hours :

$| R_1 - R_3 |$ = 42 - 15

= 27

Since $| R_1 - R_3 |$ > 11.61 there is a significant difference in the diameter of the zones of inhibition between 18 hours and 36 hours.

A comparison of nystatin at 24 hours and 36 hours:

$| R_2 - R_3 |$ = 33 - 15

= 18

Since $| R_2 - R_3 | > 11.61$ there is significant difference in the diameter of the zones of inhibition between 24 hours and 36 hours.

# CHAPTER FIVE

## DISCUSSION OF RESULTS

**5.1 The efficacy of *Arctium lappa* in 62% ethanol (v/v) as compared to 62% ethanol only (negative control) in the inhibition of the *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.**

### 5.1.1 Observations at 18 hours

*Arctium lappa* in 62% ethanol (v/v) did produce zones of inhibition at 18 hours; it did inhibit the *in vitro* growth of *Candida albicans*. There was a trend towards a difference (P = 0.05) between the diameters of the zones of inhibition of *Arctium lappa* in 62% ethanol (v/v) and the 62% ethanol (v/v) alone. As the 62% ethanol (v/v) was the control, it can thus be said that the *Arctium lappa* inhibited the *in vitro* growth of *Candida albicans*.

### 5.1.2 Observations at 24 hours and 36 hours.

The diameter of the zones of inhibition produced by *Arctium lappa* in 62% ethanol (v/v) decreased in size from 18 hours to 24 hours, as did that of 62% ethanol (v/v). The zone diameter of *Arctium lappa* in 62% ethanol (v/v) and 62% ethanol (v/v) only, decreased to a constant value of 5mm; this is the diameter of the filter paper disc. There was growth of *Candida albicans* up to the edge of the filter paper disc. Thus it can be concluded that *Arctium lappa* in 62% ethanol (v/v) and 62% ethanol (v/v) did not inhibit the *in vitro* growth of *Candida albicans* at 24 hours. The reason for the decrease in size of the zones was probably due to the regrowth of the organism because of the

evaporation of the test and control substances. There was no difference in the diameter of the zones of inhibition at 24 hours and 36 hours. At 24 hours (P = 1.00) and 36 hours (P = 1.00) the size of the diameter of the zones of inhibition of the test and control substances were the same (5mm). The results obtained indicate that *Arctium lappa* in 62% ethanol (v/v) (P = 1.00) and 62% ethanol (v/v) (P = 1.00) did not inhibit the *in vitro* growth of *Candida albicans* with regard to observations at 24 hours and 36 hours.

## 5.2 The efficacy of *Calendula officinalis* in 62% ethanol (v/v) in comparison to 62% ethanol (v/v) only (negative control) in the inhibition of the *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

### 5.2.1 Observations at 18 hours

*Calendula officinalis* in 62% ethanol (v/v) did produce a zone of inhibition at 18 hours; it did inhibit the *in vitro* growth of *Candida albicans*. However, there was no significant difference (P = 0.10) between the diameters of the zones of inhibition of *Calendula officinalis* in 62% ethanol (v/v) and 62% ethanol (v/v) only. Therefore *Calendula officinalis* in 62% ethanol (v/v) did not significantly inhibit the *in vitro* growth of *Candida albicans*.

### 5.2.2 Observations at 24 hours and 36 hours.

The diameter of the zones of inhibition produced by *Calendula officinalis* in 62% ethanol (v/v) decreased in size from 18 hours to 24 hours, as did that of 62% ethanol (v/v). There was no difference in the diameter of the zones of

inhibition (P = 1.00) of the test and control substance at 24 hours and 36 hours. Both substances had a mean zone size of 5 mm. As this was the diameter of the filter paper disc, it can thus be said that the organism grew back to the edge of the disc. Therefore neither of these substances significantly inhibited the *in vitro* growth of *Candida albicans* at 24 hours and 36 hours. The reason for the zone sizes decreasing was probably due to the fact that ethanol evaporates rapidly.

## 5.3 The efficacy of *Echinacea purpurea* in 62% ethanol (v/v) in comparison to 62% ethanol (v/v) only (negative control) in the inhibition of the *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

### 5.3.1 Observations at 18 hours

*Echinacea purpurea* in 62% ethanol (v/v) did produce a zone of inhibition at 18 hours; it did inhibit the in vitro growth of *Candida albicans*. However, there was no significant difference (P = 0.12) between the diameters of the zones of inhibition of *Echinacea purpurea* in 62% ethanol (v/v) and 62% ethanol (v/v) only. Therefore at 18 hours *Echinacea purpurea* in 62% ethanol (v/v) did not significantly inhibit the *in vitro* growth of *Candida albicans*.

### 5.3.2 Observations at 24 and 36 hours

The zones of inhibition of *Echinacea purpurea* in 62% ethanol (v/v) and 62% ethanol (v/v) decreased significantly from 18 hours to 24 hours, and there was no significant difference between them (P = 1.00). The decrease in the

size of the zones of inhibition were probably due to the regrowth of the organism and evaporation of the test and control substances. The zone sizes decreased to a constant value of 5mm. This constant zone diameter of 5mm was also observed at 36 hours. Therefore *Echinacea purpurea* in 62% ethanol (v/v) did not significantly inhibit the *in vitro* growth of *Candida albicans* at 24 hours and 36 hours.

## 5.4 The efficacy of *Arctium lappa* in 62% ethanol (v/v) as compared to nystatin (positive control) in the inhibition of the *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

### 5.4.1 Observations at 18 hours, 24 hours and 36 hours

In comparison to *Arctium lappa* in 62% ethanol (v/v), nystatin produced significantly greater zones of inhibition. The results indicate that P = 0.00 at the different time intervals. There was a significant difference between the zones of inhibition of the test and control substance. The size of the zones of inhibition produced by nystatin (15.7mm) was the same as the size (16mm) produced in Valcic et al 1999).

## 5.5 The efficacy of *Calendula officinalis* in 62% ethanol (v/v) in comparison to nystatin (positive control) in the inhibition of the *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

### 5.5.1 Observations at 18 hours, 24 hours and 36 hours

Nystatin produced a significantly greater zone of inhibition (P = .000) than *Calendula officinalis.*

## 5.6 The efficacy of *Echinacea purpurea* in 62% ethanol (v/v) in comparison to nystatin in the inhibition of the *in vitro* growth of *Candida albicans* terms of the disc diffusion test.

Nystatin produced a significantly greater zone of inhibition (P = .000) than *Echinacea purpurea.*

## 5.7 The comparison of the efficacies of the herbal extracts (*Arctium lappa, Calendula officinalis* and *Echinacea purpurea*) to each other in the inhibition of the *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

### 5.7.1 Comparison between *Arctium lappa* in 62% ethanol (v/v) and *Calendula officinalis* in 62% ethanol (v/v)

The results of this study indicate that *Calendula officinalis* in 62% ethanol (v/v) did not significantly inhibit the *in vitro* growth of *Candida albicans*, whereas *Arctium lappa* in 62% ethanol (v/v) showed a trend towards a difference. Statistically however, a comparison between *Arctium lappa* in 62% ethanol (v/v) and *Calendula officinalis* in 62% ethanol (v/v) yields, P = 0.589, P = 1.00, P = 1.00 at 18 hours 24 hours and 36 hours respectively. The results indicate that there is no significant difference between these herbal extracts. Thus, a

discussion of the differences in zone sizes between these herbal extracts is superfluous.

### 5.7.2 Comparison between *Arctium lappa* in 62% (v/v) ethanol and *Echinacea purpurea* in 62% ethanol (v/v)

The results of this study indicate that *Echinacea purpurea* in 62% ethanol (v/v) does not significantly inhibit the *in vitro* growth of *Candida albicans*, whereas *Arctium lappa* in 62% ethanol (v/v) shows a trend towards a difference. Statistically however, a comparison between *Arctium lappa* in 62% ethanol (v/v) and *Echinacea purpurea* in 62% ethanol (v/v) yields, P = 0.884, P = 1.00, P = 1.00 at 18 hours, 24 hours and 36 hours respectively. The results indicate that there is no significant difference between these herbal extracts. Thus, a discussion of the differences in zone sizes between these herbal extracts is superfluous.

### 5.7.3 Comparison between *Calendula officinalis* in 62% ethanol (v/v) and *Echinacea purpurea* in 62% ethanol (v/v)

The results of this study indicate that *Calendula officinalis* in 62% ethanol (v/v) and *Echinacea purpurea* in 62% ethanol (v/v) did not significantly inhibit the *in vitro* growth of *Candida albicans*. A comparison between *Calendula officinalis* in 62% ethanol (v/v) and *Echinacea purpurea* in 62% ethanol (v/v) yields, P= 0901, P = 1.00, P = 1.00 at 18 hours 24 hours and 36 hours respectively. Thus the discussion of the difference in the zone sizes between the two herbal extracts is superfluous.

## 5.8 Summary

The only herbal extract to demonstrate a trend towards a significant inhibitory effect on *Candida albicans in vitro* was *Arctium lappa* as observed at 18hours (P = 0.05). Statistically none of the other herbal extracts demonstrated a significant inhibitory effect on *Candida albicans in vitro*. In comparison to the herbal extracts, nystatin demonstrated a superior effect reinforcing its pre-eminence as an antifungal agent.

## 5.9 General Discussion

The statistical results of this study indicate that none of the herbal extracts except for *Arctium lappa* in 62% ethanol (v/v) at 18 hours had a significant antifungal effect.

The raw data indicates that the other herbal extracts which contain the constituents of the herb in 62% ethanol produced zones of inhibition that were smaller than those produced by the 62% ethanol (v/v) only. There is no statistical difference between the herbal extracts and 62% ethanol (v/v) except *Arctium lappa* in 62% ethanol (v/v) at 18 hours. Possible reasons for the herbal extracts producing smaller zones of inhibition than the 62% ethanol (v/v) are that the herbal constituents and the ethanol in combination produce an inadditive effect instead of a possible synergistic effect that one may expect. The results obtained at 18 hours for the herbal extracts may be due to the ethanol and not the herbal constituents, except for *Arctium lappa*. Ethanol is most antimicrobial between 50% and 70% (Ketchim, 1988: 183). The results obtained may however be due to chance.

In general the methodology utilized in the study is not refined enough and it would be preferred if a more refined approach with more frequent viewing at the early stages is warranted. Re-dosage could be considered to see if the inhibition is sustained.

The use of the disc diffusion test to evaluate the antifungal effects of nystatin, corroborates with Valcic et al. (1999). The zone size (16mm) corroborates with the zone sizes of the present study (15.35mm).

Herbal literature claims that the herbal extracts used in this study are antifungal. However, this laboratory research does not confirm these statements, which should lead to a re-evaluation of the validity of such statements. However there are other herbs that have laboratory verified activity. Vosloo (2002) has evaluated the efficacy of *Thymus vulgaris* tincture as an antimicrobial agent. The results of the study for Reid (2002) also produces positive results. However it is possible that *in vivo* effects could also be more noticeable but this can only be evaluated in a controlled clinical trial.

The negative results obtained for *Calendula officinalis* are similar to the results obtained by McFadden (1995). Both studies indicate that *Calendula officinalis* does not demonstrate inhibitory activity against the *in vitro* growth of *Calendula officinalis.*

# CHAPTER SIX

## 6.1 Conclusions

The results from statistical tests indicate that none of the herbal extracts except, *Arctium lappa* in 62% ethanol (v/v) at 18 hours had a significant antifungal effect. On the basis of these results utilization of these herbal extracts as antifungal agents in clinical practice is not warranted.

## 6.2 Recommendations

6.1.1 Further studies should consider the mean fungicidal concentration measurements of the herbal extracts.

6.1.2 Controlled clinical trials to test these herbal extracts *in vivo*.

# LIST OF REFERENCES

British Homeopathic Association. 1985. German Homeopathic Pharmacopoeia. Stuttgant Deutscher Potheker Verlag. Translation of the 1st edition, 1978 edition, 4th supplement.

Cappuccino, J.G. and Sherman, N. 1992. Microbiology a Laboratory Manual. 3rd edition. New York: Benjamin and Cummings Publishing Company.

Chang, S.R .,Yeung, H.W., 1988. Inhibition of Growth of Human Immunodefiency Virus by Crude Extracts of Chinese Medicinal Herbs. Antiviral Research, **9**: 163-176.

Chuang, S. C., Lin, C.C., Yang, J.J., Ujie, T, 1996. Anti-inflammatory and Radical Scavenger effects of *Arctium lappa*. American Journal of Chinese Medicine, **24** (2): 127-137.

Chevalliar, A. 1996. The Encyclopedia of Medicinal plants. London: Dorling Kinndersley Publishing Corporation.

Coeugniet, E.G. and Kuhnast, R. 1986. Recurrent candidiasis: adjuvant immunotherapy in different formulations of Echinacin. Therapie roche, **36**: 3352-3358.

D'Amelio, L.F., Wagner, B., Azimuddin, S., Sutyak, J.P., Hammond, J.S. 1995. Antibiotic patterns associated in fungal colonization in critically ill surgical patients. American Surgeon, **61** (12): 1049-53.

Della, L.R., Tubaro, A., Sosa, S., Becker, H., Saar, Issac, O. 1994. The Role Of Triterpenoids in the Topical Anti-inflammatory Activity of *Calendula officinalis* flowers. Planta Medica, **60** (6): 516-520.

Denning, D.W., Evans, E.G., Kibbler, C.C., Richardson, M.D., Roberts, M.M., Rogers, T.R., Warnock, D.W., Warren, R.E. 1995. Fungal Nail Disease: a guide to good practice (report of a Working Group of British Society for Medical Mycology). British Medical Journal, **311** (7015): 1277-81.

Diehl, K.B. 1996. Topical antifungal agents: an update. American Family Physician, **54** (5): 1687-1692.

Dixon, D.M., McNeil, M.M., Cohen, M.L., Gellin, B.G., La Montage, J.R. 1996. Fungal Infection: a growing threat. Public Health Reports, **3**: 226-35.

Dugaid, J.P., Marmion, B.P., Swain, R.H.A. 1978. Medical Microbiology. London: Livingstone Publishers. No. of pages: 666.

Edwards, C.R.W., Bouchier, I.A.D. and Haslett C. 1995. Davidson's Principles and Practice of Medicine. 17th Edition. New York: Pearson Professional Limited.

Ernst, E. 2000. Herbal Medicines: Where is the Evidence? British Medical Journal, **321** (7258): 395 – 6.

Fisher, L.D. and van Belle, G. 1993. Biostatistics: A Methodology for the Health Sciences. New York: John Whiley & Sons.

Fong, I.W., Lauerel, M., Burford-Mason, A. 1997. Asymptomatic oral carriage of *Candida albicans* in patients with HIV infection. Clinical Investigative Medicine, **20** (2): 85-93.

German Pharmaceutical Association. 1991. Homoeopathisches Arrzneibur. Frankfurt: German Pharmaceutical Publishers.

Goodman, A.G. and Gilman, L.S. 1980. The Pharmacological Basis of Therapeutics. 7th edition. London: Collier Macmillan Publishers.

Goorney, B., Roberts, J. 1995. Management of genital candidiasis. Working Group of the British Society for Medical Mycology. British Medical Journal, **310** (6989): 1241 – 4.

Greenwood, D., Slack, R., Peutherer, J. 1992. Medical Microbiology. New York: Churchill Livingstone Publishers.

Grimm, W., Muller, H.H. 1999. A randomized controlled trial of the effect of fluid extract of *Echinacea purpurea* on the incidence & severity of colds & respiratory infections. American Journal of Medicine, **106**: 138 – 143.

Gurumadhva, S. 1991. *Calendula* & *Hypericum*; Two homoeopathic drugs promoting wound healing in rats. Filoterapia, **62**: 508 – 510.

Hedderwick, S. and Kauffman, C.A. 1997. Opportunistic fungal infections: superficial and systemic candidiasis. Geriatrics, **52** (10): 50 – 4, 59.

Hickey, M. and King, C. 1997. Common families of flowering plants. Cambridge: University Press.

Hind, J.N. 1996. Royal Botanical Garderns, Kew: *Compositae* (online). Available from http: // www.rbgkew:org.uk/scihort/compositave.html (Accessed 28/08/2001).

Hoads, J. 1996. Healing with Herbs, Dublin: Gill and Macmillan Ltd.

Hobbs, C. 1990. Burdock: A Lion In Porcupine's Clothes (online). Available from http: // www.healthy.net/asp/templates/articles.asp?id=872&Healer Title=Herbal Medicine.html (Accessed 25/04/2001)

Hoffmann, D. 2000a. Burdock (online). Available from http: // www.healthy net/asp/template/article/asp? Page Type Article &id =1627 (Accessed on 25/04/2001).

Hoffmann, D. 2000b. *Calendula* (online). Available from http: // www.healthynet/asp/template/article.asp? Page Type = article &id = 1631 (Accessed on 25/04/2001)

Hoffmann, D. 1996. The Complete Illustrated Holistic Herbal. Dorset: Element Books Limited.

Howard, B. (ed.) 1994. Clinical and Pathogenic Microbiology. St. Louis: Mosby Publishers.

Iwu, M.M. 1993. Handbook of African Medicinal Plants. Boco Raton: CRC Press Incorporated.

Jawetz, E., Joseph, M., Adelberd, E., Brooks G. and Butel, L. 1989. Medical Microbiology. Connecticut: Appleton and Lange.

Kaegi, E. 1998. Uncoventional Therapies of cancer: Essiac. Canadian Medical Association Journal, **158** (7): 876 – 906.

Kant, S. 2000. Fungal Infections. Update: The Journal of Continuing Education for General Practitioners, **10**: 22-26.

Ketchum, P.A. 1988. Microbiology Concepts and Applications. New York: Wiley and Sons Incorporated.

Lawrence, H.M. 1963. Taxonomy of Vascular Plants. New York: The Macmillan Company.

Lipman, J. and Saadia, R. 1997. Fungal infections in critically ill patients. British Medical Journal, **315** (7103): 266 – 7.

Lisiak, M., Klszejko, C., Marcinkowski, Z. 2000. Yeast identification in vulvovaginal candidiasis: Susceptibility to Nystatin. Ginkel Poland. Sept; **71** (9): 959-963.

Lord, E. 2001. The Medicine Chest of *Calendula officinalis* (online). Available from http: // www.herbalhealthplants.com/article 1008 html. Accesssed (28/08/2001)

McFadden, R. 1995. Comparison of the Inhibitory Effects of Various Plant Extracts on the Growth of *Candida albicans – in vitro*. English Journal of Herbal Medicine, 1 (3): 5 – 20.

McLaughlin, G. 1992. *Echinacea*. Australian Journal of Medical Herbalism. **4** (4): 104 -111.

Meyers, M., Jawetz, E., Goldfien, A. 4th Edition. 1974. Review of Medical Pharmacology. California: Lange Publishers.

Moore, C,W. 1998. The *Candida* Syndrome: Health Nemesis/Myth? Natural Life, **60**: 9-12.

Murray, M.T. 1995. The Healing Power of Herbs. 2nd Edition. Rocklin, Calif: Prima Publishing.

Nassoura,Z., Ivatury, R.R., Simon R.J. 1993. Candiduria as an early marker of disseminated infection in critically ill surgical patients: The role of fluconazole therapy. Journal of General Trauma, **35**: 290 – 5.

Ness, J., Sherman F.T., Pan, C.X. 1999. Alternative medicine: what the data say about common herbal therapies. Geriatrics, **54** (10): 33 – 8, 40, 43.

Oxoid Manual. 4th edition. 1979. Hampshire: Oxoid Limited.

Reid, K. 2002. The efficacy of certain *Labiatiae* species herbal extracts in the inhibition of the *in vitro* growth of *Candida albicans* as compared to nystatin. M. Tech (Hom). Technikon Natal, Durban.

Reisacher, W.R., Finn, D.G., Stern, J., Zeifer, B., Cocker, R. 1999. Manifestations of AIDS in the head and neck. Southern Medical Journal, **92** (7): 684 – 97.

Roesler, J., Steinmuller, C., Kidderlen, A., Emmendorffer, A., Wagner, H. 1991. Polysaccharides isolated from plant cell cultures of *Echinacea purpurea* enhance the resistance of immmunosuppressed mice against systemic with *Candida albicans* and Listeria monocytogenes. International Journal of Immunopharmacology, **13** (1): 27-37.

Reynolds, E.F. and Prasad, A.B. 1989. Martindale: The Extra Pharmacopoeia. 28th Edition. London: The Pharmaceutical Press.

Ringdahl, E.N. 2000. Treatment of recurrent vulvovaginal candidiasis. American Family Physician, **61** (11): 3306 – 12.

Robertson, M.B. 1995. Research in Herbal Medicine – A Personal Perspective. English Journal of Herbal Medicine, **1** (3): 21 – 25.

Rowen, J.L., Atkins, J.T., Levy, M.L., Baer, S.C., Baker, C.J. 1995. Invasive fungal dermatitis in the < or = 1000 gram neonate. Paediatrics, **95** (5): 682 – 7.

Sailors, D.M., Barone, G.W., Gagne, P.J., Eidt, J.F., Ketel, B.L., Barnes, R.W. 1996. Candida arteritis : are GI endoscopic procedures a source of vascular infections? American Surgeon, **62** (6): 472 – 7.

Schar, C. 1999. Nature's Remedies: *Echinacea*. London: Souvenir Press Limited.

Smidt, K. 2001. Personal communication to S. Ramlachan, 23 March 2001.

Stary, F. 1998. The natural guide to Medicinal Herbs and Plants. 3rd edition. Twickenham: Tiger Books International PLC.

Valcic, S., Montenegro, G., Mujica, A., Avilla, G., Franzblau, S., Singh, M.P., Maiese, W.M., Timmermann, B.N. 1999. Phytochemical, Morphological and Biological Investigations of Propolis from Central Chile. Verlag der Zeitschrift fur Naturforshcung, **54c**: 406-416.

Vickers, A., Zollman, C. 1999. ABC of complementary medicine: Herbal Medicine. British Medical Journal, **319** (7216): 1050 – 3

Vosloo, C. 2002. The efficacy of *Thymus vulgaris* tincture as an antibacterial agent. M. Tech (Hom). Technikon Natal, Durban.

Wilcox, C.M. and Monkemuller, K.E. 1998. Diagnosis and management of esophageal disease in the acquired immunodefiency syndrome. Southern Medical Journal, **91**(11): 1002-1008. 348.

Williams, L., Home, V. 1995. A comparative study of some essential oils for potential use in topical applications for the treatment of the yeast *Candida albicans*. Australian Journal of Medical Herbalism, **7** (3): 57-62.

Wren, R.C. 1988. Potter's Encyclopedia of Botanical Drugs and Preparations. London: C.W. Daniel Company Limited.

Yamamura, D.L., Rotstein, C., Nicolle, L.E., Ioannou, S. 1999. Candidemia at selected Canadian sites: results from the Fungal Disease Registry, 1992 – 1994. Fungal Disease Registry of the Canadian Infectious Disease Society. Canadian Medical Association Journal, **160** (4): 493 – 9.

# LIST OF APPENDICES

## APPENDIX A

Raw data

## APPENDIX B

### PHOTOGRAPHY OF CULTURES

Plate B 1 - The antifungal effects of the test and control substances for agar plates 1 - 5 for observations at 18 hours.

Plate B 2 - The antifungal effects of the test and control substances for agar plates 6 - 10 for observations at 18 hours.

Plate B 3 - The antifungal effects of the test and control substances for agar plates 11 - 15 for observations at 18 hours.

Plate B 4 - The antifungal effects of the test and control substances for agar plate 3 for observations at 18 hours.

**KEY**

1 - The effects of *Arctium lappa* tincture for observations at 18 hours.

2 - The effects of *Calendula officinalis* tincture for observations at 18 hours.

3 - The effects of *Echinacea purpurea* tincture for observations at 18 hours.

4 - The effects 62% ethanol (v/v) for observations at 18 hours.

5 - The effects of nystatin for observation at 18 hours.

| Tests & Control | Zone diameter (mm) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TEST 1 | | | TEST 2 | | | TEST 3 | | | TEST 4 | | | TEST 5 | | |
| | 18hrs | 24hrs | 36hrs | 18 hrs | 24 hrs | 36hrs | 18 hrs | 24 hrs | 36hrs | 18hrs | 24hrs | 36hrs | 18 hrs | 24 hrs | 36hrs |
| 1) *Arctium Lappa* 62%(v/v) | 7.6 | 5 | 5 | 8.8 | 5 | 5 | 8.8 | 5 | 5 | 7.8 | 5 | 5 | 7.6 | 5 | 5 |
| 2) *Calendula officinalis* 62%(v/v) | 8.5 | 5 | 5 | 8 | 5 | 5 | 8.3 | 5 | 5 | 9.7 | 5 | 5 | 7.8 | 5 | 5 |
| 3) *Echinacea purpurea* 62%(v/v) | 6.6 | 5 | 5 | 7.3 | 5 | 5 | 8.3 | 5 | 5 | 8.2 | 5 | 5 | 7.3 | 5 | 5 |
| 4) Ethanol 62% (v/v) | 8.8 | 5 | 5 | 8.2 | 5 | 5 | 10.3 | 5 | 5 | 8.2 | 5 | 5 | 9 | 5 | 5 |
| 5) Nystatin | 14.3 | 14.3 | 11.2 | 13.3 | 12.8 | 10.7 | 11.6 | 11.5 | 8.6 | 16.3 | 15.3 | 14.3 | 14.7 | 14.3 | 12.7 |

| | TEST 6 | | | TEST 7 | | | TEST 8 | | | TEST 9 | | | TEST 10 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 18hrs | 24hrs | 36hrs | 18hrs | 24hrs | 36hrs | 18hrs | 24hrs | 36hrs | 18hrs | 24hrs | 36hrs | 18hrs | 24hrs | 36hrs |
| 1) *Arctium Lappa* 62%(v/v) | 7.8 | 5 | 5 | 9.7 | 5 | 5 | 8.6 | 5 | 5 | 7.7 | 5 | 5 | 9 | 5 | 5 |
| 2) *Calendula officinalis* 62%(v/v) | 9.3 | 5 | 5 | 8 | 5 | 5 | 8.5 | 5 | 5 | 8.5 | 5 | 5 | 8.8 | 5 | 5 |
| 3) *Echinacea pupurea* 62%(v/v) | 8.2 | 5 | 5 | 8.2 | 5 | 5 | 8.8 | 5 | 5 | 8.5 | 5 | 5 | 8.5 | 5 | 5 |
| 4) Ethanol 62% (v/v) | 8.5 | 5 | 5 | 10.3 | 5 | 5 | 8.8 | 5 | 5 | 8.6 | 5 | 5 | 9.5 | 5 | 5 |
| 5) Nystatin | 13 | 12.2 | 10 | 15.3 | 15.3 | 12 | 15 | 15 | 12.3 | 15.3 | 14.7 | 14.2 | 16 | 16 | 13.3 |

| | TEST 11 | | | TEST 12 | | | TEST 13 | | | TEST 14 | | | TEST 15 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 18hrs | 24hrs | 36hrs | 18hrs | 24hrs | 36hrs | 18hrs | 24hrs | 36hrs | 18hrs | 24hrs | 36hrs | 18hrs | 24hrs | 36hrs |
| 1) *Arctium Lappa* 62% (v/v) | 8.7 | 5 | 5 | 5 | 5 | 5 | 8.2 | 5 | 5 | 8.6 | 5 | 5 | 8.7 | 5 | 5 |
| 2) *Calendula officinalis* 62%(v/v) | 8.3 | 5 | 5 | 9 | 5 | 5 | 8.8 | 5 | 5 | 8.3 | 5 | 5 | 5 | 5 | 5 |
| 3) *Echinacea pupurea* 62% (v/v) | 9.3 | 5 | 5 | 8.6 | 5 | 5 | 9 | 5 | 5 | 8.7 | 5 | 5 | 10.7 | 5 | 5 |
| 4) Ethanol 62% (v/v) | 11 | 5 | 5 | 8.8 | 5 | 5 | 10.7 | 5 | 5 | 9.3 | 5 | 5 | 10.3 | 5 | 5 |
| 5) Nystatin | 16.7 | 16.7 | 14.3 | 17.3 | 17.3 | 15 | 20.3 | 18.3 | 16.7 | 15.7 | 15 | 13 | 16 | 15.7 | 13 |



## APPENDIX A - RAW DATA

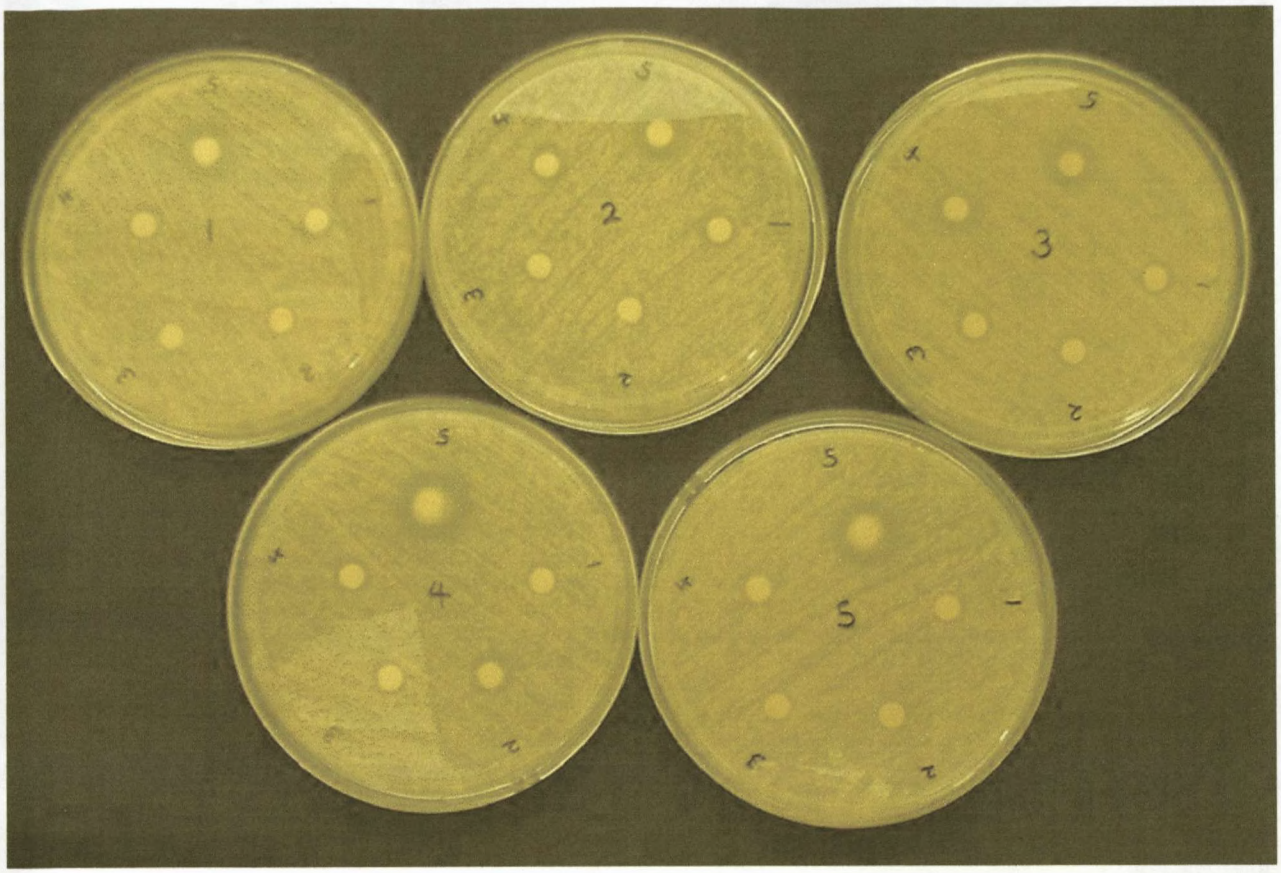


**Plate B 1 – The antifungal of the test and control substances for agar plates 1 – 5 for observations at 18 hours**

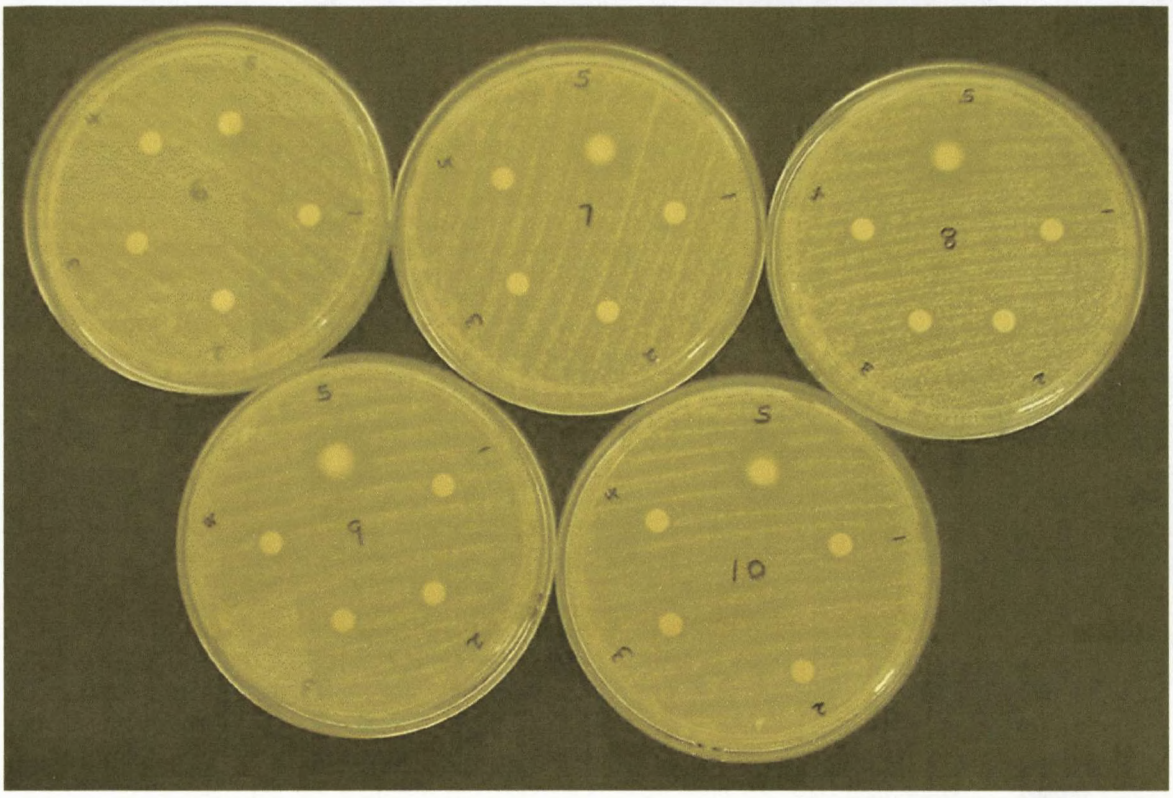


**Plate B2 – The antifungal effects of the test and control substances for Agar plates 6 – 10 for observations at 18 hours**

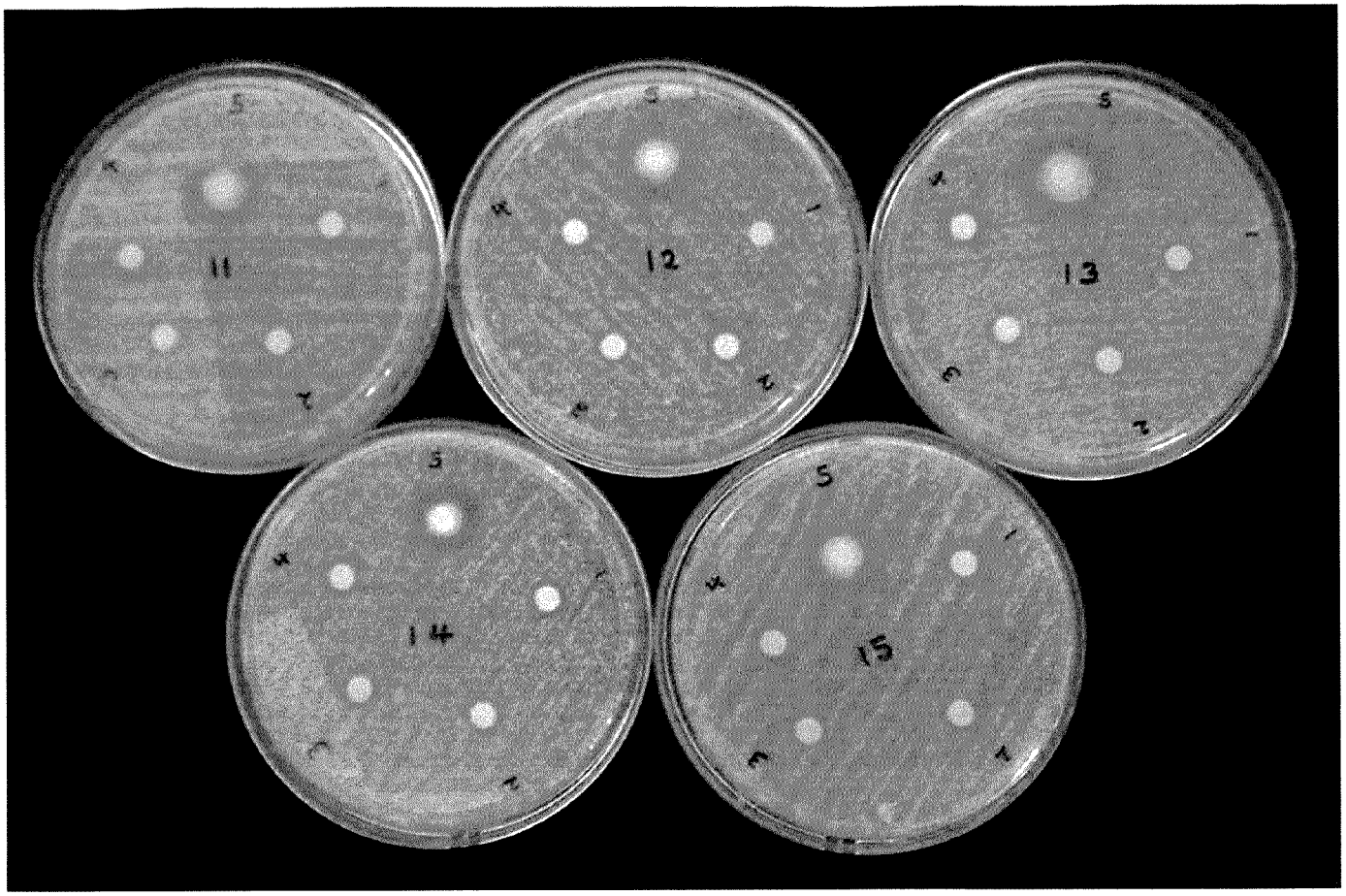


**Plate B 3 – The antifungal effects of the test and control substances for agar plates 11 – 15 for observations at 18 hours**

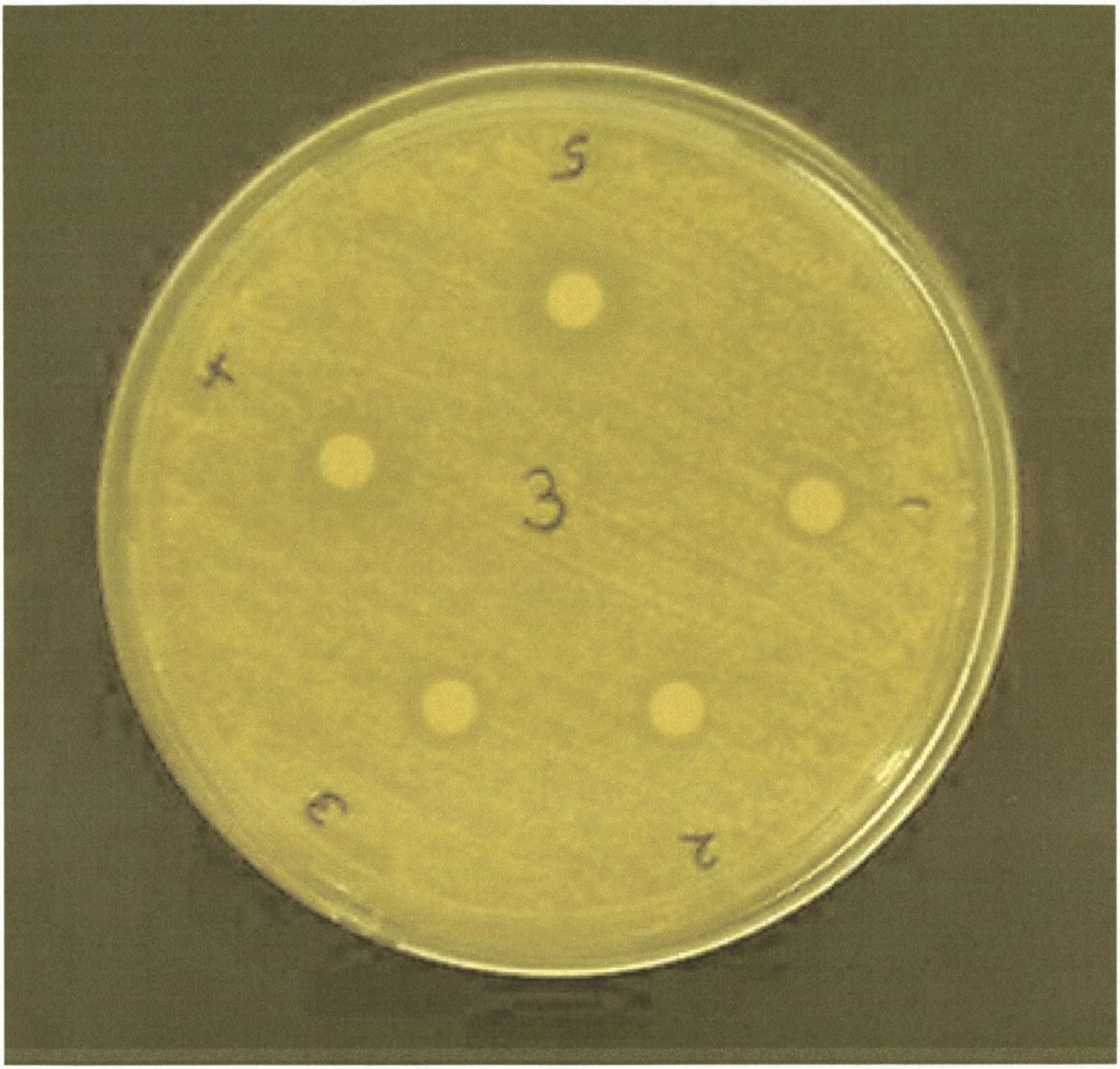


**Plate B 4 - The antifungal effects of the test and control substances for agar plate 3 for observations at 18 hours**