THE EFFICACY OF *THYMUS VULGARIS* TINCTURE AS AN ANTIBACTERIAL AGENT

By

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A dissertation in partial compliance with the requirements for a
Master's Degree in Technology : Homoeopathy
at Technikon Natal

I, Chiquita Vosloo do hereby declare that this dissertation represents my own work in both conception and execution.

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28-01-2002

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DEDICATION

This dissertation is dedicated to my parents, for their endless love and support. And to Mrs Rhona Duncan, who planted the seed.

ACKNOWLEDGMENTS

The author would like to thank the following people for their invaluable advice and assistance.

Dr Richard Steele	Supervisor
Mrs Danile Macdonald	Co-supervisor.
Mrs Rena Brijlal	Laboratory Technician
Mr Kavanal Thomas	Statistician
Mr Ron Gauldie	
Dr David Naude	
Mrs Samantha Langford	
Ms Shavashni Ramlachan	
Ms Kim Reid	
Ms Claire Stubbs	

ABSTRACT

The aim of this study was to establish the effect of *Thymus vulgaris* tincture prepared in 43% ethanol and 70% ethanol in comparison to 43% ethanol and 70% ethanol only upon *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Escherichia coli* and *Enterococcus faecalis,* using a disc diffusion method, and to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Thymus vulgaris* tincture in respect of these bacteria.

Mueller-Hinton agar plates were streaked with saline test cultures adjusted to the 0.5 McFarland Equivalence Turbidity Standard. Five sterile filter paper discs 5mm in diameter were placed on each plate. These filter paper discs were impregnated with 10µl of the test or control substances using a micropipette. The plates were incubated at 37°C for 18 hours.

The diameters of the zones of inhibition of the bacterial growth around the discs were measured. This data was used to look for inter-group change by means of the Mann-Whitney Test between the test and control subgroups in both group A and group B. The means and standard deviations of each of the groups were compared in order to look for possible trends if the p-value of each group was insignificant.

Thymus vulgaris tincture in 43% ethanol produced significant inhibitory effects in comparison to the 43% ethanol control upon all the bacteria tested. *Thymus vulgaris* tincture in 70% ethanol produced significant inhibitory effects in comparison to the

70% control upon all the bacteria tested. It was found that the ethanol alone had very little or no appreciable inhibitory effect on the six types of bacteria tested. This could be due to rapid evaporation of the ethanol, which resulted in insufficient time to cause inhibition of the bacteria streaked on the plate. That the test substances, tinctures in ethanol, caused significant inhibition of the bacteria even after this supposed evaporation of their ethanol component, leads to the conclusion that it was the *Thymus vulgaris* extract portion of the tincture that exhibited this inhibitory effect.

Due to the prohibitive cost of sterile horse serum the MIC and subsequent MBC tests were only conducted once. Therefore, the results are tentative. It was found that the MIC for the bacteria tested was between 5% and 2.5% v/v for test group A (*Thymus vulgaris* tincture in 43% ethanol) and 5% v/v or above for control group A (ethanol 43%). For test group B (*Thymus vulgaris* tincture in 70% ethanol), the MIC for all the bacteria tested was found to be between 10 % and 2.5% v/v, while the MIC for control group B (ethanol 70%) was between 20% and 5% v/v.

Results of the experiments in which the zones of inhibition were measured showed that *Thymus vulgaris* tincture in both 43% and 70% ethanol had a significant effect on the bacteria tested in comparison to the control. All the strains of bacteria were sensitive to *Thymus vulgaris* tincture.

There was no significant difference between the effects of the two tinctures, although they were made up differently. Thus, it was concluded that in this experiment no significant difference existed between the antibacterial properties of *Thymus vulgaris*

tincture made from fresh plant material and that of *Thymus vulgaris* tincture produced from dried plant material, and between the two alcohol percentages used.

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DEFINITIONS OF TERMS

Tincture: an alcoholic or water-alcohol solution, usually referring to a preparation from herbal materials (Blumenthal et al., 1998: 639.)

Antibacterial agent: an agent that kills bacteria or inhibits their growth (Prescott, Harley and Klein, 1999: G3.)

Antimicrobial agent: an agent that kills microorganisms or inhibits their growth (Prescott, Harley and Klein, 1999: G3.)

Bactericide: an agent that kills bacteria (Prescott, Harley and Klein, 1999: G4.)

Antiseptic: chemical agents applied to tissue to prevent infection by killing or inhibiting pathogens (Prescott, Harley and Klein, 1999: G3.)

Minimum Inhibitory Concentration: the least amount of antimicrobial that will inhibit visible growth of an organism after overnight incubation (Collee et al., 1996: 159.)

Minimum Bactericidal Concentration: the lowest drug concentration that kills a particular pathogen (Prescott, Harley and Klein, 1999: 681.)

Zone of Inhibition: area around an impregnated filter paper disc in which there is no bacterial growth (Atlas, 1995: 364.)

CHAPTER ONE

1.0 INTRODUCTION

1.1 Overview

Due to the enormous increase in the number of multi-drug resistant strains of bacteria, the subsequent increase in nosocomial infections and the escalation of the import, research and development and manufacturing costs of allopathic drugs, the benefits of establishing alternatives to the current antibacterials becomes clear. *Thymus vulgaris* has been used for its antiseptic properties for centuries and while much is known of the antibacterial effects of the essential oil of thyme, and thymol, little research has been conducted on the antimicrobial effects of the tincture of this common garden herb.

In the early 1990's, the director of the World Health Organisation (W.H.O.) declared that conventional Western medicine would not be able to meet the health needs of the world adequately by the turn of the century. This brought about the concept of "Health for All by 2000" in which the Director General of W.H.O. endorsed a plan to utilise traditional medical systems in each country. (Griggs, 1991: 11.) In developing countries the use of medicinal plants helps to reduce imports of drugs, thus boosting economic self-reliance. Furthermore, local products tend to be more readily accepted than those obtained abroad. (Akerele, 1993: 390.) Due to the increase in immunodeficiency states in the poorer section of the population it has become necessary to investigate a non-toxic alternative antimicrobial substance that can be used in cases of immunodeficiency with specific reference to long-term usage.

The aim of this study was to establish the efficacy of the antibacterial properties of *Thymus vulgaris* tincture upon *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* in order to determine its value in antimicrobial applications.

Thymus vulgaris is indigenous to the Southern European countries bordering on the Mediterranean, but is cultivated throughout the world. The name 'thyme' is believed to derive from the Greek term meaning 'to fumigate', the Greeks using it for incense as well as medicinally. (Evans, 1991:118.)

Contemporary herbalists recommend *Thymus vulgaris* externally for wound disinfection and internally for indigestion, sore throat, laryngitis, cough, whooping cough, and nervousness (Little, 1994:50). Thyme is markedly antiseptic, it modifies the intestinal flora, it improves appetite and is resolutive and suppressant for spasmodic coughs. Externally thyme is an excellent application for wound healing. (Rowson, 1976:134.)

While most *Thymus vulgaris* preparations are harmless when used in a low dose, and no cases of poisoning have been reported, the essential oil is highly toxic when ingested (Talalaj and Czechowicz, 1989:301).

The active constituent of *Thymus vulgaris* is volatile oil (2-3%), containing mainly thymol and carvacrol (up to 70%), borneol (15%), cineole, tannins (10%), saponins, and flavonoids (Talalaj and Czechowicz, 1989: 300).

Streptococcus pyogenes, a group A β-hemolytic streptococcus is the most virulent streptococcus species for humans, causing pharyngitis, tonsillitis, wound and skin infections, septicemia, scarlet fever, pneumonia, rheumatic fever and glomerulonephritis (Beers, et al., 1999:1150).

Pathogenic staphylococci are ubiquitous; they are carried in the anterior nares of about 30% of healthy adults and on the skin of about 20%. Newborns and nursing mothers are predisposed to staphylococcal infections, as are patients with influenza, chronic bronchopulmonary disorders (e.g. cystic fibrosis, pulmonary emphysema), leukaemia, neoplasms, transplants, prostheses or other foreign bodies, surgical incisions, diabetes mellitus, and indwelling catheters. Patients receiving adrenal steroids, irradiation, immunosuppressants, or antitumor chemotherapy are also at risk. (Beers et al., 1999:1147.)

Staphylococcus aureus, a coagulase-positive species, can be the cause of many pustular skin infections, wound infections, toxic shock syndrome, scalded skin syndrome, as well as infections in almost every region of the body (Prescott, Harley & Klein, 1999: 784).

Staphylococcus epidermidis, a common coagulase-negative species, is increasingly a cause of nosocomial bacteremia associated with catheters and other foreign bodies (Beers et al., 1999:1148).

Pseudomonas aeruginosa is an opportunistic pathogen with an innate resistance to many antibiotics and disinfectants (Greenwood, Slack, Peutherer, 1992: 345).

Pseudomonas infections can develop in many anatomic sites, including skin, subcutaneous tissue, bone, ears, eyes, urinary tract and heart valves (Beers et al., 1999:1173).

Escherichia coli, while part of the normal gastro-intestinal tract flora, may become a major cause of watery, inflammatory, or bloody diarrhoea. Urinary tract, hepatobiliary, peritoneal, cutaneous, and pulmonary infections also occur. This organism is also an opportunistic pathogen, causing disease in patients who have defects in host resistance because of other disease or who have received treatment with corticosteroids, radiation, antineoplastic drugs or antibiotics. (Beers et al., 1999: 1158.)

Enterococcus faecalis, a group D streptococcus is one of the most virulent of the group D streptococci and causes endocarditis, urinary tract infections, intra-abdominal infections, cellulitis, and wound infections as well as concurrent bacteremia (Beers et al., 1999:1151).

A recent study into the antimicrobial activity of the essential oil of *Thymus vulgaris* found that all the thyme essential oils examined, demonstrated a good degree of bacteriostatic activity against the microorganisms tested (Marino, Bersani and Comi, 1999:1022). In another study conducted by Dorman and Deans (2000) *Thymus vulgaris* essential oil was found to have the greatest spectrum of antibacterial action of all the volatile oils tested.

1.2 Problem Statement

The purpose of this study was to investigate the efficacy of the antibacterial properties of *Thymus vulgaris* tincture against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* using a disc diffusion method, and to measure the MIC and MBC in order to determine it's value in antimicrobial applications.

1.3 Subproblems

1.3.1 Subproblem One

To compare the efficacy of *Thymus vulgaris* tincture in 43% ethanol to the 43% ethanol control against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* in terms of the size of the zones of inhibition.

1.3.2 Subproblem Two

To compare the efficacy of *Thymus vulgaris* tincture in 70% ethanol to the 70% ethanol control against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* in terms of the size of the zones of inhibition.

1.3.3 Subproblem Three

To compare the efficacy of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol against *Streptococcus pyogenes*, *Staphylococcus*

aureus, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* in terms of the diameter of the zones of inhibition.

1.3.4 Subproblem Four

To compare the efficacy of *Thymus vulgaris* tincture in 43% and 70% ethanol to the 43% and 70% ethanol control against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* in terms of the MIC.

1.3.5 Subproblem Five

To compare the efficacy of *Thymus vulgaris* tincture in 43% and 70% ethanol to the 43% and 70% ethanol control against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* in terms of the MBC.

1.4 Hypotheses

1.4.1 Hypothesis One

Thymus vulgaris tincture in 43% ethanol will have no significant antibacterial effects on *Streptococcus pyogenes*.

1.4.2 Hypothesis Two

Thymus vulgaris tincture in 43% ethanol will have no significant antibacterial effects on *Staphylococcus aureus*.

1.4.3 Hypothesis Three

Thymus vulgaris tincture in 43% ethanol will have no significant antibacterial effects on *Staphylococcus epidermidis.*

1.4.4 Hypothesis Four

Thymus vulgaris tincture in 43% ethanol will have no significant antibacterial effects on *Pseudomonas aeruginosa.*

1.4.5 Hypothesis Five

Thymus vulgaris tincture in 43% ethanol will have no significant antibacterial effects on *Escherichia coli.*

1.4.6 Hypothesis Six

Thymus vulgaris tincture in 43% ethanol will have no significant antibacterial effects on *Enterococcus faecalis.*

1.4.7 Hypothesis Seven

Thymus vulgaris tincture in 70% ethanol will have no significant antibacterial effects on *Streptococcus pyogenes.*

1.4.8 Hypothesis Eight

Thymus vulgaris tincture in 70% ethanol will have no significant antibacterial effects on *Staphylococcus aureus.*

1.4.9 Hypothesis Nine

Thymus vulgaris tincture in 70% ethanol will have no significant antibacterial effects on *Staphylococcus epidermidis.*

1.4.10 Hypothesis Ten

Thymus vulgaris tincture in 70% ethanol will have no significant antibacterial effects on *Pseudomonas aeruginosa.*

1.4.11 Hypothesis Eleven

Thymus vulgaris tincture in 70% ethanol will have no significant antibacterial effects on *Escherichia coli.*

1.4.12 Hypothesis Twelve

Thymus vulgaris tincture in 70% ethanol will have no significant antibacterial effects on *Enterococcus faecalis.*

1.4.13 Hypothesis Thirteen

There will be no significant difference between the antibacterial effects of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on *Streptococcus pyogenes.*

1.4.14 Hypothesis Fourteen

There will be no significant difference between the antibacterial effects of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on *Staphylococcus aureus.*

1.4.15 Hypothesis Fifteen

There will be no significant difference between the antibacterial effects of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on *Staphylococcus epidermidis*.

1.4.16 Hypothesis Sixteen

There will be no significant difference between the antibacterial effects of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on *Pseudomonas aeruginosa*.

1.4.17 Hypothesis Seventeen

There will be no significant difference between the antibacterial effects of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on *Escherichia coli*.

1.4.18 Hypothesis Eighteen

There will be no significant difference between the antibacterial effects of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on *Enterococcus faecalis*

1.5 Delimitations

1.5.1 This study was limited to only six species of bacteria viz. *Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis.*

1.5.2 This study was limited to a specific species of *Thymus* species viz. *Thymus vulgaris*.

1.5.3 Only the following growth media were used:

A) Mueller-Hinton Agar

B) Nutrient Broth

C) Brain-Heart Infusion Broth

D) Sterile Horse Serum

1.5.4 Only *Thymus vulgaris* tinctures produced in 43% and 70% ethanol were used as test substances.

1.5.5 The incubation temperature for cell growth was 37° C.

1.5.6 This was an *in vitro* study.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Herbal medicine is the use of whole plants, or parts thereof, for the treatment of disease and the maintenance of good health (Thomas, 1996:68).

Today, there is a wide range of dispensable forms of plant drugs with tinctures being the most favoured due to their ability to remain sterile which is attributed to their alcohol content (Weiss, 1991:1).

Throughout the 19th and up to the mid 20th centuries important herbal research was being undertaken in Europe and the United States, but the development of powerful synthetic medicines that had the power to virtually wipe out many terrible diseases lead to a rapid decline in interest in the old ways. Though it was a serious mistake to abandon centuries of medical experience with natural medicines, it would be an even graver mistake to overlook the role that proper use of the scientific method can play in making great advancements in the area of herbal medicine. (Mowrey, 1986: v-vii.)

2.2 *Thymus vulgaris*

Thymus vulgaris is a member of the Labiatae family, along with wild thyme, and mint (Blumenthal et al., 1998: 615).

2.2.1 Habitat and Cultivation

Thymus vulgaris is native to the Iberian Peninsula, the maquis in southern France, the western coast and south of Italy, and also in Greece, where it grows on rocky slopes as one of the main components of the discontinuous tracts of hard-leaved plants, so-called *'tomillares'* (Stary, 1998: 194; Weiss, 1991: 208). Thyme is widely cultivated in the United States (Cruden, 1997: 161). Spain accounts for 90% of the world production of commercial thyme oil (Evans, 1996: 263).

Thymus vulgaris is a perennial low-growing plant, reaching a foot in height at most. The roots are woody and fibrous, and the stems are woody, with lots of branches. The tiny, narrow greenish-grey leaves appear in opposite pairs, with a pair of minute leaflets at the base of the stalk. (Evans, 1991: 118.) The fruits consist of four nutlets and all parts of the plant are aromatic (Bunney, 1992: 233). It prefers light chalky soils, a dry situation and is harvested in the summer (Chevallier, 1998:98). Commercial cultivation is satisfactory only in warm regions. The plants are collected before flowering if only the leaves are required; otherwise, they are collected when in flower. (Rowson, 1976:134.)

2.2.2 History, Folklore and Traditional Uses

The name thyme is believed to derive from the Greek term meaning 'to fumigate', the Greeks using it for incense as well as medicinally (Evans, 1991:118). Others derive the name from the Greek word *thumus*, signifying courage, the plant being held in ancient and medieval days to be a great source of invigoration, its cordial qualities inspiring courage (Grieve, 1978: 809). It was an emblem of activity, bravery and energy, in the days of chivalry it was custom for ladies to embroider a bee hovering over a sprig of thyme on the scarves they presented to their knights (Grieve, 1978: 809).

Dioscorides and Theophrastus both describe the virtues of thyme in reducing intestinal spasms, fighting infections and improving digestion. Pliny said that burning thyme put to flight all venomous creatures. (Evans, 1991:118.)

Lady Northcote (in *The Herb Garden*) says that among the Greeks, thyme denoted graceful elegance; 'to smell of thyme' was an expression of praise, applied to those whose style was admirable (Grieve, 1978: 809).

The Romans also used thyme medicinally as a cough remedy, digestive aid, and treatment for intestinal worms (Little, 1994:50). The antiseptic and preservative properties of thyme were known to the ancient Egyptians who used the oil for embalming (Bunney, 1992: 281).

In the South of France, wild thyme was a symbol of extreme Republicanism, tufts of it being sent with the summons to a Republican meeting (Grieve, 1978:809).

Thyme has inspired many poets over the years; Shakespeare's *A Midsummer Night's Dream*, the play which is richest in herbal lore, carries the famous lines beginning, "I know a bank whereon the wild thyme blows", and many others have alluded to it. Since the days of Charlemagne, it was obligatory to grow thyme in the garden. (Evans, 1991: 118.)

As the centuries passed, it was used as an antiseptic during plagues (Little, 1994: 50). Thyme walks were often found in old herb gardens. It was believed that when walked upon the spreading plants released their antiseptic properties into the air and these were said to combat plague and other infections. (Roberts, 1983: 22.)

By the late 17th century, apothecary shops were selling thyme oil as a topical antiseptic under the name of oil of origanum. From the mid 19th century through World War I, thyme enjoyed great popularity as an antiseptic. (Little, 1994: 50.)

2.2.3 Constituents

Thymus vulgaris contains around 2% of volatile oil containing phenols (carvacrol, thymol, and p-cymol), monoterpene hydrocarbons and sesquiterpenes. A number of chemical races, e.g. 'thymol' and 'carvacrol' types are known and the phenolic content is held largely responsible for the antiseptic, antitussive and expectorant properties of the drug. The flavonoids of the leaves may be responsible for the spasmolytic activity of the herb. (Evans, 1996: 263.) The mode of action of phenolic compounds is generally thought to involve interference with functions of the cytoplasmic membrane, including proton motive forces and active transport (Marino, Bersani & Comi, 1999: 1021-1022).

Thymol is an antiseptic, spasmolytic, expectorant, anthelmintic, calmative. Carvacrol is a marked spasmolytic. (Wren, 1994: 266.)

The percentage composition of the essential oil of *Thymus vulgaris* harvested at the start of the flowering season as cited by Marino, Bersani and Comi (1999: 1023), is listed in table 2.1.

Table 2.1 Percentage composition of the essential oil of *Thymus vulgaris*

Compound	% Composition
α-Pinene	1.12
A-Thujone	2.00
Camphene	0.59
1-Octen–3 –ol	0.75
Myrcene	3.31
α-Terpinene	3.16.
p-Cymene	10.64
1,8-Cineol	1.16
Γ-Terpinene	30.95
Cis-*p*-menten-1-ol	0.75
Camphor	0.33
Linalool	2.57
Borneol	0.68
Terminen-4-ol	1.26
Thymol	33.74
Carvacrol	1.73

2.2.4 Preparations and Common Uses

2.2.4.1 The Essential Oil

The essential oil of *Thymus vulgaris* is the most common preparation; it is distilled from the fresh herb. The essential oil, due to its toxicity, is diluted to 5% v/v maximum with carrier oil. (Chevalier, 1998: 99.) It is widely used in aromatherapy for its mood enhancing properties in the treatment of low spirits, fatigue, mental stress and premenstrual tension (Pierce, 1999: 631).

An extract of the volatile oil in thyme – thymol – appears in numerous cough syrups, gas remedies, counterirritant skin preparations, mouthwashes (it is the main ingredient in Listerine®), antifungal medicines, dental preparations (e.g. Colgate® Herbal Toothpaste) and cosmetics (Pierce, 1999: 631), as well as inhalations for the treatment of respiratory infections (Evans, 1991: 118).

The chemotypes of *Thymus vulgaris* essential oil from plants grown from seed, or collected in the wild, are diverse in their properties. They fall into two main groups, 'red' thymes being skin irritant and 'sweet' thymes being without hazard – even on children. (Price, 1993: 77.)

Red thyme oil is strongly antiseptic, therefore extremely useful in cases of colds, flu, and sore throats etc. In aromatherapy, red thyme essential oil tonics are effective in severe cases of depression or fatigue. Red thyme essential oil can be irritant to the skin because of its high phenol content. (Price, 1993: 77.)

Sweet thyme essential oil contains alcohol as its main constituent; it is antibacterial and antifungal (this makes sweet thymes useful against *Candida albicans*). Sweet thyme essential oil is used in aromatherapy to make a tonic that has effects on the nervous system and is immunostimulant. (Price, 1993: 77.)

Several research trials investigating the antimicrobial activity of thymol and essential oil of *Thymus vulgaris* have been published.

Kulevanova et al. (2000), investigated the antimicrobial activity of essential oils of several Macedonian *Thymus vulgaris* L. species. Agar diffusion and broth dilution methods were used and the inhibition of growth examined on three Gram-positive bacteria, *Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus pneumoniae*. It was found that despite a great variation in essential oil composition, all the samples examined possessed strong antibacterial activity. The zones of inhibition were between 10-54 mm in diameter. MIC's ranging from 0.012-0.1% were measured.

A study conducted by Cosentino et al. (1999) determined the chemical composition and antimicrobial properties of *Thymus vulgaris* essential oils. The antimicrobial activity of four essential oil samples from Sardinian *Thymus vulgaris* was determined against a panel of standard reference strains and multiple strains of food-derived spoilage and pathogenic bacteria, using a broth dilution method. The results of the antimicrobial assay showed that the essential oils of *Thymus vulgaris* native to the area (Sardinia) have an antimicrobial activity that was comparable to other essential oils of *Thymus vulgaris*. The study also confirmed that the antimicrobial property of the essential oils of *Thymus vulgaris* is related to their high phenol content. The results of this study confirmed the possibility of using *Thymus vulgaris* essential oils or some of their components in food systems to prevent the growth of food borne bacteria and extend the shelf life of processed foods.

Several antimicrobial effects of thymol were investigated on *Porphyromonas gingivalis, Selenomonas artemidis* and *Streptococcus sobrinus* by Shapiro and Guggenheim (1995). The MIC and MBCs were investigated. It was found that thymol

caused an extremely rapid efflux of intracellular constituents and it was postulated that the cause of the antimicrobial activity of thymol was due to membranotropic effects. It was also found that thymol induced a decline in intracellular ATP in *Streptococcus sobrinus* that could be attributed to this leakage. The decline in intracellular ATP observed in *Porphyromonas gingivalis* was attributed to a thymol-induced inhibition of the ATP-generating pathways.

In 1994 Didry, Dubreuil and Pinkas investigated the antimicrobial activity of thymol, carvacrol, cinnamaldahyde and euganol by micromethods on eight oral bacteria. All the compounds were found to have inhibitory effects, alone and in combinations.

In 1995, Twetman, Hallgren and Petersson investigated the effect of an antibacterial dental varnish containing 1% thymol and 1% chorhexidine on the levels of *Streptococcus mutans* in plaque adjacent to bonded orthodontic brackets in 18 children. The split-mouth technique with a placebo varnish control was used. The proportion of mutans streptococci within the plaque microflora was significantly ($p < 0.05$-0.01) lower on the test sides than on the opposite sides at the one-week and one-month examinations. The results suggest that topical applications of the antibacterial varnish can effectively inhibit *Streptococcus mutans* proliferation in plaque.

A further study conducted by Twetman and Petersson in 1999 investigated the effects of the same antibacterial varnish on the incidence of interdental caries in 110 children (8-10 years) anticipated to be at caries risk. It was found that the reduction of caries incidence and progression was strongly dependant on the use of antibacterial varnish. It was concluded that the suppression of *Streptococcus mutans* in interdental plaque

might be an important event in the prevention of approximal caries development in children at risk.

2.2.4.2 Dried *Thymus vulgaris*

Dried leaves of thyme are powdered and used as a snuff to clear a blocked nose or to stem the flow in a bleeding nose (Roberts, 1983: 23).

Infusions are also a popular way of prescribing thyme; a cup of boiling water is poured over 10 ml dried thyme and allowed to steep for 10 minutes (Hoffman, 1992: 237). This should be drunk three times a day (Hoffman, 1992: 237) or throughout the day in small frequent doses (Cruden, 1997: 161) or used as a gargle for sore throats and mouth infections (Little, 1994: 50).

Dried thyme in capsule form is also available and a maximum of 2g per day is recommended by Chevalier (1998: 99).

2.2.4.3 Tinctures of *Thymus vulgaris*

Fresh plant material is preferred for the preparation of tinctures although dried thyme is also used (Lilje, 2001). Tincture doses vary; Cruden (1997:161) recommends 10-20 drops three times a day and Hoffman (1992: 237) suggests a maximum of 8ml a day in 2-4ml doses.

Both tinctures and infusions are recommended by contemporary herbalists for disinfecting wounds, eliminating skin parasites, reducing aches and pains, and treating fungal infections such as athlete's foot (Pierce, 1999: 631). Tinctures are also used in

the production of cough syrups, gargles, antiseptic mouthwashes and compresses (Evans, 1991: 118, Weiss, 1991: 209).

Reid (2001) conducted an experiment to determine the efficacy of *Thymus vulgaris* tincture in 43% ethanol vs. a 43% ethanol only control, as an anticandidal agent. The zones of inhibition were measured and the results analysed using the Mann-Whitney U test. The p-value was 0.008 indicating that the *Thymus vulgaris* tincture had a statistically significant inhibitory effect on *Candida albicans*.

The fresh herb boiled in a litre of water and cooled, strained and rubbed into the scalp every day is recommended to prevent and arrest hair loss (Roberts, 1983: 23). Two drops of the essential oil in half a cup of carrier oil massaged into the scalp is recommended by Viagas (1995: 38) in the treatment of alopecia.

Thymus vulgaris preparations are widely recommended in the treatment of respiratory infections, especially whooping cough (Weiss, 1991: 209), non-specific urethritis, gonorrhoea, leucorrhoea, and trichomonas, urinary tract infections, dysentery, gastro-enteritis, low blood pressure, anaemia (Curtis, 1996: 120), boils, abscesses, excessive menstruation, headaches and hysteria (Roberts, 1983: 23).

Other studies examining the antimicrobial properties of herbal tinctures using microbiological methods include Ramlachan (2001) and Langford (2001).

Ramlachan (2001) investigated the anticandidal properties of the 62% ethanol tinctures of three Compositae species (*Arctium lappa, Calendula officinalis* and

Echinacea purpurea) as compared to a positive control of nystatin, and a negative control of 62% ethanol only. A disc diffusion method was used and the zones of inhibition measured. It was found that these tinctures had no appreciable anticandidal effects. The average p value was 1.000.

Langford (2001) investigated the efficacy of *Calendula officinalis* tincture as an antimicrobial agent. A disc diffusion method was used and the zones of inhibition measured. In this study, however the quantity of test and control substance impregnated on each disc was not standardised. The results of this study found that *Calendula officinalis* tincture in 30% and 60% ethanol exhibit significant inhibition on the growth of *Streptococcus pyogenes, Pseudomonas aeruginosa* and *Enterococcus faecalis*. The average p value was 0.037.

2.3 *Streptococcus pyogenes*

2.3.1 Classification

Streptococcus pyogenes belongs to the genus *Streptococcus* of the family Streptococcaceae (Prescott, Harley and Klein, 1999: 503).

2.3.2 Morphology and Identification

Streptococci are Gram-positive bacteria arranged in chains of varying length; each cell is 1.0μm in diameter, non-motile, non-sporing and may be capsulate. The majority are facultative anaerobes, but there are species that are strictly anaerobic. All have an anaerobic type of metabolism and are catalase-negative. (Greenwood, Slack and Peutherer, 1992: 211-212.) *Streptococcus pyogenes* tends to form small (0.5-

1.0mm) translucent colonies surrounded by a relatively large zone of beta haemolysis (Howard et al., 1994: 264). The temperature range for growth is 22-42°C with an optimum of 35-37°C (Cheesbrough, 1985: 229).

2.3.3 Epidemiology

Streptococcus spp. are widely distributed in nature, being found in water, dust, vegetation, milk and milk products (Cheesbrough, 1985:228). *Streptococcus pyogenes* is widely distributed among humans, but usually people are asymptomatic carriers (Prescott, Harley and Klein, 1999: 770).

The spread of *Streptococcus pyogenes* is mediated by direct contact, fomites or airborne droplet infection. Group A β-haemolytic streptococci (*S. pyogenes*) are responsible for 95% of human streptococcal infections. (Kumar and Clark, 1998: 22.) When highly virulent strains appear in schools, they can cause sharp outbreaks of sore throats and scarlet fever. Due to the cumulative build up of antibodies to may different *S. pyogenes* serotypes over the years, outbreaks among adults are less frequent. (Prescott, Harley & Klein, 1999: 770.)

2.4.3 *Streptococcus pyogenes* Infections

Streptococcus pyogenes, a group A β-hemolytic streptococcus is the most virulent streptococcus species for humans, causing pharyngitis, tonsillitis, wound and skin infections, septicemia, scarlet fever, pneumonia, rheumatic fever and glomerulonephritis (Beers et al., 1999:1150). According to Cheesbrough (1985: 228) otitis media, peritonsillar abscess and puerperal sepsis also result from *Streptococcus pyogenes* infections.

Serious complications may arise following an immunological response to a streptococcal infection. The most serious are:

- **Acute glomerulonephritis**, this follows a streptococcal infection of the skin. Such infection is common in developing countries, especially in rural areas.
- **Rheumatic fever**, which usually follows a respiratory streptococcal infection. This is the most serious complication because damage to heart valves and muscle can lead to chronic rheumatic heart disease. In developing countries especially those of Africa, South America, and Central America rheumatic heart disease arising from streptococcal infection has been identified as a major cause of death among school-age children. (Cheesbrough, 1985:228.)
- **Invasive *Streptococcus pyogenes* Infections** the development of which appears to depend on the presence of specific virulent strains (M-1 and M-2 serotypes) and predisposing host factors (surgical or nonsurgical wounds, diabetes, and other underlying medical problems). A life-threatening infection begins when invasive *Streptococcus pyogenes* strains penetrate a mucous membrane or take up residence in a skin lesion such as a bruise. This infection can quickly lead either to necrotizing fasciitis that destroys the sheath covering skeletal muscles or to myositis, the inflammation and destruction of skeletal muscle and fat tissue. (Prescott, Harley and Klein, 1999:771.)
- **Toxic Shock-Like Syndrome,** since 1986 it has been recognised that invasive *Streptococcus pyogenes* infections can also trigger a toxic shock-like syndrome (TSLS), characterized by a precipitous drop in blood pressure, multiple organ failure, and very high fever. TSLS is caused by an invasive *Streptococcus pyogenes* that produces one or more of the streptococcal pyrogenic exotoxins. (Prescott, Harley and Klein, 1999:771.) Patients are

usually otherwise healthy children or adults with skin and soft tissue infections (Beers et al., 1999:1150).

2.4 *Staphylococcus aureus*

2.4.1 Classification

Staphylococcus aureus belongs to the genus *Staphylococcus* of the family Staphylococcaceae (Prescott, Harley and Klein, 1999: 503).

2.4.2 Morphology and Identification

Staphylococcus aureus are facultative anaerobic, non-motile, Gram-positive cocci that usually form irregular clusters. They are catalase positive, slime producing and oxidase negative, ferment glucose anaerobically and have teichoic acid in their cell walls. (Prescott, Harley and Klein, 1999: 503, 782.) *Staphylococcus aureus* forms small, smooth, cream coloured (occasionally white) colonies that are 1-2mm in diameter (Cheesbrough, 1985: 192). Temperature range for growth is 10-42°C, with an optimum of 35-37°C (Cheesbrough, 1985: 226).

2.4.3 Epidemiology

Approximately 20% of all staphylococcal infections are autogenous. Transmission is most frequently by direct contact with an infected individual but may be by air or via fomites. (Kumar and Clark, 1998: 21.) *Staphylococcus aureus* is demonstrating increasing resilience to many commonly used antibiotics (Mansouri, 1999: 377).

Pathogenic *Staphylococcus aureus* is ubiquitous; it is carried in the anterior nares of about 30% of healthy adults and on the skin of about 20%. Newborns and nursing mothers are predisposed to staphylococcal infections, as are patients with influenza, chronic bronchopulmonary disorders (e.g. cystic fibrosis, pulmonary emphysema), leukaemia, neoplasms, transplants, prostheses or other foreign bodies, surgical incisions, diabetes mellitus, and indwelling catheters. Patients receiving adrenal steroids, irradiation, immunosuppressants, or antitumor chemotherapy are also at risk. (Beers et al., 1999:1147.)

2.4.4 *Staphylococcus aureus* Infections

Staphylococcus aureus ranks among the most important bacterial pathogens, causing a wide variety of suppurative diseases (e.g. superficial and deep abscesses, osteomyelitis, mastitis) and toxinoses (food poisoning, toxic shock syndrome) (Palmer, 1998: 125S).

Coagulase-positive staphylococci like *Staphylococcus aureus* can be the cause of pimples, impetigo, boils and carbuncles, wound infections and abscesses pharyngitis, laryngitis, bronchitis, pneumonia, nephritis, meningitis, endocarditis, osteomyelitis, enteritis and enterotoxin poisoning, as well as widespread seeding if the lymph nodes become infected (Prescott, Harley and Klein, 1999: 784).

Major infections include:

- **Food Poisoning.** This results from ingestion of food contaminated with preformed heat-stable enterotoxins A, B, C, D and E in varying combinations. *Staphylococcus aureus* accounts for approximately 5% of all food poisoning in the UK. Contamination is usually due to an infected individual. (Kumar and Clark, 1998: 21.) Foodstuffs such as canned food, processed meats, milk and cheese favour the growth of *Staphylococcus aureus*. The illness is manifest within six hours of ingestion of contaminated food and affects virtually all individuals who have eaten such food. Unlike food poisoning that is due to other organisms, staphylococcal food poisoning is characterized by the presence of persistent vomiting. Abdominal discomfort, diarrhoea and a mild fever may also be present. (Kumar and Clark, 1998: 21.)
- **Toxic Shock Syndrome.** Toxic shock syndrome is a syndrome caused by staphylococcal exotoxins, characterised by high fever, vomiting, diarrhoea, confusion, and skin rash that may rapidly progress in severe and intractable shock. Toxic shock syndrome occurs predominantly in menstruating women who use tampons. After widespread publicity of the role played by tampons and diaphragms as well as the withdrawal of some tampons from the market, the incidence in women dropped precipitously. About 15% occur postpartum or as postoperative staphylococcal wound infections, which frequently appear insignificant. Cases have also been reported in association with influenza, osteomyelitis and cellulitis. (Beers et al., 1999:1149.)
- **Staphylococcal Scalded Skin Syndrome.** Staphylococcal Scalded Skin Syndrome (SSSS) also known as Ritter-Lyell Syndrome, is an acute, widespread erythema and epidermal peeling (Beers et al., 1999:798), and is

caused by strains of *Staphylococcus aureus* that carry a plasmid-borne gene for the exfoliative toxin or exfoliatin (Prescott, Harley and Klein, 1999: 784). SSSS almost always occurs in infants, children less than six years old, and immunosuppressed adults or adults with renal failure (Beers et al., 1999: 798).

2.5 *Staphylococcus epidermidis*

2.5.1 Classification

Staphylococcus epidermidis belongs to the genus *Staphylococcus* of the genus *Staphylococcaceae* (Prescott, Harley and Klein, 1999: 503).

2.5.2 Morphology and Identification

Staphylococcus epidermidis are Gram-positive cocci, colonies are usually non-haemolytic and white (Cheesbrough, 1985: 227). *Staphylococcus epidermidis* cultures can be distinguished from *Staphylococcus aureus* by their lack of clumping factor and their failure to coagulate plasma (Greenwood, Slack and Peutherer, 1992: 210).

2.5.3 Epidemiology

Streptococcus epidermidis is usually more resistant to antibiotics than *Staphylococcus aureus* (Cheesbrough, 1985: 227). Approximately 20% of all staphylococcal infections are autogenous. Transmission is most frequently by direct contact with an infected individual but may be by air or via fomites. (Kumar and Clark, 1998: 21.)

Pathogenic *Staphylococcus epidermidis* is ubiquitous; it is carried in the anterior nares of about 30% of healthy adults and on the skin of about 20%. Newborns and nursing

mothers are predisposed to staphylococcal infections, as are patients with influenza, chronic bronchopulmonary disorders (e.g. cystic fibrosis, pulmonary emphysema), leukaemia, neoplasms, transplants, prostheses or other foreign bodies, surgical incisions, diabetes mellitus, and indwelling catheters. Patients receiving adrenal steroids, irradiation, immunosuppressants, or antitumor chemotherapy are also at risk. (Beers et al., 1999:1147.)

2.5.4 *Staphylococcus epidermidis* Infections

The most common coagulase-negative staphylococcus, *Staphylococcus epidermidis* is increasingly a cause of nosocomial bacteremia associated with catheters and other foreign bodies, and is an important cause of morbidity (especially prolongation of hospitalisation) and mortality in debilitated patients (Beers et al., 1999: 1148). The organisms cause particular problems after cardiac surgery; in patients fitted with ventriculovenous cerebrospinal fluid shunts; in continuous ambulatory peritoneal dialysis; and in immunosuppressed patients(Greenwood, Slack and Peutherer, 1992: 210).

The emergence of coagulase-negative staphylococci as major pathogens reflects the increased use of implants such as cerebrospinal fluid shunts, intravascular lines and cannulae, cardiac valves, pacemakers, artificial joints, vascular grafts, and urinary catheters (Greenwood, Slack and Peutherer, 1992: 210).

2.6 *Pseudomonas aeruginosa*

2.6.1 Classification

Pseudomonas aeruginosa belongs to the genus *Pseudomonas* of the family Pseudomonadaceae (Prescott, Harley and Klein, 1999: 475).

2.6.2 Morphology and Identification

Pseudomonas aeruginosa is a Gram-negative bacillus, non-sporing, non-capsulate and usually motile by virtue of one or two polar flagella. It is strictly aerobic but can grow anaerobically if nitrate is available; a temperature of 35°C or above is preferred. (Greenwood, Slack and Peutherer, 1992: 345; Howard et al., 1994: 351.)

Pseudomonas aeruginosa colonies can occur as five distinct types ranging from dwarf colonies to large mucoid colonies; the most common colonial form is relatively large, low-convex with an irregular surface, and edge that is translucent and an oblong shape with the long axis parallel to the line of inoculum (Greenwood, Slack and Peutherer, 1992: 345-346).

Pseudomonas aeruginosa cultures are usually recognised by the yellow-green pyocyanin pigment it produces. If the culture is left at room temperature, this yellow-green intensifies. Cultures have a distinctive sweet grape-like odour due to 2-aminoacetaophenone production. (Cheesbrough, 1985: 265; Greenwood, Slack and Peutherer, 1992: 345.)

2.6.3 Epidemiology

Pseudomonas species can be found in water, soil, sewage and vegetation and is a competent and hardy saprophyte. They can also be found in the intestinal tract. (Cheesbrough, 1985: 264; Greenwood, Slack and Peutherer, 1992: 348.) The ability of the species to persist and multiply, particularly in moist environments, and on moist equipment (e.g. humidifiers) in hospital wards, bathrooms and kitchens, is of particular importance in cross-infection control (Greenwood, Slack and Peutherer, 1992: 348). *Pseudomonas aeruginosa* has become a major cause of nosocomial infections over the past 40 years, currently accounting for up to 20% of these infections. This increased incidence has been largely attributed to the widespread use of antibiotics; the resistance of *P. aeruginosa* to most antimicrobial agents has allowed this organism to flourish while organisms that are more susceptible have been suppressed. (Howard et al., 1994: 361.)

2.6.4 *Pseudomonas aeruginosa* Infections

Pseudomonas aeruginosa infection rarely occurs in persons with normal defences, but occurs frequently in patients who are granulocytopenic or immunosuppressed. Other predisposing factors include the presence of extensive burns, immunologic immaturity, prior or concomitant antibiotic therapy, intravenous drug abuse, the presence of an indwelling foreign device or cystic fibrosis. (Howard et al., 1994: 361.)

Pseudomonas aeruginosa is associated with the chronic pulmonary disease of patients with cystic fibrosis, that is the leading cause of morbidity and mortality in these patients (Howard et al., 1994: 361).

Pseudomonas aeruginosa causes approximately 70% of the cases of otitis externa. This is frequently observed in swimmers ("swimmers ear") and is one of the few *P. aeruginosa* infections to occur in healthy people. The most serious type of ear infections caused by *P. aeruginosa* is malignant otitis externa. Most frequently seen in diabetic patients this condition now appears to be an emerging clinical entity in children with chronic illness or immunosuppression. (Howard et al., 1994: 361.)

Another infection by *Pseudomonas aeruginosa,* found in a healthy host, is folliculitis, a dermatologic infection characterized by a syndrome of malaise, mastitis, otitis external, and rash (Howard et al., 1994: 361).

Pseudomonas aeruginosa septicemia may occur in patients who are immunosuppressed, have suffered extensive burns or in infants; the mortality rate associated with septicemia is the highest for any bacterial nosocomial pathogen (Howard et al., 1994: 361).

This organism is also responsible for the formation of corneal ulcers generally seen in eye trauma involving a foreign body, if not treated properly or sometimes despite treatment; the ulcer may progress to panophthalmitis (an inflammation involving all the tissues of the eyeball) and blindness. *Pseudomonas aeruginosa* has also been associated with conditions such as meningitis, urinary tract infections, endocarditis and osteomyelitis. (Howard et al., 1994: 361.)

2.7 *Escherichia coli*

2.7.1 Classification

Escherichia coli belongs to the genus *Escherichia* of the family Enterobacteriaceae (Prescott, Harley and Klein, 1999: 478).

2.7.2 Morphology and Identification

Escherichia coli are Gram-negative, aerobic, motile bacteria and some strains produce a polysaccharide capsule. They grow well on non-selective media where they form smooth, colourless colonies 2-3mm in diameter. *Escherichia coli* grow over a wide range of temperatures from 15°C to 45°C. (Greenwood, Slack and Peutherer, 1992: 323.)

2.7.3 Epidemiology

Escherichia coli forms part of the normal microbial flora of the intestinal tract of humans and animals. It can also be found in water, soil and vegetation. (Cheesbrough, 1985: 254.) It is an opportunistic pathogen that causes disease in patients who have defects in host resistance as a result of other diseases (usually chronic) or are on treatment with corticosteroids, radiation, antineoplastic drugs or antibiotics (Beers et al., 1999: 1015).

2.7.4 *Escherichia coli* Infections

Escherichia coli causes approximately 90% of all acute urinary tract infections in non-hospitalised patients without urologic abnormalities and are responsible for 30% of nosocomial urinary tract infections (Howard et al., 1994: 322).

It is also responsible for wound infections, cellulitis, appendicitis, peritonitis, and infections of the gall bladder, as well as bacteraemia, diarrhoea and meningitis especially of the newborn (Cheesbrough, 1985: 254).

2.8 *Enterococcus faecalis*

2.8.1 Classification

Enterococcus faecalis is a member of the genus *Enterococcus* and the family Streptococcaceae (Hardie and Whiley, 1997: 7S).

2.8.2 Morphology and Identification

Enterococcus faecalis is a Gram-positive coccus that occurs in either pairs or chains (Howard et al., 1994: 267).

As with other *Streptococcus* spp. *Enterococcus faecalis* thrives on complex media and is capable of growing in both aerobic and anaerobic conditions (Howard et al., 1994: 260). They can be identified as enterococci by rapid litmus milk reduction test. On MacConkey agar, enterococci produce distinctive small dark red colonies. (Cheesbrough, 1985: 230.)

2.8.3 Epidemiology

Enterococcus faecalis is normally resident in the intestinal tracts of humans and most other animals (Prescott, Harley and Klein, 1999: 503). Some strains have been isolated from soil, food, water and plants. Their ability to grow and survive under a wide range of environmental conditions, including extremes of temperature and salt

concentrations, probably accounts for the almost ubiquitous distribution of the genus. (Hardie and Whiley, 1997: 8S.)

2.8.4 *Enterococcus faecalis* Infections

Enterococcus faecalis is an opportunistic pathogen that can cause urinary tract infections and endocarditis (Prescott, Harley and Klein, 1999: 502). Of particular concern is the increasing prevalence of enterococci in hospital-acquired infections and their increasing levels of resistance to antimicrobial agents (Hardie and Whiley, 1997: 8S).

Enterococci are often found in intra-abdominal and pelvic wound infections, but since these are usually polymicrobial, it is difficult to assess their role in such conditions (Hardie and Whiley, 1997: 8S). Bacteraemias due to enterococci are common, often occurring in elderly patients with serious underlying medical conditions or in immunocompromised individuals who have undergone antimicrobial therapy (Hardie and Whiley, 1997: 8S).

Infective endocarditis infections due to *Enterococcus faecalis* are more usual in older men with prostatic disease, in women with genitourinary infections, or following pelvic surgery (Kumar and Clark, 1998: 711). According to Hardie and Whiley (1997: 8S) *Enterococcus faecalis* is responsible for a significant proportion of cases of bacterial endocarditis, accounting for 5-20% of reported cases.

Enterococcus faecalis is also known to cause neonatal, central nervous system and respiratory tract infections on occasions, although these are comparatively rare

(Hardie and Whiley, 1997: 8S.) *Enterococcus faecalis* is also associated with septicemia, cholecystitis and peritonitis (Greenwood, Slack and Peutherer, 1992: 221).

CHAPTER THREE

3.0 METHODOLOGY

3.1 The Data and Their Interpretation

3.1.1 The Data

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

3.1.1.1 The Primary Data

3.1.1.1.1 Zones of Inhibition

Results of the experiment determining the effects of *Thymus vulgaris* tincture in 43% and 70% ethanol on *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* in comparison to 43% and 70% ethanol only by measurement of the diameter of the zones of inhibition.

3.1.1.1.2 Minimum Inhibitory Concentration

Results of the experiments determining the effects of *Thymus vulgaris* tincture in 43% and 70% ethanol on *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* in comparison to 43% and 70% ethanol only by measurement of the MIC.

3.1.1.1.2 Minimum Bactericidal Concentration

Results of the experiments determining the effects of *Thymus vulgaris* tincture in 43% and 70% ethanol on *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* in comparison to 43% and 70% ethanol only by measurement of the MBC.

3.1.1.2 The Secondary Data

Research articles from journal publications, books, and manuals.

3.2 Criteria Governing the Admissibility of Data

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

3.3 Materials and Methods

3.3.1 Preparation of Media

Mueller-Hinton agar, nutrient broth and brain-heart infusion broth were used in the study. Each medium was prepared according to the manufacturers specifications (BioLab, nd., Oxoid, nd). After preparation, all media were autoclaved to ensure sterility.

3.3.2 Preparation of Filter Paper Discs

Round discs of 5mm diameter were punched from filter paper 0.2mm thick, placed into an Schott bottle, and autoclaved at 121°C for 15 minutes to ensure sterility.

3.3.3 Preparation of *Thymus vulgaris* Tinctures

The *Thymus vulgaris* tincture in 43% ethanol (Batch No. 10019) was produced by Parceval (Pty) Ltd. according to the German Homoeopathic Pharmacopoeia Method 2a (British Homoeopathic Association, 1991) resulting in a tincture of 1: 2 concentration (i.e. for every one part of fresh *Thymus vulgaris* plant material there was two parts of ethanol).

The *Thymus vulgaris* tincture in 70% ethanol (Batch No. Exp.094) was produced by Parceval (Pty) Ltd. according to the German Homoeopathic Pharmacopoeia Method 4a (British Homoeopathic Association) resulting in a tincture of 1: 10 concentration (i.e. for every one part of dried *Thymus vulgaris* plant material there was ten parts of ethanol).

Because different source materials were used (dried and fresh plant material) and the moisture content of fresh *Thymus vulgaris* was taken into account, the two tinctures while made up in different quantities of ethanol were of equivalent strengths (Lilje, 2001).

3.3.4 Preparation of Inoculum

Single colonies (from the Technikon Natal Biotechnology Laboratory stock cultures) of each of the bacteria to be tested were used to inoculate separate bottles containing nutrient broth and incubated for 24 hours at 37 °C.

3.3.5 Preparation of Cultures

The six overnight nutrient broth cultures were used to streak six Mueller-Hinton agar plates and again incubated for 24 hours at 37 °C.

3.3.6 Preparation of Saline Test Cultures

A few individual colonies from the overnight Mueller-Hinton agar cultures were suspended in sterile saline solution and the solution adjusted to the 0.5 McFarland Equivalence Turbidity Standard (REMEL, nd).

3.3.7 Experimental Procedure to Determine Zones of Inhibition

All the agar plates were labelled with the names of the test culture and either test or control group to be tested on that plate.

A sterile cotton swab was dipped into a saline test culture and loaded with a fixed volume. Excess inoculum was removed by pressing the saturated swab against the inner wall of the culture tube. Using the swab, the surfaces of the plates were streaked first in a horizontal direction and then vertically and diagonally to ensure heavy growth over the entire surface. This procedure was followed four times for each bacterium producing one streaked plate for each test and control group per culture, 24 streaked plates in total.

With sterile forceps 5 sterile filter paper discs were distributed over the agar surface of each plate. Each filter paper disc was then impregnated (using a micropipette) with 10 µl of the *Thymus vulgaris* tincture or the respective ethanol concentration that corresponded to the label on that agar plate.

All the plate cultures were then incubated in an inverted position for 24 hours at 37°C. Following incubation; the plates were examined for the presence of growth inhibition as indicated by a zone free of bacterial growth surrounding each disc. The diameter of these zones was measured using a clear plastic measuring ruler with one-millimetre markings.

The above procedure was performed in triplicate.

3.3.8 Experimental Procedure to Determine Minimum Inhibitory Concentration.

Twenty-four sets of twelve 5ml glass bottles were labelled one to twelve and a letter assigned to each test and control substance for each bacterium, e.g. the twelve bottles for the test *Thymus vulgaris* tincture 43% on *Streptococcus pyogenes* were labelled one to twelve 'A'.

Two millilitres of a brain heart infusion broth with 25% sterile horse serum culture medium was micropipetted into all bottles labelled '1' to '12'. Into all bottles labelled '1' 3.2ml of sterile distilled water was placed. To this was added 0.8ml of the respective test or control substance (this produces a 20% v/v concentration of *Thymus vulgaris* tincture or ethanol in bottle '1'). 2ml of this solution was then placed into bottle number '2'. After thorough mixing 2ml of this was removed to bottle '3'. This procedure was repeated for all sets of bottles. After mixing, the last 2ml removed from bottle '11' was discarded leaving bottle '12' containing only culture medium.

Test cultures of the bacteria to be tested were prepared (using the plate cultures previously prepared) in sterile saline solution and adjusted to the 0.5 McFarland

Equivalence Turbidity Standard. A Pasture pipette was used to place one drop of the test culture corresponding to the label on each set of twelve bottles into each bottle.

All bottles were incubated at 37°C for 18 hours.

3.3.9. Experimental Procedure to Determine Minimum Bactericidal Concentration

A sterile culture loop was used to plate out the contents of each bottle onto Mueller-Hinton agar plates. Seventy-two agar plates were each divided into four sections by a horizontal and a vertical line. Each of these quarters was labelled with a number and a letter, resulting in one-quarter plate for each 5ml bottle used in the experiment determining the MIC. The sterile loop was dipped into a bottle and used to streak the quarter plate with the corresponding label. This procedure was followed for all two hundred and eighty eight bottles resulting in 72 streaked agar plates. The agar plates were then incubated at 37°C for 24 hours. The quarter showing no bacterial growth after incubation is taken to be the MBC.

3.4 Statistical Procedures

3.4.1 Subproblem One

3.4.1.1 Procedure 1.1

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and 43% ethanol on the growth of *Streptococcus pyogenes* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and 43% ethanol only on the growth of *Streptococcus pyogenes* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  The null hypothesis H_0 states that there is no difference in diameter of the zone of inhibition between the *Thymus vulgaris* tincture in 43% ethanol and the 43% ethanol only with respect to the variable of comparison at the $\alpha = 0.05$ level of significance. The alternative hypothesis H_1, states that there is a difference at the same level of significance.

  H_o: $M_1 = M_2$

  H_1: $M_1 \neq M_2$

- Decision rule

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

Reject H_0 if $P < \alpha$

Accept H_0 if $P \geq \alpha$

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

3.4.1.2 Procedure 1.2

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and 43% ethanol on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and 43% ethanol only on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure 1.1.

- Decision rule

  As per Procedure 1.1.

3.4.1.3 Procedure 1.3

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and 43% ethanol on the growth of *Staphylococcus epidermidis* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and 43% ethanol only on the growth of *Staphylococcus epidermidis* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure 1.1.

- Decision rule

  As per Procedure 1.1.

3.4.1.4 Procedure 1.4

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and 43% ethanol on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and 43% ethanol only on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure 1.1.

- Decision rule

  As per Procedure 1.1.

3.4.1.5 Procedure 1.5

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and 43% ethanol on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and 43% ethanol only on the growth of *Escherichia coli* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure 1.1.

- Decision rule

  As per Procedure 1.1.

3.4.1.6 Procedure 1.6

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and 43% ethanol on the growth of *Enterococcus faecalis* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and 43% ethanol only on the growth of *Enterococcus faecalis* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure 1.1.

- Decision rule

  As per Procedure 1.1.

3.4.2 Subproblem Two

3.4.2.1 Procedure 1.7

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 70% ethanol and 70% ethanol on the growth of *Streptococcus pyogenes* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 70% ethanol and 70% ethanol only on the growth of *Streptococcus pyogenes* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  The null hypothesis H_0 states that there is no difference in diameter of the zone of inhibition between the *Thymus vulgaris* tincture in 70% ethanol and the 70% ethanol only with respect to the variable of comparison at the $\alpha = 0.05$ level of significance. The alternative hypothesis H_1, states that there is a difference at the same level of significance.

  H_0: $M_1 = M_2$

  H_1: $M_1 \neq M_2$

- Decision rule

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

  Reject H_0 if $P < \alpha$

  Accept H_0 if $P \geq \alpha$

3.4.2.2 Procedure 1.8

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 70% ethanol and 70% ethanol on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 70% ethanol and 70% ethanol only on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure1.7.

- Decision rule

  As per Procedure 1.7.

3.4.2.3 Procedure 1.9

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 70% ethanol and 70% ethanol on the growth of *Staphylococcus epidermidis* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 70% ethanol and 70% ethanol only on the growth of *Staphylococcus epidermidis* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure1.7.

- Decision rule

  As per Procedure 1.7.

3.4.2.4 Procedure 1.10

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 70% ethanol and 70% ethanol on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 70% ethanol and 70% ethanol only on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure1.7.

- Decision rule

  As per Procedure 1.7.

3.4.2.5 Procedure 1.11

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 70% ethanol and 70% ethanol on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 70% ethanol and 70% ethanol only on the growth of *Escherichia coli* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure1.7.

- Decision rule

  As per Procedure 1.7.

3.4.2.6 Procedure 1.12

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 70% ethanol and 70% ethanol on the growth of *Enterococcus faecalis* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 70% ethanol and 70% ethanol only on the growth of *Enterococcus faecalis* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure1.7.

- Decision rule

  As per Procedure 1.7.

3.4.3 Subproblem Three

3.4.3.1 Procedure 1.13

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Streptococcus pyogenes* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Streptococcus pyogenes* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  The null hypothesis H_0 states that there is no difference in diameter of the zone of inhibition between the *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol with respect to the variable of comparison at the $\alpha = 0.05$ level of significance. The alternative hypothesis H_1, states that there is a difference at the same level of significance.

  H_o: $M_1 = M_2$

  H_1: $M_1 \neq M_2$

- Decision rule

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

  Reject H_0 if $P < \alpha$

  Accept H_0 if $P \geq \alpha$

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

3.4.3.2 Procedure 1.14

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure 1.13.

- Decision rule

  As per Procedure 1.13.

3.4.3.3 Procedure 1.15

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Staphylococcus epidermidis* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Staphylococcus epidermidis* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure 1.13.

- Decision rule

  As per Procedure 1.13.

3.4.3.4 Procedure 1.16

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure 1.13.

- Decision rule

  As per Procedure 1.13.

3.4.3.5 Procedure 1.17

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Escherichia coli* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure 1.13.

- Decision rule

  As per Procedure 1.13.

3.4.3.6 Procedure 1.18

Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Enterococcus faecalis* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the inhibitory effect of *Thymus vulgaris* in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Enterococcus faecalis* in terms of the average diameter of the zones on inhibition.

- Hypothesis testing

  As per Procedure 1.13.

- Decision rule

  As per Procedure 1.13.

3.4 Procedure 2 Comparison Using Bar Charts

Visual summaries of the analytical findings are given by means of bar charts (Figure 4.1 – 4.3). Average readings were used to construct the bar charts represented.

3.5 Statistical Package

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

CHAPTER FOUR

4.0 RESULTS

4.1 Criteria Governing the Admissibility of Data

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

4.2 Statistical Analysis of Results

4.2.1 Procedure 1.1–1.6: Intergroup Comparison Between *Thymus vulgaris* tincture in 43% ethanol and 43% ethanol only

See Table 4.1 and Figure 4.1

Table 4.1 Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and 43% ethanol on the growth of *Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis* in terms of the average diameter of the zones of inhibition.

***Thymus vulgaris* tincture in 43% ethanol vs. 43% ethanol only.**	**Bacteria**	**P-value**	**H_0**
Procedure 1.1	*S. pyogenes*	0.015	Reject
Procedure 1.2	*S. aureus*	0.007	Reject
Procedure 1.3	*S. epidermidis*	0.005	Reject
Procedure 1.4	*P. aeruginosa*	0.005	Reject
Procedure 1.5	*E. coli*	0.005	Reject
Procedure 1.6	*E. faecalis*	0.005	Reject

Interpretation of results for procedures 1.1–1.6: The null hypothesis is rejected since $P < \alpha = 0.05$, indicating that there is a statistically significant difference in the diameter of the zones of inhibition produced by *Thymus vulgaris* tincture in 43% ethanol and those produced by 43% ethanol only.

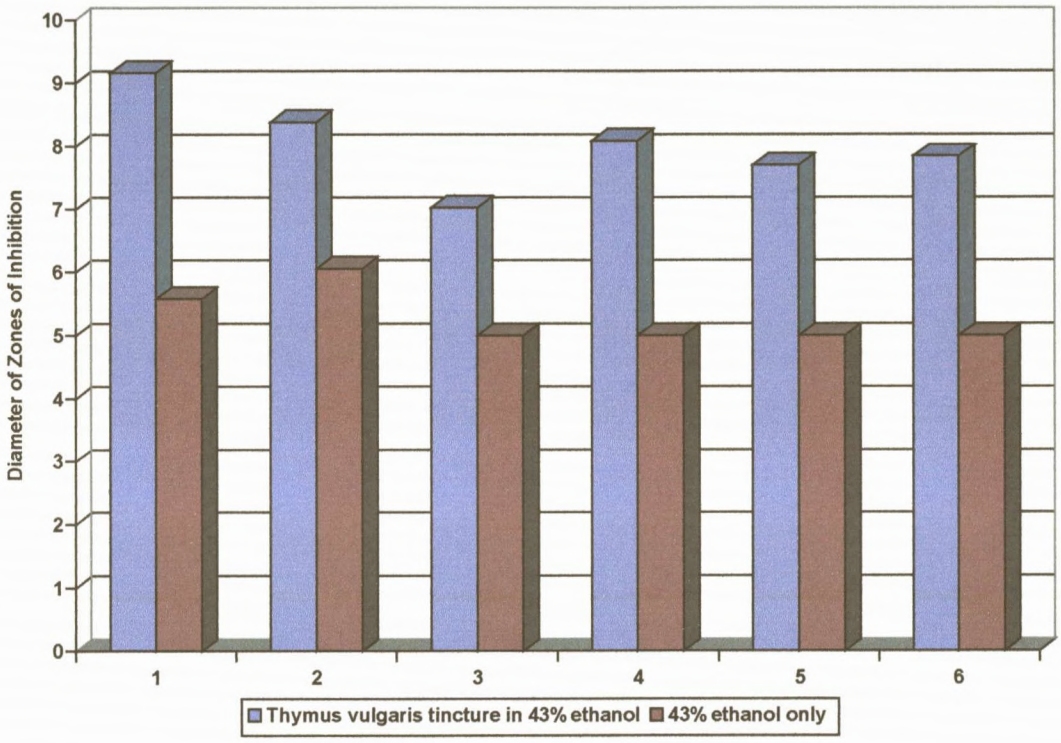


Figure 4.1 Comparison between *Thymus vulgaris* tincture in 43% ethanol and 43% ethanol only. KEY: 1 – *Streptococcus pyogenes* 2 – *Staphylococcus aureus* 3 – *Staphylococcus epidermidis* 4 – *Pseudomonas aeruginosa* 5 – *Escherichia coli* 6 – *Enterococcus faecalis*

4.2.2 Procedure 1.7–1.12: Intergroup Comparison Between *Thymus vulgaris* tincture in 70% ethanol and 70% ethanol only

See table 4.2 and Figure 4.2

Table 4.2 Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 70% ethanol and 70% ethanol on the growth of *Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis* in terms of the average diameter of the zones of inhibition.

***Thymus vulgaris* tincture in 70% ethanol vs. 70% ethanol only.**	**Bacteria**	**P-value**	**H_0**
Procedure 1.7	*S. pyogenes*	0.008	Reject
Procedure 1.8	*S. aureus*	0.005	Reject
Procedure 1.9	*S. epidermidis*	0.005	Reject
Procedure 1.10	*P. aeruginosa*	0.005	Reject
Procedure 1.11	*E. coli*	0.005	Reject
Procedure 1.12	*E. faecalis*	0.005	Reject

Interpretation of results for procedures 1.7 – 1.12: The null hypothesis is rejected since $P < \alpha = 0.05$, indicating that there is a statistically significant difference in the diameter of the zones of inhibition produced by *Thymus vulgaris* tincture in 70% ethanol and those produced by 70% ethanol only.

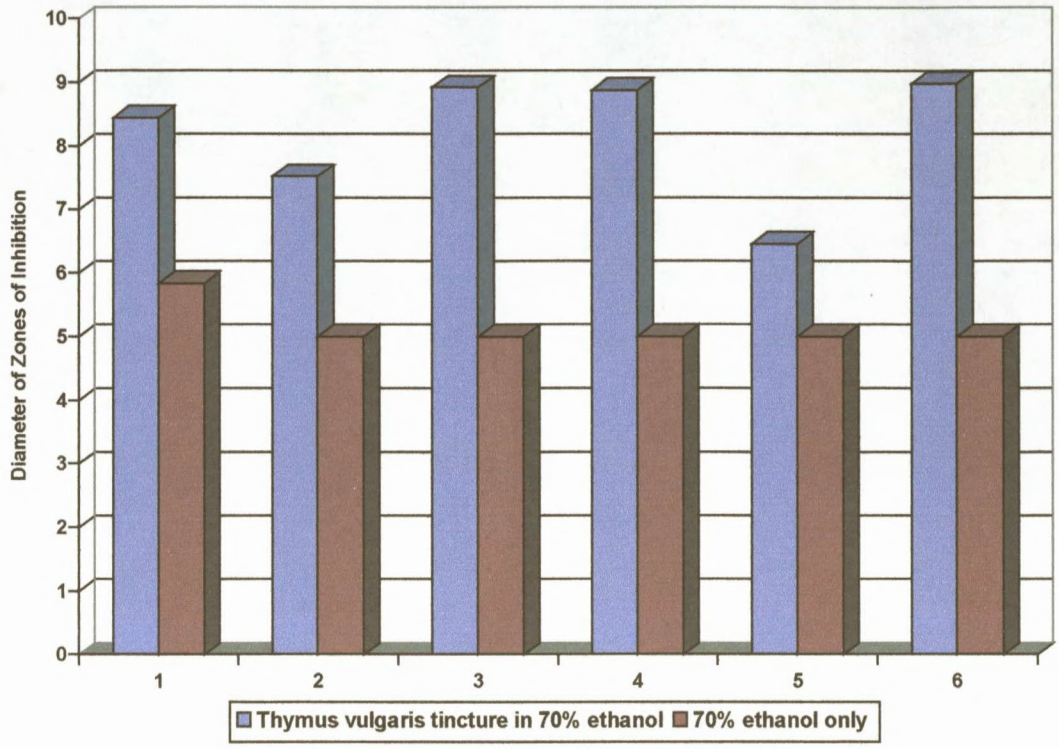


Figure 4.2 Comparison between *Thymus vulgaris* tincture in 70% ethanol and 70% ethanol only. KEY: 1 – *Streptococcus pyogenes* 2 – *Staphylococcus aureus* 3 – *Staphylococcus epidermidis* 4 – *Pseudomonas aeruginosa* 5 – *Escherichia coli* 6 – *Enterococcus faecalis*

4.2.3 Procedures 1.13–1.18: Intergroup Comparison Between *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol

See Table 4.3 and Figure 4.3

Table 4.3 Intergroup comparison between the inhibitory effect of *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris* tincture in 70% ethanol on the growth of *Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis* in terms of the average diameter of the zones of inhibition.

***Thymus vulgaris* tincture in 43% ethanol vs. Thymus vulgaris tincture in 70% ethanol.**	**Bacteria**	**P-value**	**H_0**
Procedure 1.13	*S. pyogenes*	0.173	Accept
Procedure 1.14	*S. aureus*	0.596	Accept
Procedure 1.15	*S. epidermidis*	0.076	Accept
Procedure 1.16	*P. aeruginosa*	0.248	Accept
Procedure 1.17	*E. coli*	0.074	Accept
Procedure 1.18	*E. faecalis*	0.073	Accept

Interpretation of results for procedures 1.13 – 1.18: The null hypothesis is accepted since $P \geq \alpha = 0.05$, indicating that there is no difference in the diameter of the zones of inhibition produced by *Thymus vulgaris* tincture in 43% ethanol and those produced by *Thymus vulgaris* tincture in 70% ethanol.

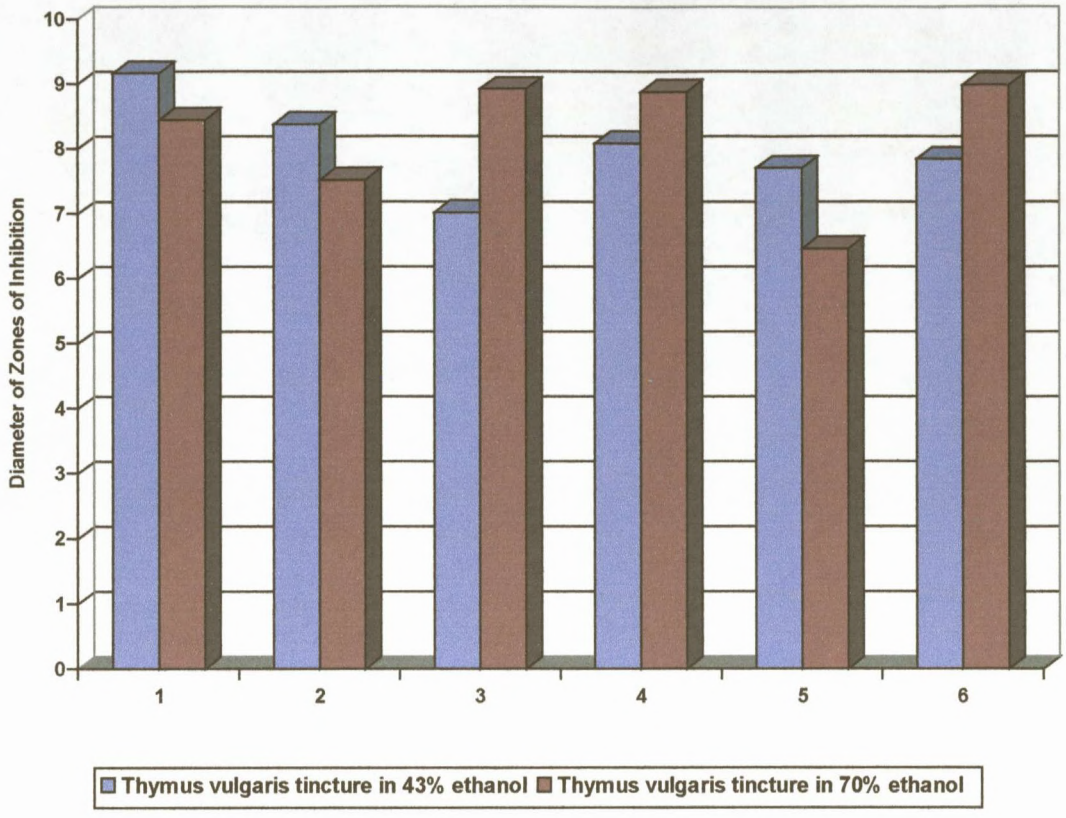


Figure 4.3 Comparison between *Thymus vulgaris* tincture in 43% ethanol and *Thymus vulgaris*. KEY: 1 – *Streptococcus pyogenes* 2 – *Staphylococcus aureus* 3 – *Staphylococcus epidermidis* 4 – *Pseudomonas aeruginosa* 5 – *Escherichia coli* 6 – *Enterococcus faecalis*

4.3 Minimum Inhibitory Concentration

4.3.1 *Thymus vulgaris* tincture in 43% ethanol vs. 43% ethanol only

The MIC of *Thymus vulgaris* tincture in 43% ethanol was half that of the MIC 43% ethanol only for all the bacteria tested. For *Staphylococcus aureus* and *Pseudomonas aeruginosa* the MIC of the *Thymus vulgaris* tincture in 43% ethanol was 2.5% v/v, while that of 43% ethanol only was 5%v/v. For *Staphylococcus epidermidis* and *Streptococcus pyogenes* the MIC of the *Thymus vulgaris* tincture in 43% ethanol was 5% v/v, while that of 43% ethanol only was 10%v/v. For *Escherichia coli* and *Enterococcus faecalis* the MIC of the *Thymus vulgaris* tincture in 43% ethanol was 10% v/v, while that of 43% ethanol only was 20%v/v.

4.3.2 *Thymus vulgaris* tincture in 70% ethanol vs. 70% ethanol only

There was no difference in the MIC of *Thymus vulgaris* tincture in 70% ethanol and that of 70% ethanol only, for the bacteria tested. The MIC for *Staphylococcus aureus* and *Pseudomonas aeruginosa* was 2.5% v/v, that of *Staphylococcus epidermidis* and *Streptococcus pyogenes* was 5% v/v and for *Escherichia coli* and *Enterococcus faecalis* it was 10% v/v.

4.4 Minimum Bactericidal Concentration

4.4.1 *Thymus vulgaris* tincture in 43% ethanol vs. 43% ethanol only

There was no difference in the MBC of *Thymus vulgaris* tincture in 43% ethanol and that of 43% ethanol only, for the bacteria tested. The MBC for *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli and Enterococcus faecalis* was 20% v/v, and for *Streptococcus pyogenes* it was 10% v/v.

4.4.2 *Thymus vulgaris* tincture in 70% ethanol vs. 70% ethanol only

The MBC of *Thymus vulgaris* tincture in 70% ethanol was half that of the MBC of 70% ethanol only, for *Streptococcus pyogenes, Staphylococcus aureus* and *Escherichia coli.* The MBC of the *Thymus vulgaris* tincture in 70% ethanol was 5% v/v, while that of 70% ethanol only was 10%v/v for *Streptococcus pyogenes.* The MBC of the *Thymus vulgaris* tincture in 70% ethanol was 10% v/v, while that of 70% ethanol only was 20%v/v for *Staphylococcus aureus and Escherichia coli.*

There was no difference in the MBC of *Thymus vulgaris* tincture in 70% ethanol and that of 70% ethanol only, for *Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Enterococcus faecalis.* The MBC for *Pseudomonas aeruginosa* was 10% v/v and that for *Staphylococcus epidermidis* and *Enterococcus faecalis* was 20% v/v.

CHAPTER FIVE

5.0 DISCUSSION

The results of this study investigating the antibacterial effect of *Thymus vulgaris* tincture in 43% and 70% ethanol indicate that *Thymus vulgaris* tincture has a statistically significant inhibitory effect on the growth of the bacterial strains tested i.e. *Streptococcus pyogenes* (43% P = 0.015; 70% P = 0.008), *Staphylococcus aureus*(43% P = 0.007; 70% P = 0.005), *Staphylococcus epidermidis*(43% P = 0.005; 70% P = 0.005), *Pseudomonas aeruginosa* (43% P = 0.005; 70% P = 0.005), *Escherichia coli*(43% P = 0.005; 70% P = 0.005) and *Enterococcus faecalis*(43% P = 0.005; 70% P = 0.005) in comparison to the controls of 43% and 70% ethanol only.

It was found that the ethanol alone had very little or no appreciable inhibitory effect on the six types of bacteria tested. This could be due to rapid evaporation of the ethanol, there not being sufficient time to cause inhibition of the bacteria streaked on the plate. That the test substances - tinctures in ethanol - caused significant inhibition of the bacteria even after this supposed evaporation of their ethanol component, leads the researcher to conclude that it is the *Thymus vulgaris* extract portion of the tincture solution that exhibited this inhibitory effect.

While some authors suggest that fresh plant material is preferred for the manufacture of tinctures (Lilje, 2001), a comparison of the results from this experiment found that no statistical difference existed in the efficacy of *Thymus vulgaris* tincture made from fresh plant material (*Thymus vulgaris* tincture in 43% ethanol) and that made from

dried plant material (*Thymus vulgaris* tincture in 70% ethanol), in terms of bacterial growth inhibition.

Due to the prohibitive cost of sterile horse serum the M IC and subsequent MBC tests were only conducted once. Therefore, the results are tentative and may indicate a possible trend only. Until further experiments are conducted, yielding multiple results, and with a more gradual dilution gradient, no firm conclusions can be reached as to the MIC and MBC of *Thymus vulgaris* tincture. It is however possible to state that an antibacterial trend has been observed in the MIC and MBC of *Thymus vulgaris* tinctures. It is also encouraging to note that the MICs fell within the v/v percentage of herbal tinctures normally used in the manufacture of creams and ointments, 10% or less (British Homoeopathic Association, 1991: 40, Hopkins, 1999: 26). This suggests a future application of *Thymus vulgaris* tincture as a component of antibacterial creams and ointments.

During the experiments determining the diameter of the zones of inhibition, two types of contaminant bacterial growth were observed on examination of a number of the agar plates. Firstly, contamination by unidentified bacilli was seen over the entire agar surface in a number of plates. This contaminant bacterial growth was evenly distributed indicating that the contamination may have come from stock cultures used to produce the test cultures before inoculation broths were prepared. It is of note that the *Thymus vulgaris* tinctures had an equally inhibitory effect on these contaminant bacteria, the zones of inhibition on contaminated plates being clear of all bacterial growth. The second type of contamination observed was that of bacterial growth immediately around the filter paper discs within the zone of inhibition. This was only

the *Thymus vulgaris* tinctures had an equally inhibitory effect on these contaminant bacteria, the zones of inhibition on contaminated plates being clear of all bacterial growth. The second type of contamination observed was that of bacterial growth immediately around the filter paper discs within the zone of inhibition. This was only observed in a few plates and seldom for more than one disc on a plate. This disc contamination is likely to have been caused by a contamination of the forceps used to place each disc on the agar surface. Such contamination may have been due to insufficient flaming of these forceps between disc placements.

The results of this study provide empirical support for the assertion by herbalists through the ages that *Thymus vulgaris* has healing and antibacterial effects (Bunney, 1992: 281; Cruden, 1997: 161; Evans, 1991: 118; Hoffman, 1992: 237; Little, 1994: 50; Roberts, 1983: 22-23.)

This study complements the many thymol and essential oil of *Thymus vulgaris* studies (Cosentino et al., 1999; Didry, Dubreuil and Pinkas, 1994; Kulevanova et al., 2000; Marino, Bersani and Comi, 1999; Shapiro and Guggenheim, 1995; Twetman, Hallgren and Petersson, 1995 and Twetman and Petersson, 1999) thus contributing to the body of knowledge on *Thymus vulgaris* extracts.

The results of this study complement those of Reid (2001) who demonstrated that *Thymus vulgaris* tincture has a significant inhibitory effect on the growth of *Candida albicans* ($P = 0.008$).

CHAPTER FIVE

5.0 DISCUSSION

The results of this study investigating the antibacterial effect of *Thymus vulgaris* tincture in 43% and 70% ethanol indicate that *Thymus vulgaris* tincture has a statistically significant inhibitory effect on the growth of the bacterial strains tested i.e. *Streptococcus pyogenes* (43% P = 0.015; 70% P = 0.008), *Staphylococcus aureus*(43% P = 0.007; 70% P = 0.005), *Staphylococcus epidermidis*(43% P = 0.005; 70% P = 0.005), *Pseudomonas aeruginosa* (43% P = 0.005; 70% P = 0.005), *Escherichia coli*(43% P = 0.005; 70% P = 0.005) and *Enterococcus faecalis*(43% P = 0.005; 70% P = 0.005) in comparison to the controls of 43% and 70% ethanol only.

It was found that the ethanol alone had very little or no appreciable inhibitory effect on the six types of bacteria tested. This could be due to rapid evaporation of the ethanol, there not being sufficient time to cause inhibition of the bacteria streaked on the plate. That the test substances - tinctures in ethanol - caused significant inhibition of the bacteria even after this supposed evaporation of their ethanol component, leads the researcher to conclude that it is the *Thymus vulgaris* extract portion of the tincture solution that exhibited this inhibitory effect.

While some authors suggest that fresh plant material is preferred for the manufacture of tinctures (Lilje, 2001), a comparison of the results from this experiment found that no statistical difference existed in the efficacy of *Thymus vulgaris* tincture made from fresh plant material (*Thymus vulgaris* tincture in 43% ethanol) and that made from

dried plant material (*Thymus vulgaris* tincture in 70% ethanol), in terms of bacterial growth inhibition.

A possible methodological weakness in this study was the use of stock bacteria rather than individual stains. The advantage of individual strains is that it allows for the precise replication of antibacterial studies.

Due to the prohibitive cost of sterile horse serum the M IC and subsequent MBC tests were only conducted once. Therefore, the results are tentative and may indicate a possible trend only. Until further experiments are conducted, yielding multiple results, and with a more gradual dilution gradient, no firm conclusions can be reached as to the MIC and MBC of *Thymus vulgaris* tincture. It is however possible to state that an antibacterial trend has been observed in the MIC and MBC of *Thymus vulgaris* tinctures. It is also encouraging to note that the MIC's fell within the v/v percentage of herbal tinctures normally used in the manufacture of creams and ointments, 10% or less (British Homoeopathic Association, 1991: 40, Hopkins, 1999: 26). This suggests a future application of *Thymus vulgaris* tincture as a component of antibacterial creams and ointments.

During the experiments determining the diameter of the zones of inhibition, two types of contaminant bacterial growth were observed on examination of a number of the agar plates. Firstly, contamination by unidentified bacilli was seen over the entire agar surface in a number of plates. This contaminant bacterial growth was evenly distributed indicating that the contamination may have come from stock cultures used to produce the test cultures before inoculation broths were prepared. It is of note that

the *Thymus vulgaris* tinctures had an equally inhibitory effect on these contaminant bacteria, the zones of inhibition on contaminated plates being clear of all bacterial growth. The second type of contamination observed was that of bacterial growth immediately around the filter paper discs within the zone of inhibition. This was only observed in a few plates and seldom for more than one disc on a plate. This disc contamination is likely to have been caused by a contamination of the forceps used to place each disc on the agar surface. Such contamination may have been due to insufficient flaming of these forceps between disc placements.

The results of this study provide empirical support for the assertion by herbalists through the ages that *Thymus vulgaris* has healing and antibacterial effects (Bunney, 1992: 281; Cruden, 1997: 161; Evans, 1991: 118; Hoffman, 1992: 237; Little, 1994: 50; Roberts, 1983: 22-23.)

This study complements the many thymol and essential oil of *Thymus vulgaris* studies (Cosentino et al., 1999; Didry, Dubreuil and Pinkas, 1994; Kulevanova et al., 2000; Marino, Bersani and Comi, 1999; Shapiro and Guggenheim, 1995; Twetman, Hallgren and Petersson, 1995 and Twetman and Petersson, 1999) thus contributing to the body of knowledge on *Thymus vulgaris* extracts.

The results of this study complement those of Reid (2001) who demonstrated that *Thymus vulgaris* tincture has a significant inhibitory effect on the growth of *Candida albicans* ($P = 0.008$).

CHAPTER SIX

6.0 CONCLUSION

The purpose of this study was to investigate the efficacy of the antibacterial properties of *Thymus vulgaris* tincture as compared to a control against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* using a disc diffusion method, and measuring the MIC and MBC in order to determine it's value in antimicrobial applications.

The results of the experiments in which the zones of inhibition were measured showed that *Thymus vulgaris* tincture in both 43% and 70% ethanol has a significant effect on the bacteria tested in comparison to the control. It is concluded that the strains of each bacteria used are sensitive to, and their growth is inhibited by, *Thymus vulgaris* tincture.

There was no significant difference between the effects of the two tinctures, although they were made up differently. Thus, it is concluded that in this experiment no significant difference existed between the antibacterial properties of *Thymus vulgaris* tincture made from fresh plant material and that of *Thymus vulgaris* tincture produced from dried plant material, or between tinctures made up in different alcohol concentrations.

Thymus vulgaris has a role to play in primary and tertiary healthcare regarding control and treatment of infections caused by the bacteria examined in this study.

6.1 Recommendations for Further Studies

1. Tinctures used should all be produced from either fresh or dried plant material.

2. The effect of *Thymus vulgaris* tincture on many strains of one microorganism.

3. Further investigation of the MIC of herbal tinctures should use a more gradual dilution gradient, and include at least five repetitions so as to be able to use statistical tests.

4. Ethanol used as the control should ideally be derived from the same batch as that used to make up the tincture in the first place.

5. *In vivo* trial of *Thymus vulgaris*.

6. Comparative clinical trials using *Thymus vulgaris*.

7. Comparison between the antibacterial efficacy *Thymus vulgaris* tincture, essential oil and infusion.

8. The bacterial strains used should be typed.

REFERENCES

Akerele, O. 1993. Nature's medicinal bounty: don't throw it away. World Health Forum. **14**: 390-395.

Atlas, R.M. 1995. Principles of Microbiology. St Louis: Mosby

Beers, M.H., Berkow, R., Bogon, R.M., Fletcher, A.J., Bondy, P.K., Dilts, P.V., Drossman, D.A., Falling, L.J., Frenkel, E.P., Gabbard, G.O., Hoeckelman, R.A., Mandell, G.L., Plum, F., Rossi, G.V., Tanser, P.H. (eds). 1999. The Merck Manual of Diagnosis and Therapy.

BioLab Diagnostics (Pty) Ltd. nd. [Bottle Label, Instructions] Brain Heart Infusion Broth. Midrand, South Africa.

Blumenthal, M., Busse, W.R., Goldberg, A., Gruenwald, J., Hall, T., Klein, S., Riggins, C.W., Rister, R.S. (eds). 1998. The Complete German E Monographs Therapeutic Guide to Herbal Medicines. Austin: American Botanical Council.

British Homoeopathic Association. 1991. German Homoeopathic Pharmacopoeia. Stuttgarnt Deutscher Potheker Verlag. Translation of the 1st Edition, 1978 Edition, 4th Supplement.:London

Bunney, S. (ed). 1992. The Illustrated Encyclopedia of Herbs. London: Chancellor Press.

Cheesbrough, M. 1985. Medical Laboratory Manuel for Tropical Countries. Oxford: Butterworth Heinemann.

Chevallier, A. 1998. Phytotherapy, Fifty Vital Herbs. Guilford: Amberwood Publishing Ltd.

Collee, J.G., Fraser, A.G., Marmion, B.P., Simmons, A. (eds). 1996. Practical Medical Microbiology. 14th Edition. New York: Churchill Livingstone.

Cosentino, S., Tuberoso, C. I., Pisano, B., Satta, M., Mascia, V., Arzedi, E., Palmas, F.1999. *In-vitro* antimicrobial activity and chemical composition of Sardinian Thymus essential oils. Letters in Applied Microbiology. **29**, 2, 130-5. [Online] Available from: http://medline.cos.com/cgi-bin/getRec?99429116 (Accessed on 18/10/2001)

Cruden, L. 1997. Medicine Grove: A Shamanic Herbal. Rochester: Destiny Books.

Curtis, S. 1996. Essential Oils. London: Aurum Books Ltd.

Didry, N., Dubreuil, L., Pinkas, M. 1994. Activity of thymol, carvacrol, cinnaldehyde and euganol on oral bacteria. Pharm Acta Helv. **69**(1): 25-28. Translated. [Online]. Available from: http://medline.cos.com/cgi-bin/getRec?95024243 (Accessed on (18/10/2001)

Dorman, H. J. D., Deans, S. G. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology. **88**: 308-316.

Evans, M. 1991. Herbal Plants History and Uses. London: Studio Editions.

Evans, W.C. 1996. Trease and Evans' Pharmacognosy. 14th Edition. London: WB Saunders Company Ltd.

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

Greenwood, D., Slack, R., Peutherer, J. (eds). 1992. Medical Microbiology. 14th Edition. Edinburgh: Churchill Livingstone.

Grieve, M. 1978. A Modern Herbal. Harmondsworth: Penguin Books Ltd.

Griggs, B. 1991. Green Pharmacy, the History and Evolution of Western Herbal Medicine. Rochester: Healing Arts Press.

Hardie, J.M., Whiley, R.A. 1997. Classification and overview of the genera Streptococcus and Enterococcus. Journal of Applied Microbiology Symposium Supplement. **83**:1S-11S.

Hoffman, D. 1992. The New Holistic Herbal. 3rd Edition. Shaftesbury: Element Books Ltd.

Hopkins, C. R., 1999. Homoeopharmaceutical IV, Practical Manual. [unpublished handout]. Department of Homoeopathy, Technikon Natal.

Howard, B.J., Keiser, J.F., Smith, T.F. Weissfeld, A.S., Tilton, R.C. 1994. Clinical and Pathogenic Microbiology. 2nd Edition. St Louis: Mosby.

Kulevanova, S., Kaftandzieva, A., Dimitrovska, A., Stefkov, G., Grdanoska, T., Panovski, G. 2000. Investigation of antimicrobial activity of essential oils of several Macedonian Thymus L. species (Lamiaceae). Bollentino Chimico Farmaceutico. **139**(6): 276-80.Translated. [Online] Available from: http://medline.cos.com/cgi-bin/getRec?21082175 (Accessed on 18/10/2001)

Kumar, P., Clark. M. (eds). 1998. Clinical Medicine. 4th Edition. Edinburgh: WB Saunders.

Langford, S., 2001. The Efficacy of *Calendula officinalis* tincture as a local antimicrobial agent. M. Tech (Hom). Technikon Natal, Durban. [In process].

Lilje, W.A., 2001. Interviewed by C. Vosloo. Telephone Interview. 22 October 2001 16h50.

Little, B. 1994. Herbal Health. Melbourne: Thomas C. Lothian Pty Ltd

Mansouri, S. 1999. Inhibition of Staphylococcus aureus mediated by extracts of Iranian plants. Pharmaceutical Biology. **37**(5): 375-377.

Marino, M., Bersani, C., Comi, G. 1999. Antimicrobial activity of the essential oils of *Thymus vulgaris* L. measured using a Bioimpedometric Method. Journal of Food Protection. **62**(9): 1017-1023.

Mowrey, D.B. 1986. The Scientific Validation of Herbal Medicine. New Canaan: Keats Publishing Inc.

Oxoid. nd. [Bottle Label, Instructions] Mueller-Hinton Agar. Hampshire.

Palmer, M. 1998. Staphylococcal alpha toxin. Journal of Applied Microbiology. **85**: 125S – 126S.

Pierce, A. 1999. The American Pharmaceutical Association Practical Guide to Natural Medicines. New York: William Morrow and Company Inc.

Prescott, L. M., Harley, J. P., Klein, D. A. 1999. Microbiology. 4th Edition. Boston: McGraw-Hill.

Price, S. 1993. The Aromatherapy Workbook. London: Hammersmith.

Ramlachan, S., 2001. The efficacy of certain Compositae species (*Arctium lappa*, *Calendula officinalis* and *Echinacea purpurea*) herbal extracts as compared to Nystatin, in the inhibition of *Candida albicans*. M. Tech (Hom). Technikon Natal, Durban. [In process].

REMEL, Technical Information. nd. McFarland Equivalence Turbidity Standards TI No. 20410 [Package Insert]. Sante Fe, Lenexa.

Reid, K., 2001. The efficacy of certain Labiatae species herbal extracts in the inhibition of the *in vitro* growth of *Candida albicans* as compared to nystatin. M. Tech (Hom). Technikon Natal, Durban. [In process].

Roberts, M. 1983. Margaret Roberts' Book of Herbs: The Medicinal and Culinary Used of Herbs in South Africa. Bergvlei: Jonathan Ball Publishers.

Rowson, J.M.1976. Medicinal Plants and Their Uses. London: W Foulsham & Co. Ltd.

Shapiro, S., Guggenheim, B. 1995. The action of thymol on oral bacteria. Journal of Oral Microbiology and Immunology. **10**, 4. 241-246. [Online] Available from: http://medline.cos.com/cgi-bin/getRec?96191966 (Accessed on 18/10/2001)

Starý, F. 1998. The Natural Guide to Medicinal Herbs and Plants. Twickenham: Tiger Books International.

Talalaj, S.T., Czechowicz, A.S. 1989. Herbal Remedies: Harmful and Beneficial Effects. Melbourne: Hill of Content.

Thomas, H. (ed). 1996. The Encyclopedia of Alternative Health and Natural Remedies. Carlton Books Limited.

Twetman, S., Hallgren A., Peters son, L.G. 1995. Effect of an antibacterial varnish on *Streptococcus mutans* in plaque from enamel adjacent to orthodontic appliances. Journal of Caries Research. **29**,3. 188-191. [Online]. Available from: http://medline.cos.com/cgi-bin/getRec?95346970 (Accessed on 18/10/2001)

Twetman, S., Petersson, L.G. 1999. Interdental caries incidence and progression in relation to mutans streptococci suppression after chlorhexidine-thymol varnish treatments in schoolchildren. Acta Odontologica Scandinavica. 57(3): 1440148. Translated. [Online]. Available from: http://medline.cos.com/cgi-bin/getRec?99408157 (Accessed on 18/10/2001)

Viagas, B.G. 1995. Natural Remedies for Common Complaints. London: Judy Piatkus Limited.

Weiss, R.F. 1991. Herbal Medicine. England: Beaconsfield Publishing Ltd.

Wren, R.C. 1994. Potter's New Cylopaedia of Botanical Drugs and Preparations. Saffron Walden: C.W. Company Limited.

APPENDIX A

Effects of Test and Control Substances on *S. pyogenes*

TABLE 4.4

Effects of *Thymus vulgaris* tincture in 43% ethanol (Test Group A) & 43% ethanol (Control Group A) on *Streptococcus pyogenes*.

S. pyogenes	`1	2	3	4	5		1	2	3	4	5
TRIAL 1	9	9	8	11	9		6	11	5	5	5
TRIAL 2	6	8	8	8	-		5	6	5	5	5
TRIAL 3	6	12	10	10	13		5	5	6	5	5
AVERAGE	7	9.6	8.6	9.6	11		5.3	7.3	5.3	5	5

TABLE 4.5

Effects of *Thymus vulgaris* tincture in 70% ethanol (Test Group B) & 70% ethanol (Control Group B) on *Streptococcus pyogenes*.

S. pyogenes	`1	2	3	4	5		1	2	3	4	5
TRIAL 1	6	6	11	9	12		6	6	6	5	5
TRIAL 2	12	12	13	14	13		6	6	6	6	6
TRIAL 3	10	9	12	11	10		6	6	6	6	6
AVERAGE	9.3	9	12	11.3	11.6		6	6	6	5.6	5.6

APPENDIX B

Effects of Test and Control Substances on *S. aureus*

TABLE 4.6

Effects of *Thymus vulgaris* tincture in 43% ethanol (Test Group A) & 43% ethanol (Control Group A) on *Staphylococcus aureus.*

S. aureus	`1	2	3	4	5		1	2	3	4	5
TRIAL 1	12	10	9	6	-		6	6	6	6	7
TRIAL 2	8	9	7	6	10		6	6	6	6	6
TRIAL 3	7	6	6	10	-		6	6	6	6	6
AVERAGE	9	8.3	7.3	7.3	10		6	6	6	6	6.3

TABLE 4.7

Effects of *Thymus vulgaris* tincture in 70% ethanol (Test Group B) & 70% ethanol (Control Group B) on *Staphylococcus aureus.*

S. aureus	`1	2	3	4	5		1	2	3	4	5
TRIAL 1	8	9	8	8	7		5	5	5	5	5
TRIAL 2	9	9	7	6	6		5	5	5	5	5
TRIAL 3	7	7	7	7	8		5	5	5	5	5
AVERAGE	8	8.3	7.3	7	7		5	5	5	5	5

APPENDIX C

Effects of Test and Control Substances on *S. epidermidis*

TABLE 4.8

Effects of *Thymus vulgaris* tincture in 43% ethanol (Test Group A) & 43% ethanol (Control Group A) on *Staphylococcus epidermidis.*

S. epidermidis	`1	2	3	4	5		1	2	3	4	5
TRIAL 1	-	8	8	6	8		5	5	5	5	5
TRIAL 2	7	-	6	10	8		5	5	5	5	5
TRIAL 3	5	5	7	-	7		5	5	5	5	5
AVERAGE	6	6.5	7	8	7.6		5	5	5	5	5

TABLE 4.9

Effects of *Thymus vulgaris* tincture in 70% ethanol (Test Group B) & 70% ethanol (Control Group B) on *Staphylococcus epidermidis.*

S. epidermidis	`1	2	3	4	5		1	2	3	4	5
TRIAL 1	11	-	8	-	6		5	5	5	5	5
TRIAL 2	10	11	8	11	9		5	5	5	5	5
TRIAL 3	10	11	6	6	-		5	5	5	5	5
AVERAGE	10.3	11	7.3	8.5	7.5		5	5	5	5	5

APPENDIX D

Effects of Test and Control Substances on *P. aeruginosa*

TABLE 4.10

Effects of *Thymus vulgaris* tincture in 43% ethanol (Test Group A) & 43% ethanol (Control Group A) on *Pseudomonas aeruginosa*.

P. aeruginosa	1	2	3	4	5		1	2	3	4	5
TRIAL 1	7	8	6	6	-		5	5	5	5	5
TRIAL 2	9	7	8	8	10		5	5	5	5	5
TRIAL 3	11	10	7	6	9		5	5	5	5	5
AVERAGE	9	8.3	7	6.6	9.5		5	5	5	5	5

TABLE 4.11

Effects of *Thymus vulgaris* tincture in 70% ethanol (Test Group B) & 70% ethanol (Control Group B) on *Pseudomonas aeruginosa*.

P. aeruginosa	1	2	3	4	5		1	2	3	4	5
TRIAL 1	9	8	10	10	9		5	5	5	5	5
TRIAL 2	8	8	9	7	10		5	5	5	5	5
TRIAL 3	10	10	9	7	-		5	5	5	5	5
AVERAGE	9	8.6	9.3	8	9.5		5	5	5	5	5

APPENDIX E

Effects of Test and Control Substances on *E. coli*

TABLE 4.12

Effects of *Thymus vulgaris* tincture in 43% ethanol (Test Group A) & 43% ethanol (Control Group A) on *Escherichia coli.*

E. coli	1	2	3	4	5		1	2	3	4	5
TRIAL 1	7	7	9	9	10		5	5	5	5	5
TRIAL 2	7	-	-	9	7		5	5	5	5	5
TRIAL 3	6	6	6	10	9		5	5	5	5	5
AVERAGE	6.6	6.5	7.5	9.3	8.6		5	5	5	5	5

TABLE 4.13

Effects of *Thymus vulgaris* tincture in 70% ethanol (Test Group B) & 70% ethanol (Control Group B) on *Escherichia coli.*

E. coli	1	2	3	4	5		1	2	3	4	5
TRIAL 1	6	7	6	7	6		5	5	5	5	5
TRIAL 2	6	7	7	6	6		5	5	5	5	5
TRIAL 3	9	7	6	-	6		5	5	5	5	5
AVERAGE	7	7	6.3	6	6		5	5	5	5	5

APPENDIX F

Effects of Test and Control Substances on *E. faecalis*

TABLE 4.14

Effects of *Thymus vulgaris* tincture in 43% ethanol (Test Group A) & 43% ethanol (Control Group A) on *Enterococcus faecalis.*

E. faecalis	1	2	3	4	5		1	2	3	4	5
TRIAL 1	13	11	6	-	-		5	5	5	5	5
TRIAL 2	6	6	8	7	7		5	5	5	5	5
TRIAL 3	9	8	10	7	7		5	5	5	5	5
AVERAGE	9.3	8.3	7.6	7	7		5	5	5	5	5

TABLE 4.15

Effects of *Thymus vulgaris* tincture in 70% ethanol (Test Group B) & 70% ethanol (Control Group B) on *Enterococcus faecalis.*

E. faecalis	1	2	3	4	5		1	2	3	4	5
TRIAL 1	11	9	10	9	8		5	5	5	5	5
TRIAL 2	10	8	9	7	10		5	5	5	5	5
TRIAL 3	10	10	6	9	9		5	5	5	5	5
AVERAGE	10.3	9	8.3	8.3	9		5	5	5	5	5

APPENDIX G

Effects of Test and Control Substances Measured after Minimum Inhibitory Concentration Test

TABLE 4.16

Results of Minimum Inhibitory Concentration test: *Streptococcus pyogenes*

Antibacterial / control tested	Minimum Inhibitory Concentration
Thymus vulgaris 43%	5%
Ethanol 43%	10%
Thymus vulgaris 70%	5%
Ethanol 70%	5%

TABLE 4.17

Results of Minimum Inhibitory Concentration test: *Staphylococcus aureus*

Antibacterial / control tested	Minimum Inhibitory Concentration
Thymus vulgaris 43%	2.5%
Ethanol 43%	5%
Thymus vulgaris 70%	2.5%
Ethanol 70%	2.5%

TABLE 4.18

Results of Minimum Inhibitory Concentration test: *Staphylococcus epidermidis*

Antibacterial / control tested	**Minimum Inhibitory Concentration**
Thymus vulgaris 43%	5%
Ethanol 43%	10%
Thymus vulgaris 70%	5%
Ethanol 70%	5%

TABLE 4.19

Results of Minimum Inhibitory Concentration test: *Pseudomonas aeruginosa*

Antibacterial / control tested	Minimum Inhibitory Concentration
Thymus vulgaris 43%	2.5%
Ethanol 43%	5%
Thymus vulgaris 70%	2.5%
Ethanol 70%	2.5%

TABLE 4.20

Results of Minimum Inhibitory Concentration test: *Escherichia coli*

Antibacterial / control tested	Minimum Inhibitory Concentration
Thymus vulgaris 43%	10%
Ethanol 43%	20%
Thymus vulgaris 70%	10%
Ethanol 70%	10%

TABLE 4.21

Results of Minimum Inhibitory Concentration test: *Enterococcus faecalis*

Antibacterial / control tested	**Minimum Inhibitory Concentration**
Thymus vulgaris 43%	10%
Ethanol 43%	20%
Thymus vulgaris 70%	10%
Ethanol 70%	10%

APPENDIX H

Effects of Test and Control Substances Measured After Minimum Bactericidal Concentration Test

TABLE 4.22

Results of Minimum Bactericidal Concentration test: *Streptococcus pyogenes*

Antibacterial / control tested	Minimum Bactericidal Concentration
Thymus vulgaris 43%	10%
Ethanol 43%	10%
Thymus vulgaris 70%	5%
Ethanol 70%	10%

TABLE 4.23

Results of Minimum Bactericidal Concentration test: *Staphylococcus aureus*

Antibacterial / control tested	Minimum Bactericidal Concentration
Thymus vulgaris 43%	20%
Ethanol 43%	>20%
Thymus vulgaris 70%	10%
Ethanol 70%	20%

TABLE 4.24

Results of Minimum Bactericidal Concentration test: *Staphylococcus epidermidis*

Antibacterial / control tested	Minimum Bactericidal Concentration
Thymus vulgaris 43%	20%
Ethanol 43%	20%
Thymus vulgaris 70%	20%
Ethanol 70%	20%

TABLE 4.25

Results of Minimum Bactericidal Concentration test: *Pseudomonas aeruginosa*

Antibacterial / control tested	Minimum Bactericidal Concentration
Thymus vulgaris 43%	20%
Ethanol 43%	20%
Thymus vulgaris 70%	10%
Ethanol 70%	10%

TABLE 4.26

Results of Minimum Bactericidal Concentration test: *Escherichia coli*

Antibacterial / control tested	Minimum Bactericidal Concentration
Thymus vulgaris 43%	20%
Ethanol 43%	20%
Thymus vulgaris 70%	10%
Ethanol 70%	20%

TABLE 4.27

Results of Minimum Bactericidal Concentration test: *Enterococcus faecalis*

Antibacterial / control tested	**Minimum Bactericidal Concentration**
Thymus vulgaris 43%	20%
Ethanol 43%	20%
Thymus vulgaris 70%	20%
Ethanol 70%	20%

APPENDIX I SPSS Printout

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
S.pyo, 43%	ID	1.00	5	7.80	39.00
		2.00	5	3.20	16.00
		Total	10		

Test Statistics[2]

	S.pyo, 43%
Mann-Whitney U	1.000
Wilcoxon W	16.000
Z	-2.424
Asymp. Sig. (2-tailed)	.015
Exact Sig. [2*(1-tailed Sig.)]	.016[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
S.pyo, 70%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	S.pyo, 70%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.652
Asymp. Sig. (2-tailed)	.008
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
S.aur, 43%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	S.aur, 43%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.703
Asymp. Sig. (2-tailed)	.007
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
S.aur, 70%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	S.aur, 70%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.795
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
S.epi, 43%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	S.epi, 43%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.785
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
S.epi, 70%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	S.epi, 70%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.785
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
P.aer, 43%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	P.aer, 43%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.785
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
P.aer, 70%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	P.aer, 70%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.785
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
E.coli, 43%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	E.coli, 43%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.785
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
E.coli, 70%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	E.coli, 70%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.805
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
E.fae, 43%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	E.fae, 43%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.795
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
E.fae, 70%	ID	1.00	5	8.00	40.00
		2.00	5	3.00	15.00
		Total	10		

Test Statistics[2]

	E.fae, 70%
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.825
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
43% vs 70% S.pyo	ID	1.00	5	4.20	21.00
		2.00	5	6.80	34.00
		Total	10		

Test Statistics[2]

	43% vs 70% S.pyo
Mann-Whitney U	6.000
Wilcoxon W	21.000
Z	-1.362
Asymp. Sig. (2-tailed)	.173
Exact Sig. [2*(1-tailed Sig.)]	.222[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
43% vs 70% S.aur	ID	1.00	5	6.00	30.00
		2.00	5	5.00	25.00
		Total	10		

Test Statistics[2]

	43% vs 70% S.aur
Mann-Whitney U	10.000
Wilcoxon W	25.000
Z	-.530
Asymp. Sig. (2-tailed)	.596
Exact Sig. [2*(1-tailed Sig.)]	.690[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
43% vs 70% S.epi	ID	1.00	5	3.80	19.00
		2.00	5	7.20	36.00
		Total	10		

Test Statistics[2]

	43% vs 70% S.epi
Mann-Whitney U	4.000
Wilcoxon W	19.000
Z	-1.776
Asymp. Sig. (2-tailed)	.076
Exact Sig. [2*(1-tailed Sig.)]	.095[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
43% vs 70% P.aer	ID	1.00	5	4.40	22.00
		2.00	5	6.60	33.00
		Total	10		

Test Statistics[2]

	43% vs 70% P.aer
Mann-Whitney U	7.000
Wilcoxon W	22.000
Z	-1.156
Asymp. Sig. (2-tailed)	.248
Exact Sig. [2*(1-tailed Sig.)]	.310[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
43% vs 70% E.coli	ID	1.00	5	7.20	36.00
		2.00	5	3.80	19.00
		Total	10		

Test Statistics[2]

	43% vs 70% E.coli
Mann-Whitney U	4.000
Wilcoxon W	19.000
Z	-1.786
Asymp. Sig. (2-tailed)	.074
Exact Sig. [2*(1-tailed Sig.)]	.095[1]

1. Not corrected for ties.
2. Grouping Variable: ID

NPar Tests

Mann-Whitney Test

Ranks

			N	Mean Rank	Sum of Ranks
43% vs 70% E.fae	ID	1.00	5	3.80	19.00
		2.00	5	7.20	36.00
		Total	10		

Test Statistics[2]

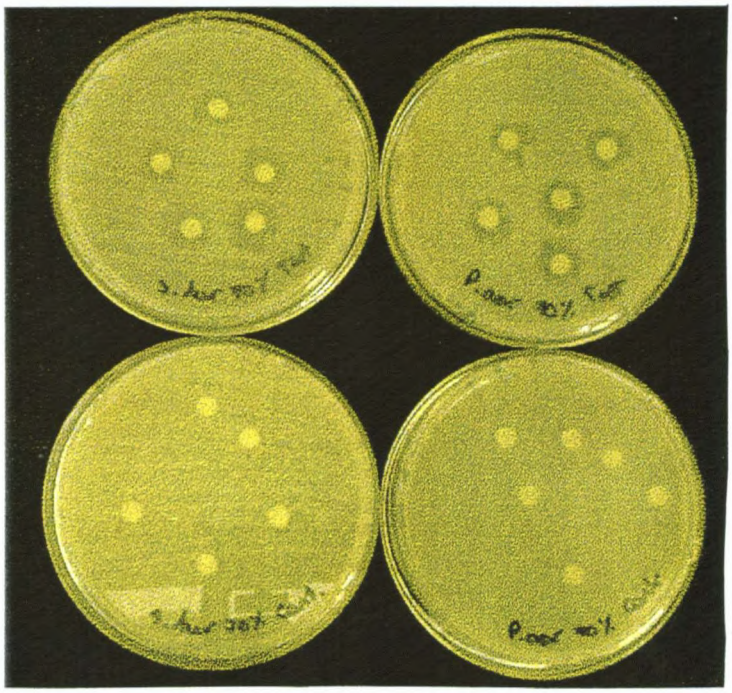
	43% vs 70% E.fae
Mann-Whitney U	4.000
Wilcoxon W	19.000
Z	-1.792
Asymp. Sig. (2-tailed)	.073
Exact Sig. [2*(1-tailed Sig.)]	.095[1]

1. Not corrected for ties.
2. Grouping Variable: ID

APPENDIX J Photography of cultures

Photograph A

The **Upper Left Plate** shows a positive result, clear zones of inhibition around the filter paper disc. This plate is an example of the inhibitory effect exhibited on the Gram-positive bacterium *Staphylococcus aureus*. The **Lower Left Plate** is the ethanol only control of the above plate, showing a negative result, no growth inhibition at all. The **Upper Right Plate** is another example of a positive result, in this case the bacterium is Gram-negative *Pseudomonas aeruginosa*. The **Lower Right Plate** shows the complete lack of growth inhibition exhibited by the control group.



Photograph B

The plate on the **Left** is an example of the contamination a large area of the plate surface. The plate on the **Right** shows both types of contamination seen in this study. The contamination of the entire surface as well as the contamination immediately surrounding the disc within the zone of inhibition.