

A STUDY COMPARING THE ANTI-MICROBIAL EFFECTIVENESS OF A WATER BASED PREPARATION OF *ECHINACEA PURPURA* TO THAT OF AN ETHANOL BASED PREPARATION ON THE IN *VITRO* GROWTH OF *CANDIDA ALBICANS* AND *ESCHERICHIA COLI*.

by

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

I, Veeran Ramesh Singh do declare that this mini-dissertation represents my
own work in both conception and execution.

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DEDICATION

I dedicate this study to my mother, Sheena Singh.

Your love, support, sacrifices and hard work has brought me to where I am today.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude and appreciation to the following persons:

Dr Ingrid Couchman

Supervisor

Professor Suren Singh

Co-Supervisor

Mrs Rehna Brijlal

Lab Technician

(Department of Biotechnology)

Dr David Naude

Department of Homeopathy

Dr Russel Hopkins

Department of Homeopathy

Mr Kemraj Singh

Mr Deena Naidoo

Abstract

The purpose of this study was to compare the effect of a water-based extraction of *Echinacea purpura* (*E. purpura*) on the *in vitro* growth of *Candida albicans* (*C. albicans*) and *Escherichia coli* (*E. coli*) to a control of distilled water in terms of the size of the zones of inhibition. It also sought to compare the results to those obtained from experiments using ethanol-based tinctures of *Echinacea purpura*.

According to Vosloo (2002) and Reid (2002), 62% ethanol-based tinctures have anti-microbial effects. Due to this fact, this study sought to determine whether the herb itself possesses anti-microbial properties or if ethanol is the active ingredient. The anti-microbial effect of a water-based extraction of *Echinacea purpura* against *C. albicans* and *E. coli* was therefore assessed using the Kirby-Bauer Anti-microbial Sensitivity test. In addition, a 62% ethanol tincture of *Echinacea purpura* was tested on *E. coli* and *C. albicans*. The tube dilution method was used to establish the minimum inhibitory concentration of the water-based extraction of *Echinacea purpura*. This was compared to the minimum inhibitory concentration of the 62% ethanol tincture of *Echinacea purpura*. The water-based extraction of *Echinacea purpura* was obtained from Parceval (Pty) Ltd, Wellington, and was prepared according to an adjusted version of method 4a of the German Homeopathic Pharmacopoeia.

In carrying out the Kirby-Bauer Anti-microbial Sensitivity test, blank antibiotic discs were placed on Mueller-Hinton agar (MHA) plates, which were streaked with *E. coli*, and Sabouraud's dextrose agar (SDA) plates, which were streaked with *C. albicans*. Thereafter, 10 µl of the test and control substances were pipetted onto the blank antibiotic discs. The plates were incubated and observations were made after 24 hours for the presence of growth inhibition areas, which were indicated by a clear zone surrounding each disc. The zones were measured using a pair of Vernier callipers.

In carrying out the tube dilution method, varying dilutions of the test and control substances were pipetted into tubes containing liquid cultures of *C. albicans* and *E.coli*. The tubes were incubated for 24 hours at 37°C, and were thereafter inspected for growth inhibition using McFarlands 0.5 turbidity standard as a comparison.

Non – parametric statistical analysis in the form of the Mann Whitney U Test were applied to perform the relevant inter-group analyses, and significance levels were set at 0.05. Data entry and analysis were done using SPSS for Window®, version 9.

From the results obtained, the *Echinacea purpura* water-based extraction did not produce significant zones of inhibition on either *C. albicans* or *E. coli*. No zones of inhibition were evident with the distilled water control. The *Echinacea purpura* 62% (v/v) ethanol-based tincture did not produce significant zones of inhibition on either *C. albicans* or *E. coli*, while the 62% ethanol control did produce significant zones of inhibition on *C. albicans* and to a greater extent, *E. coli*.

Since no previous *in vitro* antimicrobial studies have been done on water-based extractions at The Durban Institute of Technology, comparisons were not possible with the results obtained from this study.

The results obtained from the tests on the *Echinacea purpura* 62% (v/v) ethanol-based tincture is, however open to comparison; the results are in contrast to previous studies (Vosloo, 2002, Reid 2002, and Budhree, 2003) using 62% herbal tinctures. It was hypothesised that this discrepancy could be due to previous studies using filter paper discs (Whatman® number 4) as opposed to the blank antibiotic discs used in this study. A drop test was therefore carried out whereby the antimicrobial and anti-candidal effect of filter paper discs (Whatman® number 4) impregnated with 10 µl of test substance were compared to the antimicrobial and anti-candidal effect of 10 µl drops of the test substance.

The results showed there to be no significant difference between the filter paper discs (Whatman® number 4) and the 10 µl drops of the test substance.

The tube dilution tests on the *Echinacea purpura* 62% (v/v) ethanol-based tincture indicated complete inhibition of bacterial and candidal growth across all dilution ranges.

The tube dilution tests on the *Echinacea purpura* water-based extraction were paradoxical in that the more the test substance was diluted the greater was its apparent antimicrobial and anti-candidal effects. It was hypothesised that this could have been due to contamination of the original test substance. A test to determine if contamination was present was therefore carried out whereby the *Echinacea purpura* water-based extraction was streaked on SDA and MHA plates, and incubated for 24 hours. The results indicated that that there was indeed contamination of the test substance.

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DEFINITION OF TERMS

Achalasia- A neurogenic esophageal disorder characterized by esophageal peristalsis and esophageal sphincter relaxation (Berkow, et al. 1999: 1459).

Anaphylaxis- An acute IgE-mediated systemic reaction that occurs in a previously sensitized person who receives the sensitizing antigen (Berkow, et al. 1999: 1053).

Angioedema- A deep swelling due to edematous areas in the deep dermis and subcutaneous tissue and mucous membranes (Berkow, et al. 1999: 1055).

Dysphagia- A subjective awareness of difficulty in swallowing (Berkow, et al. 1999: 228).

Ethnobotany- The study of plants used by “primitive and aboriginal” people (Corrigan, 1994).

Odynophagia- Pain during swallowing (Berkow, et al. 1999: 229).

CHAPTER ONE

Introduction

1.1 Overview

Herbal Medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. A herb is a plant or plant part valued for its medicinal, aromatic or savoury qualities. Herbs produce and contain a variety of chemical substances that act upon the body (Herb Palace. 2004).

The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous people's traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional Oriental, and Native American Indian medicine (Hobbs, C. 1996).

Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed (Herb Palace. 2004). Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. For example, vincristine (an anti-tumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants (Hobbs, C. 1996).

Prior to the discovery and subsequent synthesis of antibiotics, the herb Echinacea (which comes from the plant commonly known as purple coneflower) was one of the most widely prescribed medicines in the United States. For centuries, herbalists prescribed Echinacea to fight infection. Today, research

confirms that the herb boosts the immune system by stimulating the production of disease-fighting white blood cells (Herb Palace. 2004).

Numerous studies have been done to demonstrate the effectiveness of herbal extracts as anti-microbial agents. In one study done in India, ethanolic extracts of 45 Indian medicinal plants traditionally used in medicine were studied for their anti-microbial activity against certain drug resistant bacteria and the yeast *Candida albicans*. Of these, 40 plant extracts showed varied levels of anti-microbial activity against one or more test bacteria. Anti-candidal activity was detected in 24 plant extracts. Overall, broad-spectrum anti-microbial activity was observed in 12 plants (Ahmad, I., Beg, A.Z., 2001: 113-123).

Rotblatt, 2002, outlined various points as to why herbal remedies are to be favoured:

- Historical value and acceptance suggest effectiveness.
- Many current medicines are derived from herbs.
- Several herbs have achieved acceptable therapeutic status based on randomized, controlled trials.
- Many criticisms against herbs apply equally to orthodox drugs, but drugs are more toxic.
- Many drugs cause harm to, or even kill, patients; herbs are much safer in general.
- Herbs may treat conditions that orthodox medicines cannot help.
- Herbs can be used as adjuncts to regular drugs in integrative therapeutic regimens.

Candida species are commensal organisms that colonize the normal gastrointestinal tract and sometimes the skin. Unlike other systemic mycoses, *Candida* is an endogenous organism and is not generally acquired from the surrounding environment. Infections due to *Candida* sp account for about 80% of all major systemic fungal infections. *Candida* is now the fourth most prevalent

organism found in bloodstream infections and is the most common cause of fungal infections in immuno-compromised people. The frequency of nosocomial candidiasis has raised at least five fold in the 1980's, making it one of the most common hospital acquired infections. Although often a benign self-limited problem, it may be associated with excess mortality of $\geq 40\%$ (i.e., deaths attributable to candidiasis rather than to underlying diseases) and prolongation of hospitalizations (Berkow, R., and Beers, M.H., 1999: 1220).

According to Berkow *et al.* (1999), *E. coli* is a known opportunistic pathogen in patients who have defects in host resistance as a result of other diseases (eg. Cancer, diabetes, cirrhosis), or who have received treatment with corticosteroids, anti-neoplastic drugs, or anti-biotics. It is these very people, especially those suffering from liver disorders, who cannot utilise alcohol-based extractions, hence the relevance of this study. It is because of the above that all alcohol containing medicinal preparations carries clearly visible warnings giving information regarding the alcohol content of the product in order to safeguard patients with alcohol-related diseases, disorders of liver function or epilepsy, and other high risk patients from the incorrect intake of alcohol even in the smallest amounts.

The development of anti-microbial resistant strains of *E. coli* is escalating even in acute uncomplicated cystitis, an infection that has traditionally been simple to treat. Judicious use of antibiotics and development of novel non-anti-microbial-based methods of prevention of UTI are important strategies to help slow the progression of resistance (Gupta, K 2003: 243-259).

In a prospective cohort study of 71 children younger than 10 years of age who had diarrhea caused by a strain of *E. coli* to assess whether antibiotic treatment in these children affects the risk of the hemolytic–uremic syndrome, it was concluded that antibiotic treatment of children with *E. coli* infection increases the risk of the hemolytic–uremic syndrome (Wong, C.S, Jelacic, S., Habeeb, R.L., Watkins, S.L., and Tarr, I.P. 2000).

It is thus evident that alternatives to conventional anti-biotic therapies need to emerge.

1.2 Problem Statement

The purpose of this study was to investigate the effectiveness of *Echinacea purpurea* water based extraction in inhibiting the *in vitro* growth of *E. coli* and *C. albicans* in terms of the disc diffusion assay and the tube dilution test, and to compare the results to similar experiments using ethanol tinctures.

1.3 Subproblems

1.3.1 Subproblem One

To evaluate the effectiveness of *E. purpurea* water based extraction in inhibiting the *in vitro* growth of *E. coli* and *C. albicans* in terms of the sizes of the zones of inhibition in the disc diffusion assay, and in terms of turbidity in the tube dilution test.

1.3.2 Subproblem Two

To evaluate the effectiveness of *E. purpurea* 62% (v/v) ethanol tincture in inhibiting the *in vitro* growth of *E. coli* and *C. albicans* in terms of the sizes of the zones of inhibition in the disc diffusion assay, and in terms of turbidity in the tube dilution test.

1.3.3 Subproblem Three

To compare the effectiveness of the *E. purpurea* water based extraction with that of the *E. purpurea* 62% (v/v) ethanol tincture in inhibiting the *in vitro* growth of *E. coli* and *C. albicans*.

1.4 Hypotheses

All hypotheses are stated in the null form.

1.4.1 Hypothesis One

It is hypothesized that the *E. purpura* water based extraction will have no significant effect in inhibiting the *in vitro* growth of *E. coli* and *C. albicans* in terms of the sizes of the zones of inhibition in the disc diffusion assay, and in terms of turbidity in the tube dilution test.

1.4.2 Hypothesis Two

It is hypothesized that the *E. purpura* 62% (v/v) ethanol tincture will have no significant effect in inhibiting the *in vitro* growth of *E. coli* and *C. albicans* in terms of the sizes of the zones of inhibition in the disc diffusion assay, and in terms of turbidity in the tube dilution test.

1.4.3 Hypothesis Three

It is hypothesized that there will be no difference between the *E. purpura* water based extraction and the *purpura* 62% (v/v) ethanol tincture on the inhibition of the *in vitro* growth of *E. coli* and *C. albicans* in terms of the sizes of the zones of inhibition in the disc diffusion assay, and in terms of turbidity in the tube dilution test.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

2.1 Echinacea purpurea

2.1.1 Nomenclature

Common names: purple coneflower, snakeroot, Kansas snakeroot, black samson, scurvy root, Indian head, black susans, and hedgehog. The *Echinacea* species derives its name from the Greek *echinos* (meaning sea urchin). This refers to the prickly scales of the dried seed head portion of the flower (Corrigan, 1994: 9).

2.1.2 Botanical description

Echinacea purpurea belongs to the Echinacea genus and is a member of the Asteraceae family (Rotblatt, M., Ziment, I., 2002: 160). Plants of this genus are perennials, reaching from 2 to 3 feet in height, with vertical or horizontal roots. The stems are erect with rough, coarse hairs (Lucas, 1991: 3). The leaves are ovate to lanceolate or elliptical. A solitary flower is produced atop a long peduncle. *Echinacea purpurea* differs from others of its genus in that it has a fibrous root in comparison to others, which have a taproot system (McLaughlin, 1992). A distinct purple coneflower is another distinguishing feature (Corrigan, 1994). The flower has large spreading purple rays and a disc consisting of purple florets (Indian Spring Herbs. 2003). (Appendix A)

2.1.3 Habitat and Cultivation

Echinacea purpurea is native to the central parts of the United States, but is also commercially cultivated in Europe (Chevalliar, 1996: 90). The natural range of *Echinacea 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 (McLaughlin, 1992). *Echinacea purpurea* is the most widely used and most widely cultivated medicinal species of the genus (Corrigan, 1994).

2.1.4 Plant Sections Used

The portions of the plant used for medicinal purposes include the aerial portion, the whole plant, including the root, and the root itself (Pizzorno, J.E., Murray, M.T., 1999: 703-704).

2.1.5 History and Traditional Uses

Detailed ethnobotanical studies of *Echinacea* show that it was one of the most important medicinal plants used by the Native Americans. They applied it externally to wounds, burns, swellings of the lymph glands, and insect bites. The roots were chewed to cure toothache and pains in the neck (Kligler, B. 2003). Internally, it was used against headache, stomach cramps, coughs, chills, measles, and gonorrhoea. Ethnobotanical reports frequently mention its use as an antidote for rattlesnake bites, hence the name given to it. Sometimes the plant was used to demonstrate supernatural effects, or in religious ceremonies. For example, Omaha medicine men used the macerated root as a local anaesthetic to deaden sensation, so that they could remove lumps of meat from a boiling pot without flinching, thus showing their ability to perform supernatural feats. The Cheyenne chewed the root to stimulate saliva flow, which was especially helpful as a thirst quencher for participants in the Sun Dance. They also drank a tea for rheumatism, arthritis, mumps, and measles. (Corrigan, 1994: 10).

Echinacea was very popular among Eclectic physicians, who used it externally as a local antiseptic, stimulant, deodorant, and anaesthetic; and internally for “bad blood”, i.e. to correct “fluid deprivation with tendency to sepsis and malignancy” (Pizzorno, et al. 1999: 705).

Although popular in the 19th and early 20th centuries, *Echinacea*'s use declined greatly in the U.S. after the introduction of antibiotics. It was introduced in Germany in the early 1900s, where in recent years it has been avidly researched and promoted as an immune system stimulant to help the body fight infections (Rotblatt, et al. 2002: 160).

2.1.6 Chemical Constituents

According to Pizzorno et al. (1999), the important constituents from a pharmacological perspective, of *Echinacea* sp. can be divided into seven categories:

- polysaccharides- most notable being inulin.

- flavonoids- most notable being rutoside.

- caffeic acid derivatives- most notable being echinacoside, cichoric acid, chlorogenic acid, and cynarin.

- essential oils- most notable being sesquiterpene, borneol, alpha-pinene, and related aromatic compounds.

- polyacetylenes.

- alkylamides- most notable being isobutylamides.

- miscellaneous chemicals- including resins, glycoproteins, sterols, minerals, and fatty acids.

2.1.7 Mechanisms of Action

Numerous studies have shown *Echinacea purpura* to have profound immunostimulatory effects, resulting in enhanced T-cell mitogenesis, macrophage phagocytosis, antibody binding, and natural killer cell activity, as well as increased levels of circulating neutrophils. Root extracts of *Echinacea purpura* have been shown to possess interferon-like activity, as well as direct antiviral activity against many viruses including influenza, herpes, and vesicular stomatitis viruses (Werbach, M.R., Murray, M.T., 2000: 19). The virostatic action is against all viruses, but particularly against RNA (influenza) and DNA (herpes) viruses. Interferon is a protein produced in the body that has considerable antiviral activity. It is stable in acids and is produced to overcome infections in the body, being non-specific in its action. It blocks multiplication of both RNA- and DNA-containing viruses, and also of bacteria and some protozoa. It also possesses anti-neoplastic properties (Weiss, F.G., 1991: 230).

According to Murray (1995), the polysaccharides of *Echinacea purpura* 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 the cells. These polysaccharides have shown the ability to enhance macrophage phagocytosis and stimulate macrophages to produce a number of immunopotentiating compounds.

2.1.8 Current Uses and Applications

Currently, *Echinacea* is used clinically for numerous conditions, especially in infections. Clinical studies have demonstrated effectiveness in a number of infectious conditions using all three routes of administration: injectable, oral, and topical. Externally, it is used in the treatment of wounds, eczema, burns,

psoriasis, herpes, and candidiasis. (Indian Spring Herbs. 2003). Internally, the plant is used as a prophylactic at the onset of colds and flu, upper respiratory tract infections, urogenital infections, and other general infectious conditions. It is also known to have applications in the treatment of snakebites, as well as arthritis and cancer. (Pizzorno et al. 1999: 707-708).

Echinacea purpurea is effectively used in the treatment of boils and septicemia. Chronic infections such as postviral fatigue syndrome, as well as chilblains, colds, influenza, skin disorders and respiratory problems can also be treated with *Echinacea purpurea* (Chevalliar, 1996: 90). An experimental double-blind study was carried out to evaluate the efficacy of *Echinacea* in the prevention of upper respiratory tract infections. 302 volunteers received extracts from either *Echinacea purpurea*, *Echinacea angustifolia*, or placebo for 12 weeks. The results accentuate the effectiveness of *Echinacea purpurea* in the prevention of upper respiratory tract infections (Melchart, D., Walther, E., Linde, K. 1998).
(See table 2.1)

Table 2.1- The effectiveness of *E. purpura* in the prevention of upper respiratory tract infections

	<i>E. purpura</i>	<i>E. angustifolia</i>	Placebo
Time till occurrence of the first upper respiratory infection	69 Days	66 Days	65 Days
Infection rate (%age)	29.3%	32%	37.6%
%age that felt that they had benefited from the treatment	70%	78%	56%
%age experiencing side effects	10%	18%	11%

In eight European trials that evaluated *Echinacea purpura* for acute treatment of upper respiratory infections, almost every study demonstrated beneficial results for the *Echinacea purpura* compared to placebo. These trials found that *Echinacea purpura* reduced the signs or symptoms or shortened the duration of a viral upper respiratory-like illness when initiated in the first few days of symptoms and continued for 8-10 days (Rotblatt, et al. 2002: 161).

Echinacea purpura's potent immunoregulatory properties can be useful in the treatment of HIV infection, since effective treatment is likely to require both antiviral chemotherapy and reconstitution of the immune system (Werbach, et al. 2000: 42). A phase 1 trial of *Echinacea purpura* on HIV (+) individuals was conducted. Fourteen patients with CD4 counts ranging from 6-600/mm³ (mean

269) and viral loads (\log_{10}) ranging from <2.3-5.4 (mean 4.68) were enrolled. Each subject had been on a stable antiretroviral regimen or no antiretrovirals for at least the previous 12 weeks. Each received a 12-week course of *Echinacea purpura* at 1000mg three times daily. There were no clinical or laboratory toxicities noted during the study. At 12 weeks there was no significant difference in mean CD4 count compared to baseline. However, there was an overall 0.32 \log_{10} reduction viral load (mean 4.6, $p < .05$). Thus, the Echinacea was safe and associated with a significant reduction in viral load in HIV (+) individuals (Werbach, et al. 2000: 42-43).

Because of its immune enhancing effects, *Echinacea purpura* is used as additional medication in symptomatic cancer therapy, where it may help to activate physical resources and give a certain improvement in general well being. Stimulation of interferon production is also responsible for its clinical benefits (Wiess, 1991: 326). An *in vitro* study was carried out whereby acidic arabinogalactan, a highly purified polysaccharide from *Echinacea purpura*, was effective in activating macrophages to cytotoxicity against tumor cells. Furthermore, it induced macrophages to produce tumor necrosis factor, interleukin-1, and interferon-beta 2 (Werbach, et al. 2000: 163).

Echinacea purpura's effectiveness against *Candida albicans* has been demonstrated in numerous studies. In one such study, purified polysaccharides from cell cultures of *Echinacea purpura* were investigated for their ability to enhance phagocytic activity. Macrophages could be activated to produce interleukin-1, tumor necrosis factor alpha and interleukin-6, to produce increased amounts reactive oxygen intermediates and to inhibit *in vitro* growth of *Candida albicans*. *In vivo*, the substances could induce increased proliferation of phagocytes, and migration of granulocytes to the peripheral blood (Roesler, J., Steinmuller, C., Kidderlen, A., Emmendorffer, A., Wagner, H., 1991). In another study, a commercial preparation of *Echinacea purpura* administered intramuscularly on 4 successive days to 12 healthy males resulted in a rapid and

continuous increase in granulocyte phagocytosis activity against *Candida albicans* (Werbach, et al. 2000: 198).

2.1.9 Adverse Effects and Contraindications

The reported adverse effects have generally been uncommon and minor; they include abdominal upset, nausea, and dizziness. Anaphylaxis, asthma exacerbation, and angioedema have been reported in isolated cases. Persons with a history of allergy to any plant in the daisy family (including ragweed, marigold, and chrysanthemum) may be at greater risk for significant allergic reaction to *Echinacea* (Sahelian, R., 2004).

Theoretically, the immune-stimulating properties of echinacea might interfere with the use of immunosuppressive medications in patients with autoimmune disease; however, while such an effect is theoretically possible, it has not been documented in animals or humans (Rotblatt, et al. 2002: 161).

2.2 Types of Herbal Products

2.2.1 Alcohol Based Tinctures

Tinctures are traditionally made by placing the herb in an organic solvent and leaving it to soak for days to weeks. An alternative and faster method is for the solvent to be percolated through the herb (Werbach, et al. 2000: 2-3). For commercial preparations, an alcohol solvent is the most useful. Usually the alcohol used is ethyl alcohol (96.4% strength). Commercial ethyl alcohol is usually made from corn to which many people are sensitive (Gaia Garden 2004). The liquid that is obtained from the herbal material is the medicine, and the remainder of the herb is discarded. Alcohol based solvents or extracts are usually stronger than infusions or decoctions, as alcohol can extract constituents that are water-insoluble (Rotblatt, et al. 2002: 26-27). Alcohol is also a very effective natural preservative. Note that the tincture must be at least 25% alcohol to ensure sterility and that different herbs require more or less alcohol to access the different constituents (Gaia Garden 2004). Because a tincture is easily assimilated by the body, it is a very effective way to administer herbal compounds. Tinctures are concentrated and cost-effective (Herb Palace, 2004). Although often used interchangeably, technically there is a difference between a tincture and a fluid extract:

A tincture is generally defined as a solvent (e.g. alcohol) extract having a minimum dilution of 1:5.

A fluid extract is, by definition a 1:1 or 1:2 ratio or stronger. It is supposed to be about 5-10 times more potent than a tincture, and smaller doses are used (Rotblatt, et al. 2002: 26-27).

2.2.2 Water Based Extractions

There are vast amounts of literature and research currently available on herbal tinctures in general. However, literature and research on water-based extractions is presently extremely limited (Feiter, U. 2003). It is for this reason that water based extractions are a very under-utilized resource, and it is for that same reason that studies such as this are both relevant and necessary.

The methods employed to manufacture water-based extractions are infusions and decoctions. These are very similar techniques.

An infusion is used for delicate plant parts such as leaves, flowers, soft stems and fruit. The herbal material is placed in a suitable vessel and boiling water is poured over. The infusion is steeped for 5 to 15 minutes. Decoctions are generally more concentrated than infusions, and the method is useful for fibrous plant material such as root, stems, and bark. The herbal material is placed in a pan, covered with cold water and brought to the boil. It is covered and allowed to simmer for 5 to 15 minutes (Rotblatt, et al. 2002: 26-27).

An infusion or decoction may also be made cold. The plant material and water is allowed to stand at room temperature overnight before being strained off. This is useful where there are many volatile oils that may be lost if heat is used (eg. Sweet flag (*Acorus calamus*), or where there is a lot of mucilage that would cause the end product to be very thick and glutinous, for example, Marshmallow (*Althea officinalis*).

In both of these cases, the water acts as a solvent to extract only those constituents that are soluble in water. It may be usefully applied to extract tannins, bitters and glycosides, but is not appropriate for extraction of resins, volatile and non-volatile oils or alkaloids. In the case of volatile oils, although they will not actually dissolve in water, they will evaporate in the heat and they will float on the top of the water and arise in the steam (Gaia Garden 2004).

They are the safest type of extracts, however, as toxic alkaloids are usually insoluble in water. Water extracts have a short shelf life due to bacterial contamination, and thus need to be refrigerated and discarded after a few days. They are also difficult to standardize, and are often bitter or unpleasant tasting unless flavour additives are incorporated (Rotblatt, et al. 2002: 26-27).

The water-based extraction used in this study was prepared by Parceval Pharmaceuticals (Pty) Ltd, Wellington, according to the following method:

- *Echinacea purpura* herba (fresh plant parts above ground, with flower) harvested early in the morning – (8am.).
- Plant material was immediately ground in an electrical mincer and weighed into a glass jar.
- Three parts of distilled water was added to 1 part of minced plant material.
- The mix was shaken vigorously for 5 hours, pressed through 100% cotton cloth and filtered through No.1 Whatman filter paper (Feiter, U. 2003).

Situations exist where water based extractions would be more desirable than conventional alcohol tinctures. One such situation is where alcohol intake is prohibited by religion. The religion of Islam prohibits the intake of alcohol in any form. Any medicine containing alcohol, even at low concentrations, is also not permitted. It has been stated that alcohol is not seen as a medicine, but as disease (Khomeini, 2004). There are exceptions to this non-alcoholic medicine rule, as stated by a pre-eminent Muslim scholar, Dr Yusuf Al Qaradawi:

1. The Patient's life is endangered if he does not take the medication.
2. No alternative medication made from entirely halaal sources is available.
3. The medication is prescribed by a Muslim physician who is knowledgeable, as well as God fearing (Semination, 2004).

The availability of an alternative to alcohol based tinctures i.e. water based extractions, would overcome the above limitations. There have been calls to the medical and pharmacological profession in the Muslim world to replace the drugs

containing alcohol with others, which are alcohol-free. Most of the drugs containing alcohol found on the counter e.g. tonics, etc. should be replaced by alcohol-free drugs (Albar, M., 2003). Patients belonging to the Seventh Day Adventists church are also prohibited from consuming alcohol (General Conference of Seventh-day Adventists, 1989: 283).

The use of alcohol-containing medicines is also undesirable for use in babies and pregnant women (Beard, L.M., 2004). It is not known what the safe level of alcohol intake is during pregnancy (ADF, 2004). The danger of alcohol use during pregnancy is that it may cause fetal alcohol syndrome (FAS). Babies born with FAS may:

- grow slowly
- have learning problems
- have distorted facial features (Sutter Health 2003).

Commercial ethyl alcohol is usually made from corn to which many people are sensitive (Gaia Garden 2004). Grains are among the foods most frequently associated with hypersensitivity reactions and intolerances. Though the protein antigens common to allergies are not present, it could be important to avoid exposure to other components of the fermented grain distillate. Neutral spirits are usually used to make tinctures, and most neutral spirits are derived from grain alcohol. Though the congeners have been removed, there still exists in the alcohol an energy pattern that is unique to the grain from which it was derived. In certain cases of hypersensitivity a sublingual challenge with such a highly dilute form of the sensitizing agent can still produce reactions (Brinker, 2004).

Water based extractions also has methodological advantages over alcohol based tinctures in terms of antimicrobial studies. Research conducted at the University of Pretoria, by the Faculty of Medicine Research Committee, states that if an extract is to be tested for anti microbial properties, the extractant should not inhibit the bioassay procedure (Eloff, 1998: 2). Since alcohol itself possesses

antimicrobial properties, antimicrobial studies using alcohol-based tinctures can be ineffective in determining the antimicrobial properties of the actual plant substance.

2.2.3 Other Methods of Herbal Extraction

2.2.3.1 Vinegar (acettracta)

This is about 4% acetic acid which is excreted via the lungs, kidney and skin where it acts as a mild expectorant, diuretic and diaphoretic. An acettracta may be useful when administering herbs to a small child or a person with compromised liver function because vinegar is very gentle on the body. Vinegar is a reasonably good solvent but the shelf life of an acettracta is only about 3 months. Because of the unpleasant taste the medicine is frequently mixed with honey.

2.2.3.2 Glycerine (glycetracta)

This is a colourless, odourless, viscous fluid with solvent capacities somewhere between alcohol and water. A glycetracta is commonly used to preserve fresh expressed plant juices (in the ratio 1:1) and to make syrups. The taste is sweet and the shelf life is 6 to 12 months.

2.2.3.3 Fat extractions

Using fat as a solvent will extract those constituents that are fat or alcohol soluble: gums, resins, fixed and volatile oils, waxes and alkaloids. There are two methods used:

2.2.3.3.1 Enfleurage. Fresh plant material (usually flowers) is placed over a layer of fat with a low boiling point (e.g. cocoa butter) and allowed to stand for 3 days at room temperature. A mild organic solvent (alcohol) can then be used to extract the plant constituents from the fat.

2.2.3.3.2 Digestion. This is done in a similar way to enfleurage but the fat is heated to about 35⁰ C and maintained at that level for several hours to a few days. The warm oil 'digests' the plant material and draws out the fat-soluble constituents. The oil is then squeezed out of the plant material (Gaia Garden 2004).

2.3 E. coli

2.3.1 Classification

E. coli falls under the family Enterobacteriaceae, which form a large group of Gram-negative rods, whose natural habitat is the intestinal tract of humans and animals (Williams, P., Withers, H., Dodson, S., Griffen, C., 2004).

2.3.2 Morphology and Identification

E. coli are facultative anaerobic, short, straight, Gram negative bacillus that are non-sporing, usually motile with flagella, and occurs singly or in pairs in rapidly growing liquid cultures (Williams, et al. 2004). *E. coli* grows well on non-selective media, forming circular, convex, smooth colonies with distinct edges (Jawetz, et al. 1991: 204). *E. coli* readily ferments glucose, lactose and certain other sugars, producing both an acid and a gas- a property used in preliminary identification (Pattison, J.R., Gruneberg, R.N., Holten, J., Ridgway, G.L., Scott, C., 1995: 145).

2.3.3 Laboratory Diagnosis

E. coli can be recovered easily from appropriate specimens (urine, faeces, blood, pus) on lactose containing media. Colonies become visible within a day or less. Lactose fermentation, along with other biochemical tests, help to differentiate *E. coli* from other highly pathogenic Gram-negative rods (Howes, D., 2002).

2.3.4 Clinical Manifestations

Despite the fact that *E. coli* is a member of the normal flora and is not highly pathogenic, it is of great medical importance because of the frequency and potentially serious nature of the infections it causes. The organism normally lives in the small intestine without causing apparent harm. However, because of its presence in feces, it often reaches and incites disease in other parts of the body, especially the urinary tract and peritoneum (Myrvik, Q.N., Weiser, R.S. 1989: 288).

2.3.4.1 Urinary Tract Infections

The normal urinary tract is sterile and very resistant to bacterial colonization. However, urinary tract infections are the most common bacterial infection in all age groups (Simon, H., 2002). *E coli* is the most common cause of urinary tract infections and accounts for 90% of first urinary tract infections in young women. In hospitalised patients, *E coli* accounts for 50% of cases (Howes, D., 2002).

E coli enters the urinary tract by natural means or may be introduced on catheters or other instruments. Their persistence in the urinary tract is favored by anesthesia, paralysis, by any other agency that interrupts the normal voiding reflex, or by anatomic abnormalities that permit retention of urine. Although obstruction alone does not cause urinary tract infections, its presence does predispose to infection and makes infections more difficult to eradicate with medical therapy. Urine is an excellent culture medium and readily supports the growth of *E coli* (Berkow, et al. 1999: 1885).

2.3.4.2 Diarrhoeal Diseases

Diarrhea-causing *E. coli* are placed into different categories based on their virulence properties. The major categories are enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggeregative (EaggEC) (Parry, S., Palmer, S., 2002: 2-3), and enterohemorrhagic (EHEC) *E. coli* (Mackie and McCartney, 1996:366).

a) Enteropathogenic (EPEC)

Associated with acute outbreaks of diarrhoea in young children (Parry, et al. 2002: 2-3). Illness is caused by the bacteria adhering to the intestinal wall and subsequent destruction of the microvilli (Center for Food Safety & Applied Nutrition, 2003).

b) Enterotoxigenic (ETEC)

Associated with infant diarrhoea in developing countries and traveler's diarrhoea. Acute watery diarrhoea is produced by heat stable or heat labile toxins. It is acquired by consuming contaminated water or food and produces a diarrhoea similar to cholera (Howard, 1994: 322).

c) Enteroinvasive (EIEC)

Associated with dysentery-type illness by invasion of the gut epithelial cells (Parry, et al. 2002: 2-3). Clinically the illness is characterized by fever, abdominal cramps, malaise, toxemia, and watery diarrhoea consisting of blood and mucous (Center for Food Safety & Applied Nutrition, 2003)

d) Enteroaggregative (EaggEC)

Associated with persistent diarrhoea in people in developing countries. (Parry, et al. 2002: 2-3). These bacteria are emerging as potentially important causes of diarrhea in patients with AIDS (Berkow, et al. 1999: 1160).

e) Enterohaemorrhagic (EHEC)

Associated with haemorrhagic colitis and haemolytic uremic syndrome. Affects the cells lining the gastrointestinal tract and kidneys (Parry, et al. 2002: 2-3).

2.3.4.3 Neonatal Sepsis

Neonatal sepsis can be defined as an invasive bacterial infection of the bloodstream occurring in the first 4 weeks of life (Berkow, et al. 2000: 2174). Newborns may be highly susceptible to *E. coli* sepsis because they lack IgM antibodies (Jawetz, et al. 1989: 208). A newborn may be predisposed to neonatal sepsis by obstetric complications, maternal bleeding (placenta previa, abruptio placentae), toxemia, precipitous delivery, or maternal infection (particularly of the urinary tract or endometrium) (Myrvik, et al. 1989: 294). The symptoms of sepsis are not specific and vary from child to child. A lower heart rate, breathing problems, jaundice, trouble feeding, low or unstable body temperature, lethargy,

or extreme fussiness, and neurological findings (e.g., seizures, jitteriness) can all be signs of the condition (Kids Health, 2004).

2.3.4.4 Neonatal Meningitis

Neonatal meningitis is defined as inflammation of the meninges due to bacterial invasion in the first 4 weeks of life (Berkow, et al. 2000: 2182). *E. coli* accounts for about 40% of all cases of neonatal meningitis (Jawetz, et al. 1989: 208).

Neonatal meningitis most frequently results from a pre-existing bacteremia associated with neonatal sepsis. Meningitis may also result from skin lesions of the scalp that, along with developmental defects, lead to communication of the skin surface with the subarachnoid space. Rarely, direct extension to the central nervous system from a contiguous otic focus occurs (e.g. otitis media) (Berkow, et al. 2000: 2182). Symptoms of the infection in newborns aren't very specific and may include persistent crying, irritability, sleeping more than usual, lethargy, refusing to take the breast or bottle, low or unstable body temperature, jaundice, pallor, breathing problems, rashes, vomiting, or diarrhea. There may also be bulging of the fontanelle (Kids Health, 2004). Cranial nerve abnormalities (particularly those involving the 3rd, 6th, and 7th nerves) may also be present (Berkow, et al. 2000: 2182).

2.4 *C. albicans*

Candida species are ubiquitous fungi found throughout the world as normal body flora. Unfortunately, candidiasis is also the most common mycotic infection, causing a variety of diseases. Candidiasis can range from superficial disorders such as diaper rash to invasive, rapidly fatal infections in immunocompromised hosts. (O Henderson, S., 2002). Infections due to *Candida* sp account for about 80% of all major systemic fungal infections. *Candida* is now the fourth most prevalent organism found in bloodstream infections and is the most common cause of fungal infections in immuno-compromised people (Berkow et al. 1999: 1220).

2.4.1 Classification, Morphology and Identification

Candida albicans belongs to the genus *Candida* of the family Saccharomycetaceae. *C. albicans* is a Gram positive, 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 submerged growth consists of pseudomycelium. The pseudohyphae form blastophores at the nodes (Myrvik, Q.N., Weiser, R.S. 1988: 546). *C. albicans* ferments glucose and maltose, producing both acid and gas. These carbohydrate fermentations, together with colonial and morphological characteristics, differentiate *C. albicans* from other *Candida* species (Jawetz, E., Melnik, J. L., Adelberg, E. A., Brooks, G. F., Butel, L. S., and Ornston, L. N., 1991: 307).

2.4.2 Pathophysiology

Candidiasis affects a wide variety of organ systems. In immunocompetent persons, any warm, moist part of the body exposed to the environment is susceptible to infection. Common examples of this are vaginitis; vulvar rash; oral thrush; conjunctivitis; endophthalmitis; diaper rash; and infections of the nail, rectum, and other skin folds. In immunocompromised patients, systemic illnesses such as myocarditis, hepatosplenic abscess, pulmonary infection, central

nervous system infection, and chronic disease may occur (O Henderson, S., 2002).

2.4.3 Predisposing Factors

Ordinarily, *C. albicans* lives in balance with the other microorganisms in the body and merely exist there as colonists. But various factors can upset this balance and lead to the development of progressive, symptomatic disease (Chandler, F.W., Kaplan, W., Ajello, L., 1980: 42).

According to Howard et al. (1994), the following are predisposing factors to development of candidiasis:

- 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, vagotomy resulting in an increase in 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 number of phagocytic cells as a result of leukemia, irradiation, and cancer chemotherapy.
- Alterations in phagocyte functions caused by uremia, viral infections, and the use of corticosteroids and antimicrobial agents.
- Cell-mediated immunity problems arising from defects such as chronic mucocutaneous candidiasis, and DiGeorge syndrome, from using corticosteroids,

irradiation, cancer chemotherapy, and immunosuppression for transplantation, and from collagen vascular diseases.

The association of these conditions with infection is related to their predisposition to increase colonization by *C. albicans*. Antimicrobial agents, for example, inhibit the growth of normal flora. In the healthy host the normal flora are able to inhibit *C. albicans* through competitive inhibition for binding sites and nutrients, but in the presence of antimicrobial agents, the less susceptible *C. albicans* proliferates (Volk, T., 1999).

2.4.4 Candidal Infections

2.4.4.1 Oropharyngeal Candidiasis

Oral candidiasis, which is also called thrush, is a fungal infection of the mouth and/or throat. While oral candidiasis can sometimes occur without symptoms, the most common symptoms are discomfort and burning of the mouth and throat and an altered sense of taste (often described as “bad”) (Project Inform, 2003).

Whitish or yellowish patches on the lips, tongue, palate, and inside the cheeks are present. When these patches are scraped or rubbed, pinpoint areas of bleeding can be seen underneath (Kids Health, 2004). These may be accompanied by cracking, redness, soreness and swelling at the corners of the mouth. A bad case can include mouth sores (O Henderson, S., 2002).

Candida albicans infections also occur at a high rate in persons aged 65 and older. Factors that predispose these patients to the development of oral candidiasis include the use of broadspectrum antibiotics, inhaled corticosteroids and diminished cell mediated immunity.

Thrush can also extend to the esophagus. Conditions favoring fungal growth in the esophagus include treatment with broad-spectrum antibiotics, diabetes mellitus, compromised cellular immunity (AIDS, leukemia, chemotherapy), or

esophageal stasis (e.g. in achalasia or scleroderma). Patients normally complain of odynophagia or dysphagia (Berkow, et al. 1999: 234).

2.4.4.2 Genital Candidiasis

Infections of the genital tract by *Candida albicans* are very common in women, but are not usually acquired sexually (Berkow, et al. 1999: 1334). Instead, *Candida* from the patient's normal skin and intestinal flora overgrows because of the predisposing factors mentioned previously. *Candida* infection is also associated with loss of vaginal pH (which is normally maintained by the bacterial flora in the vagina) and constrictive undergarments (Centers for Disease Control and Prevention, 2004)

Symptoms include: vaginal pain, itching, or redness; a thick, white "cheesy" vaginal discharge; pain or discomfort on urination; and sometimes whitish or yellowish patches on the skin of the vaginal area (these look similar to the patches seen in the mouth of a baby with oral thrush) (Kids Health, 2004). The lesions vary from a slight eczematoid reaction with minimal erythema, to a severe disease process with pustules, excoriations, and ulcers. The whole area is greatly inflamed and pruritis is usually intense. The condition may extend to involve the perineum, the vulva, and the entire inguinal area (Rippon, 1988: 544). Patients may also present with a history of depression, anxiety, sudden mood swings, lack of concentration, headaches, drowsiness, or fatigue (O Henderson, S., 2002). This is thought to be due to toxins produced by *C. albicans* which act on the central nervous system (Di Maio, E., 2004).

Men are often asymptomatic carriers of *C. albicans* but may occasionally notice a slight urethral discharge. They may complain of irritation and soreness of the glans penis and prepuce, especially after intercourse (Berkow et al. 1999: 1334). The glans penis and prepuce may be inflamed and white cheesy material, vesicles, or erosions may be present. In severe cases, the prepuce may be edematous, causing phimosis (constriction of the foreskin). There is often a

history of vaginitis in the spouse, and the condition is probably transmitted through sexual intercourse (O Henderson, S., 2002).

2.4.4.3 Cutaneous Infections

Children of any age may develop *Candida* paronychia, an infection of the skin around the nails. Fingernails are most often affected, especially in children who spend a lot of time with their hands in water. The cuticle and skin around the nails becomes swollen, red, and sometimes painful. The fingernails may grow to be abnormally shaped or colored, or may actually lift away from the skin (Kids Health, 2004). Secondary bacterial infections are frequent and present diagnostic and therapeutic problems (Chandler, et al. 1980: 43).

Infection of the skin occurs principally in moist, warm parts of the body, such as the axilla, intergluteal folds, groin, or inframammary folds and between the toes (Jawetz, et al. 1991: 308). The lesions are typically erythematous, scaly, and moist. Pustules and vesicles may develop in the centre of the affected areas. Pruritis is a common complaint (Berkow, et al. 1999: 805).

2.4.4.4 Chronic Mucocutaneous Candidiasis

Chronic mucocutaneous candidiasis is one of the most dramatic and devastating forms of the disease. The victims of this disease are individuals with a variety of underlying genetic defects, i.e., endocrinological, haematological, immunological, and metabolic. It occurs most often in children (Chandler, et al. 1980: 43). The disease varies considerably in severity from involvement of a single nail, to generalized mucous membrane, skin and hair involvement and disfiguring granular lesions of the face and scalp (Berkow, et al. 1999: 1037).

2.4.4.5 Systemic Candidiasis

Systemic forms of candidiasis may be localised in the urinary system, liver, heart valves, meninges, spleen, bone, skin, eye, subcutaneous and other tissues, or

the infection may be disseminated and associated with septicemia (candidemia) (Greenwood, et al. 1995: 684).

Candidemia usually causes fever, but other symptoms are typically non-specific (O Henderson, S., 2002). Sometimes, a syndrome develops resembling bacterial sepsis, with a fulminating course that may include shock, oliguria, renal shutdown, and disseminated intravascular coagulation (Berkow, et al. 1999: 1221).

Candidal endocarditis presents with a clinical picture, which includes fever, murmurs, congestive cardiac failure, anemia and splenomegaly. Large vegetations are seen on the heart valves, and there is a high incidence of embolisation (O Henderson, S., 2002).

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 *Echinacea purpura* water based extraction on *Candida albicans*.
2. Results of the experiment determining the effects of *Echinacea purpura* in 62% ethanol (v/v) on *Candida albicans*.
3. Results of the experiment determining the effects of *Echinacea purpura* water based extraction on *Escherichia coli*.
4. Results of the experiment determining the effects of *Echinacea purpura* in 62% ethanol (v/v) on *Escherichia coli*.
5. Results of the experiment determining the effects of 62%(v/v) ethanol on *Candida Albicans*.
6. Results of the experiment determining the effects of distilled water on *Candida albicans*.
7. Results of the experiment determining the effects of 62%(v/v) ethanol on *Escherichia coli*.

8. Results of the experiment determining the effects of distilled water on *Escherichia coli*.
9. Results of the experiment determining the effects of a 1:2 dilution of *Echinacea purpura* water based extraction on *Escherichia coli*.
10. Results of the experiment determining the effects of a 1:3 dilution of *Echinacea purpura* water based extraction on *Escherichia coli*.
11. Results of the experiment determining the effects of a 1:5 dilution of *Echinacea purpura* water based extraction on *Escherichia coli*.
12. Results of the experiment determining the effects of a 1:10 dilution of *Echinacea purpura* water based extraction on *Escherichia coli*.
13. Results of the experiment determining the effects of a 1:2 dilution of *Echinacea purpura* water based extraction on *Candida albicans*.
14. Results of the experiment determining the effects of a 1:3 dilution of *Echinacea purpura* water based extraction on *Candida albicans*.
15. Results of the experiment determining the effects of a 1:5 dilution of *Echinacea purpura* water based extraction on *Candida albicans*.
16. Results of the experiment determining the effects of a 1:10 dilution of *Echinacea purpura* water based extraction on *Candida albicans*.
17. Results of the experiment determining the effects of a 1:2 dilution of *Echinacea purpura* 62 % (v/v) ethanol on *Escherichia coli*.
18. Results of the experiment determining the effects of a 1:3 dilution of

- Echinacea purpurea* 62 % (v/v) ethanol on *Escherichia coli*.
19. Results of the experiment determining the effects of a 1:5 dilution of *Echinacea purpurea* 62 % (v/v) ethanol on *Escherichia coli*.
 20. Results of the experiment determining the effects of a 1:10 dilution of *Echinacea purpurea* 62 % (v/v) ethanol on *Escherichia coli*.
 21. Results of the experiment determining the effects of a 1:2 dilution of *Echinacea purpurea* 62 % (v/v) ethanol on *Candida albicans*.
 22. Results of the experiment determining the effects of a 1:3 dilution of *Echinacea purpurea* 62 % (v/v) ethanol on *Candida albicans*.
 23. Results of the experiment determining the effects of a 1:5 dilution of *Echinacea purpurea* 62 % (v/v) ethanol on *Candida albicans*.
 24. Results of the experiment determining the effects of a 1:10 dilution of *Echinacea purpurea* 62 % (v/v) ethanol on *Candida albicans*.

3.1.2 The Secondary Data

Research articles from journal publications, books and manuals.

3.2 Criteria governing admissibility of data.

Only data gathered from experiments conducted by the researcher at the Durban Institute of Technology: Steve Biko Campus, Department Of Biotechnology microbiology laboratory was used.

3.3 Materials and Methods

3.3.1 Preparation of the Water-Based Extraction of *Echinacea purpura*

Echinacea purpura was prepared by Parceval Pharmaceuticals (Pty) Ltd according to an adjusted method HAB 4a of the German Homoeopathic Pharmacopoeia:

- *Echinacea purpurea* herba (fresh plant parts above ground, with flower) harvested early in the morning – (8am.).
- Plant material immediately minced in electrical mincer and weighed into a glass jar.
- 3 parts distilled water added to 1 part minced plant material.
- The mix was shaken vigorously for 5 hours, pressed through 100% cotton cloth and filtered through No 1 Whatman filter paper (Feiter, U. 2003).

The *Echinacea purpura* water-based extraction was refrigerated throughout the entire course of this experiment.

3.3.2 Preparation of the Ethanol Tincture of *Echinacea purpura*

Echinacea purpura tincture (Batch no. 03RF70JM – expiry date: 11/2006), was prepared in 62% ethanol by Natura Homoeopathic Laboratories, according to method HAB 4a of the German Homoeopathic Pharmacopoeia. (Singh, V.2004)

3.3.3 Preparation of Distilled Water Control

Distilled water was obtained from the Homoeopathic Department Laboratory at Durban Institute of Technology.

3.3.4 Preparation of 62% (v/v) Ethanol Control

The 62% (v/v) ethanol was prepared according to the German Homeopathic Pharmacopoeia standards (British Homeopathic Association, 1985: 5): 65,90ml of 96% (v/v) ethanol was 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.

3.3.5 Blank Susceptibility Discs

Blank susceptibility disc were used and not the traditional Whatman filter paper discs no 4, as recommended by Invernizzi (2002). The discs were purchased from Mast Diagnostics (expiry date - 01/02/07- lot. no – 124986).

3.3.6 Preparation of the Media for *Escherichia coli*

The media was prepared according to the manufacturer's directions (Oxoid Manual, 1979) as follows:

1. 11.4g of Mueller-Hinton agar powder was weighed out and added to 300ml of distilled water in a screw top flask (to make 12 plates).
2. A magnetic stirrer was added to the bottom of the flask to aid the dissolving process.
3. The mixture was then autoclaved at 121°C for 15 minutes.
4. The flask was allowed to cool whilst placed on a magnetic stirring machine. This ensured adequate mixing and prevented the mixture from solidifying.
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 plates.
 - 5.2. Each plate was poured to a depth of +/- 4mm.

5.3. The plates were stacked and allowed to cool and solidify.

5.4. The plates were visually examined for contamination.

3.3.7 Preparation of the Media for *Candida albicans*

The media was prepared according to the manufacturer's directions (Oxoid Manual, 1979) as follows:

1. 19.5g of Sabouraud dextrose agar powder was weighed and added to 300ml 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.
3. The mixture was then autoclaved at 121°C for 15 minutes.
4. The flask was allowed to cool whilst placed on a magnetic stirring machine. This ensured adequate mixing and prevented the mixture from solidifying.
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 plates.
 - 5.2. Each plate was poured to a depth of +/- 4mm.
 - 5.3. The plates were stacked and allowed to cool and solidify.
 - 5.4. The plates were visually examined for contamination.

3.3.8 Preparation of Inoculum

3.3.8.1 *Escherichia coli*

1. A stock culture of *E. coli* was provided by the Department of Biotechnology, Durban Institute of Technology. A single colony was streaked onto a Mueller-Hinton agar plate and allowed to incubate for 24 hours at 37°C in an incubator.
2. A few individual colonies from the overnight cultures were suspended in 10ml of saline solution using sterile techniques.
3. The culture was swirled to allow even distribution of the culture.
4. 1ml of the liquid medium was aseptically pipetted into a test tube containing 9ml of saline solution.
5. The culture was then vortexed to enable adequate mixing.
5. The culture was made up to an organism suspension equivalent to McFarland 0.5 turbidity standard.

3.3.8.2 *Candida albicans*

1. Single colonies of *Candida albicans*, obtained from the Durban Institute of Technology Biotechnology Laboratory stock cultures, were used to inoculate a Sabouraud- dextrose agar plate and allowed to incubate for 24 hours at 37°C in an incubator.
2. A few individual colonies from the overnight culture were suspended in 10ml of Sabouraud- dextrose liquid medium using sterile techniques.

3. The culture was swirled to allow even distribution of the culture.
4. 1ml of the liquid medium was aseptically pipetted into a test tube containing 9ml of saline solution.
5. The culture was then vortexed to enable adequate mixing.
6. The culture was made up to an organism suspension equivalent to McFarland 0.5 turbidity standard.

3.3.9 Inoculation of plates

C. albicans

The inoculation of six plates with *C. albicans* was done using the following sterile techniques:

1. A sterile cotton swab was dipped into the well-mixed saline test culture prepared above and excess inoculum was removed by pressing the saturated swab against the inner wall of the culture tube.
2. Using the swab, the entire agar surface of the plate was streaked, first in a horizontal direction and then vertically to ensure a uniform growth over the entire surface.

E.coli

The inoculation of six plates with *E.coli* was done using the following sterile techniques:

1. A sterile cotton swab was dipped into the well-mixed saline test culture prepared above and excess inoculum was removed by pressing the saturated swab against the inner wall of the culture tube.

2. Using the swab, the entire agar surface of the plate was streaked, first in a horizontal direction and then vertically to ensure a uniform growth over the entire surface.

3.3.10 Impregnation and placement of disc on the inoculated media

The blank susceptibility discs were placed over the agar surface by means of sterile forceps. Each disc was gently pressed down to ensure that the discs adhere to the agar surface. A pre-measured amount of 10 microlitres of the test substances and the controls were pipetted onto separate discs using a micropipette.

3.3.11 Incubation of plates

Inoculated plates were incubated in an inverted position for 24 hours at 37°C. After incubation, the plates were examined for the presence of growth inhibition, which was indicated by a clear zone surrounding each disc. The susceptibility of an organism to a substance was determined by the size of the zone, which was measured using vernier calipers. Each test for both groups was conducted using three replicates to ensure consistency and provide statistically viable data.

3.3.12 Preparation of *Echinacea purpura* dilutions

Water based extraction

- **Preparation of *Echinacea purpura* 1:2 dilution**

1ml of *Echinacea purpura* water-based extraction was pipetted using a sterile micropipette to a 10-ml screw-top clear vial containing 1 ml of distilled water. The bottle lid was fastened and the bottle was gently agitated to mix the dilution evenly (Singh, S. 2003).

- **Preparation of *Echinacea purpura* 1:3 dilution**

1ml of *Echinacea purpura* water-based extraction was pipetted using a sterile micropipette to a 10-ml screw-top clear vial containing 2 ml of distilled water.

The bottle lid was fastened and the bottle was gently agitated to mix the dilution evenly (Singh, S. 2003).

- **Preparation of *Echinacea purpura* 1:5 dilution**

1ml of *Echinacea purpura* water based extraction was pipetted using a sterile micropipette to a 10-ml screw-top clear vial containing 4 ml of distilled water. The bottle lid was fastened and the bottle was gently agitated to mix the dilution evenly (Singh, S. 2003).

- **Preparation of *Echinacea purpura* 1:10 dilution**

1ml of *Echinacea purpura* water based extraction was pipetted using a sterile micropipette to a 10-ml screw-top clear vial containing 9ml of distilled water. The bottle lid was fastened and the bottle was gently agitated to mix the dilution evenly (Singh, S. 2003).

Ethanol tincture

- **Preparation of *Echinacea purpura* 1:2 dilution**

1ml of *Echinacea purpura* 62% (v/v) tincture was pipetted using a sterile micropipette to a 10-ml screw-top clear vial containing 1 ml of distilled water. The bottle lid was fastened and the bottle was gently agitated to mix the dilution evenly (Singh, S. 2003).

- **Preparation of *Echinacea purpura* 1:3 dilution**

1ml of *Echinacea purpura* 62%v/v tincture was pipetted using a sterile micropipette to a 10-ml screw-top clear vial containing 2 ml of distilled water. The bottle lid was fastened and the bottle was gently agitated to mix the dilution evenly (Singh, S. 2003).

- **Preparation of *Echinacea purpura* 1:5 dilution**

1ml of *Echinacea purpura* 62%v/v tincture was pipetted using a sterile micropipette to a 10-ml screw-top clear vial containing 4 ml of distilled water. The bottle lid was fastened and the bottle was gently agitated to mix the dilution evenly (Singh, S. 2003).

- **Preparation of *Echinacea purpura* 1:10 dilution**

1ml of *Echinacea purpura* 62%v/v tincture was pipetted using a sterile micropipette to a 10-ml screw-top clear vial containing 9 ml of distilled water. The bottle lid was fastened and the bottle was gently agitated to mix the dilution evenly (Singh, S. 2003).

3.3.13 Determination of Minimum Inhibitory Concentration

- 8 test tubes, each containing 2ml of *E. coli* liquid culture with an organism suspension equivalent to McFarland 0.5 turbidity standard were prepared.
- 8 test tubes, each containing 2ml of *C. albicans* liquid culture with an organism suspension equivalent to McFarland 0.5 turbidity standard were prepared.
- 2ml of *Echinacea purpura* water based extraction 1:2 dilution was pipetted into one of the *E. coli* liquid culture tubes.
- 2ml of *Echinacea purpura* water based extraction 1:3 dilution was pipetted into one of the *E. coli* liquid culture tubes.
- 2ml of *Echinacea purpura* water based extraction 1:5 dilution was pipetted into one of the *E. coli* liquid culture tubes.

- 2ml of *Echinacea purpurea* water based extraction 1:10 dilution was pipetted into one of the *E. coli* liquid culture tubes.
- 2ml of *Echinacea purpurea* ethanol tincture 1:2 dilution was pipetted into one of the *E. coli* liquid culture tubes.
- 2ml of *Echinacea purpurea* ethanol tincture 1:3 dilution was pipetted into one of the *E. coli* liquid culture tubes.
- 2ml of *Echinacea purpurea* ethanol tincture 1:5 dilution was pipetted into one of the *E. coli* liquid culture tubes.
- 2ml of *Echinacea purpurea* ethanol tincture 1:10 dilution was pipetted into one of the *E. coli* liquid culture tubes.
- 2ml of *Echinacea purpurea* water based extraction 1:2 dilution was pipetted into one of the *C. albicans* liquid culture tubes.
- 2ml of *Echinacea purpurea* water based extraction 1:3 dilution was pipetted into one of the *C. albicans* liquid culture tubes.
- 2ml of *Echinacea purpurea* water based extraction 1:5 dilution was pipetted into one of the *C. albicans* liquid culture tubes.
- 2ml of *Echinacea purpurea* water based extraction 1:10 dilution was pipetted into one of the *C. albicans* liquid culture tubes.
- 2ml of *Echinacea purpurea* ethanol tincture 1:2 dilution was pipetted into one of the *C. albicans* liquid culture tubes.

- 2ml of *Echinacea purpurea* ethanol tincture 1:3 dilution was pipetted into one of the *C. albicans* liquid culture tubes.
- 2ml of *Echinacea purpurea* ethanol tincture 1:5 dilution was pipetted into one of the *C. albicans* liquid culture tubes.
- 2ml of *Echinacea purpurea* ethanol tincture 1:10 dilution was pipetted into one of the *C. albicans* liquid culture tubes.
- All 16 tubes were incubated for 24 hours at 37°C.
- The tubes were inspected for growth inhibition using McFarlands 0.5 turbidity standard as a comparison.

3.3.14 Procedure for Test for Contamination

1. An SDA and an MHA plate were obtained from the Durban Institute of Technology Department of Microbiology Laboratory
2. Each plate was inoculated with the water based extraction of *Echinacea purpurea* in the following manner:
 - A sterile cotton swab was dipped into the *Echinacea purpurea* water based extraction and excess inoculum was removed by pressing the saturated swab against the inner wall of the culture tube.
 - Using the swab, the entire agar surface of the plate was streaked, first in a horizontal direction and then vertically to ensure a uniform growth over the entire surface.
3. The plates were incubated in an inverted position for 24 hours at 37 °C

4. The plates were visually examined for the presence or absence of growth.
5. The colonies that were present on each plate were fixed onto slides by the following method:
 - A loopful of organisms was mixed in a drop of water that was placed on a slide.
 - The mixture was smeared to form a thin layer of organisms of between 15-20mm in diameter.
 - The smear was left to air-dry.
 - The slide was rapidly passed through a flame with the smear side up for purposes of heat fixation.
 - The validity of this process was confirmed by placing the slide on the back of the hand, thereby checking that the slide was not overheated and damage caused to the organisms.
6. The slides were then subjected to the Gram staining method as follows.
 - The slides were flooded completely with crystal violet for 60 seconds.
 - The crystal violet was washed off with clean water.
 - Excess water was tipped off the slide, which was thereafter completely flooded with iodine.
 - The iodine was washed off with clean water.
 - The slide was decolourised with acetone-alcohol for a few seconds and washed off promptly with clean water.
 - The slide was thereafter flooded with the neutral safranin for 1 minute.
 - The safranin was washed off with clean water.
 - The excess water was tipped off and the slides were left on a draining rack to air-dry.

7. The slides were examined microscopically, first on 45x magnification, and then 100x oil immersion lens to look for bacteria and cells (Cheesbrough, M. 1984: 31).

3.3.15 Drop Test Procedure

1. Two SDA and two MHA plates were obtained from the Durban Institute of Technology Department of Biotechnology microbiology laboratory.
2. The SDA plates was inoculated with *C. albicans*, and the MHA plates were inoculated with *E. coli* as follows:
 - A sterile cotton swab was dipped into the well-mixed saline test culture prepared above and excess inoculum was removed by pressing the saturated swab against the inner wall of the culture tube.
 - Using the swab, the entire agar surface of the plate was streaked, first in a horizontal direction and then vertically to ensure a uniform growth over the entire surface.
3. Whatman® filter paper number 4 was used. The filter paper was punched out into discs of 5mm in diameter. These discs were placed in a jar and autoclaved at 121°C for 15 minutes to ensure sterilization.
4. Each of the four plates was divided in halves with a permanent marker. Each half was labeled as either experiment or control.
5. Two Whatman® filter paper number 4 discs where placed on one of each of the SDA and MHA plates, one disc on the experiment side, and one on the control side by means of a sterile forceps. Ten µl of *Echinacea purpura* 62% (v/v) ethanol tincture was pipetted onto the experimental filter paper

- discs. Ten μl of 62% (v/v) ethanol was pipetted onto the control filter paper discs.
6. Ten μl of *Echinacea purpurea* 62% (v/v) ethanol tincture was pipetted directly onto the experimental sides of the remaining MHA and SDA plates. Ten microlitres of 62% (v/v) ethanol was pipetted onto the control sides of the remaining MHA and SDA plates.
 7. The plates were incubated in an inverted position for 24 hours at 37°C.
 8. The plates were visually examined for growth inhibition.

3.3.16 Statistical Procedures

Procedure 1.1

Intergroup comparison between *Echinacea purpurea* water based extraction and distilled water on *E. coli*.

The Mann-Whitney Test was used to compare *Echinacea purpurea* water based extraction and distilled water on *E. coli*.

•Hypothesis testing

The null hypothesis H_0 , states that there is no difference in diameter of the zone of inhibition between *Echinacea purpurea* water based extraction and distilled water on *E. coli* / *C. albicans* with respect to the variable 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).

Procedure 1.2

Intergroup comparison between *Echinacea purpura* water based extraction and distilled water on *C. albicans*.

The Mann-Whitney Test was used to compare *Echinacea purpura* water based extraction and distilled water on *C. albicans*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 1.3

Intergroup comparison between *Echinacea purpura* water based extraction and *Echinacea purpura* 62% (v/v) ethanol tincture on *E. coli*.

The Mann-Whitney Test was used to compare *Echinacea purpura* water based extraction and *Echinacea purpura* 62% (v/v) ethanol tincture on *E. coli*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 1.4

Intergroup comparison between *Echinacea purpura* water based extraction and *Echinacea purpura* 62% (v/v) ethanol tincture on *C. albicans*.

The Mann-Whitney Test was used to compare *Echinacea purpura* water based extraction and *Echinacea purpura* 62% (v/v) ethanol tincture on *C. albicans*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 1.5

Intergroup comparison between *Echinacea purpura* 62% (v/v) ethanol tincture and 62% (v/v) ethanol on *E. coli*.

The Mann-Whitney Test was used to compare *Echinacea purpura* 62% (v/v) ethanol tincture and 62% (v/v) ethanol on *E. coli*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 1.6

Intergroup comparison between *Echinacea purpura* 62% (v/v) ethanol tincture and 62% (v/v) ethanol on *C. albicans*.

The Mann-Whitney Test was used to compare *Echinacea purpura* 62% (v/v) ethanol tincture and 62% (v/v) ethanol on *C. albicans*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 2.1

Intergroup comparison between 1:2 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:2 dilution of *Echinacea purpurea* water based extraction on *E. coli*.

The Mann-Whitney Test was used to compare the 1:2 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:2 dilution of *Echinacea purpurea* water based extraction on *E. coli*.

•Hypothesis testing

The null hypothesis H_0 , states that there is no difference in turbidity between *Echinacea purpurea* water based extraction and distilled water on *E. coli* / *C. albicans* with respect to the variable 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).

Procedure 2.2

Intergroup comparison between 1:2 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:2 dilution of *Echinacea purpura* water based extraction on *C. albicans*.

The Mann-Whitney Test was used to compare the 1:2 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:2 dilution of *Echinacea purpura* water based extraction on *C. albicans*.

- Hypothesis testing

As per procedure 2.1

- Decision rule

As per procedure 2.1

Procedure 2.3

Intergroup comparison between 1:3 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:3 dilution of *Echinacea purpura* water based extraction on *E. coli*.

The Mann-Whitney Test was used to compare the 1:3 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:3 dilution of *Echinacea purpura* water based extraction on *E. coli*.

- Hypothesis testing

As per procedure 2.1

- Decision rule

As per procedure 2.1

Procedure 2.4

Intergroup comparison between 1:3 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:3 dilution of *Echinacea purpura* water based extraction on *C. albicans*.

The Mann-Whitney Test was used to compare the 1:3 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:3 dilution of *Echinacea purpura* water based extraction on *C. albicans*.

- Hypothesis testing

As per procedure 2.1

- Decision rule

As per procedure 2.1

Procedure 2.5

Intergroup comparison between 1:5 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:5 dilution of *Echinacea purpura* water based extraction on *E. coli*.

The Mann-Whitney Test was used to compare the 1:5 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:5 dilution of *Echinacea purpura* water based extraction on *E. coli*.

- Hypothesis testing

As per procedure 2.1

- Decision rule

As per procedure 2.1

Procedure 2.6

Intergroup comparison between 1:5 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:5 dilution of *Echinacea purpura* water based extraction on *C. albicans*.

The Mann-Whitney Test was used to compare the 1:5 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:5 dilution of *Echinacea purpura* water based extraction on *C. albicans*.

- Hypothesis testing

As per procedure 2.1

- Decision rule

As per procedure 2.1

Procedure 2.7

Intergroup comparison between 1:10 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:10 dilution of *Echinacea purpura* water based extraction on *E. coli*.

The Mann-Whitney Test was used to compare the 1:10 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:10 dilution of *Echinacea purpura* water based extraction on *E. coli*.

- Hypothesis testing

As per procedure 2.1

- Decision rule

As per procedure 2.1

Procedure 2.8

Intergroup comparison between 1:10 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:10 dilution of *Echinacea purpura* water based extraction on *C. albicans*.

The Mann-Whitney Test was used to compare the 1:10 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:10 dilution of *Echinacea purpura* water based extraction on *C. albicans*.

- Hypothesis testing

As per procedure 2.1

- Decision rule

As per procedure 2.1

Procedure 3.1

Intergroup comparison between *Echinacea purpura* 62% (v/v) ethanol tincture disc and 62% (v/v) ethanol disc on *E. coli*.

The Mann-Whitney Test was used to compare *Echinacea purpura* 62% (v/v) ethanol tincture disc and 62% (v/v) ethanol disc on *E. coli*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 3.2

Intergroup comparison between *Echinacea purpurea* 62% (v/v) ethanol tincture disc and 62% (v/v) ethanol disc on *C. albicans*.

The Mann-Whitney Test was used to compare *Echinacea purpurea* 62% (v/v) ethanol tincture disc and 62% (v/v) ethanol disc on *C. albicans*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 3.3

Intergroup comparison between *Echinacea purpurea* 62% (v/v) ethanol tincture disc and *Echinacea purpurea* 62% (v/v) ethanol tincture drop on *E. coli*.

The Mann-Whitney Test was used to compare *Echinacea purpurea* 62% (v/v) ethanol tincture disc and *Echinacea purpurea* 62% (v/v) ethanol tincture drop on *E. coli*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 3.4

Intergroup comparison between *Echinacea purpurea* 62% (v/v) ethanol tincture disc and *Echinacea purpurea* 62% (v/v) ethanol tincture drop on *C. albicans*.

The Mann-Whitney Test was used to compare *Echinacea purpurea* 62% (v/v) ethanol tincture disc and *Echinacea purpurea* 62% (v/v) ethanol tincture drop on *C. albicans*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 3.5

Intergroup comparison between 62% (v/v) ethanol disc and 62% (v/v) ethanol drop on *E. coli*.

The Mann-Whitney Test was used to compare 62% (v/v) ethanol disc and 62% (v/v) ethanol drop on *E. coli*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 3.6

Intergroup comparison between 62% (v/v) ethanol disc and 62% (v/v) ethanol drop on *C. albicans*.

The Mann-Whitney Test was used to compare 62% (v/v) ethanol disc and 62% (v/v) ethanol drop on *C. albicans*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 3.7

Intergroup comparison between *Echinacea purpura* 62% (v/v) ethanol tincture drop and 62% (v/v) ethanol drop on *E. coli*.

The Mann-Whitney Test was used to compare *Echinacea purpura* 62% (v/v) ethanol tincture drop and 62% (v/v) ethanol drop on *E. coli*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

Procedure 3.8

Intergroup comparison between *Echinacea purpura* 62% (v/v) ethanol tincture drop and 62% (v/v) ethanol drop on *C. albicans*.

The Mann-Whitney Test was used to compare *Echinacea purpura* 62% (v/v) ethanol tincture drop and 62% (v/v) ethanol drop on *C. albicans*.

- Hypothesis testing

As per procedure 1.1

- Decision rule

As per procedure 1.1

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, Durban Institute of Technology was used.

4.2 Statistical Analysis of Results

4.2.1 Procedure 1.1 - 1.2

See Table 4.1

Table 4.1 Intergroup comparison between *Echinacea purpura* water based extraction and distilled water on *E. coli* and *C. albicans* (Mann-Whitney Test).

	1.1 <i>E. coli</i>	1.2 <i>C. albicans</i>
P-Value	1.000	1.000

- Interpretation of results for Procedure 1.1

P = 1.000

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between *Echinacea purpura* water based extraction and distilled water on *E. coli*.

- Interpretation of results for Procedure 1.2

P = 1.000

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between *Echinacea purpura* water based extraction and distilled water on *C. albicans*.

4.2.2 Procedure 1.3 - 1.4

See Table 4.2

Table 4.2 Intergroup comparison between *Echinacea purpura* water based extraction and *Echinacea purpura* 62% (v/v) ethanol tincture on *E. coli* and *C. albicans*.

	1.3 <i>E. coli</i>	1.4 <i>C. albicans</i>
P-Value	1.000	1.000

- Interpretation of results for Procedure 1.3

P = 1.000

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between *Echinacea purpurea* water based extraction and *Echinacea purpurea* 62% (v/v) ethanol tincture on *E. coli*.

- Interpretation of results for Procedure 1.4

P = 1.000

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between *Echinacea purpurea* water based extraction and *Echinacea purpurea* 62% (v/v) ethanol tincture on *C. albicans*.

4.2.3 Procedure 1.5 – 1.6

See Table 4.3

Table 4.3 Intergroup comparison between *Echinacea purpurea* 62% (v/v) ethanol tincture and 62% (v/v) ethanol on *E. coli* and *C. albicans*.

	1.5 <i>E. coli</i>	1.6 <i>C. albicans</i>
P-Value	0.317	0.317

- Interpretation of results for Procedure 1.5

P = 0.317

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between *Echinacea purpurea* 62% (v/v) ethanol tincture and 62% (v/v) ethanol on *E. coli*.

- Interpretation of results for Procedure 1.6

P = 0.317

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between *Echinacea purpurea* 62% (v/v) ethanol tincture and 62% (v/v) ethanol on *C. albicans*.

4.2.4 Procedure 2.1 – 2.2

See Table 4.4

Table 4.4 Intergroup comparison between the 1:2 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:2 dilution of *Echinacea purpurea* water based extraction on *E. coli* and *C. albicans*.

	2.1 <i>E. coli</i>	2.2 <i>C. albicans</i>
P-Value	0.317	0.317

- Interpretation of results for Procedure 2.1

P = 0.317

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in turbidity between the 1:2 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:2 dilution of *Echinacea purpurea* water based extraction on *E. coli*.

- Interpretation of results for Procedure 2.2

P = 0.317

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in turbidity between the 1:2 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:2 dilution of *Echinacea purpurea* water based extraction on *C. albicans*.

4.2.5 Procedure 2.3 – 2.4

See Table 4.5

Table 4.5 Intergroup comparison between the 1:3 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:3 dilution of *Echinacea purpurea* water based extraction on *E. coli* and *C. albicans*.

	2.3 <i>E. coli</i>	2.4 <i>C. albicans</i>
P-Value	0.317	0.317

. • Interpretation of results for Procedure 2.3

$$P = 0.317$$

$$\alpha = 0.05$$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in turbidity between the 1:3 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:3 dilution of *Echinacea purpurea* water based extraction on *E. coli*.

. • Interpretation of results for Procedure 2.4

$$P = 0.317$$

$$\alpha = 0.05$$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in turbidity between the 1:3 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:3 dilution of *Echinacea purpurea* water based extraction on *C. albicans*.

4.2.6 Procedure 2.5 – 2.6

See Table 4.6

Table 4.6 Intergroup comparison between the 1:5 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:5 dilution of *Echinacea purpurea* water based extraction on *E. coli* and *C. albicans*.

	2.5 <i>E. coli</i>	2.6 <i>C. albicans</i>
P-Value	0.317	0.317

- Interpretation of results for Procedure 2.5

P = 0.317

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in turbidity between the 1:5 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:5 dilution of *Echinacea purpurea* water based extraction on *E. coli*.

- Interpretation of results for Procedure 2.6

P = 0.317

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in turbidity between the 1:5 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:5 dilution of *Echinacea purpurea* water based extraction on *C. albicans*.

4.2.7 Procedure 2.7 – 2.8

See Table 4.7

Table 4.7 Intergroup comparison between the 1:10 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:10 dilution of *Echinacea purpurea* water based extraction on *E. coli* and *C. albicans*.

	2.7 <i>E. coli</i>	2.8 <i>C. albicans</i>
P-Value	0.317	0.317

- Interpretation of results for Procedure 2.7

P = 0.317

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in turbidity between the 1:10 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:10 dilution of *Echinacea purpurea* water based extraction on *E. coli*.

- Interpretation of results for Procedure 2.8

$P = 0.317$

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in turbidity between the 1:10 dilution of *Echinacea purpurea* 62% (v/v) ethanol tincture and 1:10 dilution of *Echinacea purpurea* water based extraction on *C. albicans*.

4.2.8 Procedure 3.1 – 3.2

See Table 4.8

Table 4.8 Intergroup comparison between *Echinacea purpurea* 62% (v/v) ethanol tincture disc and 62% (v/v) ethanol disc on *E. coli* and *C. albicans*.

	3.1 <i>E. coli</i>	3.2 <i>C. albicans</i>
P-Value	0.317	0.317

- Interpretation of results for Procedure 3.1

$P = 0.317$

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between the *Echinacea purpurea* 62% (v/v) ethanol tincture disc and 62% (v/v) ethanol disc on *E. coli*.

- Interpretation of results for Procedure 3.2

$P = 0.317$

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between the *Echinacea purpurea* 62% (v/v) ethanol tincture disc and 62% (v/v) ethanol disc on *C. albicans*.

4.2.9 Procedure 3.3 – 3.4

See Table 4.9

Table 4.9 Intergroup comparison between *Echinacea purpurea* 62% (v/v) ethanol tincture disc and *Echinacea purpurea* 62% (v/v) ethanol tincture drop on *E. coli* and *C. albicans*.

	3.3 <i>E. coli</i>	3.4 <i>C. albicans</i>
P-Value	0.317	1.000

- Interpretation of results for Procedure 3.3

$P = 0.317$

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between the *Echinacea purpurea* 62% (v/v) ethanol tincture disc and *Echinacea purpurea* 62% (v/v) ethanol tincture drop on *E. coli*.

- Interpretation of results for Procedure 3.4

$P = 1.000$

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between the *Echinacea purpurea* 62% (v/v) ethanol tincture disc and *Echinacea purpurea* 62% (v/v) ethanol tincture drop on *C. albicans*.

4.2.10 Procedure 3.5 – 3.6

See Table 4.10

Table 4.10 Intergroup comparison between 62% (v/v) ethanol disc and 62% (v/v) ethanol drop on *E. coli* and *C. albicans*.

	3.5 <i>E. coli</i>	3.6 <i>C. albicans</i>
P-Value	0.317	0.317

- Interpretation of results for Procedure 3.5

P = 0.317

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between 62% (v/v) ethanol disc and 62% (v/v) ethanol drop on *E. coli*.

- Interpretation of results for Procedure 3.6

P = 0.317

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between 62% (v/v) ethanol disc and 62% (v/v) ethanol drop on *C. albicans*.

4.2.11 Procedure 3.7 – 3.8

See Table 4.11

Table 4.11 Intergroup comparison between *Echinacea purpura* 62% (v/v) ethanol tincture drop and 62% (v/v) ethanol drop on *E. coli* and *C. albicans*.

	3.7 <i>E. coli</i>	3.8 <i>C. albicans</i>
P-Value	0.317	1.000

- Interpretation of results for Procedure 3.7

P = 0.317

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between *Echinacea purpurea* 62% (v/v) ethanol tincture drop and 62% (v/v) ethanol drop on *E. coli*.

- Interpretation of results for Procedure 3.8

$P = 1.000$

$\alpha = 0.05$

The null hypothesis is accepted since $P \geq \alpha$. Thus, there is no significant difference in diameter of the zones of inhibition between *Echinacea purpurea* 62% (v/v) ethanol tincture drop and 62% (v/v) ethanol drop on *C. albicans*.

CHAPTER FIVE

DISCUSSION OF RESULTS

5.1 Subproblem One

The results indicate that the *E. purpura* water based extraction used in this study had no effect on the *in vitro* growth of *E. coli* and *C. albicans* in terms of the sizes of the zones of inhibition in the disc diffusion assay. No growth inhibition was exhibited, resulting in a p-value of 1.000 when compared to the negative control using the Mann-Whitney U test.

Statistically, none of the dilutions of the *E. purpura* water based extraction exhibited significant growth inhibition of *E. coli* and *C. albicans* in terms of turbidity in the tube dilution tests. P-values of .317 were obtained across all dilution ranges when compared to the corresponding dilutions of *E. purpura* 62% (v/v) ethanol tincture using the Mann-Whitney U test.

Visually, however, the dilutions of the *E. purpura* water based extraction did exhibit a trend towards growth inhibition of *E. coli* and *C. albicans*. The growth inhibition was paradoxical in that the more the test substance was diluted the greater was its apparent antimicrobial and anti-candidal effects. This was subsequently discovered to have been due to contamination of the original test substance.

The failure of the statistical results to correspond with the visual results is due to the sample size not being large enough to produce statistically significant results (see recommendations).

5.2 Subproblem Two

The results indicate that the *E. purpura* 62% (v/v) ethanol tincture used in this study had no effect on the *in vitro* growth of *E. coli* and *C. albicans* in terms of the sizes of the zones of inhibition in the disc diffusion assay. No growth inhibition was exhibited, resulting in a p-value of .317 when compared to the negative control using the Mann-Whitney U test.

Statistically, none of the dilutions of the *E. purpura* 62% (v/v) ethanol tincture exhibited significant growth inhibition of *E. coli* and *C. albicans* in terms of turbidity in the tube dilution tests. P-values of .317 were obtained across all dilution ranges when compared to the corresponding dilutions of the *E. purpura* water based extraction using the Mann-Whitney U test.

Visually, however, all the dilutions of the *E. purpura* 62% (v/v) ethanol tincture did produce complete growth inhibition of *E. coli* and *C. albicans*. The failure of the statistical results to correspond with the visual results is due to the sample size not being large enough to produce statistically significant results (see recommendations).

5.3 Subproblem Three

The results indicate that there is no statistically significant difference between the *E. purpura* 62% (v/v) ethanol tincture and the *E. purpura* water based extraction used in this study in terms of the sizes of the zones of inhibition in the disc diffusion assay. A p-value of 1.000 was obtained when the *E. purpura* 62% (v/v) ethanol tincture was compared to the *E. purpura* water based extraction using the Mann-Whitney U test.

The results indicate that there is no statistically significant difference between the corresponding dilutions of the *E. purpura* 62% (v/v) ethanol tincture and the *E. purpura* water based extraction. P-values of .317 were obtained across all

5.6 General Discussion

From the results obtained, the *Echinacea purpurea* water-based extraction did not produce significant zones of inhibition on either *C. albicans* or *E. coli*, neither did the distilled water control. The *Echinacea purpurea* 62% (v/v) ethanol-based tincture did not produce significant zones of inhibition on either *C. albicans* or *E. coli*, while the 62% ethanol control did produce significant zones of inhibition on *C. albicans* and to a greater extent, *E. coli*. It should be noted that zones that contained even single, minute colonies were counted as 0.

Since no previous *in vitro* antimicrobial studies have been done on water-based extractions at The Durban Institute of Technology, no comparisons can be made with the results obtained from this study.

The results obtained from the tests on the *Echinacea purpurea* 62% (v/v) ethanol-based tincture is, however open to comparison. The results are in contrast to previous studies (Vosloo, 2002, Reid 2002, and Budhree, 2003) using 62% herbal tinctures. It was hypothesised that this discrepancy could have been due to previous studies using Whatman® filter paper number 4 discs as opposed to the blank antibiotic discs used in this study. This hypothesis was supported by the results of the drop vs. disc test, which showed there to be no significant difference between the Whatman® filter paper number 4 discs and the 10 microlitre drops of the test substance.

The results of the tube dilution tests on the *Echinacea purpurea* 62% (v/v) ethanol-based tincture indicated complete inhibition of bacterial and candidal growth across all dilution ranges.

The results of the tube dilution tests on the *Echinacea purpurea* water-based extraction were paradoxical in that the more the test substance was diluted the greater was its apparent antimicrobial and anti-candidal effects. It was hypothesised that this could have been due to contamination of the original test

substance. The results of the contamination test confirmed that there was indeed contamination of the test substance. This contamination could also have had an effect on the results of the disc diffusion tests.

Another variable that could have had an effect on the results of this study is that the sample size was not large enough to produce statistically significant results. The amount of 10 μl that was used to impregnate the blank susceptibility discs may also have been too small.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The purpose of this study was to compare the effect of a water-based extraction of *Echinacea purpurea* (*E. purpurea*) on the *in vitro* growth of *Candida albicans* (*C. albicans*) and *Escherichia coli* (*E. coli*) to a control of distilled water in terms of the size of the zones of inhibition and turbidity. It also sought to compare the results to those obtained from experiments using ethanol-based tinctures of *Echinacea purpurea*.

The *Echinacea purpurea* water-based extraction proved to be ineffective in inhibiting the *in vitro* growth of *Candida albicans* and *Escherichia coli* in terms of the size of the zones of inhibition.

The *Echinacea purpurea* 62% ethanol-based tincture proved to be ineffective in inhibiting the *in vitro* growth of *Candida albicans* and *Escherichia coli* in terms of the size of the zones of inhibition.

There was no significant statistical difference between the *Echinacea purpurea* water-based extraction and the *Echinacea purpurea* 62% ethanol-based tincture in inhibiting the *in vitro* growth of *E. coli* and *C. albicans* water in terms of the size of the zones of inhibition and turbidity.

6.2 Recommendations

1. This study should be repeated using a larger sample size in order to validate the results found.
2. This study should be repeated with observations being made at 18, 24 and 36 hours.
3. The study should be repeated using water-based extractions that have been sterilized by gamma radiation.
4. The study should be repeated using water-based extractions produced by the Wala method.
5. The study should be repeated using herbs in dried or powdered form.
6. Studies should be done on decoctions and infusions.
7. Studies should be done on alternate means of herbal extractions i.e. vinegar, glycerine, and fat extractions.
8. Studies should be done on gemmotherapeutics.
9. A study should be done whereby the manufacture of the herbal product is carefully monitored from harvesting to the final product. This will ensure greater control over factors like contamination.

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LIST OF APPENDICES

APPENDIX A

Pictures

APPENDIX B

Raw data

APPENDIX C

SPSS Tables

APPENDIX A

Pictures



PURPLE CONEFLOWER



TUBE DILUTIONS (ROH TINCTURE)

↓ ↓ ↓ ↓ ↓

CONTROL 1:2 1:3 1:5 1:10

TUBE DILUTIONS (H2O EXTRACTS)

↓ ↓ ↓ ↓ ↓

CONTROL 1:2 1:3 1:5 1:10

APPENDIX B

Raw data

TUBE DILUTIONS GROUP A (H2O)

E COLI

1:2 - 3+

1:3 - 3+

1:5 - 2+

1:10 - 1+

TUBE DILUTIONS GROUP B (ROH)

E COLI

1:2 - 0

1:3 - 0

1:5 - 0

1:10 - 0

CANDIDA ALBICANS

1:2 - 3+

1:3 - 3+

1:5 - 2+

1:10 - 1+

CANDIDA ALBICANS

1:2 - 0

1:3 - 0

1:5 - 0

1:10 - 0

PLATES GROUP A (H2O)

E COLI

TEST

CONTROL

P1

0

0

P2

0

0

P3

0

0

PLATES GROUP B (ROH)

E COLI

TEST

CONTROL

P1

0

8,5mm

P2

0

9mm

P3

0

7,5mm

CANDIDA ALBICANS (H2O)

P1

0

0

P2

0

0

P3

0

0

CANDIDA ALBICANS (ROH)

P1

0

8mm

P2

0

0

P3

0

0

0.5 MACFALLANS = 3+ GROWTH

DISC SIZE 7mm

VISUAL TURBIDITY

0 NO GROWTH
1+ SCANTY
2+ MODEST
3+ HEAVY
4+ VERY HEAVY

Disc and Drop Tests

C. ALBICANS

DISC TEST

Experiment	0.0mm
Control 1.	8.5mm
Control 2.	8.5mm

DROP TEST

Experiment	0.0mm
Control 1.	0.0mm
Control 2.	0.0mm

E. COLI

DISC TEST

Experiment	7.5mm
Control 1.	10mm
Control 2.	8mm

DROP TEST

Experiment	0.0mm (initial = 8)
Control 1.	0.0mm (initial = 11)
Control 2.	0.0mm (initial =10)

APPENDIX C

SPSS tables

Intergroup comparison between *Echinacea purpura* water based extraction and distilled water on *E. coli* and *C. albicans* (Mann-Whitney Test).

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.500	.500
Wilcoxon W	1.500	1.500
Z	.000	.000
Asymp. Sig (2-tailed)	1.000	1.000
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

Intergroup comparison between *Echinacea purpura* water based extraction and *Echinacea purpura* 62% (v/v) ethanol tincture on *E. coli* and *C. albicans*.

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.500	.500
Wilcoxon W	1.500	1.500
Z	.000	.000
Asymp. Sig (2-tailed)	1.000	1.000
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

Intergroup comparison between *Echinacea purpura* 62% (v/v) ethanol tincture and 62% (v/v) ethanol on *E. coli* and *C. albicans*.

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.000	.000
Wilcoxon W	1.000	1.000
Z	-1.000	-1.000
Asymp. Sig (2-tailed)	.317	.317
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

Intergroup comparison between the 1:2 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:2 dilution of *Echinacea purpura* water based extraction on *E. coli* and *C. albicans*.

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.000	.000
Wilcoxon W	1.000	1.000
Z	-1.000	-1.000
Asymp. Sig (2-tailed)	.317	.317
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

Intergroup comparison between the 1:3 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:3 dilution of *Echinacea purpura* water based extraction on *E. coli* and *C. albicans*.

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.000	.000
Wilcoxon W	1.000	1.000
Z	-1.000	-1.000
Asymp. Sig (2-tailed)	.317	.317
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

Intergroup comparison between the 1:5 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:5 dilution of *Echinacea purpura* water based extraction on *E. coli* and *C. albicans*.

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.000	.000
Wilcoxon W	1.000	1.000
Z	-1.000	-1.000
Asymp. Sig (2-tailed)	.317	.317
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

Intergroup comparison between the 1:10 dilution of *Echinacea purpura* 62% (v/v) ethanol tincture and 1:10 dilution of *Echinacea purpura* water based extraction on *E. coli* and *C. albicans*.

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.000	.000
Wilcoxon W	1.000	1.000
Z	-1.000	-1.000
Asymp. Sig (2-tailed)	.317	.317
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

Intergroup comparison between *Echinacea purpura* 62% (v/v) ethanol tincture disc and 62% (v/v) ethanol disc on *E. coli* and *C. albicans*.

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.000	.000
Wilcoxon W	1.000	1.000
Z	-1.000	-1.000
Asymp. Sig (2-tailed)	.317	.317
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

Intergroup comparison between *Echinacea purpura* 62% (v/v) ethanol tincture disc and *Echinacea purpura* 62% (v/v) ethanol tincture drop on *E. coli* and *C. albicans*.

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.000	.000
Wilcoxon W	1.000	1.000
Z	-1.000	.000
Asymp. Sig (2-tailed)	.317	1.000
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

Intergroup comparison between 62% (v/v) ethanol disc and 62% (v/v) ethanol drop on *E. coli* and *C. albicans*.

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.000	.000
Wilcoxon W	1.000	1.000
Z	-1.000	.000
Asymp. Sig (2-tailed)	.317	.317
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

Intergroup comparison between *Echinacea purpura* 62% (v/v) ethanol tincture drop and 62% (v/v) ethanol drop on *E. coli* and *C. albicans*.

Test Statistics^b

	E.COLI	C.ALBICANS
Mann Whitney U	.000	.500
Wilcoxon W	1.000	1.500
Z	-1.000	.000
Asymp. Sig (2-tailed)	.317	1.000
Exact Sig. [2*(1-tailed Sig)]	1.000^a	1.000^a

- a. Not corrected for ties.
- b. Grouping Variable: Test substance

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