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A COMPARATIVE STUDY TO ESTABLISH THE EFFECT OF HOMOEOPATHIC *ECHINACEA PURPUREA* AND CONVENTIONAL NYSTATIN ON THE GROWTH OF *CANDIDA ALBICANS* IN VITRO.

Jodi Elizabeth Sutherland

A COMPARATIVE STUDY TO ESTABLISH THE EFFECT OF HOMOEOPATHIC *ECHINACEA PURPUREA* AND CONVENTIONAL NYSTATIN ON THE GROWTH OF *CANDIDA ALBICANS* IN VITRO.

Jodi Elizabeth Sutherland

DECLARATION

I, Jodi Elizabeth Sutherland, declare that this dissertation is my own work. It is being submitted in partial fulfilment of the requirements for the Degree Masters in Technology, Homoeopathy (M. Tech. Hom.) at the Technikon Witswatersrand, Johannesburg and has not been submitted at any other teaching institution.

_____   _____
Signature of Candidate   Date

Approved for final submission

_____   _____
Supervisor: Mr. A. van den Berg   Date
(MSc. Med.)

_____   _____
Co-Supervisor: Dr. B. van Olden   Date
(D. Hom., D. Hom. Med., N.D., D.O.)

ABSTRACT

The aim of this study was to determine and compare the effect of *Echinacea purpurea*, prepared in homoeopathic form, to conventional Nystatin as an antifungal agent.

The *Echinacea purpurea* used was in 2D, 6D, 15D and 30D homoeopathic potencies prepared in 20% ethanol.

Twenty clinical isolates of *Candida albicans* were used to eliminate the possibility of mutation and the possible development of resistance patterns.

Candida albicans was inoculated into nutrient broth. The experimental broth tubes consisted of a control, unmedicated nutrient broth, one tube for each of the various potencies used in the experiment, and one tube of sterile nutrient broth to calibrate the spectrophotometer. After 24 hours the optical density was measured to monitor the growth. This procedure was done in triplicate for each of the clinical isolates and the results were recorded and statistically analysed.

The one way analysis of variance which was conducted showed that the normality and equal variance tests passed. The Dunnett's method of comparison indicated that while there was a statistically significant difference between the control and the potentised *Echinacea purpurea*, there was no statistically significant difference between the various potencies.

Candida albicans was streaked onto plates of Sabouraud agar. Each plate was divided into 5 segments, one for each medicated disc of the various potencies of *Echinacea purpurea* and one for Nystatin. After a 24 hour incubation period at 37°C the zones of inhibition around the medicated discs were measured using calipers. This procedure was done in triplicate for each of the clinical isolates and the results were recorded and statistically analysed.

Statistical analysis showed that the Nystatin had a more noticeable effect on the *Candida albicans* compared to the *Echinacea purpurea*, and that the statistical significance between the various potencies was minimal.

DEDICATION

This dissertation is dedicated to my family
for all their love and support,
without them I would not be where I am today.

ACKNOWLEDGEMENTS

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CHAPTER ONE

1.0 INTRODUCTION

1.1 The Problem Statement

1.1.1 *Candida albicans*

Candida albicans is an oval, budding yeast that produces a pseudomycelium both in culture, tissues and exudates (1). It is a member of the normal flora of the mucous membranes in the respiratory tract, gastrointestinal tract, female genital tract and oral cavities (1). It occurs in the mouth and faeces of 20%-30% of healthy persons (2), where it produces no signs or symptoms. However it may gain dominance when the normal flora is disturbed causing candidiasis (3). Infection is thus usually endogenous, but occasionally can be exogenous and thus contagious (2). Infection is generally dependent on a weakening of the immune system, e.g. by diabetes, leukaemia, iron-deficiency anaemia, neonatal debility, senility, alcoholism, drug addiction, antibiotic therapy and cortisone therapy (2). In the 1990s intestinal candidiasis is affecting all age groups, thus suggesting that our immune systems are at risk (4). In man, *Candida albicans* produces superficial infections of the skin (dermatocandidiasis) and mucous membranes (oral and vaginal thrush); it also causes broncho-pulmonary infections and, though less frequently, septicaemia and deep, blood-borne infections such as meningitis and pyelonephritis (2).

1.1.2 Nystatin

Nystatin is a conventional antimycotic antibiotic used for treating cutaneous and mucocutaneous infections caused by *Candida albicans* and other Candida species (5). It is either fungistatic or fungicidal depending on the drug concentration; the presence of blood, pus or tissue fluid that reduces activity; and the susceptibility of the fungus (6). Its antifungal activity is dependent on its binding to a sterol moiety in the membrane of the sensitive fungi (7). With the marked increase in the number of immunocompromised patients, e.g. AIDS patients, fungi are emerging as predominant pathogens and unfortunately with the indiscriminate use of antifungal agents to combat these infections, reports of resistance to antifungal agents have proliferated (8). Besides the possibility of Candida resistance developing to Nystatin (5), the adverse effects associated with these drug courses are discouraging. A viable alternative medical approach to treating infections

is desirable for two reasons: a) the conventional approach is mainly curative and not preventative b) the possibility of side-effects. Alternative medicines are more patient-orientated viewing the patient holistically (4). Medicine which recognises and treats the different levels of being enables a better understanding of the origins of many illnesses and is thus able to be both curative and preventative (4). Alternative natural medication is also for the most part free of side-effects (4).

1.1.3 Natural alternative medication

Although antibiotics can be life saving drugs (4), repeated courses can disturb the internal homeostasis and thus the immune system (9). It is therefore hoped that viable alternatives can be found to provide the restoration of health by eradicating the disease in its entirety, via the shortest, most reliable and least harmful route (10).

1.1.4 *Echinacea purpurea*

The root of the medicinal herb *Echinacea purpurea* is recognised for its ability to fight infection and to stimulate the immune system (4). *Echinacea purpurea* is a potent natural antibiotic, and thus a safe alternative to synthetic antibiotics (11). It is effective against a wide range of microbes, including many viruses, bacteria and fungi (4,11).

The aim of this study was to determine and compare the effect of *Echinacea purpurea* prepared in homoeopathic form to Nystatin as an antifungal agent on the growth of *Candida albicans*. This was done by determining any mycostatic or mycocidal effect of the potentised *Echinacea purpurea* and comparing this with Nystatin.

This study was an attempt to establish the area of action of homoeopathic *Echinacea purpurea* in effecting a response in a *Candida albicans* infection.

The study was formulated to determine:

1. whether *Echinacea purpurea* had an effect on *Candida albicans in vitro*,

2. and to provide motivation for further research as to where and how homoeopathic substances effect a response *in vivo* in the treatment of disease

1.2 Antibiotic Therapy

Antibiotics were initially developed in the 1940s. Since then a whole range of antibiotics have been produced, and are now among the most commonly prescribed drugs in the world (4). They are used to treat infection because they act by destroying, or controlling the causative organism (4).

There are four serious complications of antibiotic therapy:

1. Induction of antibiotic resistance:

Although antibiotic resistance appeared shortly after the development of antibiotics, the magnitude of the problem is increasing as both the formation and the transmission of antibiotic-resistant strains increase (12). Antibiotics can act by either inhibiting protein synthesis, cell wall formation, nucleotide or intermediary metabolism or disorganising membrane function (6).

A pathogen becomes resistant to an antibiotic used to treat it by,

- *Natural resistance*

- natural resistance which is genetically determined and depends upon the absence of a metabolic process affected by the antibiotic used in treatment. Natural resistance may be characteristic of a species or may be confined to a particular strain within a species.

- *Acquired resistance*

- mutation: spontaneous or induced.

- adaptation which presupposes that organisms contain low concentrations of antibacterial destructive enzymes, or the potential for synthesising such enzymes, and that lethal enzyme concentrations are "triggered" through enzyme induction following antibiotic exposure.

- infectious (multiple) drug resistance involves the transfer of genetic material coding for resistance from a resistant to a sensitive strain and/or species (6).

Resistant pathogens cause an antibiotic to be ineffective by either destroying or modifying the antibiotic or preventing the antibiotic from recognising or accessing its target (12). The development of cross resistance to related drugs and even between chemically dissimilar drugs, further complicates the situation (6).

The development of resistance is directly correlated with the level of antibiotic use (12). Prophylactic and empiric use of antibiotics as well as the increased use of broad-spectrum antibiotics and the general use of antibiotics in the community are major contributors to increased resistance (12). In the past, to combat antibiotic resistance a different antibiotic was used, but new types of antibiotics are running out (12).

Lessons learned in the treatment of bacteria also apply to fungi: prolonged use of an antimicrobial agent will result in resistant organisms (8). The escalating resistance to antifungal agents has prompted aggressive searches for new modes of therapy (8).

3

Twenty clinical isolates of *Candida albicans* have been used in this study in an attempt to negate the problem of possible resistance patterns.

2. Effects of the placement flora:

Candida species and other opportunistic yeasts or moulds increase in the gastrointestinal tract during the period of drug therapy as they are no longer being controlled by the organisms that succumb to the antibiotic. With their ability to now multiply uncontrollably and, if potentially pathogenic, they may start a disease process (9). Such secondary infections are often caused by *Candida albicans* following prolonged and extensive broad-spectrum antibiotic treatment (9).

3. Toxicity:

"No drug is free of toxic effects" (7). The adverse effects may be trivial or serious and, they may appear promptly or gradually. Some toxic effects of drugs are actually an extension of the desired effects and can be reduced or controlled by correct adjustment of the dosage (7). Often the desired and undesired effects of a drug are different manifestations of the same primary action and are thus inseparable (7). The effect of a drug that is sought in one patient may become an undesired effect in another when the drug is used for a different purpose (7). Some toxic effects occur only in certain patients or in combination with other drugs (7). With the introduction of drugs of greater and broader efficacy the problem with drug toxicity is increasing (7). Blood dyscrasias, hepatotoxicity and nephrotoxicity, teratogenic effects, behavioural toxicity, drug dependence or addiction, drug poisoning and drug allergies are just a few examples of drug-induced diseases (7). These adverse effects not only arise because of the inherent toxicity of the drugs and the limitations of the methods for early detection of toxicity, but also because of the excessive dosage of drugs that are prescribed (7).

4. Hypersensitivity (allergy):

Hypersensitivity (allergic) reactions represent the largest group of adverse drug reactions (6). To cause an allergic reaction the drug or drug metabolite(s) (haptene), acting as antigens, must combine strongly with a tissue or plasma protein. This complex is processed by the reticuloendothelial system and ultimately produces an antibody. Antibodies react with the complex and the haptene, in an antigen-antibody reaction. (6,13). The way in which antigen-antibody reactions cause their adverse effects differ according to the type of

reaction (13). While some reactions may involve haemolysis, others may involve the release of antihistamine, prostaglandins, serotonin, and kinins causing, for example, asthma or urticaria (13).

The manifestations of allergic drug reactions are numerous and include the full spectrum from immediate to delayed allergic responses (7). Skin reactions range from a mild rash to severe exfoliative dermatitis (7,13). Vascular responses range from acute urticaria and angioderma to severe arteritis (7,13). Drug fever is typically manifested by fever, leucocytosis, arthralgia and dermatitis, closely resembling serum sickness (7). Fever rhinitis, asthma, and anaphylactic shock are other common allergic reactions (7,13).

An allergic reaction usually occurs only after prior exposure to an antigen, however a person with no known history of receiving the drug may develop an allergic reaction on initial administration of it due to prior environmental exposure to a related chemical that stimulated antibody production (13).

The potential for an allergic response may remain forever after initial exposure, due to the antibodies that have formed in the blood. Extremely small quantities of medication may provoke the reaction, and the severity of the reaction does not always relate to the dose of drug or the route of administration (13). However in some cases re-exposure of the patient to the original drug may not elicit an allergic response at all (6).

Patients with an allergic diathesis are more likely to develop a drug reaction (13).

Antibodies against the offending drug or its metabolites are not always demonstrated in the patient (6), and often the drug or drug metabolite(s) that can act as haptens are not detected in usual biotransformation studies (7).

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CHAPTER TWO

2.0 REVIEW OF BASIC HOMOEOPATHIC PRINCIPLES

2.1 The Work of Hahnemann

Within the total context of medicine, homoeopathy may be defined as a form of regulatory therapy that aims at influencing autoregulation with the aid of a drug that relates to the way the individual patient reacts (1).

The founder of homoeopathy, Christian Friederich Samuel Hahnemann (1755-1843) based his practical medical treatment on three principles:

- Drugs tested on healthy subjects.

- Individual disease picture.

- Law of Similars (*Similia similibus curentur*), which means that an illness should be treated by a substance capable of producing similar symptoms to those suffered by the patient. This forms the basis of homoeopathy (1).

2.2 The Drug

Homoeopathy obtains data relating to drug actions from:

- Drug tests on healthy humans

- Data from toxicology and pharmacology

- Clinical use

- Use and experimental studies on animals

The totality of these data is known as the "drug picture" or "the sum and essence of the morbid elements a drug is able to produce" (1).

2.3 The Preparation of Homoeopathic Medicines

Homoeopathic remedies are derived from plant, animal, mineral extracts and only a few from synthetic compounds (1).

When homoeopathic remedies are derived from soluble substances, such as animal or plant extracts, the raw materials are dissolved in an ethanol/water mixture that contains 90% ethanol made in distilled water (this ratio may vary depending on the substance). This is then left to stand for 2-4 weeks, being shaken occasionally and finally strained through a press. The resulting mixture is known as a mother tincture or tincture (φ) (2).

Insoluble substances, such as gold or copper, are first ground in lactose, in a process known as trituration, up to a 3c potency, before they are diluted in the same way as naturally soluble substances (2).

Hahnemann developed a specific technique that involved a combination of serial dilution and succussion in the preparation of his remedies. Hahnemann called the drugs prepared by this method potencies or dynamizations (3).

To produce different potencies of a remedy the mother tincture is diluted in an ethanol/ water mixture according to either the decimal (x or DH) or centesimal (c or CH) scale (3). In the decimal scale the dilution factor is 1:10 and in the centisimal scale it is 1:100 (3). One drop of the mother tincture is diluted, to raise the level of the potency, into 9 or 99 drops of 40% ethanol/water solution, and then the dilution is succussed with great force 100 times, thus adding kinetic energy to the solution. One drop of the succussed dilution is then added to 9 or 99 drops of fresh solvent; this is then succussed 100 times again, and diluted as before. This process can be carried out literally forever, always increasing the therapeutic power while nullifying the toxic properties (3).

According to the laws of chemistry, there is a limit to how many serial dilutions can be made before losing the original substance in the mixture. This limit is known as Avogadro's number, and it roughly corresponds to a homoeopathic potency of 24x (or 12c). Thus any potency above 24x (or 12c) contains no molecule of the original substance, however potencies far beyond this limit continue to increase in power (3).

There is no available explanation for this phenomenon, yet it appears that some form of energy that is contained in a limited form in the original substance is released and transmitted to the molecules of the solvent by this technique. Once the original substance is no longer present, the remaining energy in the solvent can be continually enhanced as the solvent molecules have taken on the dynamic energy of the original substance (3).

2.4 **Potency**

Homoeopathy is a regulatory therapy that calls for individualisation in the determination of the nature and power of the stimulus (1). The toxicology based on material doses and the subtle toxicology of homoeopathic provings show a corresponding affinity of medicinal agents to the level of the disorder: organic lesions call for low potencies, functional disorders for medium, and predominantly mental states call for high potencies (1). The terms "low", "medium" and "high' potency do not refer to absolute values (1). The standard

to be used is the reactivity of the diseased organism i.e. the patient's sensivity (1). Low potencies are generally considered to be from the ϕ to 6x (4c or 5c), medium from 12x to 21x (7c or 9c) and high potencies from 15c or 30c upwards (1,4). The low decimal potencies (3x and 6x) are considered mainly for drainage purposes (4).

2.5 The Principle of the Vital Force

Hahnemann discovered that the higher the dilution/potency of a remedy the more effective its cure would be, thus he reasoned there must be some kind of a subtle energy within the body that responds to the tiny provocation's of the remedies and enables the body to heal itself (2). Hahnemann called this energy the body's "vital force", without which he believed the material organism is unable to feel, or act or maintain itself (5). The symptoms of illness are the outward manifestation of the vital force's attempt to restore homeostasis (2). The aim of homoeopathic treatment is not to remove or suppress symptoms in a specific way, as is done in conventional medicine, but to restore the total balance of the organism by stimulating the vital force to return to its healthy state (5,6).

Hahnemann considered the action of the remedy to be due to its production of an artificial disease similar to the patient's illness. This artificial disease produces a reaction from the body which in turn cures the illness. The stimulus must be accurate, and the response depends on the initial state of the organism (6).

2.6 Homoeopathic Laboratory Research

The principle of "like cures like" is the basis of homoeopathy, and therefore deserves rigorous testing by scientific means not only to answer the questions of sceptics but also to help homoeopathy to optimise the use of natural medicine. Laboratory research is able to show biological activity of homoeopathic medicines which cannot be explained as a placebo response, a common accusation of sceptics. As laboratory research seeks to assess changes in biological systems it is capable of explaining how the homoeopathic medicines may work. However very few well designed scientific trials have been conducted. Although it is not yet known how homoeopathic remedies actually work or where they work, their effect on the body as well as on groups of cells and specific organs is well recorded.

Since homoeopathic *Echinacea purpurea* has a direct effect on the yeast cells it can be assumed that the homoeopathic method of inhibiting cell growth and multiplication in *Candida albicans* infections is by direct action on the micro-organism.

2.7 Other Forms of Homoeopathic Treatment in Candidiasis

Homoeopathically the treatment of candidiasis is approached on three levels: symptomatic treatment, nosodal treatment and constitutional treatment (6,7).

Symptomatic remedies which are often used to treat acute attacks of candidiasis are Psorinum and Helonias (7).

The nosodal treatment is homoeopathically prepared *Candida albicans* (7).

Constitutional remedies are used to treat patients with chronic recurring attacks of candidiasis, which act by stimulating the vital force and thus improving the patients general wellbeing thereby raising the patient's resistance to infection and preventing further infection (6). Constitutional prescribing involves individualisation of the case, determination of the totality of the symptoms and selection of the important symptoms according to given criteria (1). A predisposition to parasitic infections is a sign of a psoric reactional mode and major remedies that have in their proving all or part of the psoric reactional mode are Sulphur, Sepia, Arsenicum album, Arsenicum iodatum, Lycopodium, Calcarea carbonica, and Psorinum (7).

Echinacea purpurea can provide symptomatic relief as it acts directly on the organism inhibiting the growth of the cells and by stimulating the immune response, *Echinacea purpurea* can raise the patient's resistance to infection, thus improving the patients wellbeing and preventing further infection.

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Verification of the Possible Ethanol Effects on *Candida albicans*

The necessary concentration of ethanol in distilled water for the homoeopathic remedy to be prepared in, without the ethanol content having any direct effect on the *Candida albicans* spp., was determined. Twenty Sabouraud agar plates were streaked with the respective clinical isolate. The plate was then divided into 8 segments, one for each of the percentages of alcohol to be tested, i.e. 20%, 35%, 45%, 60%, 70%, 80%, and 96%, and one segment for the distilled water which acted as a control.
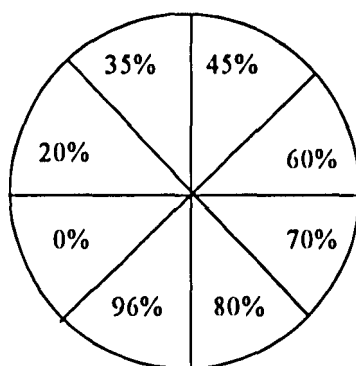
Sterile discs made from Whatman 1 filter papers (Springfield Mill, Maidstone, Kent, England), which was cut 5-6mm in diameter using a hand punch, were impregnated with ethanol/distilled water, and placed in their respective segments using sterile fine-pointed forceps. The plates were then incubated for 24 hours at 37°C. After 24 hours the growth was recorded and tabulated. (See chapter 4). This procedure was repeated in triplicate.

3.2 *Echinacea Purpurea* - The Remedy

The *Echinacea purpurea* (planta tota) was obtained from Natura Laboratories (Hazelwood, Pretoria), in the following potencies: D2, D6, D15 and D30 which were used for the remaining experiments. The procedure followed at Natura was briefly:

Echinacea purpurea D2

A mother tincture containing 60% ethanol was used to prepare a D1 in 20% ethanol. The D1 was succussed 10 times by hand. This gave a final potency of D2 in 20% ethanol.

Echinacea purpurea D6

A D3 containing 73% ethanol was used to prepare a D4 in 20% ethanol. The D4 was succussed 10 times by hand to get a D5 in 20% ethanol which in turn was succussed 10 times by hand to give the final potency of D6 in 20% ethanol.

Echinacea purpurea D15

A D12 containing 73% ethanol was used to prepare a D13 in 20% ethanol. The D13 was succussed 10 times by hand to prepare a D14 in 20% ethanol. The D14 was succussed 10 times by hand to give the final potency of D15 in 20% ethanol.

Echinacea purpurea D30

A D27 containing 73% ethanol was used to make a D28 in 20% ethanol. The D28 was succussed 10 times by hand to prepare a D29 in 20% ethanol. The D29 was succussed 10 times by hand to give the final potency of D30 in 20% ethanol.

3.3 Preparation and Acquisition of Media and Cultures

Sabouraud agar obtained from the South African Institute for Medical Research (Johannesburg, South Africa) and nutrient broth (Oxoid, Unipath LTD, Basingstroke, UK) were the media of choice.

Twenty strains of *Candida albicans*, isolated from clinically significant infections, were obtained from the South African Institute of Medical Research. These were maintained by subculturing each week onto fresh Sabouraud agar plates.

The clinical isolates were subcultured into nutrient broth which had been prepared and distributed into 20 broth tubes each containing 10ml aliquots of broth. Each broth tube had been capped and autoclaved at 121°C for 15 minutes. The inoculated nutrient broth was then used to preserve the clinical isolates by freeze drying, as per manufacturers specifications (LSL Secfroid. Supplier/serviced by Labtec, Midrand, Gauteng).

All 60 sealed glass ampoules were stored in the refrigerator (Defy. Suppliers/serviced by Gaynors, Central Johannesburg, South Africa) at 4-6°C.

3.4 Determination of the Effect of Homoeopathic *Echinacea Purpura* on *Candida albicans*

3.4.1 Determination of the standard curve

A standard curve of the optical density at 540nm of the nutrient broth against colony forming units per ml was determined. This straight line graph was formulated using five clinical isolates.

10ml aliquots of nutrient broth was dispensed into 5 broth tubes and a further 9ml aliquots was dispensed into 40 other broth tubes. The broth tubes were capped and autoclaved. The five tubes containing 10ml of nutrient broth were then inoculated with colonies from five clinical isolates and incubated at 37°C for 24 hours. After 24 hours the tubes were agitated using a vortex stirrer (Whirl-EE-Mix, vortex stirrer. Helena Laboratories (UK) Limited), in order to distribute the growth throughout the nutrient broth in the tube. 1ml was then transferred, using a sterilised 1ml pipette into the first of 8 broth tubes each containing 9ml aliquots of nutrient broth. 1ml from tube 1 was transferred to tube 2 and then 1ml from tube 2 was transferred to tube 3, this dilution procedure was repeated until tube 8, thus diluting the original inoculum though a series of broth tubes. This was repeated for each of the five clinical isolates. Optical density readings were then determined for each of the dilution series using the spectrophotometer at 540nm (Jenway 6100 Spectrophotometer, Jenway LTD Dunmow Essex England). To determine the number of colony forming units per ml for each serial dilution, 0.1 ml of each concentration was transferred onto separate Sabouraud agar plates. The Sabouraud agar plates were then incubated for 24 hours at 37°C after which the colonies were read by means of a colony counter (Lapiz Laboratory Equipments. Bacteriological digital. Media Instrument MFG. Co., Bombay. Supplied by Instrulab cc. Erand Midrand). Only the plates that produced between 0 and 300 colonies were counted as these numbers have been shown to give the most reliable indication as to the correct number of cells. The readings were then statistically analysed and represented as a standard curve. (See chapter 4).

3.4.2 Determination of the antimicrobial activity of *Echinacea purpurea*

A series of nutrient broth tubes containing the homoeopathic compound in the potencies to be tested was prepared in triplicate for each isolate. 0.5ml of the respective homoeopathic potency, 0 potency, 2D, 6D, 15D and 30D was added to 4.5ml of nutrient broth. These tubes were then inoculated with isolates and incubated at 37°C for 24 hours after which

optical density readings at 540nm were determined for each sample. Sterile nutrient broth was used as a blank.

The number of colonies in each sample was then determined by using the standard curve and plotting the optical density reading against the number colony forming units per ml.

3.5 Comparison of Allopathic Nystatin Effects Versus Homoeopathic *Echinacea purpurea* on *Candida albicans*

3.5.1 Preparation of homoeopathic antimicrobial discs

Four 25ml bottles, one for each of the homoeopathic potencies to be tested, were autoclaved. Each bottle was then filled with 100 discs made from Whatman 1 filter papers. The discs were autoclaved before being placed in the bottles using a sterile fine-pointed forceps in a laminar flow cabinet. Using a sterile pipette, 1ml of the homoeopathic remedy was then pipetted into the bottle in the laminar flow cabinet. This was repeated for each of the potencies of *Echinacea Purpurea*. It was assumed that each disc was thus impregnated with 0.01ml of the homoeopathic remedy. The bottles containing the medicated discs were then kept in a dark, cool cabinet under sterile conditions.

Premedicated discs of Nystatin were obtained from the South African Institute of Medical Research and were kept in the refrigerator at 4-6°C.

3.5.2 Determination of the antimicrobial activity of Nystatin and *Echinacea purpurea*

Twenty Sabouraud agar plates were streaked with their respective clinical isolate of *Candida albicans* using a sterile cotton-wool swab which had been dipped into a broth tube containing 4.5 ml aliquots of sterile nutrient broth which had been inoculated with the respective clinical isolate of *Candida albicans* and incubated at 37°C for 24 hours. Each plate was then divided into 5 segments, i.e. one for each of the homoeopathic potencies of *Echinacea Purpurea* and one for the Nystatin which served as a control.
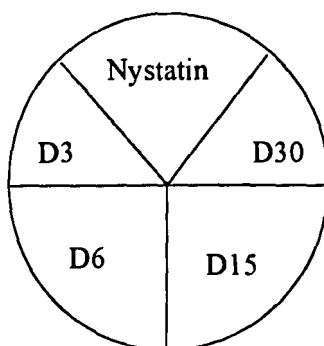
Once the medicated discs had been distributed in their respective quadrants using sterile, fine-pointed forceps, the plate was incubated at 37°C for 24 hours. After 24 hours the zone of inhibition for each of the homoeopathic potencies of *Echinacea Purpurea* and the Nystatin was measured using Vernier calipers (Mitutoyo, High precision calipers). The zone of inhibition was measured and recorded as being the largest diameter from the edge of the disc to the edge of transparency.
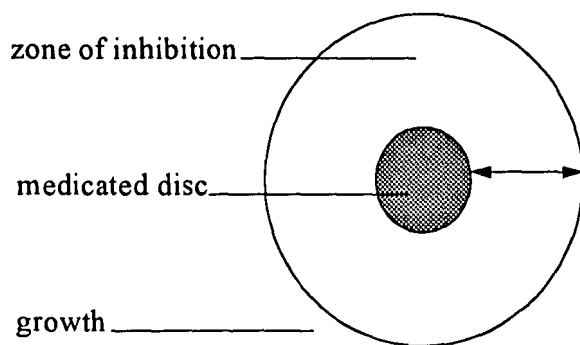
Although the Vernier calipers measure very accurately eye interpretation errors have to be considered. This procedure was repeated in triplicate for each of the 20 clinical isolates. The results were statistically analysed and presented in the form of a histogram. (See chapter 4).

3.6 Statistical Methods

The results were statistically analysed by means of the statistics program Sigma Suite by Jandel Scientific which incorporates Sigma Plot, Sigma Stat and Sigma Scan/Image.

Means and standard errors were calculated for all experiments and one and two way analysis of variance tests were carried out where appropriate to determine the true significance of the results.

3.7 Echinacea

3.7.1 Taxonomy

Echinacea is one of the members of the coneflowers, a group of native American wildflowers from the Daisy family (Asteraceae = Compositae), characterised by spiny flowering heads, with an elevated receptacle that forms the "cone" (1).

There are nine Echinacea species of which *Echinacea angustifolia, Echinacea purpurea, and Echinacea pallida* are the most commonly used (2).

3.7.2 Synonyms of *Echinacea purpurea* (L) Moench

Rudbeckia purpurea

Brauneria purpurea (1).

3.7.3 Common names

Purple Coneflower, Purple Kansas Coneflower, Black Samson, Red Sunflower, Comb Flower, Cock Up Hat, Missouri Snakeroot and Indian Head (1).

3.7.4 Chemical composition

From a pharmacological perspective the important constituents of Echinacea can be divided into seven categories: polysaccharides, flavonoids, caffeic acid derivatives, essential oils, alkylamides, polyacetylenes and miscellaneous chemicals (2).

• *Polysaccharides*

A number of immunostimulatory and mild anti-inflammatory polysaccharides have been isolated from *Echinacea purpurea*, found particularly in the aerial parts of the plant (2). Most notably is inulin which possesses significant immune-enhancing properties (2). Echinacea polysaccharides have been shown to enhance macrophage phagocytosis and stimulate macrophages to produce a number of immune-enhancing compounds e.g. tumour necrosis factor- α (TNF), interferon β_2, and interleukin 1 (2).

• *Flavonoids*

Echinacea purpurea contains numerous flavonoids such as quercetin, kaempferol, isorhamnetin, and rutin (2,3).

• *Caffeic acid derivatives*

Important caffeic acid derivatives include: echinacoside (not present in *Echinacea purpurea*), cichoric acid, chlorogenic acid, and cynarin (1,2,3).

• *Essential oils*

The major essential oil components of *Echinacea purpurea* are sesquiterpene derivatives, borneal, α- and β-pinene, limonene, germacrane D, humulene, caryophyllene, caryophyllene epoxide (2,3).

- *Alkylamides*

Alkylamides such as isobutylamid exert a tingling sensation on the tongue representing the mild anaesthetic effect of *Echinacea purpurea* (2,3).

- *Polyacetylene*

The different polyacetylenes help differentiate between species (2). Some polyacetylene compounds isolated from *Echinacea purpurea* have shown bacteriostatic and fungistatic effects (3).

- *Miscellaneous*

Other compounds isolated from Echinacea species include: the alkaloids tussilagine, isotussilagine and pyrrolizidine, resins, glycoproteins, sterols, vitamin C, monoterpenes, N-alkanes, minerals and fatty acids, (1,2,3).

3.7.5 Pharmacology

The chemistry, pharmacology and clinical applications of Echinacea have been the subject of over 350 scientific studies (2). The majority of the clinical studies have used an extract of the juice of *Echinacea purpurea* along with 22% ethanol, for preservation (2).

Local effects:

- A bacteriostatic and fungistatic effect has been described for some polyacetylene compounds isolated from the roots of *Echinacea purpurea* (3).

- By inhibiting hyaluronidase the enzyme responsible for breaking down hyaluronic acid, a major component of the ground substance that holds the cells of the body together and prevents penetration of micoorganisms, and increasing the differentiation of fibroblasts and epidermis cells in the stratum germinativum *Echinacea purpurea* promotes tissue regeneration (2,4,5).

- Due to the alkylamides and the inhibition of depolymerization of the hyalronic acid, *Echinacea purpurea* has a direct anti-inflammatory action on poorly healing wounds, chronic septic processes and inflammatory skin diseases (3,6). Thus *Echinacea purpurea* can keep an infection localised while argentophil fibers form (7).

Systemic effects:

- *Echinacea purpurea's* anti-inflammatory properties may also be due to its activation of the histogenous and haematogenous defences (4). The fact that Echinacea has a mild

direct cortisone-like effect and enhances the secretion of adrenal cortex hormones is also a contributing factor (2).

- Due to inulin Echinacea increases the levels of properdin, the normal serum globulin that stimulates the alternative complement pathway thus enhancing the movement of white blood cells (neutrophils, monocytes, and eosinophils) into areas of infection promoting the solubilization of immune complexes and the destruction of bacteria, viruses, and other microorganisms (2,8).

- Intravenous and oral ingestion of *Echinacea purpurea* has shown to cause increased levels of granulocytes in the peripheral blood due to stimulation of the bone marrow (3).

- Echinacea polysaccharides bind to the receptors on the cell surface of white blood cells stimulating the formation of T-lymphocytes or T-cells, macrophages, and natural killer cells (2,9).

By stimulating the formation of T-lymphocytes, which are responsible for cell-mediated immunity, Echinacea plays an important role in the resistance to infections caused by mould-like bacteria, yeast fungi, parasites, and viruses, and protecting against the development of cancer, auto-immune disorders and allergies (2). By binding to the surface of the T-cells the Echinacea polysaccharides promote non-specific T-cell activation, i.e. transformation, induction of interferon, and secretion of lymphokines (1,8). The result is enhanced T-cell replication, macrophage phagocytosis, antibody binding, natural killer cell activity, and increased neutrophil counts (2,8).

- Carbon clearance tests, which are used to measure systemic macrophage activation, have shown that the root extracts of *Echinacea purpurea* greatly enhance phagocytosis (2). The macrophages have been shown to destroy tumour cells in the tissue culture and inhibit *Candida albicans* infections in rats infected intravenously with a lethal dose (30,000 cells) of *Candida albicans* (2).

- Tests for antiviral activity showed that cells pretreated with *Echinacea purpurea* extracts were 50 to 80% resistant against influenza, herpes, and the vesicular stomatitis virus (10). This is possibly due to certain components blocking virus receptors on the cell surface or due to the inhibition of hyaluronidase (2). Echinacea also plays a role stimulating the helper T-cells thus promoting the production of interferon which causes the production of an intracellular protein that inhibits viral RNA transcription (8).

- *In vitro* studies have shown that *Echinacea pupurea* extracts has an anticancer activity (11). This is due to its stimulation of the macrophages to greater cytotoxic activity

against tumour cells. (Z)-1,8-pentadecadiene, not found in *Echinacea purpurea*, has been shown *in vivo*, to possess significant direct anticancer activity. The general immuno-enhancing effects of Echinacea also has an indirect effect on cancer (2).

3.7.6 Clinical studies

The effect against *Candida albicans* noted in animal studies has been confirmed in several clinical studies (2). A study by Coeugniet and Kuhnast demonstrated that the fresh-pressed juice of *Echinacea purpurea* greatly accentuates the efficacy of econazol nitrate, a topical antimycotic agent, decreasing reoccurrence from 60.5% to 16.7% (2).

Polysaccharides isolated from plant cell cultures of *Echinacea purpurea* enhance the resistance of immunosuppressed mice against systemic infections with *Candida albicans* and *Listeria monocytogenes*:

Polysaccharides isolated from large scale plant cell cultures of *Echinacea purpurea* have been shown to activate human and murine phagocytes. The influence of *Echinacea purpurea* on the non-specific immunity in immunodeficient mice was investigated. *Echinacea purpurea* was effective in activating peritoneal macrophages which were isolated from animals after the administration of cyclophosphamide or cyclosporin A. *Echinacea purpurea*-treated macrophages exhibited increased production of tumour necrosis factor-α (TNF) and enhanced cytotoxicity against tumour target WEHI 164 as well as against the intracellular parasite *Leishmania enrietti*. After cyclophosphamide-mediated reduction of leukocytes in the peripheral blood, the polysaccharides induced an earlier influx of neutrophil granulocytes as compared to PBS-treated controls. *Echinacea purpurea* treated mice, immunosuppressed with cyclophosphamide or cyclosporin, restored their resistance against lethal infections with the predominantly macrophage-dependent pathogen *Listeria monocytogenes* and predominantly granulocyte-dependent *Candida albicans*. These findings may have therapeutical implications regarding the prophylactic treatment of opportunistic infections (12).

3.7.7 Clinical applications

Echinacea has had effects in general infectious conditions such as:

-influenza and colds, especially as a prophylactic

-Candidiasis

-Streptococcal throat

-Staphylococcal infections

-urogenital infections

-upper respiratory tract infections such as whooping cough and bronchitis

-mouth and gum infections (1,2,9).

It can be used internally to treat infections anywhere in the body and can also be used for external conditions as an ointment or salve (9).

Echinacea purpurea has shown success in the treatment of skin conditions such as wounds, burns, skin ulcers, abscesses, folliculitis, psoriasis, herpes, and eczema (2,9).

Bites, stings, arthritis, leucopenia, allergies and blood and food poisoning have also been shown to benefit from *Echinacea purpurea* (1,2,9).

3.7.8 Preparations

Commercially *Echinacea purpurea* is available in the form of teas, injectibles, juices, salves, homoeopathic potencies, spray- or freeze-dried extracts in capsules and tablets, simple herbal powders in capsules and tablets, tinctures and topical creams/ointments (1,2).

Of the proven immuno-active compounds from Echinacea spp., the polyacetylenes and cichoric acid are unstable and may not occur in commercial products (1). Only the polysaccharides and alkylamides are currently accepted as being active in commercial products (1).

- Dosage:

    Acute infection: 50 drops or 300-400mg of dry extract three times daily (1).

    As a tonic: 10-25 drops daily or 1-2 capsules (1).

    External application: The dressing can be changed as often as necessary (1).

Doses between the above extremes can be taken, depending on personal needs, body weight, state of health and illness (1).

It is however thought that a maximum stimulation is reached after five days after which the ingestion should be interrupted for a few weeks (1).

3.7.9 Contraindications

AIDS is associated with wide-spread depression of the immune system, however stimulation of T-cell replication and increasing levels of TNF by Echinacea may stimulate the replication of the human-immunodeficieny virus (HIV). This is yet to be conclusively determined (2).

Homoeopathically there are no known contraindications, however precaution is suggested up to a D4 in cases of chronic progressive inflammations, leukaemia, diabetes mellitus,

pregnancy, and in cases of known hypersensitivity to plants of the Compositae family (13,14).

3.7.10 Adverse reactions
Homoeopathically none are known (14).

3.7.11 Interactions
None known (13).

3.7.12 Toxicity
Tests for acute and subacute toxicity, mutagenity, and carcinogenity showed no indications of specific toxic effects (15).

3.8 Nystatin
3.8.1 Proprietary name
Mycostatin® (16).

3.8.2 Source and chemistry
Nystatin is a polyene antibiotic obtained from *streptomyces noursei* (17). Its large conjugated, double-bond ring system is linked to an amino acid sugar, mycosamine (18).

3.8.3 Antifungal activity
Nystatin is both fungistatic and fungicidal depending on the drug concentration; the presence of blood, pus or tissue fluid that reduce activity; and the susceptibility of the fungus (17). *Candida, Cryptococcus, Histoplasma, Blastomyces, Trichophyton, Epidermophyton, and Microsporum audouini* are sensitive in vitro to concentration ranging from 1,5 to 6,5 g/ml (18). Nystatin has no effect on bacteria, protozoa, or viruses.
Antifungal activity, MIC (units/ml): *Candida albicans* - 7.8 (16).

3.8.4 Mechanism of action
Nystatin is bound by drug sensitive yeasts and fungi but not by resistant microorganisms (18). The antifungal activity of the antibiotic is dependent on its binding to a sterol moiety present in the membrane of sensitive fungi (18). As a result of interacting with the yeast plasma membrane sterols there is a release of cations from the cells causing an increase in cell permeability (19). This provokes the leakage of amino acids, sugars, and other

metabolites from the cytoplasm resultng in lysis and cell death (20). There is a direct association between the sensitivity of an organism to a polyene and the presence of sterols in the plasma membrane of the cell (20). Thus its lack of antibacterial activity may be due to the absence of sterols in bacterial cell membranes (17).

3.8.5 Absorption

There is no evidence of systemic absorption of Nystatin from the skin or mucous membranes (21), and therefore it should not be used orally to treat systemic fungal infections but rather to treat intestinal candidiasis (22).

3.8.6 Clinical uses

• Cutaneous or mucocutaneous candidal infections
• Vaginal candidiasis
• Oropharyngeal candidiasis
• Intestinal candidiasis (22).

3.8.7 Adverse effects

Mild and transitory nausea, vomiting and diarrhoea may occur after oral administration of Nystatin (18). Although Nystatin is relatively innocuous when applied topically, cutaneous irritation may develop following repeated exposure (17). Administration of Nystatin intramuscularly may complex with red blood cell sterols producing haemolytic anaemia (17). Candida resistance may also develop (21).

3.8.8 Preparations

Nystatin is available as an oral suspension, pastille, tablets, vaginal suppository and a topical cream (22).

• Directions for use of Mycostatin® B-M Squibb, Schedule 1:
  Oral suspension: Instil 1ml into the mouth 4x daily. Keep in contact with the affected area for as long as possible.

• Directions for use of Mycostatin® B-M Squibb, Schedule 4:
  Vaginal Tablets (100 000 units): Insert one tablet high into the vagina once or twice daily for 14 days.
  Vaginal Cream (100 000 units/4g): Insert one to two, 4g applications high into the vagina for 14 nights (21).

3.8.9 Prophylactic uses

Nystatin is administrated with the tetracyclines to decrease the overgrowth of yeasts and fungi in the intestines of patients predisposed to such infections (18).

The oral suspension is frequently used as a prophylaxis of oral candidiasis, which often develops in patients who are receiving immunosuppressive therapy (22).

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CHAPTER FOUR

4.0 RESULTS

4.1. **Verification of the Possible Ethanol Effects on** *Candida albicans*

Of the various concentrations of ethanol in distilled water that were tested the 20% showed the least number of zones, and was therefore assumed to be the percentage of ethanol that would have the least fungistatic or fungicidal effect on *Candida albicans* while still preserving the homoeopathic compound. See Table 1 below.

Table 1: Summary of Ethanol Results

distilled water	20%	35%	45%	60%	70%	80%	96%
no zones	no zones	15% zones	zones	zones	zones	zones	zones

Hence all the homoeopathic potencies of *Echinacea purpurea* i.e. D2, D6, D15, D30 were made up in 20% ethanol by Natura Laboratories.

4.2 The Standard Curve

A standard curve was formulated to establish the correlation between optical density readings at 540nm and the colony forming units per ml. See figure 1 below.
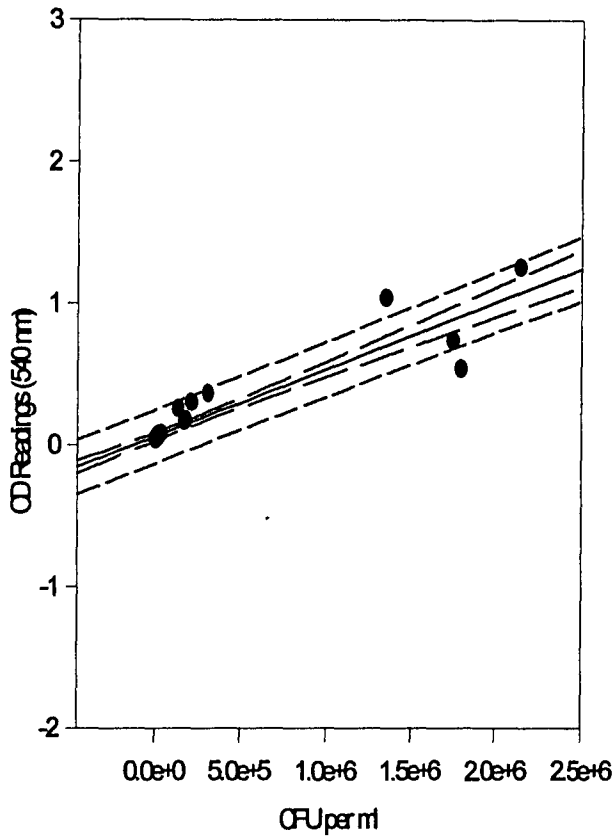
Figure 1: Colony Forming Units per ml Versus the Optical Density Readings at 540nm.

The two parameters correlated with a correlation co-efficient of $r^2 = 0.87$ to the line defined by the formula OD = mxCFU+c, where m (= 0.0575) is the gradient and c (= 4. 7 x 10^{-7}) is the cut off point.

4.3 Determination of the Antimicrobial Activity of *Echinacea purpurea*

The results of experiments conducted in triplicate involving the nutrient broth are presented as a histogram in Figure 2.
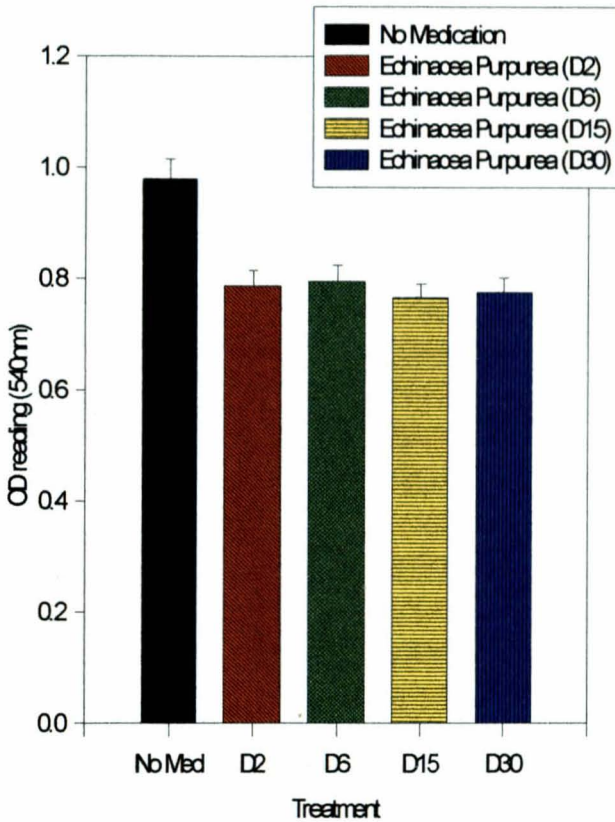
Figure 2: The Antimicrobial Activity of *Echinacea purpurea*.

According to the one way analysis of variance that was carried out, the normality test and equal variance tests passed. The summarised results of this experiment are tabulated below.

Table 2: Anova Table for the Broth Dilution Experiment

Source of variance	DF	SS	MS	F	P
Between treatments	4	6.30E-001	1.58E-001	4.59E+000	0.0020
Residual	95	3.26E+000	3.43E-002		
Total	99	3.89E+000			

Power of preformed test with alpha = 0.500 : 0.8645.

Table 3: Optical Density of Broth Cultures

	Culture	D2	D6	D15	D30
Mean	9.79E-001	7.86E-001	7.95E-001	7.71E-001	7.76E-001
Standard Deviation	1.67E-001	2.05E-001	1.95E-001	1.69E-001	1.88E-001
Standard Error	3.72E-002	4.59E-002	4.35E-002	3.77E-002	4.21E-002

The differences in the mean values among the treatment groups were found to be greater than would be expected to be found by chance, thus proving there to be a statistically significant difference (P = 1.96E-003).

To isolate the group or groups that differ from the others, a multiple comparison procedure was conducted using the Dunnett's and the Student-Newman-Keuls methods. According to both methods there was a significant difference when comparing the unmedicated culture to the cultures that were medicated with the various potencies of *Echinacea purpurea* that were used in the experiment, i.e. D2, D6, D15 and D30.

The culture and the *Echinacea purpurea* in the D15 potency showed the greatest difference in mean values, followed by the *Echinacea purpurea* in the D30 potency. The least difference in mean values was found to be between the *Echinacea purpurea* in the D6 potency and the culture.

Comparison between the various potencies of *Echinacea purpurea* showed no statistical significance as their differences in mean values were diminutive. (See figure 2 and table 2).

These findings suggest that the homoeopthically potentised *Echinacea purpurea* does in fact have an effect on the growth rate of *Candida albicans*, however because the statistically significant difference between the unmedicated and medicated broth is small it implies that the *Echinacea purpurea* has a fungistatic effect rather than a fungicidal action.

Note:

fungicidal.............................killing the fungi

fungistatic............................inhibiting the growth and reproduction of fungi

4.4 Comparison of Allopathic Nystatin Effects Versus Homoeopathic *Echinacea purpurea* on *Candida albicans*

The results of experiments conducted in triplicate involving the disk diffusion methodology are presented a histogram in figure 3.
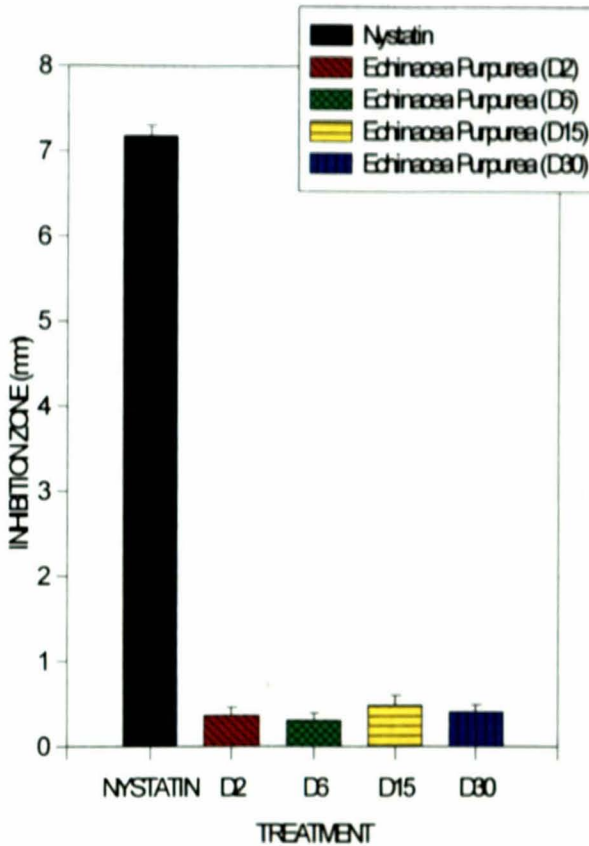
Figure 3: The Inhibitory Action of *Echinacea purpurea* on the Growth of *Candida albicans* in Comparison to Nystatin.

The one way analysis of variance that was conducted showed that the normality test failed indicating that the distribution of the data deviated from the normal distribution curve. However the Kruskal-Wallis one way analysis of variance on ranks indicated that the differences in the median values among the treatment groups were greater than would be expected by chance (P = 4.87E-010), and therefore a statistically significant difference was still found between the Nystatin and *Echinacea purpurea*. (See figure 3).

Using the Student-Newman-Keuls method as a multiple comparison procedure to isolate the group or groups that differ from the other it was found that the *Echinacea purpurea* in the D6 potency exhibited the most substantial difference in rank values when compared to Nystatin. The D2 potency of *Echinacea purpurea* also showed a marked difference in rank values when compared to Nystatin, although not to the same extent as the *Echinacea purpurea* in the D6 potency. The *Echinacea purpurea* in the D15 potency demonstrated a less marked difference in rank value followed by *Echinacea purpurea* in the 30D potency. No significant difference was found to exist among the various potencies in how they effected a response against the *Candida albicans* organism.

Table 4: Zone Diameters (mm)

	Nystatin	*D2*	*D6*	*D15*	*D30*
Mean	7.1850	0.3600	0.3050	0.4350	0.4050
Standard Deviation	0.5724	0.4672	0.3993	0.5112	0.3913
Standard Error	0.1280	0.1045	0.0893	0.1143	0.0875

These findings suggest that although the *Echinacea purpurea* may have a very slight effect in the control of *Candida albicans*, the Nystatin which is a more established and proved antifungal agent is far more effective in its antimicrobial activity.

4.5 **Legends**

List of abbreviations:

E	Exponential power
OD	Optical density
CFU	Colony forming units
DF	Degrees of freedom
SS	Sum of squares
MS	Mean squares

CHAPTER FIVE

5.0 DISCUSSION

5.1 Determination of the Antimicrobial Activity of *Echinacea purpurea*

The results as shown in chapter 4.3 show that the homoeopathically potentised *Echinacea purpurea*, in all the potencies that were tested: D2, D6, D15 and D30, demonstrated a difference in growth rates to the control after 24 hours, thus proving that *Echinacea purpurea* does have an effect on the growth rates of *Candida albicans*. The difference in the effectiveness of the various potencies is negligible.

5.2 Comparison of Allopathic Nystatin Effects Versus Homoeopathic *Echinacea purpurea* on *Candida albicans*

In the presence of conventional Nystatin the *Candida albicans* cultures demonstrated the most marked zones of inhibition, with the Nystatin exhibiting a minimum inhibitory concentration (MIC) of 7.2 units/ml, confirming that Nystatin is effective in destroying *Candida albicans*. The various potencies of *Echinacea purpurea* also showed zones of inhibition but to a less marked extent than the Nystatin, with minimum inhibitory concentrations ranging from 0.48 units/ml by D15 to 0.31 units/ml by the D6 potency. Since the potentised *Echinacea purpurea* had such a minimal effect in comparison to the Nystatin, it suggests that the *Echinacea purpurea* exhibits a fungistatic rather than a fungicidal effect.

5.3 Fungistatic *Echinacea purpurea*

Often remedies exhibit a different effect according to the dilution used, for example the homoeopathic remedy Hepar Sulphuris Calcareum is used in low potencies when it is used to promote suppuration and in higher potencies it is used to abort the suppurative process (1).

As previously mentioned in chapter two, homoeopathically low potencies are used as local organ or tissue remedies, primarily in organic disease while medium potencies are used mainly in functional disorders and high potencies are considered to work primarily on the mental sphere (2).

The homoeopathic potencies D2, D6, D15 and D30 are relatively low and are therefore considered to act essentially on the local sphere. Whilst the D2, D6, D15 potencies still

contain some of the original substance (*Echinacea purpurea*) the D30 potency according to Avogardo's constant will contain none of the original substance. Therefore it would have been expected that D2 potency would have had the most marked action followed by D6 then D15 and finally D30. However unlike orthodox medicine where the more diluted the medicine the weaker its effect, homoeopathically it is believed that the higher the dilution/potency the greater the therapeutic power of the remedy, even beyond the point of there being even one molecule of the original substance remaining. Although this assertion that by mere succussion and serial dilution the therapeutic power of a substance can be increased without limit, while nullifying toxicity, seems to violate our usual understanding of physics and chemistry, the clinical results of homoeopaths the world over, using potencies beyond Avogardo's number cannot be denied.

In both experiments the D15 had the most significant effect in stopping the growth of *Candida albicans* followed by D30. These two remedies although considered the most "diluted" of the various potencies used showed the most marked action of the organism suggesting that somehow the force of the electromagnetic field of the original substance is transferred to the solvent molecules without changing the resonant frequency.

The D6 potency had the least marked effect on the organism while the D2 potency which could be considered to contain the largest quantity of the original substance, in comparison to the other potencies, had only a slightly more fungistatic effect than the D6.

As there was inhibition of cell growth in the laboratory, it can be assumed that the *Echinacea purpurea* has a direct effect on the cells. If there had been no inhibition of cell growth, then it could be assumed that:

- factors other than the substances used influenced the body's reaction and/or
- the body's utilisation of the substances used determined its reaction in the presence of *Candida albicans* during an infection.

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The purpose of this study was to determine and compare the effect of *Echinacea purpurea* in homoeopathic potencies to Nystatin as an antifungal agent. This was done by determining any mycostatic or mycocidal effect of the homoeopathically potentised *Echinacea purpurea* and comparing this with conventional Nystatin.

Echinacea purpurea was chosen in this experiment because it is recorded as a potent natural antibiotic, effective against a wide range of microbial infections, viral, bacterial and fungal. However this medicinal herb is also well known as an immune stimulant and therefore this research was undertaken to determine whether this mycocidal action of *Echinacea purpurea* was due to a direct action on the micro-organism, or due to its effectiveness in enhancing the immune response in the body.

To compare the action of Nystatin to the possible fungicidal effect of *Echinacea purpurea* was decided on because Nystatin is known primarily as a topical fungicidal application and hence its efficiency in destroying the fungus is known to be due to a direct action on the organism's cell membrane. Hence if the *Echinacea purpurea* also demonstrated a direct action on the organism it could provide an alternative topical application without the possibility of hypersensitivity reactions or side-effects such as local irritation and contact dermatitis developing.

The graphical results did not show any significant difference between the various potencies of homoeopathic *Echinacea purpurea* in their action on the organism, however a statistical significant difference was shown between the control and the various potencies of *Echinacea purpurea* in the both studies. A significant difference was also demonstrated between the homoeopathic potencies and the Nystatin in the disc diffusion results.

Echinacea purpurea therefore has a fungistatic effect on the growth rates of *Candida albicans*, although it is to a lesser extent than Nystatin which is a fungicidal agent as well. Thus *Echinacea purpurea* could provide a viable and safe alternative to synthetically manufactured fungistatic agents.

This study provided information on how homoeopathic substances, viz. *Echinacea purpurea*, effect the response in the treatment of disease thereby making homoeopathy more acceptable to both allopathic practitioners and laymen alike, thus furthering the acknowledgement of homoeopathy.

6.2 Recommendations

It is recommended that more research in this field be continued as the homoeopathic forms of *Echinacea purpurea* have demonstrated that they are capable, though minimally, to effect cell growth.

As *Echinacea purpurea* is well researched and documented as an immune stimulate, its main sphere of action in the treatment of candidiasis may be in activating the macrophages and increasing the level of phagocytosis, by raising the levels of white blood cells such as the neutrophils, monocytes, eosinophils, and B lymphocytes. Therefore it is recommended that further studies involving *Echinacea purpurea* in the treatment of candidiasis be done *in vivo*, to determine whether this remedy is in fact more beneficial when it can act holistically.

It is therefore suggested that homoeopathically potentised *Echinacea purpurea* be given to patients with candidiasis, particularly in cases of immune deficiencies, as it will have the advantage of inhibiting fungal growth while simultaneously enhancing the immune response thus facilitating a better prognosis.

APPENDIX A

Homoeopathic Research in the Laboratory

The following experiments are examples of how homoeopathic remedies have an effect on the body, as well as on groups of cells, and specific organs:

Koopman *et al*, 1990, investigated the inhibitory effect of Viscum album on human fibroblast cell lines, mouse tumour cell lines, human carcinoma tumour cell lines and on human lymphoblastic tumour cell lines. There was no evidence that the malignant cell lines were selectively killed by the remedy. *In vivo* specific anti-tumour effects of the remedy have been described, but the *in vivo* effects may have been brought about by a mechanism differing from *in vitro* activity for instance by the stimulation of the immune system (1).

Benveniste, 1988, examined the inhibitory effects on achromasia by the homoeopathic remedy Apis Mellifica. Basophils were incubated with substantial concentrations of anti-IgE serum after treatment with either Apis Mellifica or sodium chloride. Some dilutions of Apis Mellifica inhibited basophil achromasia. Apis showed peaks of activity at 30CH and 34CH with weaker effects at 32CH and 40CH and no significant change at 36CH and 38CH. The sodium chloride dilutions produced no effects (2). Apis Mellifica, which is the honey bee prepared homoeopathically, is able to reverse the crude effects of a bee sting i.e. basophil degranulation and the other features of an acute inflammation (2).

Thyroxine (T4) is essential for the spontaneous tendency of juvenile frogs to leave the water and climb on land. Endler *et al*, 1991, added thyroxine 30X to the basins in which tadpoles were placed. Results showed that the 30X slowed down their climbing activity significantly. There was a statistical significance both in the comparison control (H20) observations as well as in the potentized solvent (30X). This also demonstrated the law of similars i.e. thyroxine caused spontaneous climbing of frogs whilst homoeopathic thyroxine suppressed this climbing activity (3). What makes this study more interesting is that additional investigations resulted in the same effect when a glass bottle of the homoeopathic doses of thyroid hormone was simply suspended in the water with the lip of the bottle above the water line. This research was repeated in several laboratories, and the results were consistent (4).

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APPENDIX B

Candida albicans

Morphology

Candida organisms grow on the surface partly as spherical or oval yeast cells (blastospores), and partly as submerged pseudomycelium of non-branching filamentous cells which divide by constriction and give rise to yeast cells by budding from division sites (1). Both forms are thin walled, Gram-positive and non-capsulated (1).

Candida albicans, the principle pathogenic member of the genus, also produces true mycelia (2). Pseudomycelium are chains of budding cells that fail to detach, thus forming a branching network that resembles true mycelia (2).

Candida albicans produces four different forms on cornmeal agar:

◊ true mycelia

◊ pseudomycelia

◊ blastospores

◊ chlamydospores (2).

Colonies composed of pseudomycelium have the soft, white character of yeasts in comparison to the cottony or woolly growth of true mycelia (2). Pseudomycelium and mycelium formation is an indication of colonisation of tissue, whereas the appearance of yeast forms alone means a saprophytic existence (3). The presence of both yeasts and mycelium in sputum, blood, urine, or stool specimens is an indication of colonisation (3), thus conditions in the body generally give both yeast and mycelial growth (1).

The blastospores of *Candida albicans* vary in size from 2 x 3 µ to 8.5 x 14 µ, and they grow in round clusters at intervals along the pseudomycelia (2). An abundance of easily assimilable nutrients and sufficient aeration encourages growth in the yeast form, whereas nutritionally poor media without fermentable carbohydrate (e.g. corn meal agar), and poorly aerated conditions and/or a high nitrogen content, favours mycelial growth and chlamydospore formation (1,3).

The large round chlamydospores are particularly adapted for maintaining vitality during starvation and other adverse conditions due to their large size (8 to 12 µ) which facilitates the storage of reserve nutritional substances (2). The chlamydospores have a thick wall with a high lipid content, which is composed of two layers, the outer is polysaccharide and the

inner is protein, protecting them from unfavourable environments (2). Terminal chlamydospores (macroconidia) growing at the end of the mycelium is a distinctive feature of *Candida albicans*, and their formation is used as a test for distinguishing between *Candida albicans* and those species of Candida that do not develop into these forms (2).

Physiology

The metabolism of *Candida* spp. cells is the same as that of other aerobic eukaryotic cells (2). They are capable of aerobic glycolysis via the hexose monophosphate pathway and of anaerobic glycolysis through the Embden-Meyerhof pathway (2). They also have the Krebs cycle enzymes and the mitochondrial enzymes for oxidative phosphorylation which involves mainly cytochromes a, a_3, b, c, and c_1 (2).

Protein synthesis involves 80S ribosomes, which dissociate into 60S and 38S sub-units (2). The physiological changes associated with or responsible for the development of mycelia or chlamydospores is not fully understood, however temperature is known to be an important factor in the formation of either mycelia and blastospores (37°C) or chlamydospores (<25°C) (2). Mycelial formation is accompanied by a suppression of the pentose phosphate pathway and the alteration of hextoses for cell wall biosynthesis (2).

Laboratory Diagnosis

Since *Candida albicans* commonly occurs as a commensal in the same situations as it causes infection, the mere demonstration of its presence is not diagnostic of infection (1). This diagnosis requires demonstration that the organism is abundantly present on several occasions, and also the exclusion of other possible causative agents (1).

• Specimens

Candida spp. appear in tissues and exudates as blastospores and mycelium (4). Either form may predominate, but both are usually present (4). The mycelial components are most often pseudomycelium but true mycelium may occur in infections (4).

Specimens consist of swabs and scrapings from surface lesions, sputum, exudates, and material from removed intravenous catheters (5). Specimens must be examined with minimum delay to avoid contamination (1).

- Microscopic examination

Microscopic examinations of sputum and exudate should be in Gram-stained smears and in unstained wet films (1). Skin and nail scrapings should be examined in a wet film with 10-20% sodium hydroxide (1), or first placed in a drop of 10% potassium hydroxide (3,5). Budding yeast cells together with long filaments are indicative of a yeast-like fungus (1). The cells and filaments stain Gram-positively and they are clearly much larger (e.g. 2-4 fold in diameter) than the commensal bacteria that are often also found in the specimens (1).

- Culture

For culture, exudate may be collected on dry swabs or, preferably, swabs soaked in Sabouraud broth (1). The cultures are grown on plates of blood agar at 37°C and on Sabouraud's glucose agar at 37°C and at 22°C, producing soft, cream-coloured colonies comprised of blastospores and budding pseudomycelia (1,4).

Specimens of faeces or sputum should be cultivated on the selective tellurite malt agar or penicillin-streptomycin blood agar in order to control the growth of bacterial contaminants. In two to three days, large creamy bacteria-like colonies develop on the Sabouraud's medium and smaller grey colonies on blood agar (1). The culture is also examined in an unstained wet film, or a Gram film, for budding yeast forms (1).

A pure culture is isolated by picking a colony from one of the primary plates or, if necessary to ensure purity, from a second plating, and grown on a slope of Sabouraud's glucose agar for 48 hours at 37°C (1).

Examinations to identify a pure culture of *Candida albicans*:

◊ The naked eye appearance of the 48 hours Sabouraud slope of *Candida albicans* growth is raised white, moist and creamy with a characteristic yeasty odour (1,5).

◊ In mediums without fermentable carbohydrate and semi-anaerobic conditions and/or a high nitrogen content the yeast elongates producing a well developed branching "tree-like" pseudomycelium which consists of hyphal cells which develop large spherical thick walled chlamydospores and clusters of smaller, oval, thin-walled blastospores at the junctions of the filamentous cells (1,3).

◊ *Candida albicans* ferments glucose and maltose, producing both acid and gas; produces acid from sucrose and fails to ferment lactose (1,5).

◊ Upon intravenous injection into rabbits or mice *Candida albicans* is the only species of *Candida* that causes widespread abscesses, particularly in the cortex of the kidney, and death in less than one week (1,5).

◊ When the yeast cells of *Candida albicans* are suspended in serum and incubated at 37°C, they produce within two to four hours a short filament measuring 1.5 x 15 μ, which has the appearance of a bean sprout (2). As the germ tubes develop so quickly, this is used as a rapid test in the identification *Candida albicans* (2).

- Serology

The increased incidence of opportunistic infections in man has made the need for serological tests for systemic infection extremely important (3). Attempts have been made to find antibody in sera from patients by testing them for their ability to agglutinate *Candida albicans* yeast cells, or to precipitate antigens from disrupted *Candida albicans* in tubes or in gel diffusion plates (2).

The immunodiffusion test is one such test that has been developed, which uses the cell sap (S antigen) (3). In this test precipitin bands form in gel only in cases of chronic mucocutaneous disease and systemic candidiasis (3). A carbohydrate extract of group A *Candida* gives positive precipitin reactions with sera of 50% of normal persons and 70% of persons with chronic mucocutaneous candidiasis (5). Various tests can detect a rise in titer of antibiotics to *Candida albicans* in systemic candidiasis (5).

The interpretation of serologic test results however remains controversial (5), because although it is rarely positive in the absence of infection it may be negative in cases where the patient has developed disseminated candidiasis and is therefore immunologically suppressed, or where a patient has had long-term immunosuppressive therapy and is no longer capable of generating a specific immune response (2,3). Efforts are now being aimed to detect circulating Candida antigens by immunologic or chemical methods in order to make a diagnosis of systemic candidiasis in patients with negative blood cultures (2).

- Skin

A Candida test is universally positive in normal adults, and is therefore not used in the diagnosis of candidiasis as such, but rather as an indicator of competent cellular immunity (5).

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Pathogenicity

Dermatocandidiasis

Intertriginous candidiasis is promoted by prolonged exposure to moisture and the lesions involve those areas of the body, usually opposed skin surfaces, that are warm and moist: axillae, groin, infra-mammary areas, interdigital clefts, umbilicus and gluteal folds, and, in infants, in the napkin area (1,6). The infection is promoted by prolonged exposure to moisture and the lesions are characterised by erythema, exudation and desquamation (1). Candida infections of the subcutaneous tissues of the digits, paronychia, and the nails, onychomycosis, usually result from continued immersion in water (6). The nail shows transverse ridges and becomes thickened, distorted and brown, and there is often involvement of the nailbed (paronychia) (3). Perlèche, an infection of the angles of the mouth, is another form of intertrigo produced by *Candida albicans* (3). Candida granulomas are associated with immune deficiencies, and are a result of a chronic Candida infection (3). Further research has also demonstrated that Candida is responsible for and involved in many forms of psoriasis (7).

Infections of the mucous membranes

Candidiasis of the mucous membranes is often referred to as thrush and it appears as creamy white patches of exudate on red, raw, inflamed surfaces of the mouth, pharynx and tongue (1,3). Thrush is a common infection in new-born infants, particularly before the development of the normal bacterial flora (3). Although the infection is usually mild and usually remains localised it may spread to other mucous membranes and to the skin causing a generalised cutaneous eruption, intertriginous lesions, and Candida granuloma (3). In adults, denture sore mouth, due to ill-fitting dentures may result in lesions under the plate, and angular chelitis may be caused by an infection with Candida (1,3).

Vaginal candidiasis results from a loss of acid pH, which is normally controlled by the commensal Lactobacilli (5,6). The vaginal discharges generally contain little or no pus and are relatively acid (pH 4.3-5.2), causing intense itching and irritation (1,5). Diabetes, antibiotic therapy, oral contraceptives or pregnancy, are common predisposing factors (5,6). Candida can also be transmitted to males during intercourse causing balantitis, a candidal infection of the glans penis which begins as vesicles on the penis that develop into patches which produce severe itching and burning, occurring particularly in uncircumcised men (6).

There may be an associated infection of the anus, pruritus ani, due to colonisation and the metabolic products of Candida as a complication of broad-spectrum antibiotics (3).

In older children and adults the appearance of thrush is associated with polyendocrine disorders, immune deficiency disorders or other serious malfunctions (3).

The disease may progress to Chronic Mucocutaneous Candidiasis which is an uncommon superficial candidal infection of the skin, nails, and oral and genital mucosa (8). Affecting individuals with immunological defects in cell-mediated immunity (4), it is typified by the onset, usually within the first year of life, of chronic occasionally granulomatous candidal infections (8). Clinical features typically manifest as persistent oral thrush with subsequent hypertrophic changes, vulvovaginal infection, cutaneous lesions, which are sometimes severely hyperkeratotic, involving the trunk, limbs, and scalp, and parenchymal involvement with accompanying nail dystrophy (8). Dermatophyte infections are frequent concomitants (8). Although recurrent, the site of involvement remains localised without spreading to deeper tissues and organ systems (4). Chronic Mucocutaneous Candidiasis is not a single disease entity but rather a final common pathway for multiple predisposing abnormalities of the immunological defects in cell-mediated immunity (8). Traditional anticandidal and antifungal therapies are not effective (8).

* Medical conditions associated with Chronic Mucocutaneous Candidiasis:

Candida endocrinopathy is associated with Chronic Mucocutaneous Candidiasis that begins in early childhood with hypoparathyroidism, hypoadrenalism, hypothyroidism, and diabetes mellitus (8). These endocrinopathies may be severe and progressive and they usually follow the onset of Chronic Mucocutaneous Candidiasis by several years (8).

Patients with abnormal iron metabolism with low serum iron and decreased iron stores, perhaps secondary to decreased iron absorption, form another subgroup of patients with Chronic Mucocutaneous Candidiasis (8).

Thymoma patients in whom the onset of Chronic Mucocutaneous Candidiasis is delayed until after the third decade of life form an important subgroup of patients with Chronic Mucocutaneous Candidiasis. They tend to have an increased incidence of myasthenia gravis, hypogammaglobulinemia, and abnormalities of the bone marrow and circulating blood elements (8).

Malabsorption, dental enamel dysplasia, chronic hepatitis, and keratoconjuctivitis have also been sporadically reported in association with Chronic Mucocutaneous Candidiasis (8).

Pulmonary candidiasis

Bronchial and pulmonary candidiasis manifests with the production of a mucoid, gelatinous sputum which contains numerous Candida organisms. *Candida albicans* is a commensal in the upper respiratory tract but is a secondary invader in the lower tract, e.g. in pulmonary tuberculosis, bronchial carcinoma and brochiectasis (1). Pulmonary candidiasis is an opportunistic infection associated with an underlying disease or treatment (3). It is most frequently seen in leukaemia and lymphoma patients on cytotoxic therapy or in patients with diseases that require immunosuppression (3). Pulmonary disease in such patients represents miliary hematogeneous spread from some other focus of infection (3).

Systemic candidiasis

Before the use of antibiotics, macrodisruptive drugs, and surgical procedures systemic candidiasis was uncommon (3). Almost every organ system can be infected by Candida - the central nervous system, causing meningitis or encephalitis, the heart, kidneys, eyes, liver and joints are the most common targets (9). Blood stream invasion, thrombophlebitis, endocarditis, or infection of the eyes can occur when *Candida albicans* is introduced intravenously (5). Neutropenia, advanced malignancies, bone marrow transplants and Hodgkin's disease are often associated with systemic candidiasis (4).

Candida has also been suggested to play a role in "leaky gut", a condition characterised by increased intestinal permeability (7). By permitting the passage of dietary polypeptides, particularly casein and gliadin, across the bowel into the blood stream and albumin leaking out into the gut lumen (10), an antibody response is initiated resulting in high levels of IgG and IgA antibodies to casein and gliadin, as well as Candida immune complexes (10). This immune response is thought to sensitize the patient to normally harmless molecules causing the patient to respond to various harmless inhalants in the environment as well as various foods. The immune system is thus compromised and the body's ability to defend against the Candida is further weakened, creating a cycle (7). It is also possible for these molecules to pass through the blood/brain barrier and produce mental symptoms as they are mistaken for neurotransmitters (7).

A recent study by Broughton and Lanson (1997), has shown that patients with chronic candidiasis and increased intestinal permeability tend to have reduced leucocyte phagocytosis which is thought to be secondary to free radical damage to the neutrophils (7).

Chronic Candida infections have also been linked to Chronic Fatigue Syndrome, Attention Deficit Disorder in children and Obsessive Compulsive Disorder (7).

Associated symptoms

Some of the common symptoms which may indicate candidiasis include:

° fatigue, lethargy, irritability, depression

° headaches, migraines

° joint pain with or without swelling, muscle pain/weakness/paralysis

° nettle-rash and hives

° irritable bowel syndrome

° adult onset allergies

° flatulence

° inability to concentrate

° decreased libido

° endometriosis or infertility

° cramps and/or other menstrual irregularities

° hypothyroidism

° acne

° abdominal pain

° recurrent cystitis

° impotence

° prostatitis (7,11)

The cause of the varied symptoms are from the 79 distinct toxins that the yeast cells release (7).

Oral, vaginal, intestinal, broncho-pulmonary, and septicaemia candidiasis often result from the treatment of other infections with broad-spectrum antibiotics, which act by eradicating the normal commensal flora defence mechanism (1,7,12). Thus the antibiotic is responsible, at least in part, for facilitating the infection with Candida (1).

Major Predisposing Factors of Candidiasis

• Weakening of the immune system due to :-

     i.   cytotoxic therapy, immunosuppressive therapy

    ii. corticosteroid therapy

    iii. antibiotic therapy - as personal medication or ingested via the flesh of cattle who have been on antibiotics

    iv. inherited/ acquired immunological defects in cell-mediated immunity

- Diabetes
- Contraceptive pill
- Acidity
- Poor nutrition - diet rich in carbohydrates, alcohol or yeast containing foods such as mushroom
- Pregnancy
- Iron deficiency anaemia
- Leukaemia
- Intravenous narcotic abuse
- General debility (1,5,9,11)

Immunity

While the stratum cornum is generally an effective barrier against most Candidal invasion *Candida albicans* can penetrate the skin causing desquamation and inflammation (8). Second in the line of defence are the humoral factors, with the cell-wall products activating the alternative complement pathway that produces potent chemotactic agents which enhance the accumulation of polymorphonuclear cells and macrophages around invading Candida organisms (8). Serum inhibitory factors and iron-free transferrin exert inhibitory influences by removing iron needed for Candida growth (8).

The disturbances of T-cell function often found in chronic mucocutaneous candidiasis suggests that cell-mediated immunity is important in the resistance to Candida infection (2). The familial tendency for mucocutaneous infections to occur in children who have abnormal T-lymphocytes suggests that there is a genetic origin in the disturbance of these cells (2). There is evidence that candidiasis occurs because the T-cell cannot recognise the antigen or produce migration inhibition factor (MIF) (2). Despite universal delayed sensitivity to Candida antigens in healthy people, those with chronic candidiasis are often anergic to its antigens, and transformation of their lymphocytes by Candida antigen is sometimes depressed. The importance of abnormal T-cells in the cause of impaired immunity to Candida is also suggested by the tendency of mucocutaneous candidiasis to occur in

patients who have frank thymic disorders such as thymomas and congenital thymic aplasia (2).

Abnormalities in the humoral immunity have not displayed a special susceptibility to Candida infection, and the ability of humoral antibody to prevent experimental candidiasis after active or passive immunisation has been inconsistent and unimpressive (2).

The natural immunity to Candida infection is thought to develop early in life when the alimentary tract initially becomes colonised by *Candida albicans* (2). The surface glycoproteins (mannoproteins and glucoproteins) are thought to stimulate both humoral and cellular immunity (2). Thus normal people develop antibodies and delayed hypersensitivity to Candida culture filtrates, which contain glycoprotein and polysaccharide antigens (2).

Candida antibodies belong to the IgA, IgG and IgM immunoglobulin sub-classes, and are all involved in direct agglutination (13). Secretory antibody, particularly secretory IgA, has been detected in human vaginal tissues, secretions and in saliva, and IgA, IgM and IgG can all react with Candida cells *in vivo* (13).

It has been found that individuals with systemic candidiasis have an average of nearly 2000% increase in IgE antibodies to Candida while patients with vaginal candidiasis have an average of over a 1000% increase, thus suggesting that IgE antibodies are significant in the defence against Candida (7). IgE antibodies in candidiasis sufferers were also elevated to other antigens suggesting that candidiasis may also increase allergic responsiveness (7).

Virulence Factors of *Candida albicans*

1. Genetics.

Candida albicans is an amyctic, diploid organism and efforts to bring about haploidization have been unsuccessful (4).

2. Adherence.

The adhesion of *Candida albicans* to the epithelial surface is the essential first step in the process leading to persistent colonisation of the gastrointestinal tract and the mucous membranes, otherwise the organism may be swept away from the surfaces which are exposed to a continuous fluid flow (2). The cell wall of *Candida albicans* contains 5 to 8 distinct layers depending on the growth conditions and the cytochemical techniques used (14). The outermost layer, which is involved in adhesion, is fibrillar-floccular and sometimes discontinuous in nature, consisting mainly of mannoprotein (14). Growth to the stationary

phase in a medium with a high concentration of galactose or sucrose promotes the formation of mannoprotein, and simultaneously enhances yeast adhesion (14). Interaction involving the protein portion of this lectin-like mannoprotein on the yeast surface and a sugar receptor on the epithelial surface is the most probable mechanism of adhesion to the epithelial surface (14). Synthesis of yeast adhesion(s) is enhanced in the presence of high concentrations of certain sugars, a factor that is likely to be important *in vivo* (14). Sucrose, glucose and maltose are common dietary sugars, and galactose can be formed in the mouth as a result of lactose degradation by oral bacteria (14). Considering that all of these sugars can promote synthesis of yeast adhesion(s) explains the clinical observation that a carbohydrate-rich diet can predispose individuals to oral candidiasis (14). Similarly, glucose-induced adhesion might contribute in the pathogenesis of vaginal thrush in diabetic and pregnant women who are known to have high concentrations of vaginal glycogen which can be converted into glucose by enzymes present in the tissues or produced by the normal flora (14).

The fibrillar protrusions may represent appendages analogous to bacterial fimbrae whose importance in adhesion is well documented (14). Fimbrae have been reported in a variety of yeast species including *Candida albicans* (14).

It is thought that the anaerobic members of the indigenous bacterial flora inhibit the colonisation of *Candida albicans* by interfering with its adhesion to mucosal surfaces (14).

3. Filament formation.

Tissue invasion by *Candida albicans* is often associated with germ tube formation and the appearance of *Candida* spp. *in vivo* is usually one of yeast cells and hyphal filaments (4). Thus both true hyphae and pseudohyphae would appear to be involved in the disease process by different species of Candida (4). The exact signals that encourage germ tube formation or promote pseudohyphae *in vivo* are unknown (4).

Filamentation alone in *Candida albicans* does not account for its pathogenicity, as the production of hyphae, indicating active growth *in vivo*, also enhances its virulence in mammalian hosts (13).

Studies using zinc-starved stationary-phase cells at 25°C were conducted to determine the factors that influence whether the cell will assume a budded or filamentous mode of growth (13). It was shown that these pluripotent cells will form buds if inoculated into fresh medium at 37°C an pH 4.5, and mycelia will form if they are inoculated at 37°C and pH 6.5

(13). Commitment to either one form occurs 20-30 minutes after cell wall evagination, while commitment to the mycelial form seems to be made possible only from stationary phase whereas buds can form from growing mycelia (13) In mycelial forms there is much more chitin and higher chitin synthetase activity (13)

4. Proteases

A number of protein degrading enzymes have been described as produced by *Candida albicans* (4) These proteases could be involved in virulence (e g by the degrading of immunoglobulins, etc.), but although genetic studies show proteases are involved in candidiasis, the exact pathogenic role is not understood (4)

5. Antigenic variability

The cell wall that gives the thallus rigidity and structure and enables the passage of nutrients into the cytoplasm and waste matter to the environment, is composed of 80-90% carbohydrate, the rest being protein and lipids and in certain instances sterol (15) The main structural polysaccharides are: cellulose, chitin, mannan and glucan (15). The important antigenic determinants in *Candida albicans* are the surface polysaccharides, such as the mannans and glucans. Mannan forms the outer layer while the glucans form the inner layer of the cell wall (2). The two sugars occur naturally as complexes of polysaccharide-proteins linked by N-acetylglucosamine (2) The arrangement of the linkages in the side chains of these fungal polysaccharides give the organism its antigenic specificity (15) The antigenic specificity of the mannans depends on the lengths of the polysaccharide side branches and the type of glycoside linkages (2) In the main chain of *Candida albicans* the mannans are polymers of mannose connected by alpha 1 to 6 linkages In the side chains the linkages are alpha 1 to 6 linkages or alpha 1 to 3 and there are six mannose units or less (2) *Candida albicans* has a pleomorphic structure and can therefore exist either as a yeast or mycelial form (15) This diversity and morphological change is important in the pathogenicity of *Candida albicans* as research has shown that *Candida albicans* is resistant to phagocytosis in its mycelial form (7,15)

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