

THE EFFICACY OF ECHINACEA ANGUSTIFOLIA TINCTURE
AS AN ANTIMICROBIAL AGENT

By

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

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ABSTRACT

The aim of this study was to establish the effect of *Echinacea angustifolia* prepared in 30% ethanol and 62% ethanol in comparison to a 30% ethanol and 62% ethanol control respectively upon *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis* in terms of the Kirby-Bauer Antimicrobial Sensitivity Test.

The diameters of the zones of inhibition of the bacteria growth around the test and control substances were measured. The *Echinacea angustifolia* tincture prepared in 30% ethanol and the 30% ethanol control yielded no effect upon the bacteria tested. However, the *Echinacea angustifolia* tincture prepared in 62% ethanol and the 62% ethanol control both had an antimicrobial effect upon the bacteria.

However, the *Echinacea angustifolia* tincture in 62% ethanol only produced a significant difference in comparison to the 62% ethanol control upon certain organisms, viz. *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis*.

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CHAPTER ONE

1.0. INTRODUCTION

1.1. OVERVIEW

With the ever-increasing cost of allopathic drugs and the rise of multi-drug resistant bacterial infections, the benefits of establishing the antimicrobial properties of more cost effective alternative remedies such as *Echinacea angustifolia* can be highly valuable in the fight against disease. In the early 1990's, the Director General 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) The aim of this study is to be able to establish the efficacy of *Echinacea angustifolia* tincture upon *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis* so as to be able to employ a suitable antimicrobial alternative to allopathic agents. Furthermore, with the increasing number of HIV infections, the antimicrobial properties of *Echinacea angustifolia* tincture may be of additional therapeutic benefit in the fight against the superficial opportunistic infections that arise in these immune compromised patients.

This study endeavoured to establish the effects of *Echinacea angustifolia* tincture in both a 30% ethanol dilution and a 62% ethanol dilution upon the above mentioned pathogens in order to determine if the tincture had any greater antimicrobial effect upon these microorganisms than ethanol of the same concentrations.

1.2. PROBLEM STATEMENT

The purpose of this study is to investigate the efficacy of the antimicrobial properties of *Echinacea angustifolia* tincture against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis* in terms of the Kirby-Bauer Antimicrobial Sensitivity Test in order to determine its uses in antimicrobial applications.

1.3. SUBPROBLEMS

1.3.1. SUBPROBLEM ONE

To compare the efficacy of *Echinacea angustifolia* tincture in 30% ethanol to the 30% ethanol control against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *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 *Echinacea angustifolia* tincture in 62% ethanol to the 62% ethanol control against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas*

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

1.4. HYPOTHESES

1.4.1. HYPOTHESIS ONE

Echinacea angustifolia tincture in 30% ethanol will have an antimicrobial effect on *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis*.

1.4.2 HYPOTHESIS TWO

Echinacea angustifolia tincture in 62% ethanol will have an antimicrobial effect on *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis* to a greater extent than 62% ethanol.

1.5. DELIMITATIONS

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

1.5.2. This study was limited to a specific subspecies of the Echinacea species viz. *Echinacea angustifolia*.

1.5.3. Only the following growth media were used :

A) Mueller-Hinton Agar

B) Nutrient Broth

1.5.4. Only *Echinacea angustifolia* tinctures produced in 30% and 62% 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.

1.6. ASSUMPTIONS

1.6.1. The First Assumption

All cultures of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis* were grown under optimal conditions.

1.6.2. The Second Assumption

The preparation of the herbal tinctures was done according to the phytotherapeutic principles as laid down in the British Pharmacopoeia.

1.7. DEFINITIONS

1.7.1. Tincture : An alcoholic or hydroalcoholic solution prepared from animal or vegetable drugs or chemical substances.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. INTRODUCTION

Herbal medicine or phytotherapy is the science of using herbal remedies to treat the ill. Plant drugs or phytopharmaceuticals are usually considered not to have any appreciable toxic effects and can therefore be safely used over a long period of time. Plant drugs do not have standardised drug principles that entirely determine the drug action of the plant but rather a complex of active principles in the plant interact with each other to produce a therapeutic action. (Weiss, 1991:1)

Today, there are a wide range of dispensable forms of plant drugs with tinctures being the most favoured due to it's ability to remain fairly sterile which can be attributed to its alcohol content. (Weiss, 1991:1)

2.2. ECHINACEA ANGUSTIFOLIA

This flowering plant is commonly referred to as the purple coneflower and belongs to the *Echinacea* species.

2.2.1. Habitat and Cultivation

Echinacea angustifolia is a perennial native to the central parts of the United States of America and is now commercially cultivated in both the United States and Europe. *Echinacea angustifolia* thrives best in rich, sandy soil and grows up to a height of 50 centimeters. It is usually cultivated only once it has been growing for four years and cultivation usually occurs in autumn. (Chevallier, 1996:90)

2.2.2. History and Traditional Uses

Echinacea angustifolia was used by the North American native tribes for a wide range of ailments and was regarded as a very versatile medicinal plant. (Ody, 1996:138) The Comanches used *Echinacea angustifolia* as a remedy for toothache and sore throats while the Sioux used it for snakebites, rabies and septic conditions. (Chevallier, 1996:90) Interest in the medicinal properties of *Echinacea angustifolia* were only initiated in the 1930's in Germany when its highly effective antibiotic actions were first noted. (Ody, 1996:138) However,

Echinacea angustifolia was introduced into Western American medicine in 1885 by Dr. H.C. Meyer who used it as a "blood purifier" (Tyler, et al. 1981:480)

2.2.3. Current Uses

Western uses of the *Echinacea* species has now varied and today *Echinacea angustifolia* is well known among herbal remedies for its immune-stimulating properties. (Chevallier, 1996:90) Research has shown that when *Echinacea angustifolia* is given internally, it enhances resistance to microorganisms, stimulates the lymphatic vascular system and fibroblastic activity. (Weiss, 1991:229) It is also known for possessing a strong activating force on the macrophage-mediated defence component of the immune system thereby increasing the body's capability to fight invading microorganisms. (McKenna, 1997:60) *Echinacea angustifolia* possesses a polysaccharide, echinacin B, which apparently forms a complex with hyaluronic acid, a component of human cell membranes. This makes the cell membranes resistant to attack by hyalurodinase, an enzyme produced by certain bacteria which prevents wound healing. (Tyler, et al. 1981:89) Of greatest relevance to this research is the antimicrobial properties that *Echinacea angustifolia* possess, mainly its bacteriostatic action, which prevents bacterial growth and reproduction. (Weiss, 1991:326)

2.2.4. Constituents

The key constituents in *Echinacea angustifolia* are the alkamides, mostly isobutylamides, caffeic acid esters, mainly echinacoside and cynarin, polysaccharides, a volatile oil, humelene, echinolone and betaine. The component that is of most importance in this research is echinacoside, which possesses bacteriostatic properties. (Weiss 1991:326)

2.2.5. Preparations

The root of *Echinacea angustifolia* is commonly used as a tincture or in capsule form for its immune stimulating properties or as a decoction for gargling in pharyngitis. The dosage for *Echinacea angustifolia* differs but not drastically and is generally taken as 10 to 40 drops of the tincture (Cruden, 1997:126), 500mg capsules three times daily or gargling with approximately 50ml of the decoction three times daily (Chevallier, 1996:90).

2.3. STREPTOCOCCUS PYOGENES

2.3.1. Classification

Streptococcus pyogenes belongs to the genus *Streptococcus* of the family Streptococcaceae.
(Howard, et al. 1995:255)

2.3.2. Morphology and Identification

Streptococcus pyogenes are gram-positive facultative aerobes that grow in chains and are also referred to as Group A beta-hemolytic streptococci because the toxins produced by these bacteria causes complete hemolysis of red blood cells. *Streptococcus pyogenes* form small, dome-shaped colonies (0.5 to 1.0mm) and require microaerophilic incubation to grow.
(Howard, et al. 1995:260 ; Wilson, et al. 1979:320)

2.3.3. Epidemiology

Streptococcus pyogenes is usually transmitted via droplet spread and with man usually being a reservoir for these organisms. Human carriers (both symptomatic and asymptomatic) harbour these organisms in their respiratory systems and shed them in their respiratory secretions. Direct and indirect contact with temporarily contaminated food or drink (like milk) also promotes their transmission. (Wilson, et al. 1979:321)

2.3.4. Growth Characteristics

Streptococcus pyogenes like most streptococci grow best on nutritionally fertile media with preference for complex media like brain-heart infusions and blood or serum agar. These facultative organisms can grow both aerobically and anaerobically but anaerobic conditions prove to be more favourable to growth rates. (Howard, et al. 1995:260) Therefore, in laboratory procedures, microaerophilic incubation (where atmospheric oxygen is decreased and carbon dioxide is increased by burning a candle in an air-tight jar containing the bacterial cultures) is favoured for growth. (Wilson, et al. 1979:320)

2.3.5. Toxins and Enzymes

Streptococcus pyogenes have numerous extracellular and cell-associated products which play a large role in the virulence of these organisms. (Howard, et al. 1995:268)

2.3.5.1. Hemolysins

These toxins are responsible for the complete hemolysis of red blood cells which are characteristic of this group of streptococci. There are two types of hemolysins, viz. Streptolysin S and Streptolysin O. (Wilson, et al. 1979:317)

Streptolysin S binds to red blood cell membranes and causes lysis by an osmotic process while Streptolysin O binds to specific cholesterol in red blood cell membranes thereby creating rod-shaped structure which result in membrane abnormalities resulting in their lysis. (Howard et al., 1995:268)

2.3.5.2. Erythogenic Toxin

This soluble toxin is released by the streptococci and travel from the site of infection via the blood stream to distant sites where it causes changes in the blood flow of the area. These toxins have an affinity for the superficial blood vessels of the skin where its action results in a diffuse reddening of the skin typical of a scarlatinal rash. (Wilson et al., 1979:317)

2.3.6. *Streptococcus pyogenes* Infections

Group A beta-hemolytic streptococci (*Streptococcus pyogenes*) are the most virulent species of streptococci. It causes pharyngitis, tonsillitis, wound and skin infections, septicemia, scarlet fever, pneumonia, rheumatic fever and glomerulonephritis. The most common type of streptococcal disease is primary pharyngeal infection with Group A beta-hemolytic streptococci. (Berkow, 1992:90) This is often treated topically by the use of mouthwashes, containing antimicrobial agents with simultaneous administration of systemic antibiotics.

However, *Streptococcus pyogenes* infections of the skin is not uncommon and include conditions like impetigo (streptococcal pyoderma), erysipelas, cellulitis and myositis.

Impetigo is a superficial cutaneous infection characterised by crusted lesions. Erysipelas and cellulitis however involve the deeper cutaneous layers where myositis is a potentially life-threatening infection of muscular tissue. The bacteria first colonise the skin and then invade the soft tissues via abrasions, minor trauma or insect bites, especially if the injured area is untreated (Howard, et al. 1995:264)

2.4. STAPHYLOCOCCUS AUREUS

2.4.1. Classification

Staphylococcus aureus belongs to the genus *Staphylococcus* of the family Micrococcaceae. (Wilson et al., 1979:93)

2.4.2. Morphology and Identification

Staphylococcus aureus are gram-positive bacteria that form smooth circular colonies that are beige to yellow in colour. *Staphylococcus aureus* are classified as a coagulase-positive staphylococci because it possesses the enzyme coagulase which has the ability to clot blood plasma. (Howard et al., 1995:245)

2.4.3. Epidemiology

Staphylococci are normally carried in the anterior nares of about 30% and on the skin of about 20% of healthy adults while hospital patients or personnel have slightly higher rates. Antibiotic-resistant strains are especially common in hospitals. Patient-to-patient transmissions via the hands of personnel are the most common and important means of spread. (Berkow et al., 1992:86)

2.4.4. Growth Characteristics

Staphylococcus aureus are facultative anaerobes and able to grow on any nutrient media.

Due to the fact that it does not have any hemolytic abilities, it does not cause hemolysis of blood cells on blood agar. (Wilson et al., 1979:519)

2.4.5. Toxins and Enzymes

Staphylococcus aureus is the primary toxin-producing type of staphylococci and produces a range of extracellular products viz. hemolysins, leukocidin, enterotoxins, exfoliatin and enzymes. (Howard et al., 1995:252)

2.4.5.1. Hemolysins

Staphylococcus aureus produce four types of hemolysins viz. alpha, beta, gamma and delta. The exact roles of each type of hemolysin is unknown but it is believed that alpha hemolysin has dermonecrotic activity as well as hemolytic abilities. (Howard et al., 1995:252)

2.4.5.2. Leukocidin

This toxin produced by *Staphylococcus aureus* is capable of causing lysis of human leukocytes and macrophages. (Howard et al., 1995:252)

2.4.5.3. Enterotoxin

There are five distinct types of enterotoxins (labelled as A through E) produced by *Staphylococcus aureus*. These toxins are responsible for staphylococcal food poisoning and conditions such as toxic shock syndrome which results when staphylococci grow and thrive within a cavity on the human body. (Howard, et al. 1995:253)

2.4.5.4. Exfoliatin

Exfoliatin is a toxin which has an epidermolytic or exfoliative effect. It causes layers within the epidermis of the skin to split thereby causing exfoliation of the skin. (Howard, et al. 1995:253)

2.4.5.5. Enzymes

There are numerous enzymes produced by staphylococci viz. lipase, fibrinolysin and a variety of proteases. However, coagulase-positive staphylococci, like *Staphylococcus aureus*, produce the enzyme coagulase, which not only causes hemolysis but also inhibits the bactericidal activity of normal serum. (Howard, et al. 1995:253)

2.4.6. Staphylococcal Infections

When considering staphylococcal infections, it is important to account for the two types of staphylococci viz. those that are coagulase-positive staphylococci and those that are coagulase negative staphylococci. Coagulase-positive staphylococci mean that the species of staphylococcus contains one or both types of coagulase enzymes which causes the production of an insoluble fibrin clot once the staphylococcus makes contact with the bloodstream while coagulase-negative staphylococci do not contain such enzymes. Of the two types of staphylococci, *Staphylococcus aureus* constitutes the largest cause of staphylococcal infections due to coagulase-positive staphylococci while *Staphylococcus epidermidis* is the largest cause of coagulase-negative staphylococci infections. Although most staphylococcal infections can be attributed to coagulase-positive staphylococci viz. *Staphylococcus aureus*, 4% of all bacterial urinary tract infections and 12% of wound infections are caused by coagulase-negative staphylococci. *Staphylococcus epidermidis* are commensal organisms that commonly occur on the skin and is also the biggest cause of coagulase-negative staphylococcal septicemia. (Howard, et al. 1995:251)

Local skin infections are by far the most common of all types of staphylococcal infections. Folliculitis is the most superficial of these skin infections. This condition follows the infection of hair follicle and is manifested by minute erythematous nodules around the follicle. The surrounding skin and deeper tissues are not affected. If folliculitis spreads to involve the subcutaneous tissue, the lesion is called a furuncle or boil which are characterised by pus. More serious invasive staphylococcal conditions are not very common in healthy individuals but can occur in persons whose host defences have been compromised. Predisposing

conditions include injury to normal skin (e.g. traumatic wounds, burns and surgical incisions), prior viral infections (e.g. measles and influenza), leukocyte defects, deficiencies in humoral immunity, presence of foreign bodies (e.g. sutures, pacemakers and IV catheters), alteration of normal flora through use of antimicrobial agents to which *S.aureus* is not susceptible and in persons with miscellaneous illnesses (including diabetes mellitus, alcoholism, coronary artery disease and various malignant tumours). The presence of these conditions can lead to serious local or contiguous infections, such as sinusitis, mastitis and carbuncles (a series of furuncles) or large, indurated and painful lesions with multiple ineffective drainage sites. They may also result in invasion of the bloodstream. From the blood, the organisms can spread to numerous body sites. (Howard, et al. 1995:247)

Neonatal infections appear usually within six weeks after birth and most commonly present as pustular or bullous skin lesions which are generally located in the axillary, inguinal or neck skin folds. Microscopic examination of the pus discloses polymorphonuclear neutrophils and staphylococci, often within leukocytes. (Berkow, et al. 1992:86)

Nursing mothers who develop breast abscesses or mastitis one to four weeks postpartum are usually infected with antibiotic-resistant staphylococci, most probably derived from the nursery via the infant. This may be due to poor aseptic techniques in the nursery. (Berkow, et al. 1992:87)

Postoperative infections ranging from stitch abscesses to extensive wound involvement commonly are due to staphylococci. These infections appear within a few days or several weeks after an operation and may be delayed in onset if the patient received antibiotics at the

time of surgery. The toxic shock syndrome may occur as a complication of a post operative staphylococcal infection. (Berkow, et al. 1992:87)

Toxic shock syndrome is a syndrome characterised by high fever, vomiting, diarrhoea, confusion and skin rash that may rapidly progress to severe and intractable shock. This syndrome was first observed in children (8 to 17 years old) in 1978 but it was not until 1980 that a large number of cases began to be recognised. It was predominantly noted in young women (aged 13 to 52 years) all of whom were menstruating and used vaginal tampons. Although the number of reported cases have dropped since both the public and tampon manufacturers became aware of the problem, cases of toxic shock syndrome in menstruating women still do occur occasionally. About 15% of cases occur in men, usually postoperatively. The exact cause of toxic shock syndrome is unknown but almost all patients have found to be infected with toxin-producing strains of *Staphylococcus aureus*. In menstruating women it has been found in the vagina and in almost every case the affected women used vaginal tampons for the menses. Presumably, women most at risk are those with pre-existing S.aureus colonisation of the vagina who also used tampons regularly for their menses. (Berkow, et al. 1992:88)

Staphylococcal scalded skin syndrome is an acute, widespread erythematous process in which the epidermis peels off. It usually occurs in infants, young children, or immunosuppressed patients. Antibiotic-resistant staphylococci produce an epidermolytic toxin (exfoliatin) that splits off the upper part of the epidermis just beneath the granular cell layer. The toxin enters the circulation and affects the skin systematically, as in scarlet fever. Staphylococcal scalded skin syndrome may occur in epidemics in nurseries, with transmission

from infant to infant on the hands of personnel. Most frequently, colonised infants are the source, although nursery personnel may be nasal carriers of S.aureus. The disease is also seen sporadically in children, usually less than six years of age, and in immunosuppressed adults.

(Berkow, et al. 1992:234)

2.5. STAPHYLOCOCCUS EPIDERMIDIS

2.5.1. Classification

Staphylococcus epidermidis belongs to the genus *Staphylococcus* of the family Micrococcaceae. (Wilson et al., 1979:93)

2.5.2. Morphology and Identification

Staphylococcus epidermidis are gram-positive bacteria that are classified as a coagulase-negative staphylococci because it does not possess the enzyme coagulase unlike *Staphylococcus aureus*. (Howard et al., 1995:245)

2.5.3. Epidemiology

Staphylococcus epidermidis is a normal commensal of the human skin but can cause local or systemic infections if introduced by contaminated sutures or instruments or by any other break in the skin. (Wilson et al., 1979:93)

2.5.4. Growth Characteristics

Staphylococcus epidermidis are facultative anaerobes and are able to grow on any nutrient media. However, on blood agar, their typical beige to yellow pigmentation becomes evident in the pale circular colonies that they form. (Wilson, et al. 1979:93)

2.5.5. Toxins and Enzymes

Staphylococcus epidermidis does not produce as many enzymes and toxins as *Staphylococcus aureus*. (Howard, et al. 1995:252)

2.5.5.1. Hemolysins

Of the four types of hemolysins viz. alpha, beta, gamma and delta produced by staphylococci, *Staphylococcus epidermidis* is only known to produce delta hemolysin of which its role in infections is unknown. (Howard, et al. 1995:252)

2.5.5.2. Exfoliatin

This is a toxin which has an epidermolytic or exfoliative effect. It causes layers within the epidermis of the skin to split thereby causing exfoliation of the skin. (Howard, et al. 1995:253)

2.5.5.3. Enzymes

There are numerous enzymes produced by staphylococci viz. lipase, fibrinolysin and a variety of proteases. However, coagulase-negative staphylococci, like *Staphylococcus epidermidis*, do not produce the enzyme coagulase, which plays a large role in blood clotting and bactericidal activity. (Howard et al., 1995:253)

2.5.6. Staphylococcal Infections

This aspect has been discussed concurrently with the Staphylococcal Infections caused by *Staphylococcus aureus* since the pathogenicity of these two microorganisms are so similar and overlap each other.

2.6. PSEUDOMONAS AERUGINOSA

2.6.1. Classification

Pseudomonas aeruginosa belongs to the genus *Pseudomonas* of the family Pseudomoadaceae. (Wilson et al., 1979:91)

2.6.2. Morphology and Identification

Pseudomonas aeruginosa are strictly aerobic, gram-negative rods that form large colonies (approximately 2mm in diameter) and produce a metallic sheen on blood agar. (Wilson et al., 1979:91 ; Howard et al., 1995:341)

2.6.3. Epidemiology

Pseudomonas aeruginosa can be found occasionally in the axilla and the anogenital areas of normal skin but rarely in stools of adults unless antibiotics are being given. The organism is commonly a contaminant of lesions populated with more virulent organisms, but occasionally it causes infection in tissues that are exposed to the external environment. (Berkow et al., 1992:116)

2.6.4. Growth Characteristics

Pseudomonas aeruginosa can grow well on any nutrient media and produce a characteristic greenish-blue pigment.(Howard et al., 1995:341)

2.6.5. Toxins and Enzymes

2.6.5.1. Proteases

The main proteases produced by *Pseudomonas aeruginosa* are elastase and a nonelastolytic alkaline proteinase. These proteases degrade a wide variety of host proteins involved in maintaining host resistance to infection and homeostasis. (Howard et al., 1995:363-364)

2.6.5.2. Exotoxin A

This is the most toxic product produced by *Pseudomonas aeruginosa*. It causes cellular damage and is toxic to macrophages. (Howard et al., 1995:363-364)

2.6.5.3. Phospholipase

The phospholipase destroys pulmonary surfactant and is also a potent inflammatory mediator. (Howard et al., 1995:363-364)

2.6.6. Pseudomonas aeruginosa Infection

The most serious infections occur in debilitated patients with diminished resistance resulting from other diseases or therapy. Pseudomonas infection can develop in many anatomic locations, including skin, subcutaneous tissue, bone, ears, eyes, urinary tract, and heart valves. The site varies with the portal of entry and the patient's particular vulnerability. In burns, the region below the epidermis can become heavily infiltrated with organisms, serving as a focus for a subsequent septicemia, an often-lethal complication of burns. Clinical presentation depends on the site involved. (Berkow, et al. 1992:116)

Pseudomonas aeruginosa septicemia may occur in infants, immunocompromised patients and burn patients with the mortality rate associated with septicemia being the highest for any bacterial nosocomial pathogen. Although not always present, ecthyma gangrenosum maybe a clue to P.aeruginosa septicemia. These skin lesions appear as a round, indurated, purple, blanched area about one centimetre in diameter with an ulcerated centre and surrounding zone of erythema. Lesions are most commonly seen on the buttocks, extremities and perineum.(Howard, et al. 1995:361)

Pseudomonas aeruginosa causes approximately 70% of the cases of otitis externa. This is frequently observed among swimmers ("swimmer's ear") and is one of the few *Pseudomonas* infections that occur in healthy people. Folliculitis, a dermatologic infection characterised by a syndrome of malaise, mastitis, otitis externa and rash may also occur in healthy hosts (folliculitis discussed above under staphylococcal infections). This condition results from

bathing in contaminated water and has been associated with the use of whirlpool baths and swimming pools.

The most serious type of the infection caused by *Pseudomonas aeruginosa* is malignant otitis externa. The condition was most frequently seen in diabetic patients but now appears to be an increasing clinical condition in children with chronic illness or immunosuppression, with 73% of cases reported since 1980. This condition begins as ordinary otitis externa but may fail to respond to therapy. The condition may spread to involve the temporal bone and to cause osteomyelitis of the base of the skull and paralysis of certain cranial nerves.

Pseudomonas aeruginosa may be responsible for the formation of corneal ulcers, generally following eye trauma involving a foreign body, but sometimes as a complication of mechanical ventilation. This infection arises from the organism harboured in the patient's respiratory tract. If not treated properly or sometimes despite treatment, the ulcer may progress to panophthalmitis (inflammation involving all the tissue of the eyeball) and blindness. Keratitis, manifesting itself as the granular epithelial keratopathy, has been reported in soft contact lens wearers. Other conditions such as meningitis, urinary tract infections, endocarditis and osteomyelitis have also been associated with *Pseudomonas aeruginosa*. (Howard, et al. 1995:361)

2.7. ESCHERICHIA COLI

2.7.1. Classification

Escherichia coli belongs to the genus *Escherichia* of the family Enterobacteriaceae. (Wilson et al., 1979:91)

2.7.2. Morphology and Identification

Escherichia coli are gram-negative rods which are facultatively anaerobic. (Wilson et al., 1979:91) These microorganisms are catalase-positive and therefore capable of hemolysis. (Howard et al., 1995:305)

2.7.3. Epidemiology

Escherichia coli normally inhabits the gastrointestinal tract. 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. (Berkow et al, 1992:101)

2.7.4. Growth Characteristics

Escherichia coli can grow on a range of nutrient media but the media of choice are usually media that inhibit the growth of gram-positive bacteria. The optimum temperature for *Escherichia coli* growth is 37°C but growth is possible at temperatures as high as 45°. (Howard et al., 1995:305)

2.7.5. Toxins and Enzymes

Escherichia coli produce two types of toxins, viz. LT (heat-labile enterotoxin) and ST (heat-stable enterotoxin). (Howard et al., 1995:325)

2.7.5.1. LT (Heat-Labile Enterotoxin)

These toxins bind to epithelial cell membranes causing these cells to hypersecrete their intracellular electrolytes and water. (Howard et al., 1995:325)

2.7.5.2. ST (Heat-Stable Enterotoxin)

These toxins bind to intestinal epithelial cells causing the production of an enzyme that causes diarrhoea. (Howard et al., 1995:325)

2.7.6. Escherichia coli Infections

Escherichia coli is also the most common cause of urinary tract infections constituting 90% of all acute bacterial urinary tract infections and 10% of all wound infections. (Howard et al, 1995:87)

Vulvovaginitis is an infectious disease and inflammatory condition affecting the vaginal mucosa and often secondarily involving the vulva. E.coli infection is the most common bacterial cause of vulvovaginitis in children and less commonly in adults. It usually causes vaginal discharge which is abnormal and has an offensive odour. Complications include salpinigits which is an infection and inflammation of the fallopian tubes, which is considered severe and can lead to infertility. (Berkow et al, 1992:1786)

Cellulitis is a diffuse, spreading, acute inflammation within solid tissues, characterised by hyperemia, leukocytic infiltration and oedema without cellular necrosis or suppuration. It is most commonly evident in the skin and subcutaneous structures but may involve deeper structures. *Escherichia coli* is one of the common causative organisms as well as *Enterococcus faecalis*. (Berkow et al, 1992:56)

2.8. ENTEROCOCCUS FAECALIS

2.8.1. Classification

Enterococcus faecalis is also referred to as *Streptococcus faecalis* and belongs to the genus *Streptococcus* of the family Streptococcaceae. (Howard et al., 1995:255)

2.8.2. Morphology and Identification

Enterococcus faecalis is a gram-positive coccus that occurs in either pairs or chains. (Wilson et al., 1979:94 ; Howard et al., 1995:258)

2.8.3. Epidemiology

Enterococcus faecalis are commensal organisms that are part of the normal intestinal flora. (Howard et al., 1979:94)

2.8.4. Growth Characteristics

Like other streptococci, *Enterococcus faecalis* thrives best on complex media and are capable of growing in both aerobic and anaerobic conditions. (Howard et al., 1995:260)

2.8.5. Toxins and Enzymes

Enterococcus faecalis do not produce any extracellular products but the presence of cellular products such as Group D carbohydrate-amino acid complex can cause immune-mediated responses by the host and this is the mechanism of pathogenicity of *Enterococcus faecalis*. (Howard, et al. 1995:269 ; Wilson, et al. 1979:318)

2.8.6. *Enterococcus faecalis* Infections

Enterococcus faecalis is one of the most common causes of infections caused by Group D streptococci and, along with *S.bovis*, are considered more pathogenic than the other Group D streptococci due to their ability to grow in 40% bile and to hydrolyze esculin. *E.faecalis* causes urinary tract infections (16% of all UTI's), abdominal sepsis, cellulitis and wound infections (13% of all wound infections) as well as concurrent bacteremia. (Berkow, et al. 1992:90 ; Howard, et al. 1995:87)

Enterococcus faecalis is also one of the organisms responsible for cellulitis which is discussed under *Escherichia coli* infections.

CHAPTER THREE

3.0. METHODOLOGY

3.1. THE DATA , TREATMENT AND THEIR INTERPRETATION

3.1.1. THE DATA

This research involves two types of data : primary and secondary. The nature of these data are as follows :

3.1.1.1. THE PRIMARY DATA

1. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 30% ethanol on *Streptococcus pyogenes*.
2. Results of the experiment determining the effects of 30% ethanol on *Streptococcus pyogenes*.
3. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 62% ethanol on *Streptococcus pyogenes*.
4. Results of the experiment determining the effects of 62% ethanol on *Streptococcus pyogenes*.

5. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 30% ethanol on *Staphylococcus aureus*.
6. Results of the experiment determining the effects of 30% ethanol on *Staphylococcus aureus*.
7. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 62% ethanol on *Staphylococcus aureus*.
8. Results of the experiment determining the effects of 62% ethanol on *Staphylococcus aureus*.
9. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 30% ethanol on *Pseudomonas aeruginosa*.
10. Results of the experiment determining the effects of 30% ethanol on *Pseudomonas aeruginosa*.
11. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 62% ethanol on *Pseudomonas aeruginosa*.
12. Results of the experiment determining the effects of 62% ethanol on *Pseudomonas aeruginosa*.
13. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 30% ethanol on *Staphylococcus epidermidis*.
14. Results of the experiment determining the effects of 30% ethanol on *Staphylococcus epidermidis*.
15. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 62% ethanol on *Staphylococcus epidermidis*.
16. Results of the experiment determining the effects of 62% ethanol on *Staphylococcus epidermidis*.

17. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 30% ethanol on *Escherichia coli*.
18. Results of the experiment determining the effects of 30% ethanol on *Escherichia coli*.
19. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 62% ethanol on *Escherichia coli*.
20. Results of the experiment determining the effects of 62% ethanol on *Escherichia coli*.
21. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 30% ethanol on *Enterococcus faecalis*.
22. Results of the experiment determining the effects of 30% ethanol on *Enterococcus faecalis*.
23. Results of the experiment determining the effects of *Echinacea angustifolia* tincture in 62% ethanol on *Escherichia coli*.
24. Results of the experiment determining the effects of 62% ethanol on *Enterococcus faecalis*.

3.1.1.2. THE SECONDARY DATA

Research articles from journal publications , books and manuals were referred to.

3.2. CRITERIA GOVERNING THE ADMISSIBILITY OF DATA

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

3.3. MATERIALS AND METHODS

3.3.1. Preparation of Media

Mueller-Hinton Agar and Nutrient Broth were the media used in the experiments. They were prepared according to the manufacturer's directions. A total of 72 agar plates containing the agar was produced for this research study.

3.3.2. Preparation of Inoculum

A single colony of each of the microorganisms being tested were introduced into separate bottles containing the nutrient broth. These cultures were then incubated for 24 hours.

3.3.3. Preparation of the Cultures

Each of the six culture of bacteria was used to aseptically streak and thereby inoculate 12 separate Mueller-Hinton agar plates per bacteria resulting in 72 inoculated agar plates.

3.3.4. Preparation of Filter Paper Discs

Filter paper was punched into discs approximately 5mm in diameter. These discs were then autoclaved at 121° C for 15 minutes to ensure that they were sterile.

3.3.5. Preparation of *Echinacea angustifolia* Tincture

Both tinctures, *Echinacea angustifolia* in 30% ethanol (Batch No. Exp. 033) and 62% ethanol (Batch No. Exp. 033) was produced by Parceval (Pty)Ltd. according to the British Homoeopathic Pharmacopoeia Method 4a resulting in tinctures of 1:10 concentrations (i.e. for every one part of *Echinacea angustifolia* root there is ten parts of ethanol).

3.3.6. Effect of *Echinacea angustifolia* in 30% ethanol on the six types of bacteria

Six agar plates containing different cultures (i.e. each plate contained a different type of bacteria being tested) was used for this part of the research. Each plate was labelled with the

name of the bacteria and as "*Echinacea angustifolia* 30% ethanol Test". Filter paper discs were immersed into the *Echinacea angustifolia* tincture in 30% ethanol and then removed with a sterile forceps. Five of these discs were then placed at different sites along the periphery of the culture on each agar plate. These plates were then incubated along with other test and control plates for 24 hours at 37° C. This is the Kirby-Bauer Method for Antimicrobial Sensitivity Testing. (Cappucino 1995:249) This procedure was done three times to ensure consistency of results.

3.3.7. Effect of 30% ethanol on the six types of bacteria

Six agar plates containing different cultures (i.e. each plate contained a different type of bacteria being tested) was used for this part of the research. Each plate was labelled with the name of the bacteria and as "30% ethanol Control". Filter paper discs were immersed into 30% ethanol and then removed with a sterile forceps. Five of these discs were then placed at different sites along the periphery of the culture on each agar plate. These plates were then incubated along with other test and control plates for 24 hours at 37° C. This is the Kirby-Bauer Method for Antimicrobial Sensitivity Testing. (Cappucino 1995:249) This procedure was done three times to ensure consistency of results.

3.3.8. Effect of *Echinacea angustifolia* in 62% ethanol on the six types of bacteria

Six agar plates containing different cultures (i.e. each plate contained a different type of bacteria being tested) was used for this part of the research. Each plate was labelled with the name of the bacteria and as "*Echinacea angustifolia* 62% ethanol Test". Filter paper discs were immersed into the *Echinacea angustifolia* tincture in 62% ethanol and then removed with a sterile forceps. Five of these discs were then placed at different sites along the periphery of the culture on each agar plate. These plates were then incubated along with other test and control plates for 24 hours at 37° C. This is the Kirby-Bauer Method for Antimicrobial Sensitivity Testing. (Cappucino 1995:249) This procedure was done three times to ensure consistency of results.

3.3.9. Effect of 62% ethanol on the six types of bacteria

Six agar plates containing different cultures (i.e. each plate contained a different type of bacteria being tested) was used for this part of the research. Each plate was labelled with the name of the bacteria and as "62% ethanol Control". Filter paper discs were immersed into 62% ethanol and then removed with a sterile forceps. Five of these discs were then placed at different sites along the periphery of the culture on each agar plate. These plates were then incubated along with other test and control plates for 24 hours at 37° C. This is the Kirby-Bauer Method for Antimicrobial Sensitivity Testing. (Cappucino 1995:249) This procedure was done three times to ensure consistency of results.

3.4. THE SPECIFIC TREATMENT OF EACH SUBPROBLEM

3.4.1. SUBPROBLEM ONE

The first subproblem was to compare the efficacy of *Echinacea angustifolia* tincture in 30% ethanol to the 30% ethanol control against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis* in terms of the size of the zones of inhibition.

Data Needed

- A. Average of the diameters of the zones of inhibition of the *Echinacea angustifolia* 30% ethanol test group.
- B. Average of the diameters of the zones of inhibition of the 30% ethanol control group.

Location of Data

The experiments were conducted at the Department of Biotechnology, Technikon Natal.

Means of Obtaining the Data

All data were collected by measuring the diameters of the zones of inhibition around each filter paper disc.

Treatment and Interpretation of Data

The average value of data collected had to be calculated.

$$\text{AVERAGE VALUE} = \frac{\text{SUM OF DIAMETERS}}{\text{NUMBER OF DISCS}}$$

The Mann-Whitney U-test will be used to perform intergroup comparisons between the test and control groups with the specified level of significance (α) being set at 0.05.

3.4.2. SUBPROBLEM TWO

The second subproblem was to compare the efficacy of *Echinacea angustifolia* tincture in 62% ethanol to the 62% ethanol control against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis* in terms of the size of the zones of inhibition.

Data Needed

- A. Average of the diameters of the zones of inhibition of the *Echinacea angustifolia* 62% ethanol test group.
- B. Average of the diameters of the zones of inhibition of the 62% ethanol control group.

Location of Data

The experiments were conducted at the Department of Biotechnology, Technikon Natal.

Means of Obtaining the Data

All data were collected by measuring the diameters of the zones of inhibition around each filter paper disc.

Treatment and Interpretation of Data

The average value of data collected had to be calculated.

$$\text{AVERAGE VALUE} = \frac{\text{SUM OF DIAMETERS}}{\text{NUMBER OF DISCS}}$$

The Mann-Whitney U-test will be used to perform intergroup comparisons between the test and control groups with the specified level of significance (α) being set at 0.05.

CHAPTER FOUR

4.0. RESULTS

4.1. Effect of *Echinacea angustifolia* tincture in 30% ethanol vs. 30% ethanol control on *Pseudomonas aeruginosa*

The *Echinacea angustifolia* tincture in 30% ethanol (Test Group A) and the 30% ethanol control (Control Group A) did not have any antimicrobial effect on *Pseudomonas aeruginosa* as would be evident by the zones of inhibition (Table 1) produced in the Kirby-Bauer Antimicrobial Sensitivity Test.

4.2. Effect of *Echinacea angustifolia* tincture in 30% ethanol vs. 30% ethanol control on *Staphylococcus aureus*

The *Echinacea angustifolia* tincture in 30% ethanol (Test Group A) and the 30% ethanol control (Control Group A) did not have any antimicrobial effect on *Staphylococcus aureus* as would be evident by the zones of inhibition (Table 3) produced in the Kirby-Bauer Antimicrobial Sensitivity Test.

4.3. Effect of *Echinacea angustifolia* tincture in 30% ethanol vs. 30% ethanol control on *Escherichia coli*

The *Echinacea angustifolia* tincture in 30% ethanol (Test Group A) and the 30% ethanol control (Control Group A) did not have any antimicrobial effect on *Escherichia coli* as would be evident by the diameter zones of inhibition (Table 5) produced in the Kirby-Bauer Antimicrobial Sensitivity Test.

4.4. Effect of *Echinacea angustifolia* tincture in 30% ethanol vs. 30% ethanol control on *Staphylococcus epidermidis*

The *Echinacea angustifolia* tincture in 30% ethanol (Test Group A) and the 30% ethanol control (Control Group A) did not have any antimicrobial effect on *Staphylococcus epidermidis* as would be evident by the diameter zones of inhibition (Table 7) produced in the Kirby-Bauer Antimicrobial Sensitivity Test.

4.5. Effect of *Echinacea angustifolia* tincture in 30% ethanol vs. 30% ethanol control on *Enterococcus faecalis*

The *Echinacea angustifolia* tincture in 30% ethanol (Test Group A) and the 30% ethanol control (Control Group A) did not have any antimicrobial effect on *Enterococcus faecalis* as would be evident by the diameter zones of inhibition (Table 9) produced in

the Kirby-Bauer Antimicrobial Sensitivity Test.

4.6. Effect of *Echinacea angustifolia* tincture in 30% ethanol vs. 30% ethanol control on *Streptococcus pyogenes*

The *Echinacea angustifolia* tincture in 30% ethanol (Test Group A) and the 30% ethanol control (Control Group A) did not have any antimicrobial effect on *Streptococcus pyogenes* as would be evident by the diameter zones of inhibition (Table 11) produced in the Kirby-Bauer Antimicrobial Sensitivity Test.

4.7. Effect of *Echinacea angustifolia* tincture in 62% ethanol vs. 62% ethanol control on *Pseudomonas aeruginosa*

The antimicrobial effect of *Echinacea angustifolia* tincture (Test Group B) produced in 62% ethanol on *Pseudomonas aeruginosa* did not display any significant statistical difference (Appendix H) in comparison to the antimicrobial effects of the control group of 62% ethanol (Control Group B) according to the diameters of the zones of inhibition produced by each group (Table 2). This can be observed in Appendix H where the p-value of this specific group was greater than the specified level of significance ($\alpha = 0.05$) divided by 2.

4.8. Effect of *Echinacea angustifolia* tincture in 62% ethanol vs. 62% ethanol control on *Staphylococcus aureus*

The antimicrobial effect of *Echinacea angustifolia* tincture (Test Group B) produced in 62% ethanol on *Staphylococcus aureus* did not display any significant statistical difference (Appendix H) in comparison to the antimicrobial effects of the control group of 62% ethanol (Control Group B) according to the diameters of the zones of inhibition produced by each group (Table 4). This can be observed in Appendix H where the p-value of this specific group was greater than the specified level of significance ($\alpha = 0.05$) divided by 2.

4.9. Effect of *Echinacea angustifolia* tincture in 62% ethanol vs. 62% ethanol control on *Escherichia coli*

The antimicrobial effect of *Echinacea angustifolia* tincture (Test Group B) in 62% ethanol on *Escherichia coli* showed a significant statistical difference (Appendix H) in comparison to the antimicrobial effects of the control group of 62% ethanol (Control Group B) as was evident by the diameter of the zones of inhibition (Table 6) caused by each substance. This can be observed in Appendix H where the p-value of this specific group was less than the specified level of significance ($\alpha = 0.05$) divided by 2.

4.10. Effect of *Echinacea angustifolia* tincture in 62% ethanol vs. 62% ethanol control on *Staphylococcus epidermidis*

The antimicrobial effect of *Echinacea angustifolia* tincture (Test Group B) in 62% ethanol on *Staphylococcus epidermidis* showed a significant statistical difference (Appendix H) in comparison to the antimicrobial effects of the control group of 62% ethanol (Control Group B) as was evident by the diameter of the zones of inhibition (Table 8) caused by each substance. This can be observed in Appendix H where the p-value of this specific group was less than the specified level of significance ($\alpha = 0.05$) divided by 2.

4.11. Effect of *Echinacea angustifolia* tincture in 62% ethanol vs. 62% ethanol control on *Enterococcus faecalis*

The antimicrobial effect of *Echinacea angustifolia* tincture (Test Group B) in 62% ethanol on *Enterococcus faecalis* showed a significant statistical difference (Appendix H) in comparison to the antimicrobial effects of the control group of 62% ethanol (Control Group B) as was evident by the diameter of the zones of inhibition (Table 10) caused by each substance. This can be observed in Appendix H where the p-value of this specific group was less than the specified level of significance ($\alpha = 0.05$) divided by 2.

4.12. Effect of *Echinacea angustifolia* tincture in 62% ethanol vs. 62% ethanol control on *Streptococcus pyogenes*

The antimicrobial effect of *Echinacea angustifolia* tincture (Test Group B) in 62% ethanol on *Streptococcus pyogenes* showed a significant statistical difference (Appendix H) in comparison to the antimicrobial effects of the control group of 62% ethanol (Control Group B) as was evident by the diameter of the zones of inhibition (Table 12) caused by each substance. This can be observed in Appendix H where the p-value of this specific group was less than the specified level of significance ($\alpha = 0.05$) divided by 2.

CHAPTER FIVE

5.0. DISCUSSION

5.1. SUBPROBLEM ONE

The *Echinacea angustifolia* tincture in 30% ethanol showed no effect on the six species of microorganisms tested viz. P.aeruginosa , S.aureus , E.coli , S.epidermidis , E.faecalis and S.pyogenes according to the diameter of the zones of inhibition that result according to the Kirby-Bauer Antimicrobial Sensitivity Test. The control group of 30% ethanol also yielded no results upon these six species of microorganisms. This would suggest that the antimicrobial component in *Echinacea angustifolia* is not present or not present in a sufficiently high concentration to produce any significant antimicrobial effect. This could possibly be due to the low ethanol concentration which is unable to extract certain alcohol-soluble components from the *Echinacea angustifolia* root source material.

The absence of any effect on the microorganisms by the 30% ethanol was expected since a 30% ethanol concentration is too low a concentration to exhibit any significant antimicrobial properties. (Ketchum, 1988:131)

5.2. SUBPROBLEM TWO

The *Echinacea angustifolia* tincture in 62% ethanol showed a significant difference in the diameters of the zones of inhibition to the 62% ethanol control for four of the six species of microorganisms tested viz. E.coli , S.epidermidis , E.faecalis and S.pyogenes. Therefore, this would suggest that these four microorganisms are sensitive to and cannot grow in the presence of the *Echinacea angustifolia* tincture in 62% ethanol.

However, for two species of microorganisms viz. P.aeruginosa and S.aureus , there was no significant difference between the diameters of the zones of inhibition between the *Echinacea angustifolia* tincture in 62% ethanol and the 62% ethanol control. This would suggest that these two microorganisms are not sensitive to and therefore not significantly affected by *Echinacea angustifolia* in 62% ethanol.

CHAPTER SIX

6.0. CONCLUSION

The purpose of this study was to investigate the efficacy of the antimicrobial properties of *Echinacea angustifolia* tincture against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis* in terms of the Kirby-Bauer Antimicrobial Sensitivity Test in order to determine its uses in antimicrobial applications.

The results of the experiments did not show any effect of *Echinacea angustifolia* in 30% ethanol on the microorganisms with the 30% ethanol control not having any effect on the microorganisms either. The *Echinacea angustifolia* tincture in 62% ethanol did have a significant difference in comparison to the 62% ethanol control but only on E.coli , S.epidermidis , E.faecalis and S.pyogenes. Conversely there was no significant statistical difference between the test and control groups on P.aeruginosa and S.aureus.

This implies that *Echinacea angustifolia* tincture in 62% ethanol is able to influence bacterial growth on certain bacteria whereas the tincture in 30% ethanol is totally ineffective.

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APPENDIX A

EFFECT OF TEST AND CONTROL SUBSTANCES ON P.AERUGINOSA

TABLE 1

Echinacea angustifolia tincture 30% ethanol (Test Group A) & 30% Ethanol (Control Group A)

P. aeruginosa	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 2	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 3	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 2

Echinacea angustifolia tincture 62% ethanol (Test Group B) & 62% Ethanol (Control Group B)

P. aeruginosa	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	12	10	11	10	10	10.6	12	9	9	10	11	10.2
TRIAL 2	10	10	8	10	10	9.6	9	6	10	6	8	7.8
TRIAL 3	12	11	10	10	9	10.4	9	9	10	11	8	9.4

APPENDIX B

EFFECT OF TEST AND CONTROL SUBSTANCES ON S.AUREUS

TABLE 3

Echinacea angustifolia tincture 30% ethanol (Test Group A) & 30% Ethanol (Control Group A)

S.aureus	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 2	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 3	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 4

Echinacea angustifolia tincture 62% ethanol (Test Group B) & 62% Ethanol (Control Group B)

S. aureus	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	11	10	10	9	8	9.6	10	10	9	9	10	9.6
TRIAL 2	9	8	10	8	10	9	9	9	8	8	10	8.8
TRIAL 3	10	8	8	10	10	9.2	10	8	8	8	6	8

APPENDIX C

EFFECT OF TEST AND CONTROL SUBSTANCES ON E.COLI

TABLE 5

Echinacea angustifolia tincture 30% ethanol (Test Group A) & 30% Ethanol (Control Group A)

E. coli	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 2	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 3	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 6

Echinacea angustifolia tincture 62% ethanol (Test Group B) & 62% Ethanol (Control Group B)

E. coli	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	12	11	12	10	11	11.2	10	10	10	11	10	10.2
TRIAL 2	11	10	11	11	11	10.8	10	9	9	10	9	9.4
TRIAL 3	11	10	12	11	11	11	10	11	10	10	9	10

APPENDIX D

EFFECT OF TEST AND CONTROL SUBSTANCES ON S.EPIDERMIDIS

TABLE 7

Echinacea angustifolia tincture 30% ethanol (Test Group) & 30% Ethanol (Control Group)

S. epidermidis	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 2	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 3	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 8

Echinacea angustifolia tincture 62% ethanol (Test Group) & 62% Ethanol (Control Group)

S. epidermidis	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	11	13	11	12	12	11.8	10	10	9	9	10	9.6
TRIAL 2	13	13	12	11	12	12.2	9	10	10	9	9	9.4
TRIAL 3	13	12	13	10	10	11.6	10	10	9	11	10	10

APPENDIX E

EFFECT OF TEST AND CONTROL SUBSTANCES ON E.FAECALIS

TABLE 9

Echinacea angustifolia tincture 30% ethanol (Test Group A) & 30% Ethanol (Control Group A)

E. faecalis	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 2	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 3	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 10

Echinacea angustifolia tincture 62% ethanol (Test Group B) & 62% Ethanol (Control Group B)

E. faecalis	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	10	10	9	11	9	9.8	8	8	8	7	8	7.8
TRIAL 2	10	10	10	9	10	9.8	9	7	7	8	8	7.8
TRIAL 3	10	8	9	10	10	9.4	8	9	8	8	9	8.4

APPENDIX F

EFFECT OF TEST AND CONTROL SUBSTANCES ON S.PYOGENES

TABLE 11

Echinacea angustifolia tincture 30% ethanol (Test Group A) & 30% Ethanol (Control Group A)

S. pyogenes	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 2	0	0	0	0	0	0	0	0	0	0	0	0
TRIAL 3	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 12

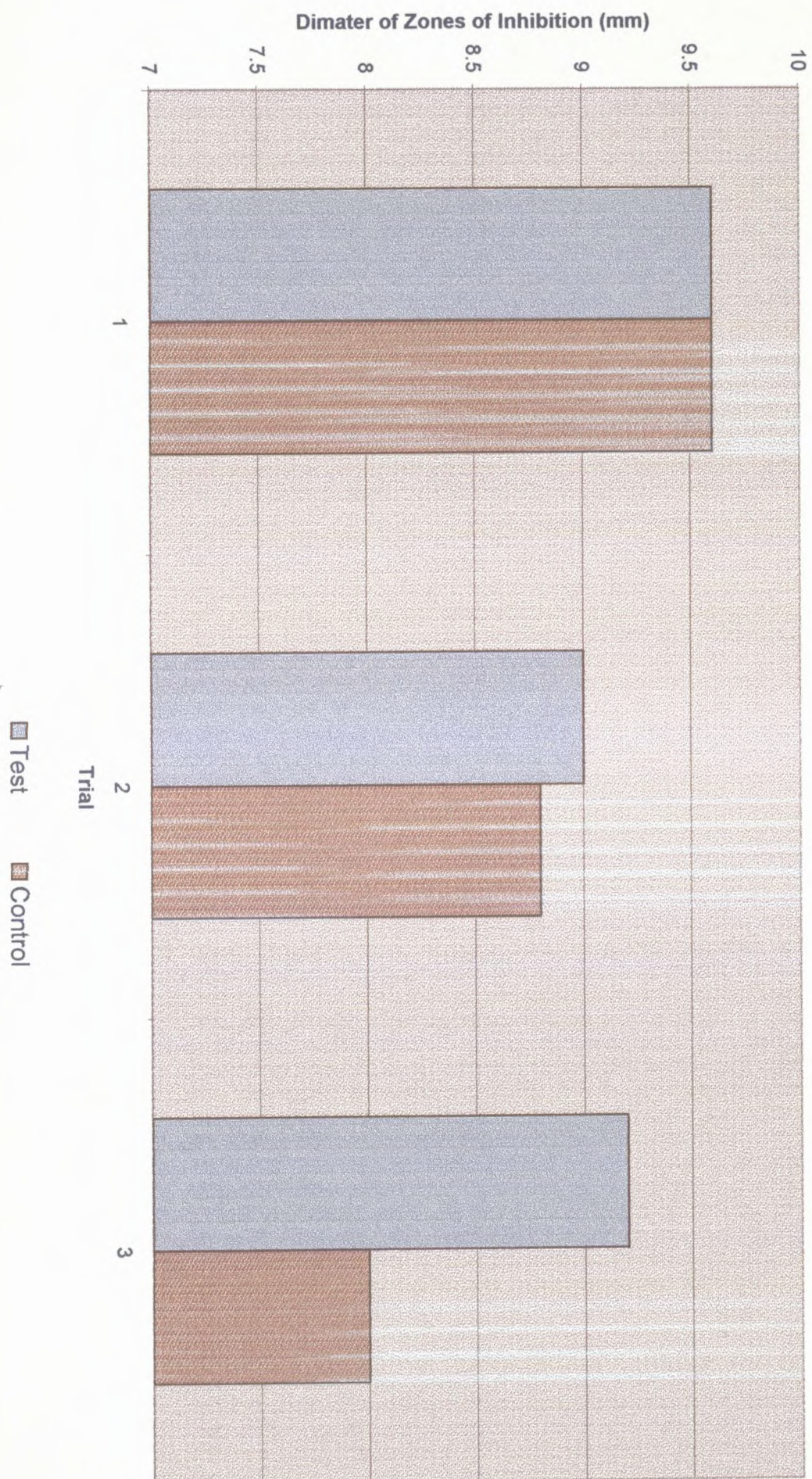
Echinacea angustifolia tincture 62% ethanol (Test Group B) & 62% Ethanol (Control Group B)

S. pyogenes	TEST (mm)						CONTROL (mm)					
	1	2	3	4	5	AV.	1	2	3	4	5	AV.
TRIAL 1	16	18	11	16	12	14.6	10	9	7	9	8	8.6
TRIAL 2	18	14	16	17	15	16	9	9	9	9	8	8.8
TRIAL 3	19	18	16	16	19	17.6	10	8	10	10	10	9.6

E.angustifolia tincture (62% ethanol) vs. 62% ethanol control on P.aeruginosa



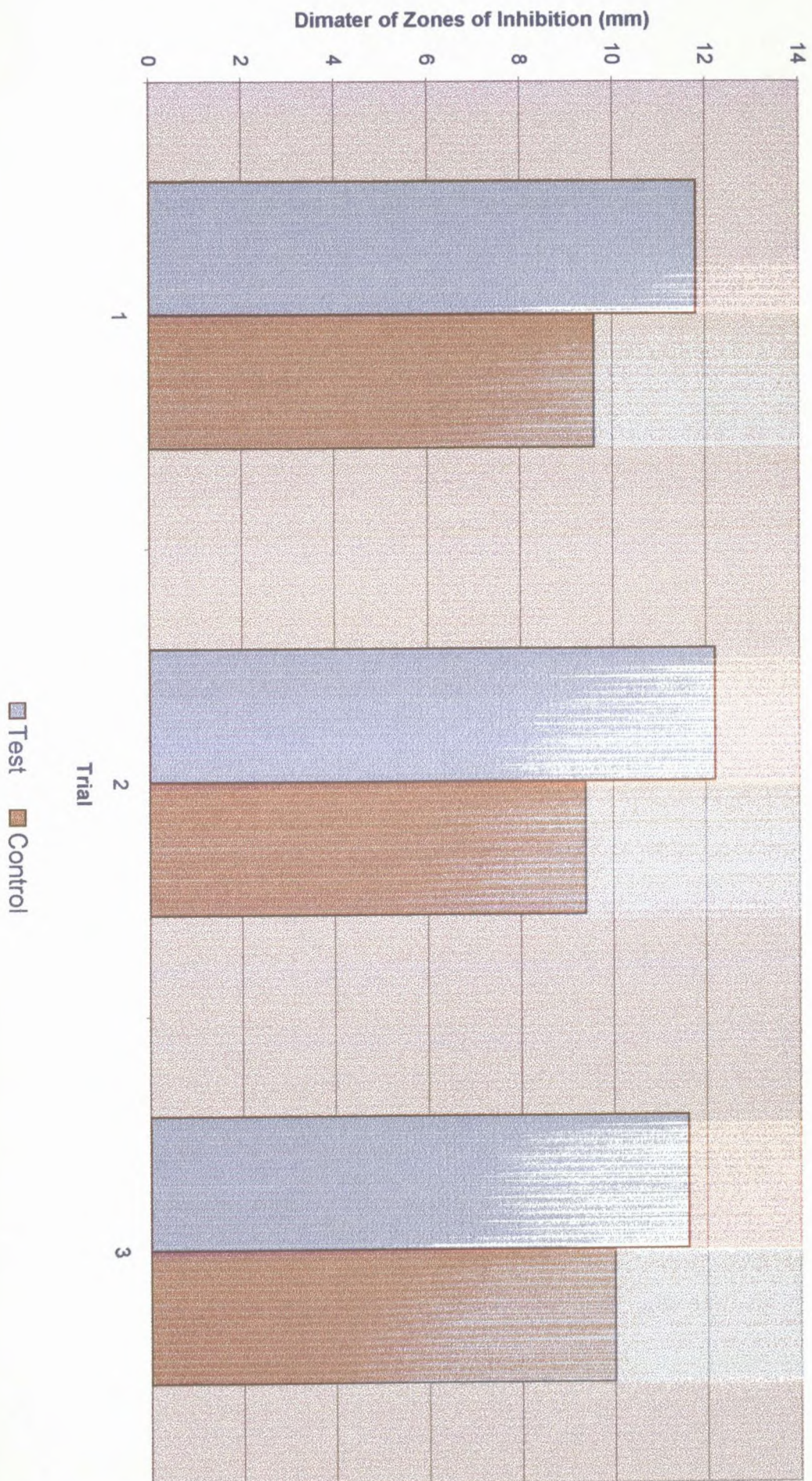
E.angustifolia tincture (62% ethanol) vs. 62% ethanol control on S.aureus



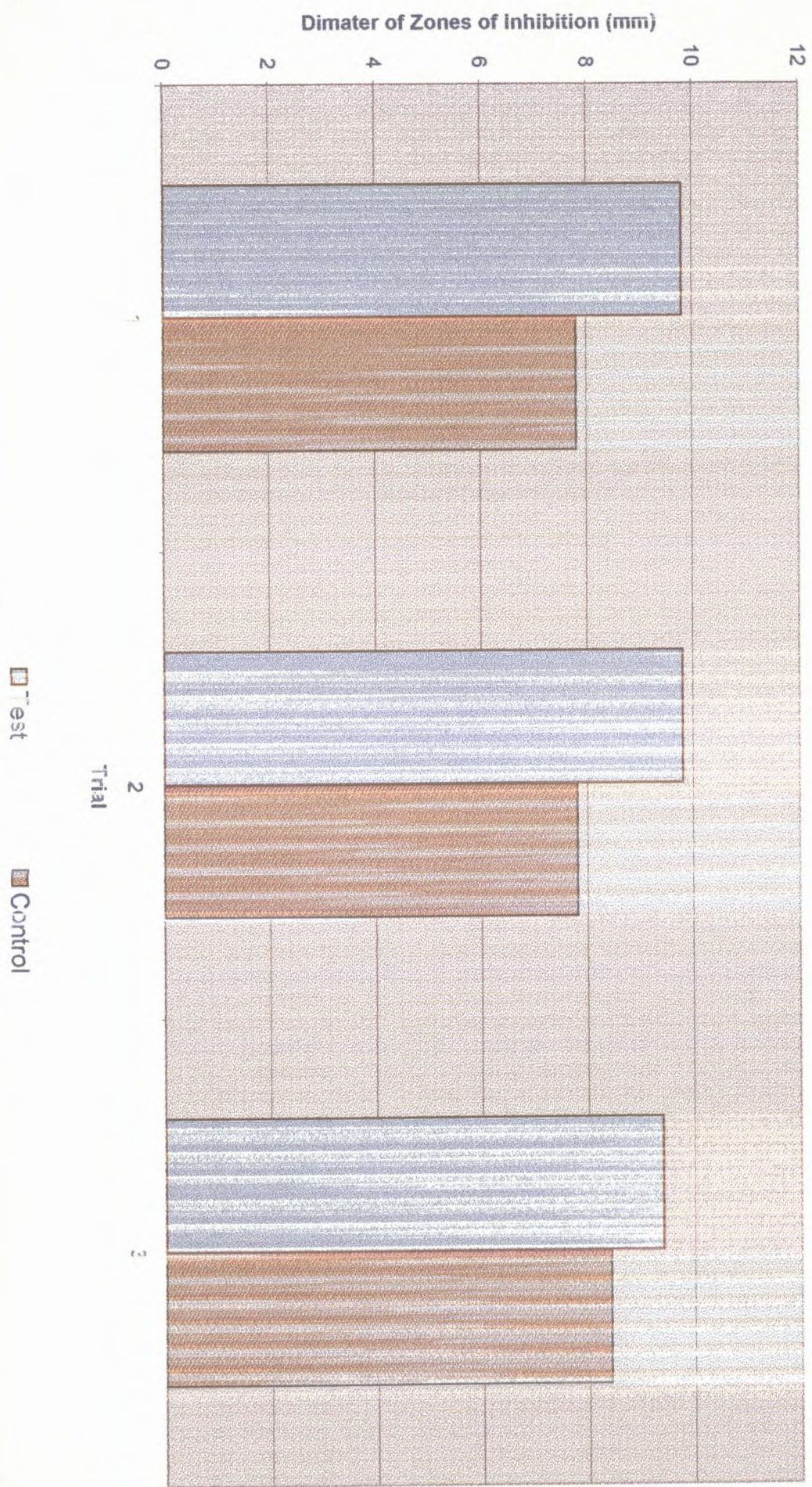
E.angustifolia tincture (62% ethanol) vs. 62% ethanol control on E.coli



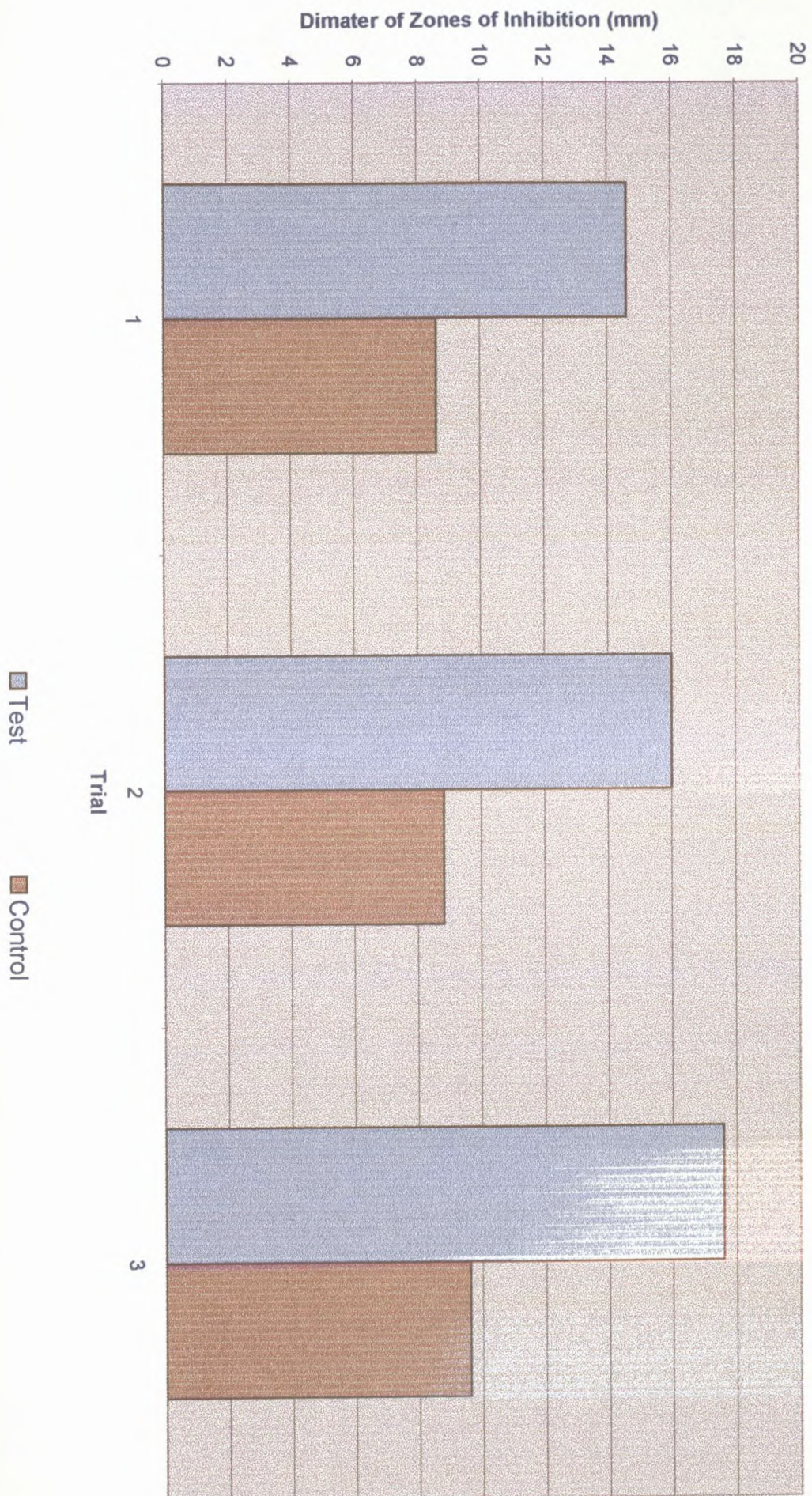
E. angustifolia* tincture (62% ethanol) vs. 62% ethanol control on *S.epidermidis



E.angustifolia tincture (62% ethanol) vs. 62% ethanol control on E.faecalis



E.angustifolia tincture (62% ethanol) vs. 62% ethanol control on S.pyogenes.



APPENDIX H

STATISTICAL ANALYSIS OF TEST GROUP B vs CONTROL GROUP B ACCORDING TO MANN-WHITNEY TEST

<u>GROUP NO.</u>	<u>BACTERIA</u>	<u>TEST AVERAGE</u>	<u>CONTROL AVERAGE</u>	<u>p-VALUE</u>
1	P.aeruginosa	10.2 mm	9.13 mm	0.045
2	S.aureus	9.26 mm	8.8 mm	0.302
3	E.coli	11 mm	9.86 mm	0.0
4	S.epidermidis	11.86 mm	9.67 mm	0.0
5	E.faecalis	9.67 mm	8 mm	0.0
6	S.pyogenes	16.07 mm	9 mm	0.0

Reject H_0 if $p \leq \alpha/2$ (2-tailed)

p-Value = Observed level of significance
(Refer to Appendix I)

α = 0.05 (Specified level of significance)

H_0 : No difference between the 2 tests being compared

H_1 : Significant difference between the 2 tests being compared

Group Number x : Test x + Control x

APPENDIX I

SPSS Printout

Mann-Whitney Test (Test vs Control)

Test Statistics

	TEST 1 vs CONTROL 1
Assymp. Sig. (2-tailed)	0.045

Test Statistics

	TEST 2 vs CONTROL 2
Assymp. Sig. (2-tailed)	0.302

Test Statistics

	TEST 3 vs CONTROL 3
Assymp. Sig. (2-tailed)	0.000

Test Statistics

	TEST 4 vs CONTROL 4
Assymp. Sig. (2-tailed)	0.000

Test Statistics

	TEST 5 vs CONTROL 5
Assymp. Sig. (2-tailed)	0.000

Test Statistics

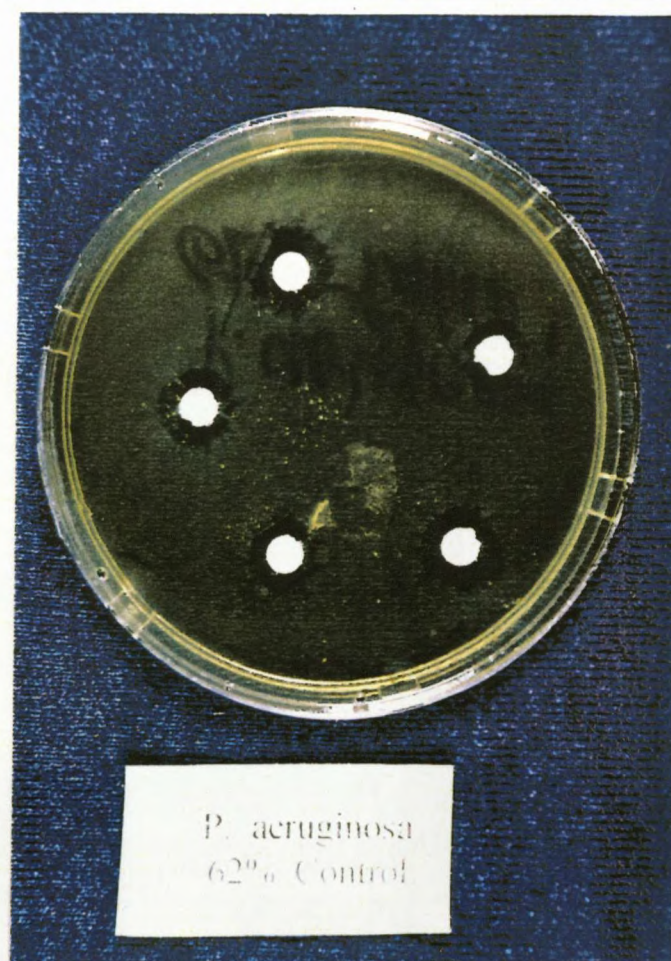
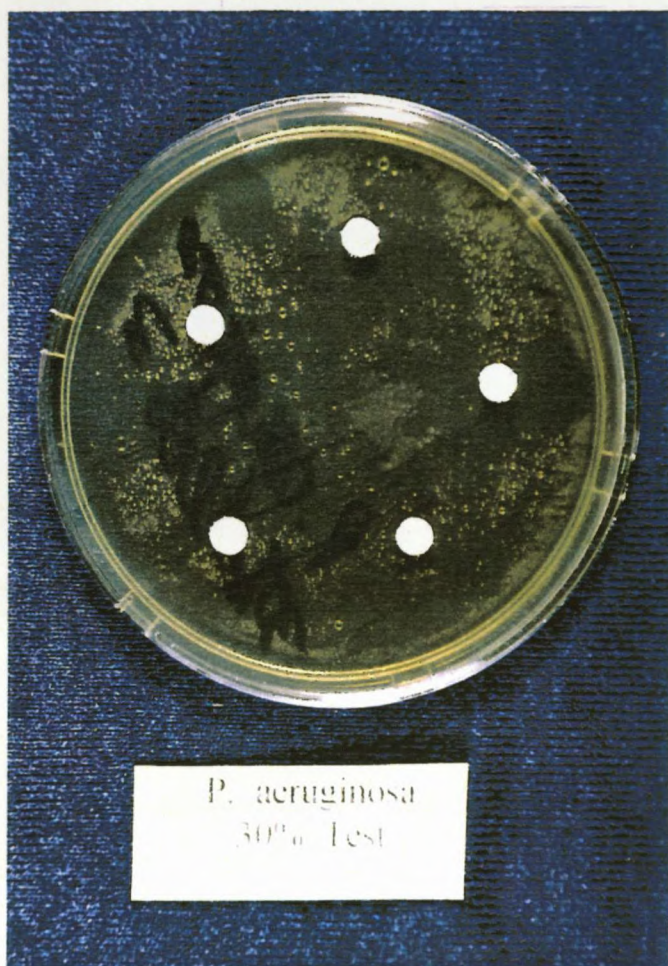
	TEST 6 vs CONTROL 6
Assymp. Sig. (2-tailed)	0.000

APPENDIX J

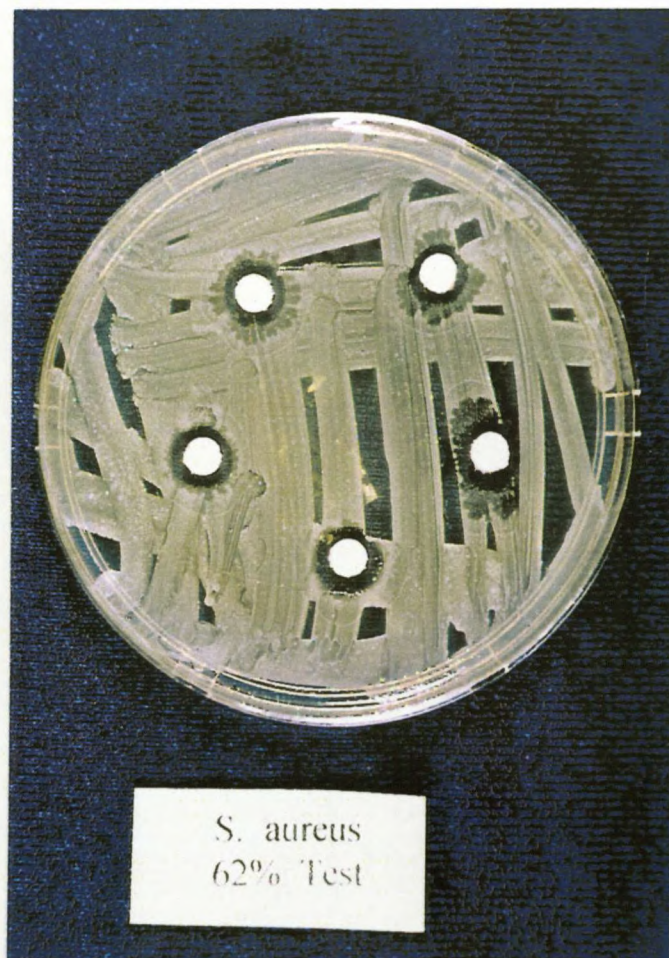
PHOTOGRAPHY OF CULTURES

- Effect of Test and Control Substances on *Pseudomonas aeruginosa*
- Effect of Test and Control Substances on *Staphylococcus aureus*
- Effect of Test and Control Substances on *Escherichia coli*
- Effect of Test and Control Substances on *Staphylococcus epidermidis*
- Effect of Test and Control Substances on *Enterococcus faecalis*
- Effect of Test and Control Substances on *Streptococcus pyogenes*

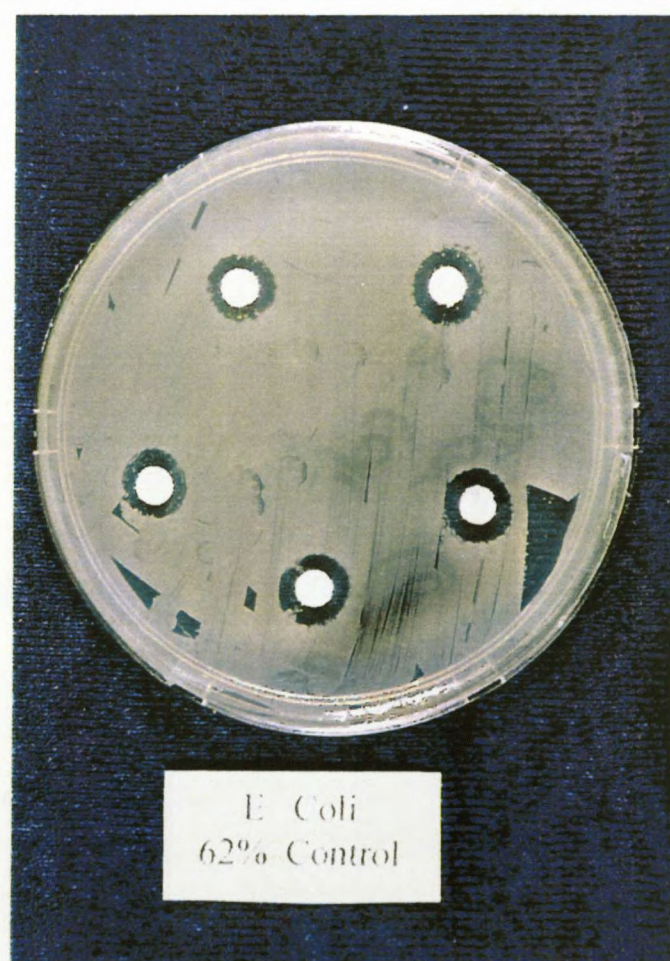
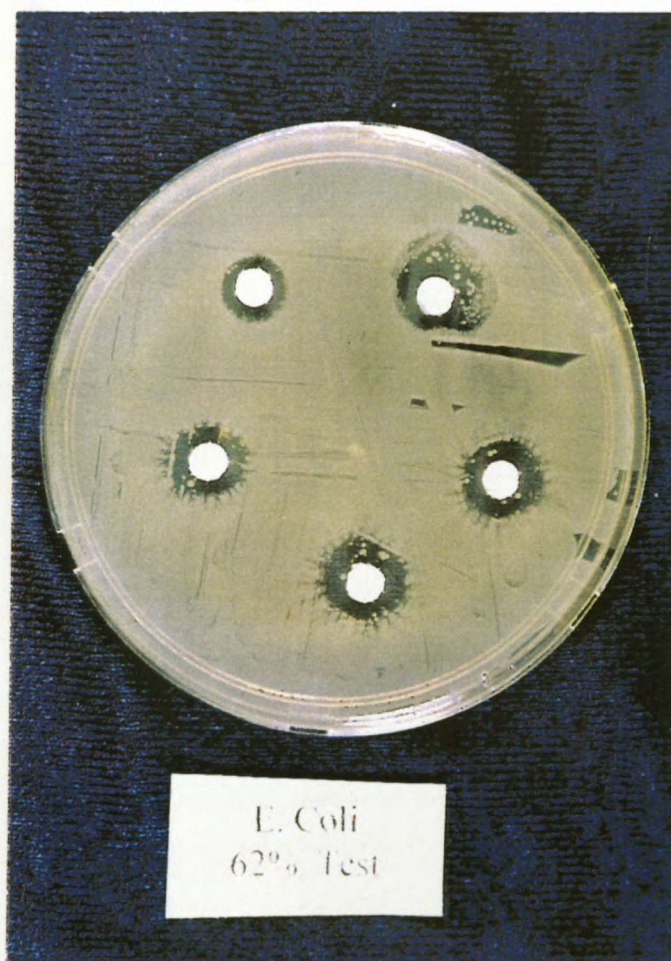
Effect of Test & Control Substances on *P.aeruginosa*



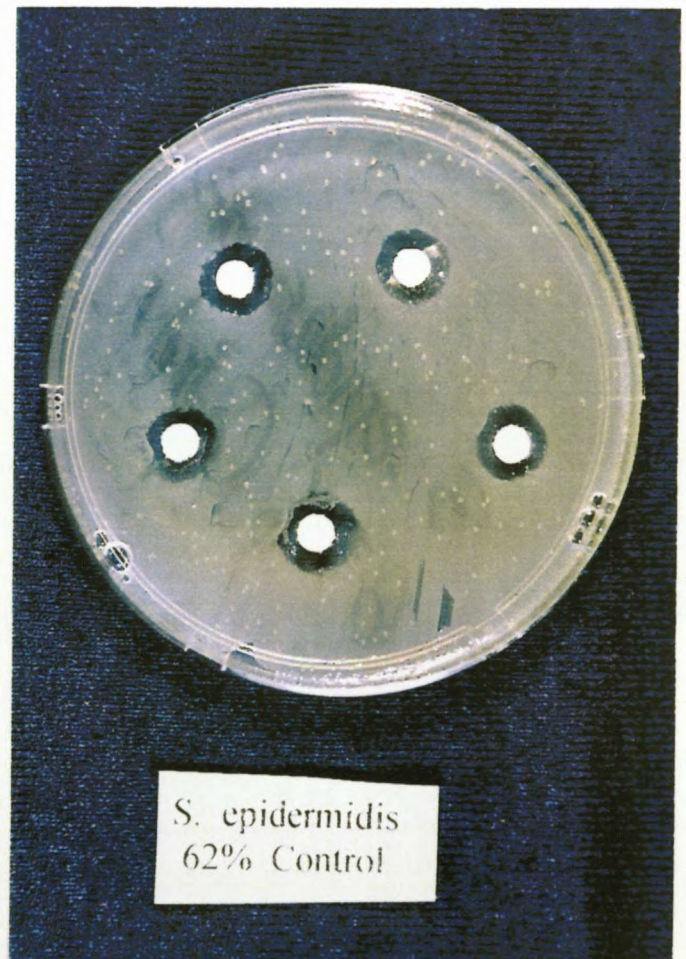
Effect of Test & Control Substances on *S.aureus*



Effect of Test & Control Substances on *E.coli*



Effect of Test & Control Substances on *S. epidermidis*



Effect of Test & Control Substances on *E. faecalis*



Effect of Test & Control Substances on *S. pyogenes*



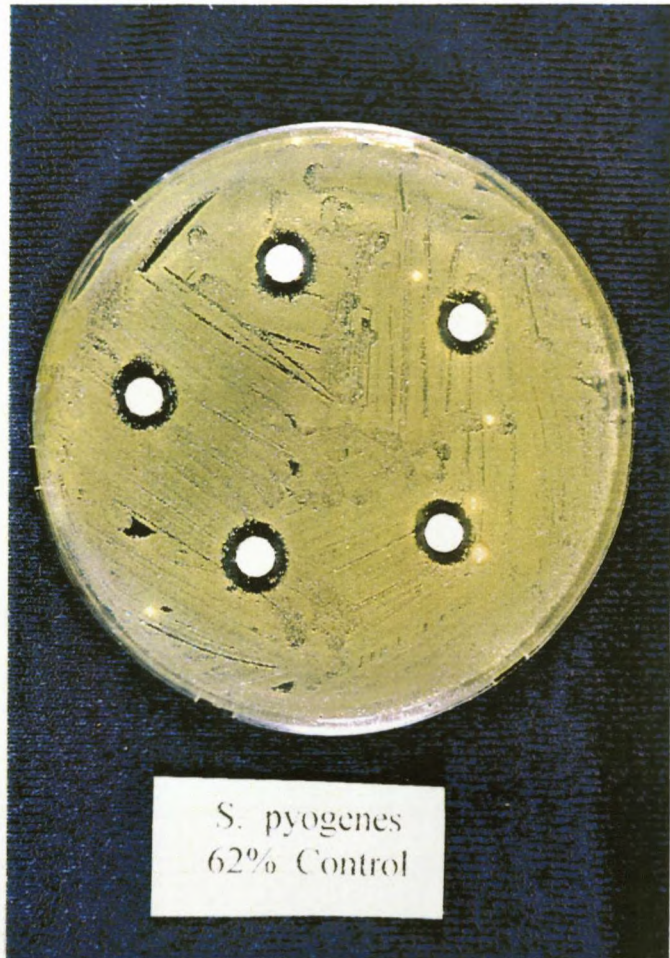
S. pyogenes
30% Test



S. pyogenes
30% Control



S. pyogenes
62% Test



S. pyogenes
62% Control