

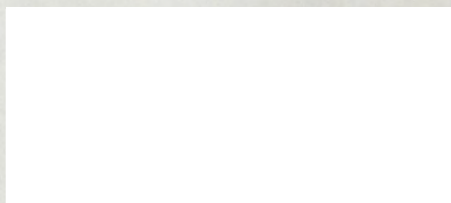
**THE EFFICACY OF CERTAIN LABIATIAE SPECIES HERBAL
EXTRACTS (*ROSMARINUS OFFICINALIS*, *SALVIA OFFICINALIS* AND
THYMUS VULGARIS) AS COMPARED TO NYSTATIN, IN THE
INHIBITION OF *IN VITRO* GROWTH OF *CANDIDIA ALBICANS*.**

BY

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Dissertation submitted in partial compliance with the requirements
of the Master's Degree in Technology: Homoeopathy in the
Faculty of health at Technikon Natal.

I, Kim Louise Reid do declare that this dissertation represents my own work
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DEDICATION

I would like to dedicate this dissertation to my parents, Alan Reid and Rachel Dobson for all their support, love and encouragement over the past six years.

ACKNOWLEDGEMENTS

I wish to express my gratitude to the following persons for their assistance in the undertaking of this dissertation:

Dr Richard Steele for all his guidance and assistance as a supervisor.

Mrs MacDonald for her assistance as a co-supervisor.

Mr Ron Gauldie for his guidance and knowledge (and all his books).

Mrs Reena Brijlal for her valued assistance as a laboratory technician.

Dr Shanie Sarawan for patiently assisting me with the SPSS statistical package.

Mr Kavarnal Thomas for his assistance with the statistics.

Miss Shavashni Ramlachan for all her support and encouragement, without which, I would not be where I am today.

Dr David Naudé for his general assistance and guidance in the field of research.

Mr Nigel Peek for showing me the ins and outs of a computer and assisting me with printing, as well supporting and encouraging me.

ABSTRACT

The purpose of this study was to determine the effect of herbal extracts of certain *Labiatae* species (*Rosmarinus officinalis*, *Salvia officinalis* and *Thymus vulgaris*) in the inhibition of *in vitro* growth of *Candida albicans* as compared to ethanol as a control and nystatin in terms of the disc diffusion test.

For this study, 15 agar plates containing Sabouraud's dextrose agar were inoculated with *Candida albicans*. Six filter paper discs were placed equidistantly apart on the surface of each agar plates. Each test or control substance was then micro-pipetted onto these discs. The plates were then incubated at 37°C. The diameters of the zones of inhibition were then measured at 18 hours, 24 hours and 36 hours.

Data entry and analysis was done using the SPSS statistical package. The Friedman test was used to compare each test or control substance at 18 hours, 24 hours and 36 hours. The Mann-Whitney-U test was used to compare the mean inhibition zones produced by the test and control substances after 18 hours, 24 hours and 36 hours of incubation. The Kruskal-Wallis test was used to compare the effects of all three herbs to each other after 18 hours, 24 hours and 36 hours of incubation. The tests were performed at $\alpha=5\%$ significance.

The results showed that *Rosmarinus officinalis* and *Salvia officinalis* were ineffective in the inhibition of the *in vitro* growth of *Candida albicans*, whilst

Thymus vulgaris was effective in the inhibition of *in vitro* growth of *Candida albicans* at 18 hours and 24 hours. Nystatin proved to be more effective than any of the herbs in the inhibition of the *in vitro* growth of *Candida albicans*.

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DEFINITION OF TERMS

Antiputrescent: An agent that prevents and combats decay or putrefaction (Lawless, 1995: 237).

Antitusive: Relieves cough (Lawless, 1995: 237).

Astringent: Causes contraction of organic tissue (Lawless, 1995: 237).

Cephalic: Remedy for disorders of the head; referring or directed towards the head (Lawless, 1995: 238).

Chloretic: Aids secretion of bile by the liver, so there is greater flow of bile (Lawless, 1995: 238).

Cholagogue: Stimulates the secretion and flow of bile into the duodenum (Lawless, 1995: 238).

Cicatrizant: An agent that promotes healing by the formation of scar tissue (Lawless, 1995: 238).

Cordial: A stimulant and tonic (Lawless, 1995: 238).

Cytophylactic: Referring to cytophylaxis – the process of increasing the activity of leucocytes in defence of the body against infection (Lawless, 1995: 238).

Diaphoretic: An agent that cause sweating (Lawless, 1995: 238).

Emmenagogue: Induces or assists menstruation (Lawless, 1995: 238).

Febrifuge: Combats fever (Lawless, 1995: 239).

Nervine: Strengthening and toning to the nerves and nervous system (Lawless, 1995: 240).

Revulsive: Relieves pain by means of the diversion of blood or disease from one part of the body to another (Lawless, 1995: 240).

Rubefacient: A substance which causes redness of the skin, possibly irritation (Lawless, 1995: 240).

Stomachic: Digestive aid and tonic, improving appetite (Lawless, 1995: 241).

Sudorific: An agent that causes sweating (Lawless, 1995: 241).

Vulnerary: An agent that helps heal wounds and sores by external application
(Lawless, 1995: 241).

CHAPTER ONE

INTRODUCTION

1.1 OVERVIEW

Candida albicans is a yeast-like fungus and is a member of the normal flora of the mouth, gastrointestinal tract and vagina but it can cause disease, usually superficial infection (Duguid *et al.*, 1978:544). Fungal infections are becoming more common due to the increasing number of susceptible people such as the elderly and immunocompromised (Kant, 2000:22).

Drug therapy is at the centre of allopathic medicine's program for yeast infections. One of several drugs is used to interrupt the normal growth progress of the fungus. These medications are taken as vaginal suppositories, creams or tablets. Possible medications include: clotrimazole, econazole nitrate, fluconazole, miconazole and nystatin. These drugs carry potential side effects such as: increased vaginal irritation, headaches, nausea and stomach pain. (Althoff *et al.*, 1997:164.) These drugs may not have serious side effects, however, hypersensitivity may be experienced and for those patients preferring non-allopathic treatment, many traditional herbal remedies can be recommended, especially as part of a therapeutic program that also seeks to reinforce the body's immune system, which normally prevents a common commensal from becoming an opportunistic pathogen (McFadden, 1995).

Herbs are the basis of many different medicinal systems around the world. Especially popular in Asia, Europe and India, herbal medicine is used by an estimated 80% of the world's population. Modern conventional medicine is based largely on herbalism. Drug companies still use herbs as a source of many pharmaceutical products: 77% of the 150 most commonly prescribed drugs are of plant origin. (Althoff *et al.*, 1997:241.)

Very little research has been done on the antimycotic nature of whole plant remedies, and the action of some herbs is speculative (McFadden, 1995).

This study investigated three herbal tinctures in an attempt to find a suitable natural alternative to possibly harmful drugs such as nystatin.

The study was conducted *in vitro* using the disc diffusion test (Capuccino and Sherman, 1992: 248). The zone of inhibition produced by each substance was used as a measure of how effective an anti-candidal agent each test substance was.

1.2 STATEMENT OF THE RESEARCH OBJECTIVE

The purpose of this study was to determine the efficacy of herbal extracts of certain *Labiatae* species (*Rosmarinus officinalis*, *Salvia officinalis* and *Thymus vulgaris*) in the inhibition of *in vitro* growth of *Candida albicans* as compared to nystatin in terms of the disc diffusion test.

1.3 OBJECTIVES

1.3.1 The first objective

The first objective was to determine the efficacy of *Rosmarinus officinalis* in 62% v/v ethanol as compared to 62% v/v ethanol only (negative control) in the inhibition of *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

1.3.2 The second objective

The second objective was to determine the efficacy of *Salvia officinalis* in 62% v/v ethanol as compared to 62% v/v ethanol only (negative control) in the inhibition of *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

1.3.3 The third objective

The third objective was to determine the efficacy of *Thymus vulgaris* in 43% v/v ethanol as compared to 43% v/v ethanol only (negative control) in the inhibition of *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

1.3.4 The fourth objective

The fourth objective was to compare the efficacy of *Rosmarinus officinalis*, *Salvia officinalis* and *Thymus vulgaris* to nystatin (positive control) in the inhibition of *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

1.3.5 The fifth objective

The fifth objective was to compare the efficacies of *Rosmarinus officinalis*, *Salvia officinalis* and *Thymus vulgaris* to each other in the inhibition of *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

CHAPTER TWO

LITERATURE REVIEW

2.1 CANDIDA ALBICANS

2.1.1 Classification

Candida albicans belongs to the Class Three Fungi. These are the Fungi Imperfecti or Deuteromycetes (Anderson and Sobieski, 1980:470-471).

2.1.2 Morphology and identification

Candida albicans is an oval budding yeast producing pseudomycelium; the budding yeast cells may resemble hyphae (pseudohyphae), which form blastospores at the nodes and chlamydospores terminally. *Candida albicans* stains Gram positive (Jawetz *et al.*, 1989:307-309). It is best grown on blood agar or Sabouraud's dextrose agar at 37°C. Colonies reach a 0,5mm diameter in 18 hours and develop into high convex, off-white colonies 1,5mm in diameter after 48 hours (Duguid *et al.*, 1978:544). *Candida albicans* ferments glucose and maltose, producing both acid and gas. It produces acid from sucrose but does not attack lactose. These carbohydrate fermentations together with colonial and morphological characteristics differentiate *Candida albicans* from other species of *Candida* (Jawetz *et al.*, 1989:307). *Candida albicans* fails to split urea; this is also a differentiating factor (Duguid *et al.*, 1978:544).

2.2 CANDIDAL INFECTIONS

Candida albicans is a usual member of the normal flora of the mouth, gastrointestinal tract and vagina (Duguid *et al.*, 1978:545). *Candida albicans* can cause infection when the normal host defences are compromised and commonly affects the mouth, female genitalia, skin and nails (Jawetz *et al.*, 1989:307-309). Infection of the mucosa with *Candida albicans* is called thrush (Duguid *et al.*, 1978:545). Infection may be localised or disseminated. Organisms may disseminate directly or haematogenously from localised lesions. (Burnett and Schuster, 1978:349.)

Candida albicans feeds on sugar, and any condition that increases the amount of sugar in the body may result in a candidal infection. If the body's pH is upset for any reason, the bacterial flora of the body that metabolise sugar do not thrive. In these circumstances, *Candida albicans* thrives. Antibiotic use affects the bacterial flora in the body and therefore predisposes to candidal infection. Changing hormone levels may result in an increase in the amount of sugar in the vagina, therefore pregnancy and the use of hormone supplements such as the oral contraceptive pill may result in candidal infection. (Balch and Balch, 2000:264.)

2.2.1 Factors predisposing to infection

- Damaged skin barriers due to maceration, wounds, abrasions, burns and intravascular catheters;
- Altered mucosal barriers can occur as a result of diabetes, antimicrobials, irradiation, smoking, cytotoxic drugs, corticosteroids, cimetidine, after a vagotomy (which increases gastric pH) and the presence of a foreign body such as dentures, contraceptive diaphragms and nasogastric tubes;
- Hormonal and nutritional balances such as: diabetes, oral contraceptives, pregnancy, menstruation, malnutrition and uraemia;
- A decreased number of phagocytes as a result of leukaemia, irradiation, chemotherapy and agranulocytosis;
- Defects in the function of the phagocytic cells caused by chronic granulomatous disease and myeloperoxidase deficiency;
- Alterations in phagocytic cells caused by uraemia, viral infections, and the use of corticosteroids and antimicrobials (Howard *et al.*, 1980:617);
- Other predisposing factors such as: neonatal debility, senility, continued exposure of the skin to moisture, debility due to alcoholism and drug addiction (Duguid *et al.*, 1978:545) as well as obesity (Jawetz *et al.*, 1989:308).

2.2.2 Types of candidiasis

2.2.2.1 Vaginal

Vaginal candidiasis is commonly known as thrush. The signs and symptoms of vaginal thrush include lesions, irritation, intense itching and a whitish discharge (Jawetz *et al.*, 1989:308). The lesions resemble either simple eczematoid dermatitis or excoriated vesicular pustules, rarely ulcerating (Burnett and Schuster, 1978:351). The discharge has a pH below 5.2 and contains pus and yeast cells (Duguid *et al.*, 1978:545). Loss of acid pH, which is normally maintained by bacterial flora, predisposes to candidal vulvovaginitis (Jawetz *et al.*, 1989:308). Increased glucose in the body fluids and blood, as well as accumulation of polysaccharides in the vagina predisposes to vaginal thrush (Burnett and Schuster, 1978:351). Diabetes, pregnancy, progesterone and antibiotic therapy predispose to infection (Jawetz *et al.*, 1989:308).

2.2.2.2 Oral

Pèrleche is candidal infection of the lips. There is symmetric erosion of the labial commissures. The upper layers of epidermis are lost and the deeper layers are red. There are deep cracks in the corners of the mouth and a grey or white membrane may develop over the corners of the mouth. (Burnett and Schuster, 1978:349.)

Oral candidiasis in infants is often referred to as thrush. It often develops eight or nine days after contact with an infected birth canal and may then be transmitted

to other infants in a ward or nursery. The lesions are flaky and whitish with a loosely adherent membrane covering the tongue, lips, gums and buccal mucous membrane and less commonly, the uvula, fauces and soft palate are involved. Beneath the membrane, the mucosa is bright red. The incidence of oral candidiasis in newborns is less than 5%. (Burnett and Schuster, 1978:349.) It is more likely to occur in bottle-fed or debilitated infants (Duguid *et al.*, 1978:545).

Oral candidiasis in adults is generally sub-acute or chronic, and rarely acute (Burnett and Schuster, 1978:351). In sub-acute oral candidiasis there are white, cream coloured or greyish plaques on the oral mucous membranes. On removing these plaques, the underlying mucosa is red and inflamed. (Burnett and Schuster, 1978:351.) In chronic oral candidiasis the buccal mucosa is dry and red. There is little or no membrane. The tongue is shiny red, dry, cracked, fissured and swollen. As the entire mucous membrane is dry and burning, patients are only able to eat bland food and may therefore develop nutritional deficiencies. (Burnett and Schuster, 1978:351.)

2.2.2.3 Gastrointestinal

Candidal infection of the alimentary canal occurs in infants. The oesophagus is the most common site. There is a whitish pseudomembrane that may occlude the oesophagus. There may be ulcerations that can cause fatal bleeding. (Burnett and Schuster, 1978:349-350.) The clinical presentation of oesophageal candidiasis may range from being asymptomatic to a complete inability to

swallow with consequent dehydration. Dysphagia is the most common symptom; odynophagia, heartburn or retrosternal pains are less common. The most common physical finding in patients with oesophageal candidiasis is oral thrush. (Wilcox and Mönkemüller, 1998:1002-1008.)

The intestines may also be infected causing: pruritis ani, diarrhea and increased yeast count in the faeces (Duguid *et al.*, 1978:545).

2.2.2.4 Respiratory

Candidal infection of the respiratory system also occurs in infants but is less common than gastrointestinal infection. The tonsils and larynx may be involved and it can resemble diphtheria. It may involve the pharynx leading to the eustachian tube and cause an ear infection. (Burnett and Schuster, 1978:350-351.) Bronchial and pulmonary involvement is rare (Duguid *et al.*, 1978:545).

2.2.2.5 Cutaneous

Next to oral candidiasis in infants, superficial cutaneous candidiasis is most common. It often begins in the perianal regions and spreads to the thighs or abdomen. Weeping lesions occur on the neck and axillary region, and the entire cutaneous surface may be involved. (Burnett and Schuster, 1978:351.)

Cutaneous candidiasis in adults is of three types: localised, generalised and allergic. The common sites are the axilla, gluteal folds, the groin, the webs of the

toes and fingers and the perianal region (Burnett and Schuster, 1978:351) as well as the inframammary folds. (Jawetz *et al.*, 1989:308.) Wetting of the skin, diabetes and trauma may predispose to infection. Lesions are characterised by erythema, exudation and desquamation. (Duguid *et al.*, 1978:545.)

2.2.2.6 Ungal

Infected nail folds are painful, swollen and red, they resemble pyogenic paronychia. Infection may lead to thickening and transverse grooving of the nails and eventual loss of the nails. (Jawetz *et al.*, 1989:308.)

2.2.2.7 Systemic

This is due to dissemination of yeast in the blood and is usually preceded by extensive cutaneous candidiasis. Clinical features include: anorexia, severe vomiting, progressive wasting, severe diarrhoea and sudden collapse. (Burnett and Schuster, 1978:351.) Systemic infections are common in drug addicts, after heart valve operations, in severe generalised disease such as leukaemia, especially when treated with cytotoxic drugs, steroids and antibiotics, and sometimes in small children with no clear predisposing conditions (Duguid *et al.*, 1978:545-546).

2.2.2.8 Other

Candida albicans can be a secondary invader of the lungs, kidneys and other organs where a pre-existing disease is present (Jawetz *et al.*, 1989:308).

2.2.3 Acquired immunodeficiency syndrome (AIDS) and candidiasis

Compared to other signs and symptoms that occur during the acute HIV-1 infection, oral candidiasis is the strongest predictor of rapid disease progression (Lachman, 1999:219). Patients with unexplained oral candidiasis have a high risk of developing AIDS, and the presence of thrush in newly diagnosed HIV-positive patients has prognostic significance (Fong *et al.*, 1997:87).

Candida albicans infection may occur at any stage of HIV infection, but it is most commonly associated with low CD4+ lymphocyte counts, and increased isolation of *Candida albicans* from the oropharynx has been correlated with low T-lymphocyte : helper-T-suppressor ratios and T-cell dysfunction. AIDS has been associated with abnormal monocyte functioning. Since monocytes have a fungicidal activity, their dysfunction combined with defects and depletion of T-lymphocytes may explain the predisposition of AIDS patients to oral and oesophageal candidiasis. Asymptomatic oral *Candida albicans* transmission has also been demonstrated in HIV-positive patients. (Fong *et al.*, 1997:85-93.)

2.3 DIAGNOSIS

Diagnosis is made on the basis of symptoms (as listed above) and laboratory analysis obtained of *Candida albicans* specimens and blood samples (Jawetz *et al.*, 1989: 308-309).

2.3.1 Specimens

Specimens may consist of swabs and scrapings from surface lesions, sputum and exudates (Jawetz *et al.*, 1989:308).

2.3.2 Microscopic examination

Specimens may be examined in Gram-stained smears for pseudohyphae and budding cells (Jawetz *et al.*, 1989:309).

2.3.3 Culture

All specimens are cultured at room temperature and at 37°C on Sabouraud's agar, typical colonies are examined for cells and budding pseudomycelia.

Candida albicans also produces chlamydospores on cornmeal agar, and this is an important differential test. (Jawetz *et al.*, 1989:309.)

2.3.4 Serology

In systemic candidiasis, a rise in the titer of antibodies to *Candida albicans* may be detected by many tests, but the interpretation of the serological findings is controversial (Jawetz *et al.*, 1989:309).

2.4 CONVENTIONAL TREATMENT

The use of drugs in candidal infections may be useful in shortening the duration of disease but the underlying cause must be identified and corrected to prevent recurrence (Duguid *et al.*, 1978:546).

In systemic candidiasis, amphotericin B is used intravenously (Duguid *et al.*, 1978:546) or ketoconazole is given orally (Jawetz *et al.*, 1989:309).

Mucocutaneous candidiasis in immunodeficient children occasionally responds to administration of transfer factor obtained from persons with active cell-mediated reactions to *Candida* (Jawetz *et al.*, 1989:309).

Local infection is commonly treated with nystatin. Other topical agents may also be employed such as: gentian violet, parahydroxybenzoic acid esters, sodium propionate and miconazole. (Jawetz *et al.*, 1989:309.)

In addition to any treatment, the cause of local lesions must be removed such as: avoiding moisture, keeping areas cool, powdered and dry and withdrawing antibiotics (Jawetz *et al.*, 1989:309).

2.4.1 Nystatin

2.4.1.1 Introduction

Candida albicans is commonly treated topically with nystatin (Diehl, 1996:1687-1692; Kant, 2000:22-26). *Candida albicans* is sensitive, *in vitro*, to nystatin (Gunderson *et al.*, 2000: 2887-2890).

2.4.1.2 History, source and chemistry

The source of nystatin is *Streptomyces noursei*, and the name of the antibiotic is derived from *New York State*. Nystatin is a polyene antibiotic and is only slightly soluble in water. (Goodman and Gilman, 1980:1222.)

2.4.1.3 Antifungal activity

Nystatin is both fungicidal and fungistatic. *Candida*, *Cryptococcus*, *Histoplasma* and *Blastomyces* are sensitive *in vitro* to it. Nystatin is too toxic for parenteral use in systemic fungal infections, and is therefore commonly used to treat topical candidiasis only. (Goodman and Gilman, 1980:1222.)

2.4.1.4 Mechanism of action

Nystatin binds to a sterol moiety in the membranes of sensitive fungi. Pores or channels form in the membrane resulting in an increased permeability of the membrane, allowing leakage of a variety of small molecules. (Goodman and Gilman, 1980:1222.)

2.4.1.5 Fungal resistance

Candida albicans shows little resistance, but other species of *Candida* become resistant to nystatin and simultaneously become resistant to amphotericin as well. This resistance is lost when the antibiotic is removed. (Goodman and Gilman, 1980:1222.)

2.4.1.6 Absorption

Topical nystatin uses the epidermis as a depot. Its greatest concentration is in the stratum corneum with subsequent distribution through all epidermal layers. Drug concentration in the dermis remains low and systemic absorption is limited. Absorption may be greater through the hair follicles, sweat glands and sebaceous glands. Epidermal hydration may increase the absorption rate. (Diehl 1996: 1687-92.) Absorption from the gastrointestinal tract is negligible and the drug appears in the faeces (Goodman and Gilman, 1980:1222).

2.4.1.7 Preparation, routes of administration and dosage

Preparations of nystatin include oral suspensions, ointments and oral and vaginal tablets. Creams, powders, ointments and suspensions contain 100 000 units of nystatin per gram. Tablets for oral therapy contain 500 000 units; vaginal tablets contain 100 000 units. The oral dose for adults for oral candidiasis is 500 000 to 1 million units three or four times daily, for children, 100 000 to 400 000 units three to four times daily. Topical application is usually made two or three times a day. Vaginal tablets (one or two) are inserted daily for fourteen days. (Goodman and Gilman, 1980:1222.)

2.4.1.8 Untoward effects

Side effects with nystatin are not common. Mild nausea, vomiting and diarrhoea may occur after oral administration. (Goodman and Gilman, 1980:1222.)

2.4.1.9 Therapeutic uses

Nystatin is mainly used to treat Candidal infections of the skin, mucus membranes and intestinal tract. Vaginitis and stomatitis caused by *Candida* are benefited by topical therapy. (Goodman and Gilman, 1980:1222.)

2.4.1.10 Prophylactic uses

Nystatin may be administered with the tetracyclines for the purpose of preventing the overgrowth of yeasts and fungi in the bowels of patients predisposed to infection with *Candida* (Goodman and Gilman, 1980:1222-3).

2.5 COMPLEMENTARY TREATMENT

Several herbs have the ability to rid the body of excess fungus (Althoff *et al.*, 1997:165). *Rosmarinus officinalis* and *Thymus vulgaris* are said to be effective against yeast infections (Althoff *et al.*, 1997:165). *Salvia officinalis* is said to be antifungal (WebMD Corporation, 2001).

2.5.1 The Labiatae family

Rosmarinus officinalis, *Salvia officinalis* and *Thymus vulgaris* all belong to the *Labiatae* or mint family of plants (Hickey and King, 1997:119). This family of plants has been found to have great medicinal potential (Githinji and Kokwaro, 1993:197-203). This plant family has a worldwide distribution, but is native to the Mediterranean region. They are used for their volatile oils, which are obtained by

distillation. These plants also have culinary uses for flavouring as well as ornamental uses. (Hickey and King, 1997:119.)

Plants from the *Labiatae* family are mainly herbs or shrubs, often with square stems. The leaves are usually opposite, simple, without stipules, often hairy, and with epidermal glands secreting volatile oils. The flowers are usually bisexual and irregular. The fruit is usually a group of four nutlets; each containing one seed, and the seed usually has little or no endoderm. (Hickey and King, 1997:119.)

Many plants in this family have antioxidant properties (Nakatani, 2000:141).

Herbs in the *Labiatae* family have been demonstrated, *in vitro*, to have an anti-HIV-1 activity (Yamasaki *et al.*, 1998:829-833).

2.5.2 *Rosmarinus officinalis*

2.5.2.1 Family

Rosmarinus officinalis belongs to the *Labiatae* family (Hoffman, 1996:136).

2.5.2.2 Common name

The common name of *Rosmarinus officinalis* is Rosemary (Hoffman, 1996:136).

2.5.2.3 Description

Rosmarinus officinalis is an evergreen shrub growing to 2 metres; it has narrow, dark green, pinelike leaves and is strongly aromatic (Chevallier, 1996:125).

2.5.2.4 Habitat and cultivation

Rosemary is native to the Mediterranean; it grows freely over most of southern Europe and is cultivated throughout the world (Chevallier, 1996:125). The leaves are gathered in the summer and are at their best during flowering time (Hoffman, 1996:136). Rosemary is propagated from seed or cutting in spring and prefers a moderately warm dry climate and a sheltered site (Chevallier, 1996:125).

2.5.2.5 Parts used

The leaves of *Rosmarinus officinalis* are used as the oil concentration is at its highest here (Chevallier, 1996:125).

2.5.2.6 Constituents

The constituents of *Rosmarinus officinalis* are 1% volatile oil (borneol, linalol, camphene, cineole, and camphor), tannins, bitter principle and resins (Hoffmann, 1996: 136), as well as: flavanoids (apigenin and diosmin), rosmarinic acid, diterpenes (picrosalvin) and rosmarinine (Chevallier, 1996:125). Twelve phenolic diterpenes have been isolated from *Rosmarinus officinalis*, of these carnosol, carnosic acid, rosmanol, epirosmanol, isorosmanol, rosmaridiphenol, rosmadial

and miltirone have been shown to have antioxidant activities (Ho *et al.*, 2000: 161).

2.5.2.7 Actions

The following actions are attributed to *Rosmarinus officinalis*: tonic, stimulant, astringent, nervine, anti-inflammatory and carminative (Chevallier, 1996: 125); aromatic, antispasmodic, antidepressive, rubefacient, parasiticide, antimicrobial and emmenagogue (Hoffmann, 1996: 136); analgesic, antioxidant, antirheumatic, antiseptic, aphrodisiac, cephalic, cholagogue, chloretic, cicatrizant, cordial, cytophylactic, diaphoretic, digestive, diuretic, fungicide, hepatic, hypertensive, restorative, stomachic, sudorific and vulnerary (Lawless, 1995: 209).

2.5.2.8 Preparations

Rosmarinus officinalis is administered as an essential oil, a tincture or an infusion (Chevallier, 1996:125).

2.5.2.9 Traditional and current uses

2.5.2.9.1 Circulatory stimulant

Rosmarinus officinalis is a warming herb; it stimulates circulation of blood to the head, improving memory and concentration. It may also ease headaches and encourage hair growth by improving circulation to the scalp. Rosemary is also thought to raise low blood pressure and is valuable for weakness and fainting

associated with deficient circulation. (Chevallier, 1996:125.)

2.5.2.9.2 Nervous problems

Rosemary is a nervine stimulant (Hoffman, 1996:136). Rosemary has been used to treat epilepsy and vertigo (Chevallier, 1996:125).

2.5.2.9.3 Restorative

Rosemary may aid the recovery from long-term stress and chronic illness. It is thought to stimulate the adrenal glands and is used for debility especially when accompanied by poor digestion and circulation. (Chevallier, 1996:125.)

2.5.2.9.4 Antioxidant

The main antioxidant constituents of *Rosmarinus officinalis* are phenolic diterpenoids. Four of these have been isolated, namely: rosmanol, epirosmanol, isorosmanol and carnosol. The effect of these constituents was evaluated in lard measured by the active oxygen method, and they were found to be more effective than BHT, a synthetic antioxidant. This study was performed in order to find effective antioxidants to delay or prevent oxidation of fats and oils. (Nakatani, 2000:141.) *Rosmarinus officinalis* also contains flavonoids that have an antioxidant effect, although not as strong (Ho, *et al.*, 2000:161).

As *Rosmarinus officinalis* has a high antioxidant activity, crude and refined extracts of it are now commercially available which are especially applicable to food stabilization (Ho, *et al.*, 2000:161).

Rosmarinus officinalis also has an effect on tumour genesis. This interlinks with its antioxidant properties. A study conducted on mice showed that topical application of rosemary extract inhibited the growth of skin tumours. Oral administration of the plant also inhibited (or prevented) the growth of lung tumours, mammary tumours and colon tumours in mice. (Ho, *et al.*, 2000:161.)

2.5.2.9.5 Antimicrobial activity

According to McFadden (1995), the essential oil of *Rosmarinus officinalis*, along with other essential oils and tinctures, was tested *in vitro* on the inhibition of colony growth of *Candida albicans*. The agar plate dilution method was used, whereby the oil was incorporated into the agar and inhibition was assessed by measuring the colony area from a single point inoculum. The mean colony size for *Rosmarinus officinalis* was 0.00, Candidal growth was significantly inhibited by the essential oil of *Rosmarinus officinalis*. The minimum inhibitory concentration of *Rosmarinus officinalis* was found to be 18430 µg/ml.

The essential oils from *Satureja montana*, *Rosmarinus officinalis*, *Thymus vulgaris* and *Calamintha nepeta* were chemically analysed and their antimicrobial and fungicidal activities were evaluated on the basis of their minimum inhibitory

concentration and minimum bactericidal concentration. All four oils were found to be biotoxic, the most active being *Calamintha* and *Thymus*. (Panizzi *et al.*, 1993:167-170).

Bacteria, filamentous fungi and yeasts were subjected to the action of *Rosmarinus officinalis* essence in a steam phase using a microatmospheric technique. It had a negative effect on microbial growth. (Larrondo *et al.*, 1995:171-172.)

Rosmarinus officinalis, along with other essential oils was tested against 41 microbial strains. The test organisms were selected on the basis of their significance regarding food spoilage and/or poisoning, common human and plant pathogens. The agar diffusion assay was performed. *Rosmarinus officinalis* displayed broad antimicrobial properties and may therefore have preservative potential for the food and cosmetic industry. (Mangena and Muyima, 1999:291-296.)

2.5.3 Salvia officinalis

2.5.3.1 Family

Salvia officinalis belongs to the *Labiatae* family (Chevallier, 1996:130).

2.5.3.2 Common name

Salvia officinalis is commonly known as sage (Chevallier, 1996:130), or as red

sage (Hoffman, 1996:138).

2.5.3.3 Description

Sage is an evergreen growing to 80 centimetres in height. It has square stems with grey-green or purple coloured leaves. (Chevallier, 1996:130.)

2.5.3.4 Habitat and cultivation

Sage is native to the Mediterranean region, but is cultivated all over the world and thrives in sunny conditions. It is grown in spring from seed and the plant is replaced after 3-4 years. (Chevallier, 1996:130.) The leaves are gathered at the beginning of flowering in late spring or early summer. The leaves are dried in the shade not above 35°C. (Hoffman, 1996:138.)

2.5.3.5 Parts used

The leaves of *Salvia officinalis* are used (Hoffman, 1996:138).

2.5.3.6 Constituents

The constituents of *Salvia officinalis* are: volatile oils (thujone), diterpene, bitters, flavanoids, phenolic acid and tannins (Chevallier, 1996:130), as well as other volatile oils (cineole, linalol, camphor, borneol, salvene and pinene), triterpenoids, oestrogenic substance and resins (Hoffmann, 1996:138). Some phenolic diterpenes have also been isolated from *Salvia officinalis* which show antioxidant activity, namely: carnosol, carnosic acid, rosmadial, rosmanol,

epirosmanol and methyl carnosate. Glycosylated phenolic compounds have also been isolated from *Salvia officinalis*. (Ho *et al.*, 2000: 161.) Ursolic acid is also present in *Salvia officinalis* (Baricevic *et al.*, 2001:125-132).

2.5.3.7 Actions

The following actions are attributed to *Salvia officinalis*: astringent, antiseptic, aromatic, carminative, reduces sweating, tonic anti-asthma remedy (Chevallier, 1996:130); spasmolytic, anticatarrhal, antimicrobial, emmenagogue, febrifuge and stimulant (Hoffmann, 1996:138). It is antibacterial, antiviral and anti fungal, and decreases secretions (WebMD Corporation, 2001).

2.5.3.8 Preparations

Salvia officinalis is commonly administered as an infusion or a tincture or the leaves maybe rubbed directly onto the skin (Chevallier, 1996:130). Decoctions are commonly used as a mouth wash (Hoffman, 1996:138). *Salvia officinalis* is also administered as an essential oil (Lawless, 1995:212).

2.5.3.9 Traditional and current uses

2.5.3.9.1 Antiseptic, anti-inflammatory and astringent

Sage has antiseptic, astringent and relaxing properties making it useful as a gargle for sore throats. It is also used to treat canker sores and sore gums. Sage's astringency makes it useful in diarrhoea treatment. (Chevallier, 1996: 130.) Sage is a classic remedy for inflammation of the mouth, tongue, gums,

throat and tonsils; its volatile oils soothe the mucous membranes. A compress of sage promotes wound healing. (Hoffman, 1996:138.)

According to Baricevic *et al.* (2001:125-132), *Salvia officinalis* is a useful topical anti-inflammatory agent, with the main anti-inflammatory constituent being ursolic acid.

2.5.3.9.2 Tonic

Sage is a digestive tonic and stimulant. It is also a nerve tonic, it helps to calm and stimulate the nervous system. (Chevallier, 1996:130.)

2.5.3.9.3 Hormonal stimulant

Sage encourages better flow of blood and is therefore helpful in treating irregular and light menstruation. Sage's hormonal action is not well understood, but it's anti-hidrotic action along with its tonic and oestrogenic effects make it useful in menopause. Sage reduces hot flushes and helps the body adapt to hormonal changes. (Chevallier, 1996:130.) Sage may also be useful in reducing breast milk production and stimulating the muscles of the uterus (Hoffman, 1996:138).

2.5.3.9.4 Asthma remedy

Sage is a traditional remedy for asthma. The leaves are still included in herbal smoking mixtures intended for use in asthmatic patients. (Chevalier, 1996:130.)

2.5.3.9.5 Alzheimer's disease

Salvia officinalis has memory improving properties as well as cholinergic properties that may be of assistance in the treatment of Alzheimer's disease (Perry *et al.*, 1999:527-534).

2.5.3.9.6 Antioxidant

Salvia officinalis is a known antioxidant (Nakatani, 2000:141; Ho *et al.*, 2000:161). According to Ho *et al.* (2000:161), carnosol, carnosic acid, rosmadiol, rosmanol, epirosmanol and methyl carsonate have been identified from sage leaves and showed antioxidant activities. Flavonoids and other phenolic compounds act as antioxidants.

2.5.3.10 Precautions

Sage should be avoided during pregnancy (Hoffman, 1996:138).

2.5.4 *Thymus vulgaris*

2.5.4.1 Family

Thymus vulgaris belongs to the *Labiatae* family (Hoffman, 1996:153).

2.5.4.2 Common name

The common name for *Thymus vulgaris* is garden thyme (Chevallier, 1996:142).

2.5.4.3 Description

Thymus vulgaris is an aromatic shrub that grows up to 40cm. It has woody stems, small leaves and pink flowers (Chevallier, 1996:142).

2.5.4.4 Habitat and cultivation

Thymus vulgaris is native to southern Europe, but is now cultivated worldwide. It is grown from seed or by root division in spring, and prefers light, chalky soil. (Chevallier, 1996:142.) The flowering stems are collected between early and late summer on a dry sunny day. The leaves are stripped off the dry stems. (Hoffman, 1996:153.)

2.5.4.5 Parts used

The leaves and flowering tops of *Thymus vulgaris* are used (Hoffman, 1996:153).

2.5.4.6 Constituents

The constituents of *Thymus vulgaris* are: volatile oil (thymol, methychavicol, cineole and borneol), flavanoids (apigenin and luteolin) and tannins (Chevallier, 1996:142), as well as other volatile oils (carvacrol, linalool and cymol), bitter principles and triterpenoids (Hoffman, 1996:153). *Thymus vulgaris* also contains cymene, terpinene, camphene, geraniol, citral and thuyanol. There are different chemotypes of *Thymus vulgaris* oil: the "thymol" and "carvacrol" types, the "thuyanol" type and the "linalool" or "citrol" types (Lawless, 1995:228).

2.5.4.7 Actions

The following actions are attributed to *Thymus vulgaris*: antiseptic, tonic, antispasmodic, expectorant, expels worms, antifungal, relieves bites and stings, helps sciatica and rheumatic pains (Chevallier, 1996:142); carminative, antimicrobial, astringent, diaphoretic, vulnerary (Hoffman, 1996:153); antioxidant, antiputrescent, antitussive, antitoxic, diuretic, emmenagogue, nervine, revulsive, rubefacient, parasiticide and stimulant (Lawless, 1995:228).

2.5.4.8 Preparations

Thymus vulgaris is commonly administered as an infusion, an essential oil or as a syrup (Chevallier, 1996:142).

2.5.4.9 Traditional and current uses

2.5.4.9.1 Infections

The tonic and antiseptic properties of *Thymus vulgaris* make it a useful tonic for the immune system for chronic, especially fungal, infections, as well as an effective remedy for chest infections, such as bronchitis, whooping cough, and pleurisy. The infusion may also be used for minor throat and chest infections.

The leaves may be chewed to relieve sore throats. (Chevallier, 1996:142.)

The volatile component of *Thymus vulgaris* is effective against Gram positive and Gram-negative bacteria grown on agar slants (Agnihotri and Vaidya, 1996:712-715).

According to McFadden (1995), the essential oil of *Thymus vulgaris*, along with other essential oils and tinctures, was tested *in vitro* on the inhibition of colony growth of *Candida albicans*. The agar plate dilution method was used, whereby the oil was incorporated into the agar and inhibition was assessed by measuring the colony area from a single point inoculum. The mean colony size for *Thymus vulgaris* was 0.00 showing that Candidal growth was significantly inhibited by the essential oil of *Thymus vulgaris*. The minimum inhibitory concentration of *Thymus vulgaris* was found to be 1140 µg/ml.

2.5.4.9.2 Asthma and hay fever

Thymus vulgaris is often prescribed along with other herbs for asthma, especially in children. The invigorating effects balance the sedative effects of many herbs used for asthma. *Thymus vulgaris* may also be useful in hay fever. (Chevallier, 1996: 142.)

2.5.4.9.3 Worms

Thymus vulgaris is commonly used to treat worms in children (Chevallier, 1996:142).

2.5.4.9.4 External uses

When applied to the skin, *Thymus vulgaris* relieves bites and stings, and helps sciatica and rheumatic aches and pains. It assists with the treatment of

ringworm, athlete's foot, thrush and other fungal infections, as well as scabies and lice. A tincture of *Thymus vulgaris* is commonly used for vaginal thrush; 40 drops can be applied 2-3 times daily. The infusion may be added to bathwater as a stimulant. (Chevallier, 1996:142.)

2.5.4.9.5 Antioxidant

Thymol and carbacrol, the major essential monoterpenes of *Thymus vulgaris* show high antioxidant activity (Nakatani, 2000:141).

2.5.4.10 Precautions

Thymus vulgaris is contraindicated in cases of hypertension (Lawless, 1995: 228).

CHAPTER THREE

METHODOLOGY

3.1 THE DATA.

The research involves two types of data: primary and secondary. The nature of the data is as follows:

3.1.1 The primary data

- 1) Results of the experiment determining the effects of *Rosmarinus officinalis* tincture in 62% v/v ethanol on *Candida albicans*.
- 2) Results of the experiment determining the effects of *Salvia officinalis* tincture in 62% v/v ethanol on *Candida albicans*.
- 3) Results of the experiment determining the effects of 62% v/v ethanol on *Candida albicans*.
- 4) Results of the experiment determining the effects of *Thymus vulgaris* tincture in 43% v/v ethanol on *Candida albicans*.
- 5) Results of the experiment determining the effect of 43% v/v ethanol on *Candida albicans*.
- 6) Results of the experiment determining the effect of a nystatin suspension on *Candida albicans*.

3.1.2 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 carried out by the researcher at the Department of Biotechnology, Technikon Natal was used.

3.3 MATERIALS AND METHOD

The basic methodology followed, unless otherwise stated is per Cappucino and Sherman (1992).

3.3.1 Preparation of media

Sabouraud's dextrose agar was the medium used in the experiments. It was prepared according to The Oxoid manual (1979), as follows:

- 1) 32,5g of Sabouraud's dextrose agar powder was weighed out.
- 2) The Sabouraud's dextrose agar powder was added to 0,5 litres of distilled water in a screw top flask.
- 3) A magnetic stirrer was then added to aid dissolution.
- 4) The mixture was then shaken until well mixed.
- 5) The mixture was then autoclaved at 121°C for 15 minutes.
- 6) The flask was then allowed to cool in a beaker of cold water, which had been placed on a magnetic stirring machine. This ensured adequate mixing and prevented the mixture from solidifying.
- 7) Once the flask had cooled enough to hold, the agar was then poured into agar plates as follows:

7.1 The top of the flask was flamed with a bunsen burner before

pouring each plate to prevent contamination;

7.2 Each plate was poured to a depth of approximately 5 millimetres;

7.3 A total of 15 plates were prepared;

7.4 The plates were stacked and allowed to cool and solidify;

7.5 They were then visually checked for contamination.

3.3.2 Preparation of the inoculum

- 1) *Candida albicans* was obtained from a culture of the organism growing on a Sabouraud's dextrose agar plate, derived from the culture collection of the Biotechnology department of Technikon Natal.
- 2) A specimen of the above culture was transferred from the plate into 10 millilitres of Sabouraud's dextrose liquid medium using sterile techniques.
- 3) This was then swirled to distribute the yeast.
- 4) 1 millilitre of this liquid medium was then pipetted into 9 millilitres of saline solution in a test tube.
- 5) The solution was then vortexed to ensure proper mixing.

3.3.3 Measurement of optical density of the inoculum

The optical density of the inoculum was measured as follows:

- 1) The Spectronic 20 spectrophotometer was switched on 15 minutes prior to use to warm up.

- 2) The wave length was set to 100 nanometres
- 3) The percentage transmission was set at zero using the knob on the left.
- 4) 10 millilitres of saline was poured into a test tube to serve as a blank. The outside was wiped and the test tube was inserted into the spectrophotometer.
- 5) The percentage transmission was set to 100% using the knob on the right (this is inversely proportional to the optical density which will therefore be zero).
- 6) The blank was then removed from the spectrophotometer.
- 7) The *Candida albicans* culture was placed into the spectrophotometer after wiping the outside of the test tube.
- 8) The absorbance or percentage transmission was recorded. The recorded value was 0,05% transmission.

This created a standardised inoculum so that the test could be replicated at any time using the same quantity of *Candida albicans* culture.

3.3.4 Preparation of filter paper discs.

Whatman® filter paper number 3 was used. The filter paper was punched into discs 5 millimetres in diameter. These discs were placed in a jar and autoclaved at 121°C for 15 minutes to ensure sterilization.

3.3.5 Preparation of herbal tinctures.

3.3.5.1 Preparation of *Rosmarinus officinalis* tincture.

Rosmarinus officinalis tincture (batch no.10016, expiry date 09/2005) was made up by Parceval (Pty) Ltd Pharmaceuticals according to the German Homoeopathic Pharmacopoeia (1985). The tincture was made up according to method HAB3a. Extracts of fresh plant were used, with 33,3 parts of fresh plant to 66,7 parts of ethanol with a final alcoholic concentration of 62% v/v. (Smidt, 2001.)

3.3.5.2 Preparation of *Salvia officinalis* tincture

Salvia officinalis tincture (batch no. 01035, expiry date 12/2004) was made up by Parceval (Pty) Ltd Pharmaceuticals according to the German Homoeopathic Pharmacopoeia (1985). The tincture was made up according to method HAB3a. Extracts of fresh plant were used, with 33,3 parts of fresh plant to 66.7 parts of ethanol with a final alcoholic concentration of 62% v/v. (Smidt, 2001.)

3.3.5.3 Preparation of *Thymus vulgaris* tincture

Thymus vulgaris tincture (batch no. 10019, expiry date 09/2005) was prepared by Parceval (Pty) Ltd Pharmaceuticals according to the German Homoeopathic Pharmacopoeia (1985). The tincture was made up according to method HAB2a. Extracts from the fresh plant were used,

with 50 parts plant to 50 parts ethanol with a final alcoholic concentration of 43% v/v.(Smidt, 2001.)

3.3.6 Preparation of controls.

3.3.6.1 Preparation of 62% v/v ethanol.

62% v/v ethanol (negative control) was made up according to the German Homoeopathic Pharmacopoeia standards (GHP, 1985:11):

65,90 millilitres of 96% v/v ethanol was diluted with sufficient water to produce 100 millilitres. The weight per millilitre was between 0.8885 and 0.9295 grams as measured with a hydrometer.

3.3.6.2 Preparation of 43% v/v ethanol

43% v/v ethanol (negative control) was made up according to the German Homoeopathic Pharmacopoeia standards (GHP, 1985: 11):

45.20 millilitres of 96% v/v ethanol was diluted with sufficient water to produce 100 millilitres. The weight per millilitre was between 0.9335 and 0.9295 grams, as measured with a hydrometer.

3.3.6.3 Preparation of nystatin

Nystatin (batch no. 006391, expiry date 09/2002) was prepared by Bristol-Myers Squibb (Pty) Ltd.

3.3.7 Inoculation of plates

Using sterile techniques, the plates were inoculated as follows:

- 1) A sterile cotton swab was dipped into the well-mixed, diluted test culture of *Candida albicans* and excess inoculum was removed by pressing the saturated swab against the wall of the test tube.
- 2) Using the swab, the entire surface of the agar was streaked. A cross was first made on the agar, and then, the plate was swabbed in three different directions. This ensured a confluent growth over the entire surface.

3.3.8 Placement and impregnation of discs

The under surface of each agar plate was premarked with letters using a marker pen. A particular letter denoted each test or control substance.

- A) *Rosmarinus officinalis* in 62% v/v ethanol
- B) *Salvia officinalis* in 62% v/v ethanol
- C) *Thymus vulgaris* in 43% v/v ethanol
- D) 62% v/v ethanol
- E) 43% v/v ethanol
- F) Nystatin

Six sterile filter paper discs were placed on each agar plate. This was done by means of a sterile needle. The discs were placed equidistant from each other in their premarked places.

A premeasured amount of 7 microlitres of each test substance and each control was pipetted onto each disc respectively.

3.3.9 Incubation

The plates were incubated at 37°C.

3.3.10 Recording of results.

The plates were examined at 18, 24 and 36 hours for the presence of growth inhibition, which was indicated by a clear zone surrounding each disc. The susceptibility of *Candida albicans* to each test or control substance was determined by the size of this zone. The zone diameters were measured in millimetres using a ruler. The measurement was performed in triplicate the average inhibition zone diameter was calculated and recorded on a table (see Appendix A). Photographs were then taken at 18 hours as a visual record.

3.4 DATA ANALYSIS

3.4.1 Sample size of the study

The sample size of the study was 15, which means each test yielded 15 data sets. The efficacy of each test and control substance was tested against *Candida albicans* 15 times to make the study statistically viable.

3.4.2 Statistical methods

3.4.2.1 Intra-group comparison of *Rosmarinus officinalis* in 62% v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare results from related samples.

(i) Hypothesis testing

The null hypothesis H_0 , states that there was no change in diameter of the zone of inhibition with regard to observations at 18 hours, 24 hours and 36 hours, at the $\alpha=0.05$ level of significance. The alternative hypothesis H_1 , states that there was a change in the diameter of the zone of inhibition with regard to observations at 18 hours, 24 hours and 36 hours, at the same level of significance.

H_0 : There was no change in the diameter of the zone of inhibition

H_1 : There was a change in the diameter of the zone of inhibition

(ii) 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 $P = \alpha$.

P is the observed significance level or probability value.

If the null hypothesis H_0 is rejected for Friedman's T test, then multiple applied, comparison procedure will have to be using the Dunn Procedure to

determine which of the times are significantly different. Let R_j and R'_j be the j^{th} and j'^{th} time rank totals.

Let α be the experiment wise error rate. Usually $\alpha = 0.10$

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

In the above formula:

b = the number of blocks

k = the number of times

z = value in the inverse normal distribution corresponding to

$(1 - [\alpha/k(k-1)])$

To compute the treatment rank totals, rank values in each block and then compute the sum of the ranks for each time.

When $k=3$, $\alpha=0.10$, $z=2.12$

(Fisher and van Belle, 1993:430).

3.4.2.2 Intra-group comparison of *Salvia officinalis* in 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare results from related samples.

(i) Hypothesis testing

As per 3.4.2.1 (i).

(ii) Decision rule

As per 3.4.2.1 (ii).

3.4.2.3 Intra-group comparison of *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare results from related samples.

(i) Hypothesis testing

As per 3.4.2.1 (i).

(ii) Decision rule

As per 3.4.2.1 (ii).

3.4.2.4 Intra-group comparison of 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare results from related samples.

(i) Hypothesis testing

As per 3.4.2.1 (i).

(ii) Decision rule

As per 3.4.2.1 (ii).

3.4.2.5 Intra-group comparison of 43% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare results from related samples.

(i) Hypothesis testing

As per 3.4.2.1 (i).

(ii) Decision rule

As per 3.4.2.1 (ii).

3.4.2.6 Intra-group comparison of nystatin with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare results from related samples.

(i) Hypothesis testing

As per 3.4.2.1 (i).

(ii) Decision rule

As per 3.4.2.1 (ii).

3.4.2.7 Inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol and 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney U test was used to compare the samples.

(i) Hypothesis testing

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

H_0 : there was no difference between the 2 groups, $M_1=M_2$.

H_1 : there was a difference between the 2 groups, $M_1 \neq M_2$.

(ii) 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 $P = \alpha$.

P is the observed significance level or probability value

(Fisher and van Belle, 1993:315).

3.4.2.8 Inter-group comparison between *Salvia officinalis* in 62% v/v ethanol and 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney U test was used to compare the samples.

(i) Hypothesis testing

As per 3.4.2.7 (i).

(ii) Decision rule

As per 3.4.2.7 (ii).

3.4.2.9 Inter-group comparison between *Thymus vulgaris* in 43% v/v ethanol and 43% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney U test was used to compare the samples.

(i) Hypothesis testing

As per 3.4.2.7 (i).

(ii) Decision rule

As per 3.4.2.7 (ii).

3.4.2.10 Inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol and nystatin with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney U test was used to compare the samples.

(i) Hypothesis testing

As per 3.4.2.7 (i).

(ii) Decision rule

As per 3.4.2.7 (ii).

3.4.2.11 Inter-group comparison between *Salvia officinalis* in 62% v/v ethanol and nystatin with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney U test was used to compare the samples.

(i) Hypothesis testing

As per 3.4.2.7 (i).

(ii) Decision rule

As per 3.4.2.7 (ii).

3.4.2.12 Inter-group comparison between *Thymus vulgaris* in 43% v/v ethanol and nystatin with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney U test was used to compare the samples.

(i) Hypothesis testing

As per 3.4.2.7 (i).

(ii) Decision rule

As per 3.4.2.7 (ii).

3.4.2.13 Inter-group comparison between *Rosmarinus officinalis* in 62% v/v and *Salvia officinalis* in 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney U test was used to compare the samples.

(i) Hypothesis testing

As per 3.4.2.7 (i).

(ii) Decision rule

As per 3.4.2.7 (ii).

3.4.2.14 Inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney U test was used to compare the samples.

(i) Hypothesis testing

As per 3.4.2.7 (i).

(ii) Decision rule

As per 3.4.2.7 (ii).

3.4.2.15 Inter-group comparison between *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney U test was used to compare the samples.

(i) Hypothesis testing

As per 3.4.2.7 (i).

(ii) Decision rule

As per 3.4.2.7 (ii).

3.4.2.16 Inter-group comparison between *Rosmarinus officinalis* in 62% v/v, *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Kruskal-Wallis non-parametric Analysis of Variance by Ranks method was used to compare the diameters of the zones of inhibition of the herbs, to each other, with regard to observations at 18 hours, 24 hours and 36 hours.

(i) Hypothesis testing

In each test, the null hypothesis states that there was no difference in diameter among the means of the herbs being compared to each other. The alternative hypothesis states that there was a difference among the means.

$$H_0 : \mu_1 = \mu_2 = \mu_3.$$

$H_1 : \mu_1 \neq \mu_2 \neq \mu_3$. (All 3 means are not equal, at least one mean differs from the rest)

(ii) 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 $P = \alpha$.

P is the observed significance level or probability value.

If the null hypothesis H_0 is rejected for Kruskal-Wallis test, then multiple comparison procedure will have to be used to determine which of the medians (test substances) are significantly different.

Let R_i and R_j be the means of the ranks of the i^{th} and j^{th} samples respectively.

Let α be the experimentwise error rate. The values of α are usually 0.15, 0.20, 0.25 depending on the value of k . (as k increases, α increases)

$$\text{If } |R_i - R_j| > Z_{(1-\alpha/k(k-1))} \sqrt{N(N+1)/12 (1/n_i + 1/n_j)},$$

then the difference $|R_i - R_j|$ is declared significant at the α level.

In the above formula:

k = the number of samples

N = the number of observations in all samples combined

z =the value in the inverse normal distribution corresponding to $(1-[\alpha/k(k-1)])$

If $k=3$, $\alpha=0.15$; $z=1.96$

If $k=4$, $\alpha=0.20$; $z=2.12$

If $k=5$, $\alpha=0.25$; $z=2.326$ etc..

(Fisher and van Belle, 1993: 430).

If there are extensive ties in the data, the inequalities will be adjusted to ensure a conservative result. The appropriate inequality for equal sample sizes is:

$$|\bar{R}_i - \bar{R}_j| \leq \frac{z \sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

where t is the number of values in the combined sample that are tied at a given rank (Daniel, 1978: 213).

where t is the number of values in the combined sample that are tied at a given rank (Daniel, 1978: 213).

3.4.2.17 Comparison using bar charts.

Visual summaries of the analytical findings are given by means of bar charts.

Mean readings were used to construct the bar charts represented.

3.4.3 Statistical package

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

CHAPTER FOUR

RESULTS

4.1 INTRODUCTION

This chapter covers the results obtained from statistical analysis of the data obtained.

4.2 PHOTOGRAPHS

The following photographs (plates 4.1-4.4) were taken after 18 hours of incubation as a visual record of the results.

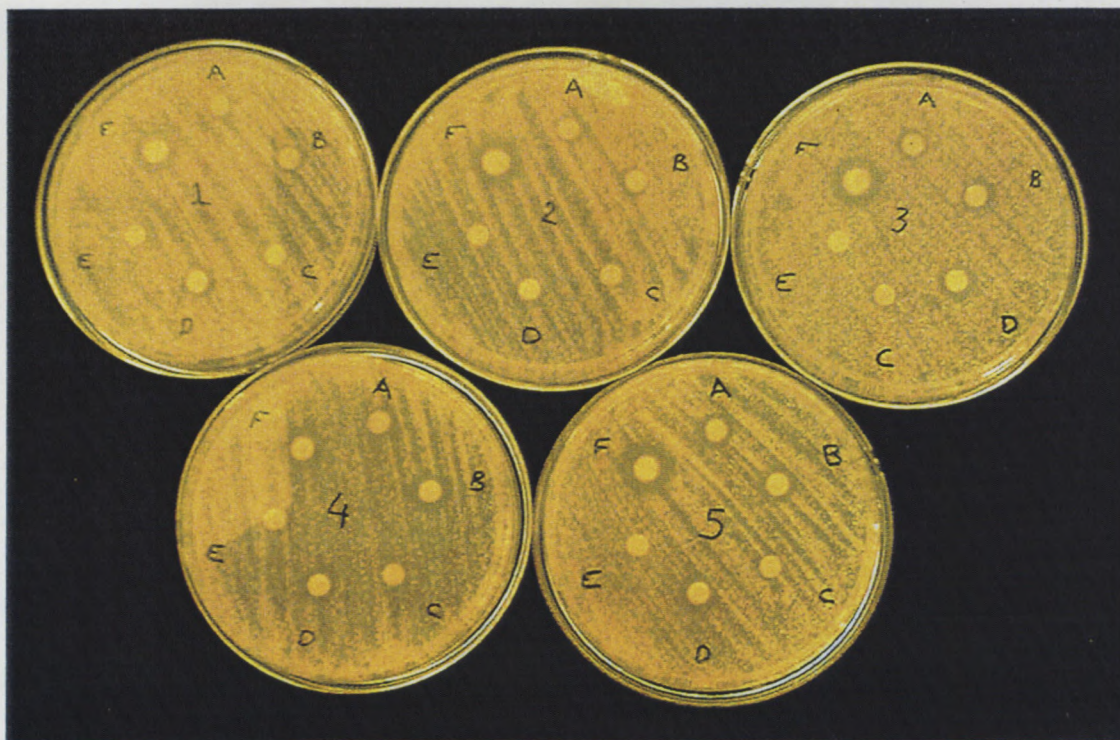


Plate 4.1 Agar plates 1-5 showing zones of inhibition around discs at 18 hours.

A= *Rosmarinus officinalis* in 62% v/v ethanol

B= *Salvia officinalis* in 62% v/v ethanol

C= *Thymus vulgaris* in 43% v/v ethanol

D= 62% v/v ethanol

E= 43% v/v ethanol

F= nystatin

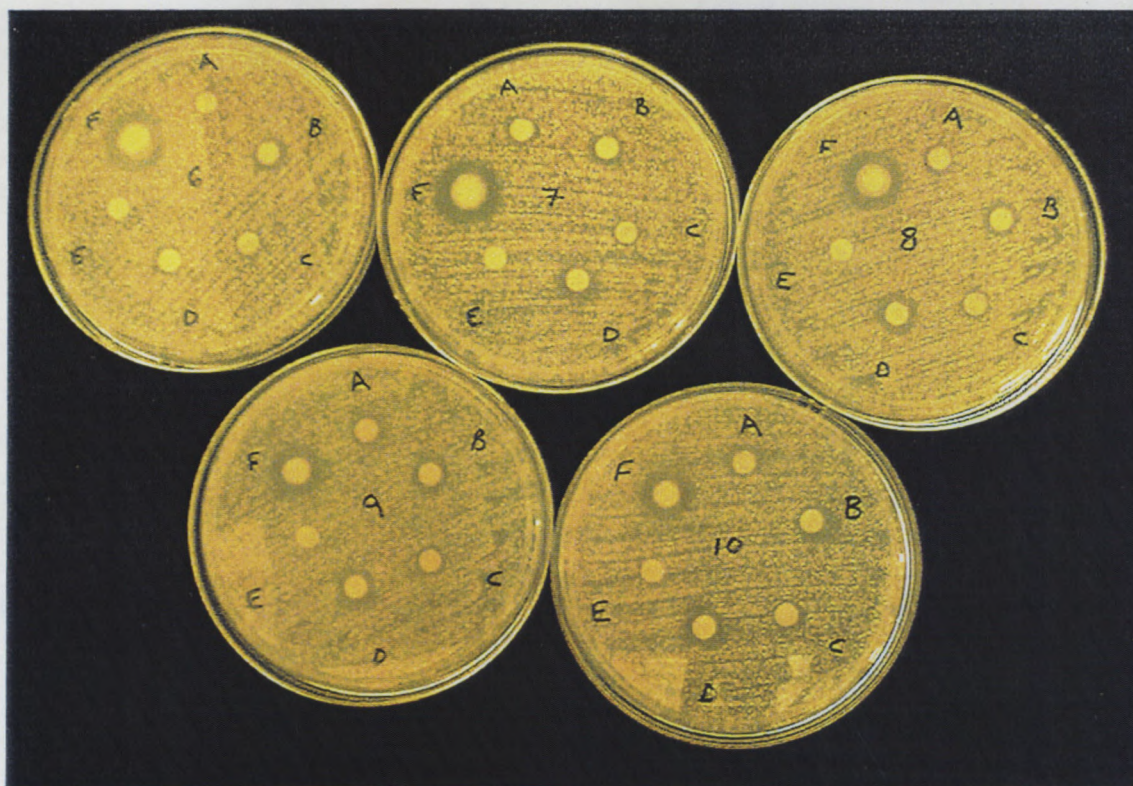


Plate 4.2 Agar plates 6-10 showing zones of inhibition around discs at 18 hours.

A= *Rosmarinus officinalis* in 62% v/v ethanol

B= *Salvia officinalis* in 62% v/v ethanol

C= *Thymus vulgaris* in 43% v/v ethanol

D= 62% v/v ethanol

E= 43% v/v ethanol

F= nystatin



Plate 4.3 Agar plates 11-15 showing zones of inhibition around discs
at 18 hours.

A= *Rosmarinus officinalis* in 62% v/v ethanol

B= *Salvia officinalis* in 62% v/v ethanol

C= *Thymus vulgaris* in 43% v/v ethanol

D= 62% v/v ethanol

E= 43% v/v ethanol

F= nystatin



Plate 4.4 Agar plate number 12 showing zones of inhibition around disc at 18 hours.

A= *Rosmarinus officinalis* in 62% v/v ethanol

B= *Salvia officinalis* in 62% v/v ethanol

C= *Thymus vulgaris* in 43% v/v ethanol

D= 62% v/v ethanol

E= 43% v/v ethanol

F= nystatin

4.3 STATISTICAL ANALYSIS OF DATA

4.3.1 The intra-group comparison of *Rosmarinus officinalis* in 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol at 18, 24 and 36 hours.

Table 4.1 Descriptive statistics for *Rosmarinus officinalis* in 62% v/v ethanol.

| TIME | N | MEAN | STD. DEVIATION | MINIMUM | MAXIMUM |
|----------|----|--------|-------------------|---------|---------|
| 18 hours | 15 | 9.3800 | 0.6910 | 8.00 | 10.80 |
| 24 hours | 15 | 6.5000 | 1.5561 | 5.00 | 8.60 |
| 36 hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |

Table 4.2 Friedman's test statistics for *Rosmarinus officinalis* in 62% v/v ethanol.

| | |
|-------------|--------|
| N | 15 |
| Chi-Square | 27.887 |
| df | 2 |
| Asymp. Sig. | .000 |

CONCLUSION: $P=.000$, therefore $P<\alpha$. Therefore the null hypothesis was rejected. The Dunn Procedure was therefore used to establish which timings were significantly different.

If $|R_j - R_i| \geq z \sqrt{\frac{bk(k+1)}{6}}$, then R_1 and R_2 are declared significant.

Table 4.3 Mean values and ranks for *Rosmarinus officinalis* in 62% v/v ethanol.

| SAMPLE NO. | 18 HOURS | 24 HOURS | 36 HOURS |
|------------|-----------|----------|----------|
| 1 | 8.36 [3] | 6 [2] | 5 [1] |
| 2 | 9.67 [3] | 5 [1.5] | 5 [1.5] |
| 3 | 9 [3] | 5 [1.5] | 5 [1.5] |
| 4 | 9.34 [3] | 5 [1.5] | 5 [1.5] |
| 5 | 9.38 [3] | 8.63 [2] | 5 [1.5] |
| 6 | 8 [3] | 5 [1.5] | 5 [1.5] |
| 7 | 9 [3] | 5 [1.5] | 5 [1.5] |
| 8 | 9.13 [3] | 5 [1.5] | 5 [1.5] |
| 9 | 9.13 [3] | 5 [1.5] | 5 [1.5] |
| 10 | 9.63 [3] | 7.67 [2] | 5 [1] |
| 11 | 10.75 [3] | 8.17 [2] | 5 [1] |
| 12 | 9.75 [3] | 7.83 [2] | 5 [1] |
| 13 | 9.38 [3] | 8.17 [2] | 5 [1] |
| 14 | 9.75 [3] | 7.83 [2] | 5 [1] |
| 15 | 10.25 [3] | 8.17 [2] | 5 [1] |

$R_1=45$ (sum total of the ranks at 18 hours)

$R_2=26.5$ (sum total of the ranks at 24 hours)

$R_3=18.5$ (sum total of the ranks at 36 hours)

$b=15$

$k=3$

$z=2.12$

If $|R_1 - R_2| \geq z \sqrt{\frac{bk(k+1)}{6}}$

$$|45-26.5| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|18.5| \geq 11.61$$

The difference between R_1 and R_2 was significant. There was a difference in diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol at 18 hours and 24 hours.

$$\text{If } |R_2 - R_3| \geq z \sqrt{bk(k+1)/6}$$

$$|26.5-18.5| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|18| \geq 11.61$$

The difference between R_2 and R_3 was significant. There was a difference between the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol at 24 hours and 36 hours.

$$\text{If } |R_1 - R_3| \geq z \sqrt{bk(k+1)/6}$$

$$|45-18.5| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|26.5| \geq 11.61$$

The difference between R_1 and R_2 was significant. There was a difference in diameters of zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol at 18 hours and 36 hours.

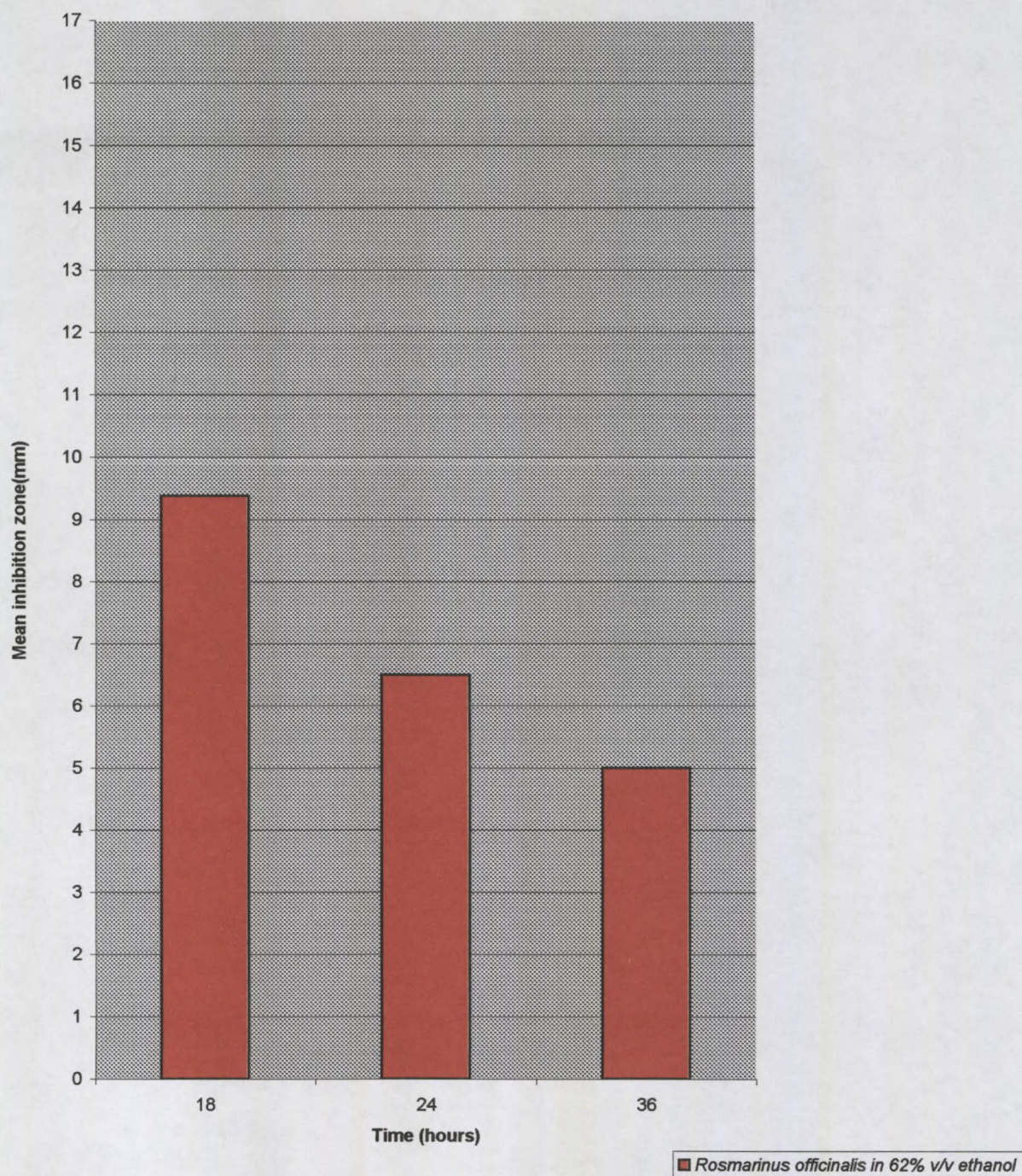


Figure 4.1 Bar chart comparing the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.2 The intra-group comparison of *Salvia officinalis* in 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol at 18, 24 and 36 hours.

Table 4.4 Descriptive statistics for *Salvia officinalis* in 62% v/v ethanol.

| TIME | N | MEAN | STD. DEVIATION | MINIMUM | MAXIMUM |
|----------|----|---------|----------------|---------|---------|
| 18 hours | 15 | 10.0400 | 0.9679 | 8.60 | 12.50 |
| 24 hours | 15 | 8.1133 | 1.1319 | 5.00 | 9.80 |
| 36 hours | 15 | 5.8467 | 1.2822 | 5.00 | 8.50 |

Table 4.5 Friedman's test statistics for *Salvia officinalis* in 62% v/v ethanol.

| | |
|-------------|--------|
| N | 15 |
| Chi-square | 29.525 |
| df | 2 |
| Asymp. Sig. | .000 |

CONCLUSION: $P=.000$, therefore $P<\alpha$. Therefore the null hypothesis was rejected. The Dunn Procedure was applied to establish which timings were significant.

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

Table 4.6 Mean values and ranks for *Salvia officinalis* in 62% v/v ethanol.

| SAMPLE NO. | 18 HOURS | 24 HOURS | 36 HOURS |
|------------|-----------|----------|----------|
| 1 | 8.63 [3] | 5 [1.5] | 5 [1.5] |
| 2 | 8.75 [3] | 8.67 [2] | 5 [1] |
| 3 | 9.38 [3] | 7.67 [2] | 5 [1] |
| 4 | 11.17 [3] | 9.75 [2] | 5 [1] |
| 5 | 10.25 [3] | 8.88 [2] | 5 [1] |
| 6 | 9.88 [3] | 8.33 [2] | 5 [1] |
| 7 | 9.13 [3] | 7.66 [2] | 5 [1] |
| 8 | 10 [3] | 7.83 [2] | 5 [1] |
| 9 | 9.67 [3] | 8.13 [2] | 5 [1] |
| 10 | 10.33 [3] | 7.83 [2] | 5 [1] |
| 11 | 12.5 [3] | 9.67 [2] | 8.5 [1] |
| 12 | 10.25 [3] | 7.67 [2] | 7.17 [1] |
| 13 | 9.83 [3] | 7.5 [2] | 7.17 [1] |
| 14 | 10.17 [3] | 9 [2] | 7.83 [1] |
| 15 | 10.63 [3] | 8 [2] | 7 [1] |

$R_1 = 45$ (sum total of ranks at 18 hours)

$R_2 = 29.5$ (sum total of ranks at 24 hours)

$R_3 = 15.5$ (sum total of ranks at 36 hours)

$b = 15$

$K = 3$

$Z = 2.12$

If $|R_1 - R_2| \geq z \sqrt{\frac{bk(k+1)}{6}}$

$$|45-29.5| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|15.5| \geq 11.61$$

The difference between R_1 and R_2 was significant. There was a difference in the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol at 18 hours and 24 hours.

$$\text{If } |R_2-R_3| \geq z \sqrt{bk(k+1)/6}$$

$$|29.5-15.5| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|18.5| \geq 11.61$$

The difference between R_2 and R_3 was significant. There was a difference in the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol at 24 hours and 36 hours.

$$\text{If } |R_1-R_3| \geq z \sqrt{bk(k+1)/6}$$

$$|45-15.5| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|29.5| \geq 11.61$$

The difference between R_1 and R_3 was significant. There was a difference in the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol at 18 hours and 36 hours.

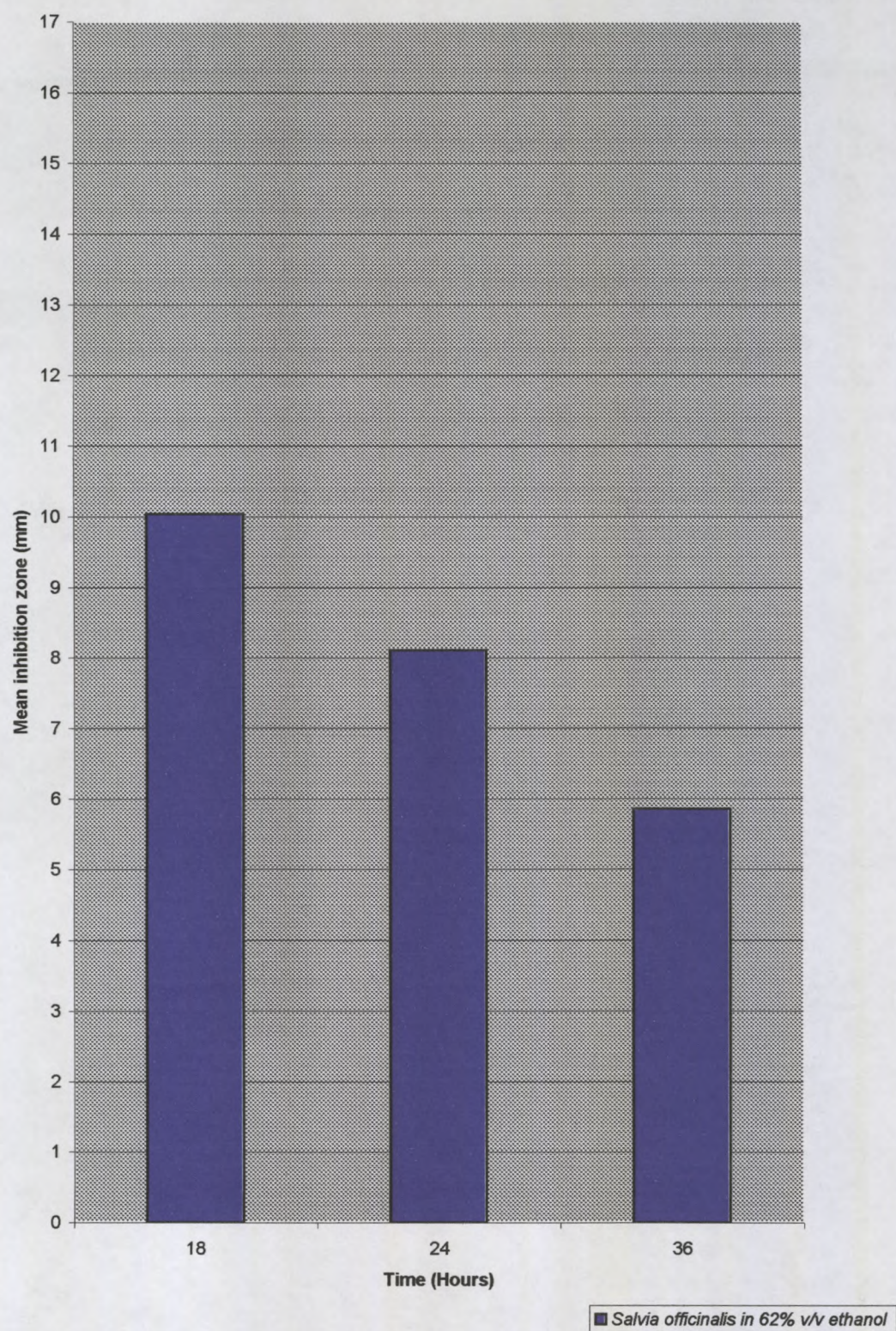


Figure 4.2 Bar chart comparing the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours.

4.3.3 The intra-group comparison of *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare the diameters of the zones of inhibition of *Thymus vulgaris* in 43% v/v ethanol at 18, 24 and 36 hours.

Table 4.7 Descriptive statistics for *Thymus vulgaris* in 43% v/v ethanol.

| TIME | N | MEAN | STD. DEVIATION | MINIMUM | MAXIMUM |
|----------|----|--------|----------------|---------|---------|
| 18 hours | 15 | 8.2200 | 1.2324 | 5.00 | 10.60 |
| 24 hours | 15 | 5.6200 | .9229 | 5.00 | 8.00 |
| 36 hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |

Table 4.8 Friedman's test statistics for *Thymus vulgaris* in 43% v/v ethanol.

| | |
|-------------|--------|
| N | 15 |
| Chi-Square | 26.000 |
| df | 2 |
| Asymp. Sig. | .000 |

CONCLUSION: $P=.000$, therefore the null hypothesis was rejected. The Dunn Procedure was applied to establish which timings were significantly different.

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

Table 4.9 Mean values and ranks for *Thymus vulgaris* in 43% v/v ethanol.

| SAMPLE NO. | 18 HOURS | 24 HOURS | 36 HOURS |
|------------|----------|----------|----------|
| 1 | 7.67 [3] | 5 [1.5] | 5 [1.5] |
| 2 | 5 [2] | 5 [2] | 5 [2] |
| 3 | 7.63 [3] | 5 [1.5] | 5 [1.5] |
| 4 | 8.17 [3] | 5 [1.5] | 5 [1.5] |
| 5 | 7.63 [3] | 5 [1.5] | 5 [1.5] |
| 6 | 7.25 [3] | 6 [2] | 5 [1] |
| 7 | 8 [3] | 5 [1.5] | 5 [1.5] |
| 8 | 8.63 [3] | 6 [2] | 5 [1] |
| 9 | 8 [3] | 6 [2] | 5 [1] |
| 10 | 8.88 [3] | 6.25 [2] | 5 [1] |
| 11 | 8.88 [3] | 8 [2] | 5 [1] |
| 12 | 8.88 [3] | 7 [2] | 5 [1] |
| 13 | 8.5 [3] | 6.66 [2] | 5 [1] |
| 14 | 9.5 [3] | 8 [2] | 5 [1] |
| 15 | 8.75 [3] | 6.83 [2] | 5 [1] |

$R_1=44$ (sum total of ranks at 18 hours)

$R_2=27.5$ (sum total of ranks at 24 hours)

$R_3=17.5$ (sum total of ranks at 36 hours)

$b=15$

$k=3$

$z=2.12$

If $|R_1 - R_2| \geq z \sqrt{bk(k+1)/6}$

$$|44-27.5| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|16.5| \geq 11.61$$

The difference between R_1 and R_2 was significant. There was a difference in the diameters of the zones of inhibition of *Thymus vulgaris* in 43% v/v ethanol at 18 hours and 24 hours.

$$\text{If } |R_2 - R_3| \geq z \sqrt{bk(k+1)/6}$$

$$|27.5-17.5| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|10| \geq 11.61$$

The difference between R_2 and R_3 was not significant. There was no difference in the diameters of the zones of inhibition of *Thymus vulgaris* in 43% v/v ethanol at 24 hours and 36 hours.

$$\text{If } |R_1 - R_3| \geq z \sqrt{bk(k+1)/6}$$

$$|44-17.5| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|26.5| \geq 11.61$$

The difference between R_1 and R_3 was significant. There was a difference in the diameters of the zones of inhibition of *Thymus vulgaris* in 43% v/v ethanol at 18 hours and 36 hours.

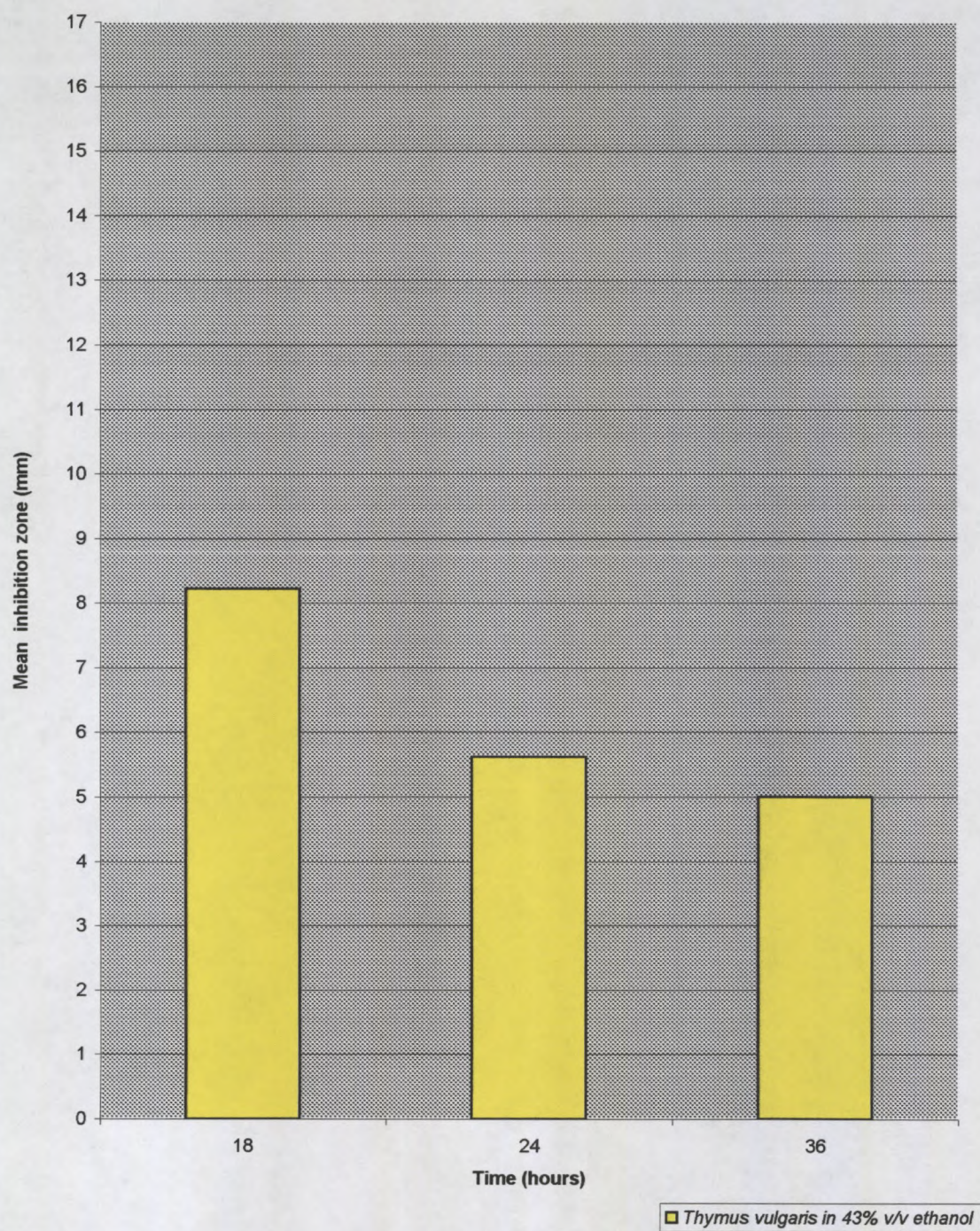


Figure 4.3 Bar chart comparing the diameters of the zones of inhibition of *Thymus vulgaris* in 43% v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.4 The intra-group comparison of 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare the diameters of the zones of inhibition of 62% v/v ethanol at 18, 24 and 36 hours.

Table 4.10 Descriptive statistics for 62% v/v ethanol.

| TIME | N | MEAN | STD. DEVIATION | MINIMUM | MAXIMUM |
|----------|----|--------|----------------|---------|---------|
| 18 hours | 15 | 9.4067 | 1.7376 | 5.00 | 11.80 |
| 24 hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |
| 36 hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |

Table 4.11 Friedman's test statistics for 62% v/v ethanol.

| | |
|-------------|--------|
| N | 15 |
| Chi-Square | 28.000 |
| df | 2 |
| Asymp. Sig. | .000 |

CONCLUSION: $P=.000$, therefore $P<\alpha$. Therefore the null hypothesis was rejected. The Dunn Procedure was applied to establish which timings were significantly different.

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

Table 4.12 Mean values and ranks for 62% v/v ethanol.

| SAMPLE NO. | 18 HOURS | 24 HOURS | 36 HOURS |
|------------|-----------|----------|----------|
| 1 | 10 [3] | 5 [1.5] | 5 [1.5] |
| 2 | 5 [2] | 5 [2] | 5 [2] |
| 3 | 9.5 [3] | 5 [1.5] | 5 [1.5] |
| 4 | 6.83 [3] | 5 [1.5] | 5 [1.5] |
| 5 | 10.25 [3] | 5 [1.5] | 5 [1.5] |
| 6 | 8.75 [3] | 5 [1.5] | 5 [1.5] |
| 7 | 8.67 [3] | 5 [1.5] | 5 [1.5] |
| 8 | 11.5 [3] | 5 [1.5] | 5 [1.5] |
| 9 | 8.33 [3] | 5 [1.5] | 5 [1.5] |
| 10 | 10 [3] | 5 [1.5] | 5 [1.5] |
| 11 | 11 [3] | 5 [1.5] | 5 [1.5] |
| 12 | 9.66 [3] | 5 [1.5] | 5 [1.5] |
| 13 | 9.13 [3] | 5 [1.5] | 5 [1.5] |
| 14 | 11.83 [3] | 5 [1.5] | 5 [1.5] |
| 15 | 10.13 [3] | 5 [1.5] | 5 [1.5] |

In the above formula:

$R_1=44$ (sum total of ranks at 18 hours)

$R_2=23$ (sum total of ranks at 24 hours)

$R_3=23$ (sum total of ranks at 36 hours)

$b=15$

$k=3$

$z=2.12$

If $|R_1 - R_2| \geq z \sqrt{\frac{bk(k+1)}{6}}$

$$|44-23| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|21| \geq 11.61$$

The difference between R_1 and R_2 was significant. There was a difference in the diameters of the zones of inhibition of 62% v/v ethanol at 18 hours and 24 hours.

$$\text{If } |R_2 - R_3| \geq z \sqrt{bk(k+1)/6}$$

$$|23-23| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|0| \geq 11.61$$

The difference between R_2 and R_3 was not significant. There was no difference in the diameters of the zones of inhibition of 62% v/v ethanol at 24 hours and 36 hours.

$$\text{If } |R_1 - R_3| \geq z \sqrt{bk(k+1)/6}$$

$$|44-23| \geq 2.12\sqrt{15 \cdot 3(3+1)/6}$$

$$|21| \geq 11.61$$

The difference between R_1 and R_3 was significant. There was a difference in the diameters of the zones of inhibition of 62% v/v ethanol at 18 hours and 36 hours.

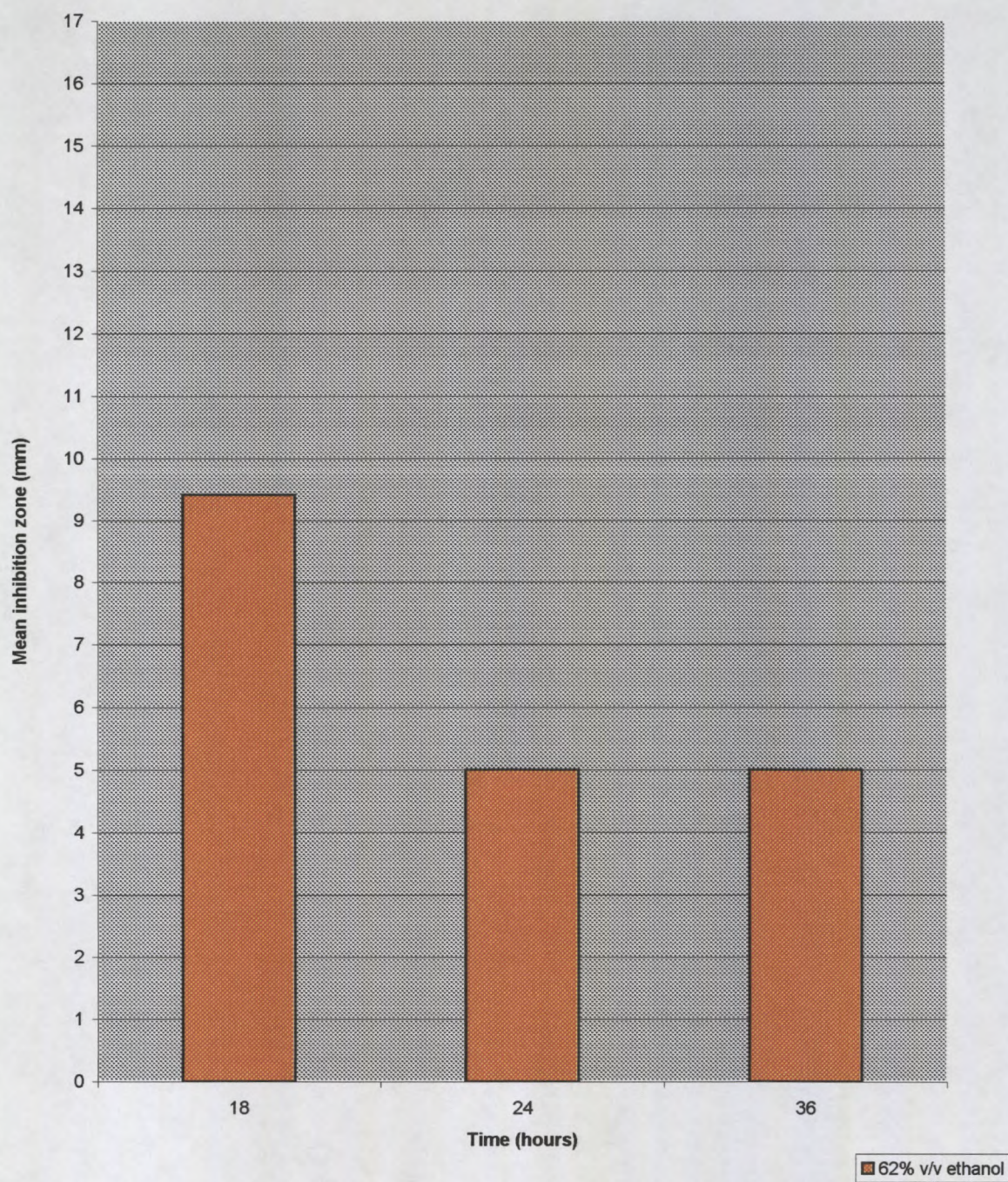


Figure 4.4 Bar chart comparing the diameters of the zones of inhibition of 62% v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.5 The intra-group comparison of 43%v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare the diameters of the zones of inhibition of 43% v/v ethanol at 18, 24 and 36 hours.

Table 4.13 Descriptive statistics for 43% v/v ethanol.

| TIME | N | MEAN | STD. DEVIATION | MINIMUM | MAXIMUM |
|----------|----|--------|----------------|---------|---------|
| 18 hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |
| 24 hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |
| 36 hours | 15 | 5.0000 | .0000 | 5.00 | 5.00 |

Table 4.14 Friedman's test statistics for 43% v/v ethanol.

| | |
|-------------|-------|
| N | 15 |
| Chi-Square | .000 |
| df | 2 |
| Asymp. Sig. | 1.000 |

CONCLUSION: $P=1.000$, therefore the null hypothesis was accepted. There was no significant difference between the groups being compared. There was no difference between the diameters of the zones of inhibition of 43% v/v ethanol at 18 hours, 24 hours and 36 hours.

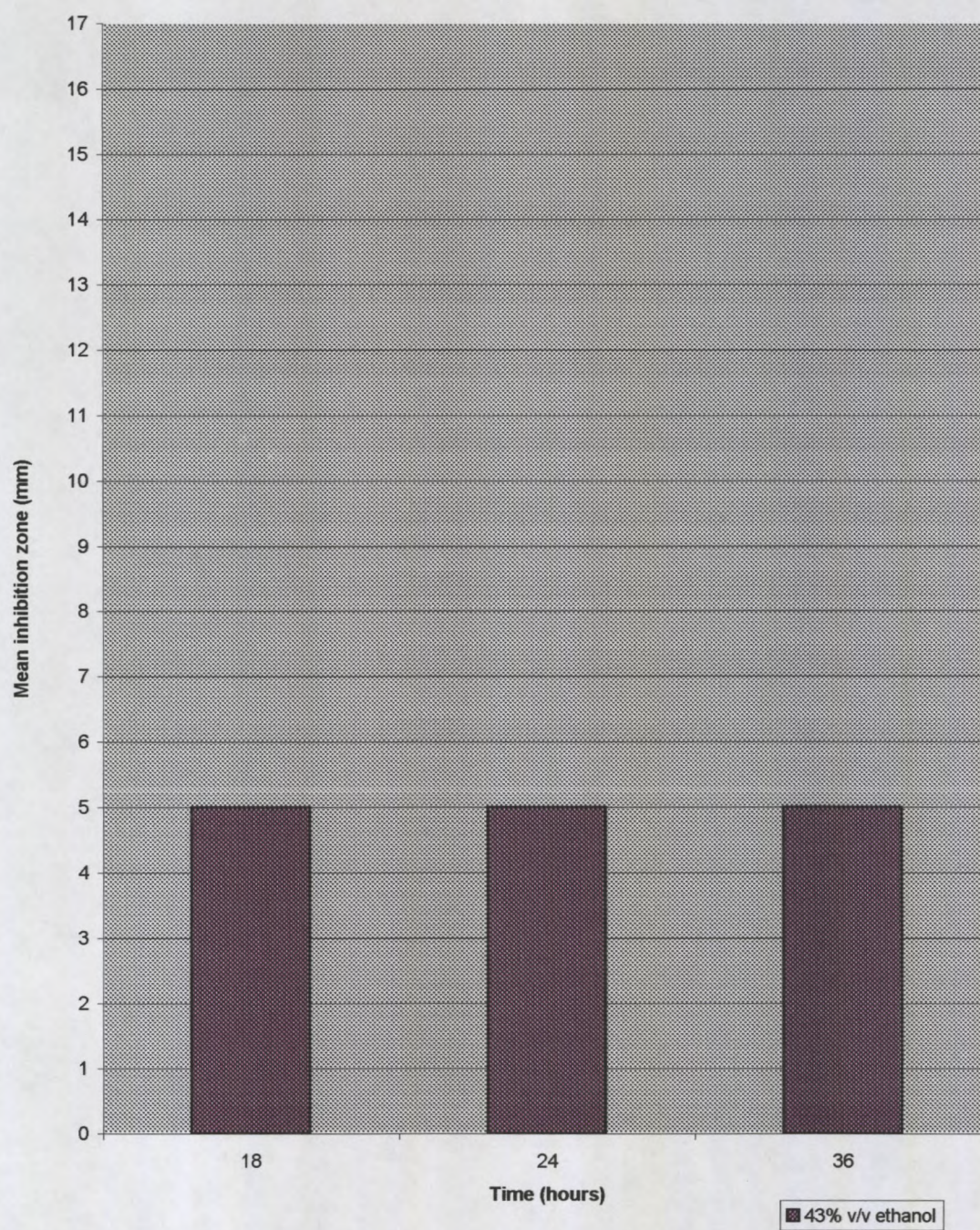


Figure 4.5 Bar chart comparing the diameters of the zones of inhibition of 43% v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.6 The intra-group comparison of nystatin with regard to observations at 18 hours, 24 hours and 36 hours

The Friedman's test was used to compare the diameters of the zones of inhibition of nystatin at 18, 24 and 36 hours.

Table 4.15 Descriptive statistics for nystatin.

| TIME | N | MEAN | STD. DEVIATION | MINIMUM | MAXIMUM |
|----------|----|---------|----------------|---------|---------|
| 18 hours | 15 | 15.1867 | 1.9246 | 12.10 | 19.00 |
| 24 hours | 15 | 13.6533 | 1.7816 | 11.30 | 16.80 |
| 36 hours | 15 | 12.4667 | 1.7108 | 9.80 | 15.30 |

Table 4.16 Friedman's test statistics for nystatin.

| | |
|-------------|--------|
| N | 15 |
| Chi-Square | 28.526 |
| df | 2 |
| Asymp. Sig. | .000 |

CONCLUSION: $P=.000$, therefore, $P<\alpha$. Therefore the null hypothesis was rejected. The Dunn Procedure was applied to establish which timings were significantly different.

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

Table 4.17 Mean values and ranks for nystatin.

| SAMPLE NO. | 18 HOURS | 24 HOURS | 25 HOURS |
|------------|-------------|-------------|-----------|
| 1 | 12.75 [2.5] | 12.75 [2.5] | 10.17 [1] |
| 2 | 13.87 [2.5] | 13.87 [2.5] | 11.33 [1] |
| 3 | 14.13 [3] | 13.17 [2] | 11.33 [1] |
| 4 | 12.13 [3] | 11.33 [2] | 9.83 [1] |
| 5 | 14.5 [3] | 12.17 [2] | 11 [1] |
| 6 | 17 [3] | 14.63 [2] | 14 [1] |
| 7 | 17.25 [3] | 16.5 [2] | 14.84 [1] |
| 8 | 16.25 [3] | 13.83 [2] | 13.5 [1] |
| 9 | 14.13 [3] | 12.25 [2] | 11.67 [1] |
| 10 | 13.5 [3] | 11.5 [2] | 11.5 [1] |
| 11 | 14.25 [3] | 11.88 [2] | 11.83 [1] |
| 12 | 15.38 [3] | 13 [2] | 12.83 [1] |
| 13 | 16.23 [3] | 15.33 [2] | 13.66 [1] |
| 14 | 16.88 [3] | 15.66 [2] | 14.33 [1] |
| 15 | 19 [3] | 16.83 [2] | 15.33 [1] |

$R_1=44$ (sum total of ranks at 18 hours)

$R_2=31$ (sum total of ranks at 24 hours)

$R_3=15$ (sum total of ranks at 36 hours)

$b=15$

$k=3$

$z=2.12$

If $|R_1 - R_2| \geq z \sqrt{bk(k+1)/6}$

$|44 - 31| \geq 2.12 \sqrt{15 \cdot 3(3+1)/6}$

$|13| \geq 11.61$

The difference between R_1 and R_2 was significant. There was a difference between the diameters of the zones of inhibition of nystatin at 18 hours and 24 hours.

$$\text{If } |R_1 - R_2| \geq z \sqrt{bk(k+1)/6}$$

$$|31-15| \geq 2.12 \sqrt{15 \cdot 3(3+1)/6}$$

$$|16| \geq 11.61$$

The difference between R_2 and R_3 was significant. There was a difference between the diameters of the zones of inhibition of nystatin at 24 hours and 36 hours.

$$\text{If } |R_2 - R_3| \geq z \sqrt{bk(k+1)/6}$$

$$|44-15| \geq 2.12 \sqrt{15 \cdot 3(3+1)/6}$$

$$|29| \geq 11.61$$

The difference between R_1 and R_3 was significant. There was a difference between the diameters of the zones of inhibition of nystatin at 18 hours and 36 hours.

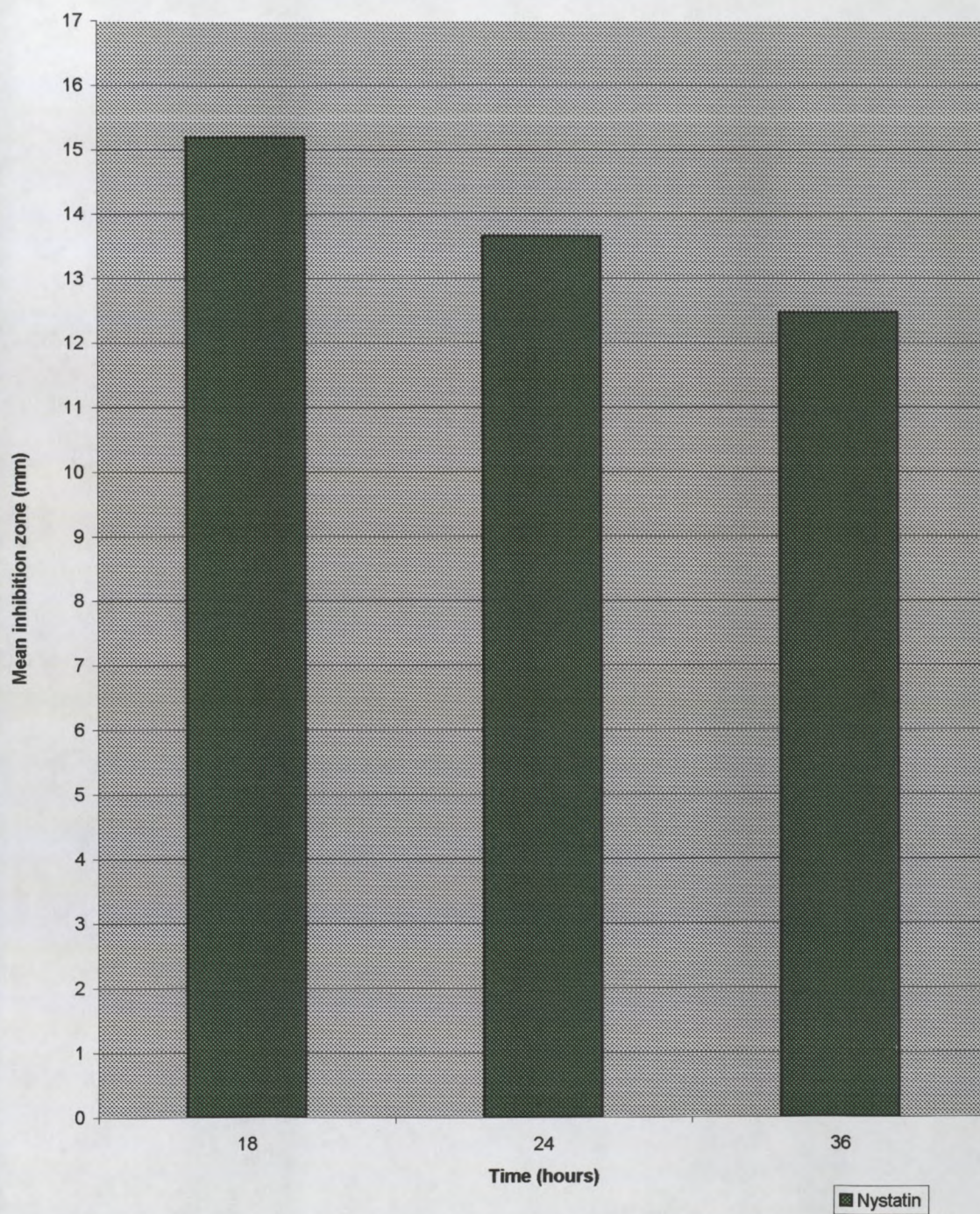


Figure 4.6 Barchart comparing the diameters of the zones of inhibition of nystatin with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.7 The inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol and 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney-U test was used to compare *Rosmarinus officinalis* in 62% v/v ethanol and 62% v/v ethanol.

Table 4.18 Inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol and 62% v/v ethanol.

| TIME | P-VALUE |
|----------|---------|
| 18 hours | 0.520 |
| 24 hours | 0.001 |
| 36 hours | 1.000 |

CONCLUSION: At 18 hours $P=0.520$, therefore, $P=\alpha$. The null hypothesis was accepted. There was no difference in diameter of the zone of inhibition between *Rosmarinus officinalis* in 62% v/v ethanol and 62% v/v ethanol.

At 24 hours, $P=0.001$, therefore, $P<\alpha$. The null hypothesis was rejected. There was a difference in diameter of the zone of inhibition between *Rosmarinus officinalis* in 62% v/v ethanol and 62% v/v ethanol.

At 36 hours, $P=1.00$, therefore, $P=\alpha$. The null hypothesis was accepted. There was no difference in diameter of the zone of inhibition between *Rosmarinus officinalis* in 62% v/v ethanol and 62% v/v ethanol.

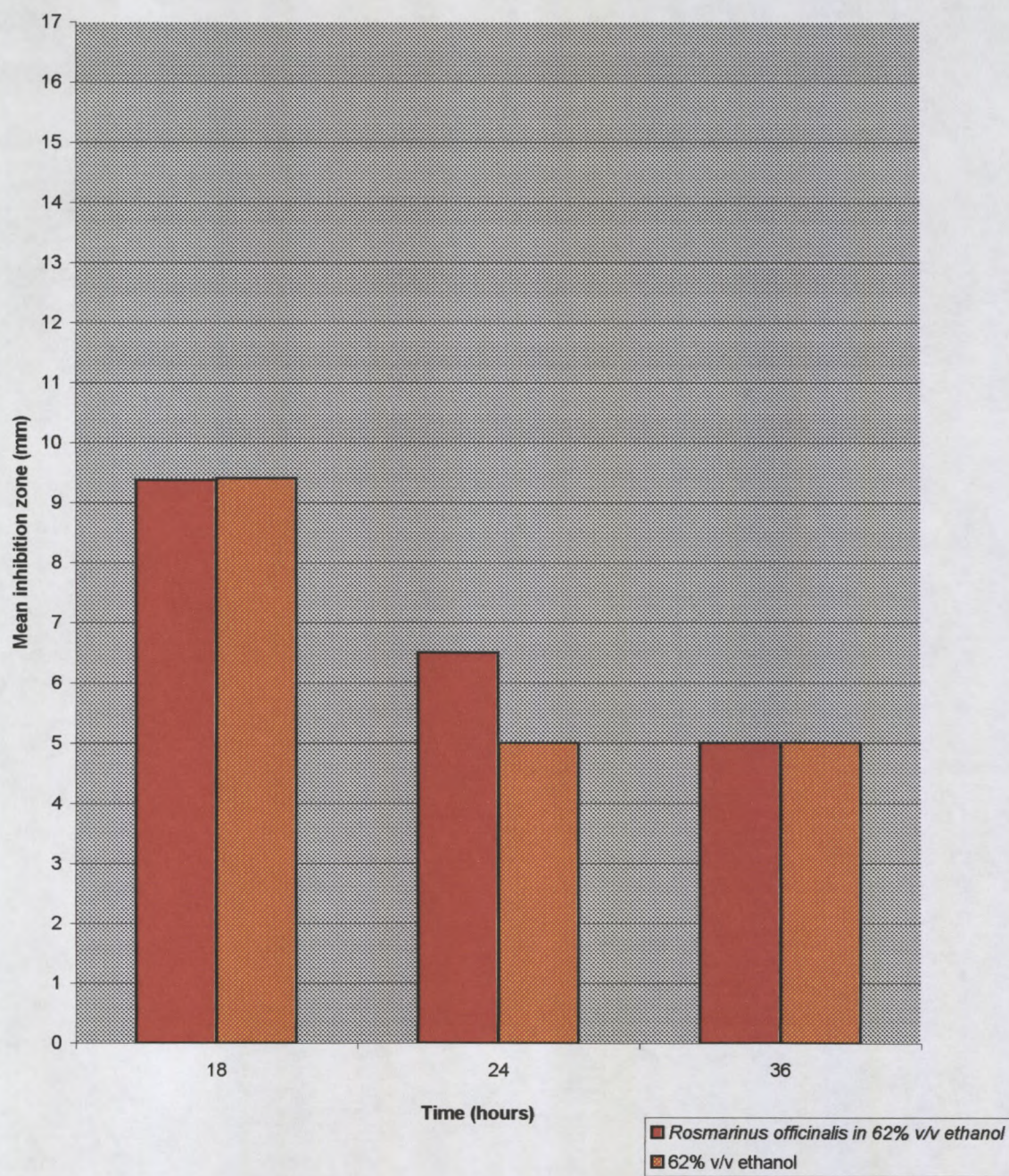


Figure 4.7 Bar chart comparing the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and 62%v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.8 The inter-group comparison between *Salvia officinalis* in 62% v/v ethanol and 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney-U test was used to compare *Salvia officinalis* in 62% v/v ethanol to 62%v/v ethanol.

Table 4.19 Intergroup comparison between *Salvia officinalis* in 62 % v/v ethanol and 62% v/v ethanol.

| TIME | P-VALUE |
|----------|---------|
| 18 hours | 0.350 |
| 24 hours | .000 |
| 36 hours | 0.017 |

CONCLUSION: At 18 hours $P=0.350$, therefore $P = \alpha$. The null hypothesis was accepted. There was no difference in diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and 62% v/v ethanol.

At 24 hours, $P=.000$, therefore, $P < \alpha$. The null hypothesis was rejected. There was a difference in diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and 62% v/v ethanol.

At 36 hours, $P=0.017$, therefore $P < \alpha$. The null hypothesis was rejected. There was a difference in diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and 62% v/v ethanol.

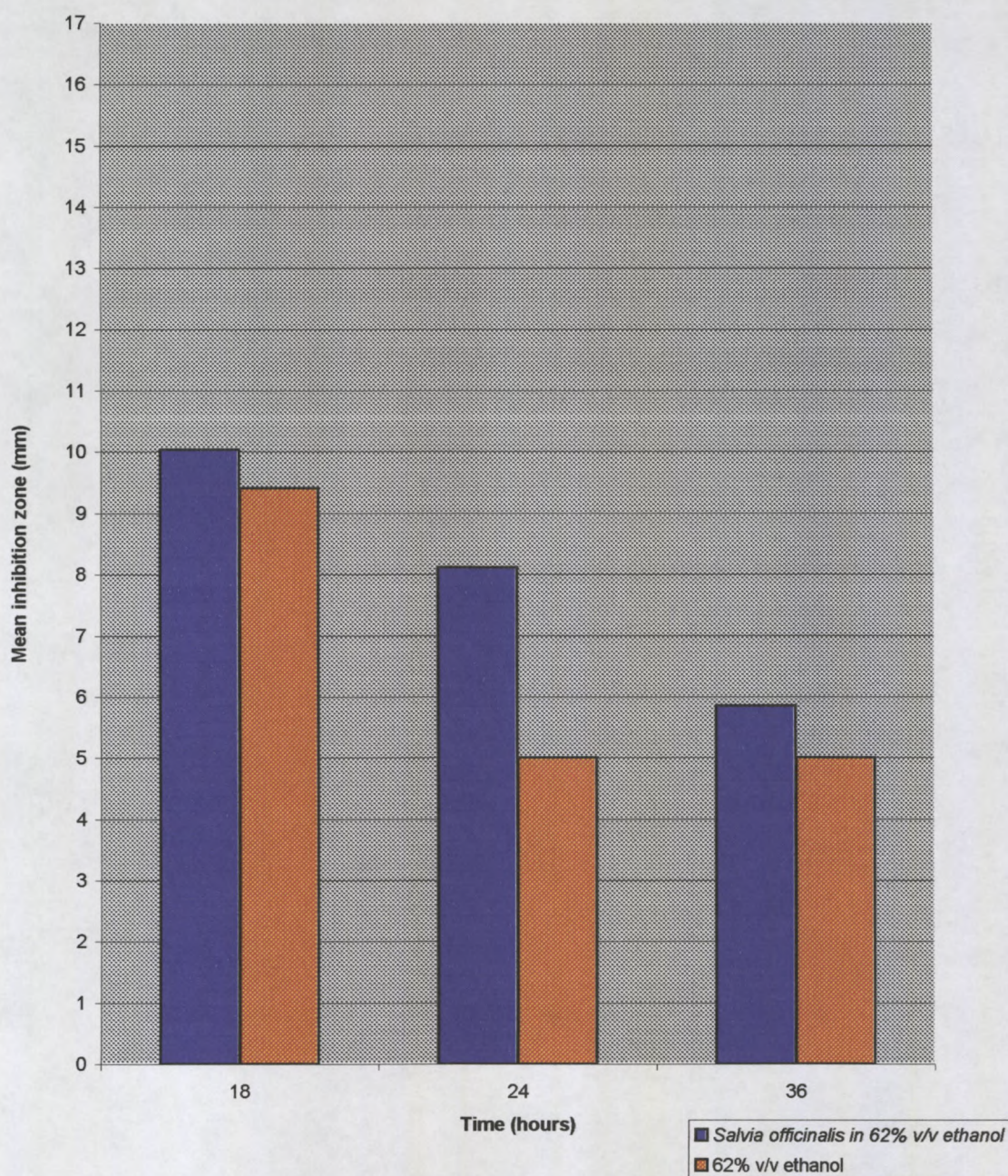


Figure 4.8 Bar chart comparing the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and 62% v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.9 The inter-group comparison between *Thymus vulgaris* in 43% v/v ethanol and 43% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney-U test was used to compare *Thymus vulgaris* in 43% v/v ethanol to 43% v/v ethanol.

Table 4.20 Inter-group comparison between *Thymus vulgaris* in 43% v/v ethanol and 43% v/v ethanol.

| TIME | P-VALUE |
|----------|---------|
| 18 hours | .000 |
| 24 hours | 0.008 |
| 36 hours | 1.00 |

CONCLUSION: At 18 hours $P=0.00$, therefore, $P < \alpha$. The null hypothesis was rejected. Therefore, there was a difference in diameters of the zones of inhibition between *Thymus vulgaris* in 43% v/v ethanol and 43% v/v ethanol.

At 24 hours, $P=0.008$, therefore $P < \alpha$. The null hypothesis was rejected.

Therefore, there was a difference in diameters of the zones of inhibition between *Thymus vulgaris* in 43% v/v ethanol and 43% v/v ethanol.

At 36 hours, $P=1.00$, therefore, $P = \alpha$. The null hypothesis was accepted.

Therefore, there is no difference in diameters of the zones of inhibition of *Thymus vulgaris* in 43% v/v ethanol and 43% v/v ethanol.

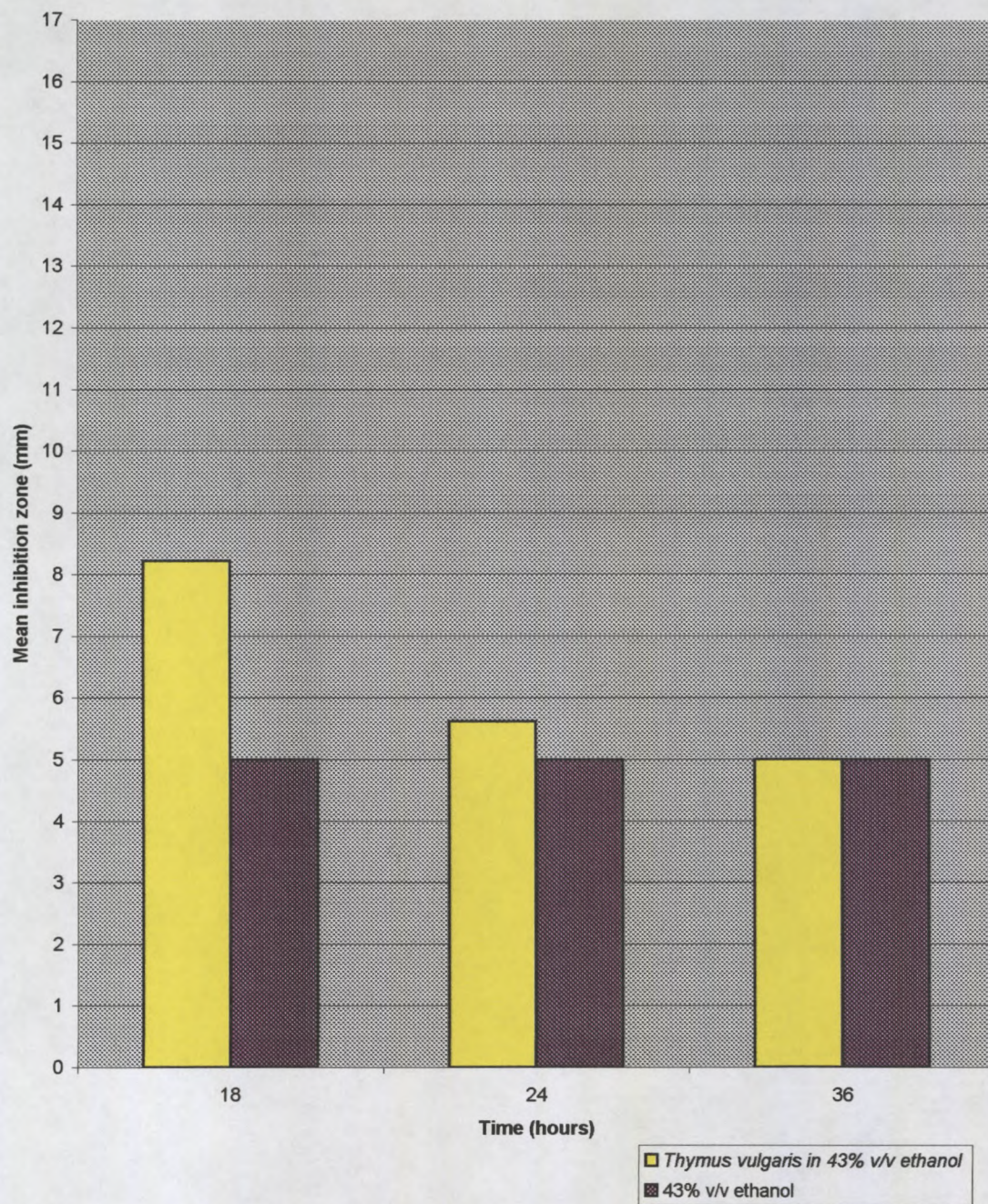


Figure 4.9 Bar chart comparing the diameters of the zones of inhibition of *Thymus vulgaris* in 43% v/v ethanol and 43% v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.10 The inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol and nystatin with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney-U test was used to compare *Rosmarinus officinalis* in 62% v/v ethanol and nystatin.

Table 4.21 inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol and nystatin.

| TIME | P-VALUE |
|----------|---------|
| 18 hours | .000 |
| 24 hours | .000 |
| 36 hours | .000 |

CONCLUSION: At 18 hours, $P=.000$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition between *Rosmarinus officinalis* in 62% v/v ethanol and nystatin.

At 24 hours, $P=.000$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition between *Rosmarinus officinalis* in 62% v/v ethanol and nystatin.

At 36 hours, $P=.000$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition between *Rosmarinus officinalis* in 62% v/v ethanol and nystatin.

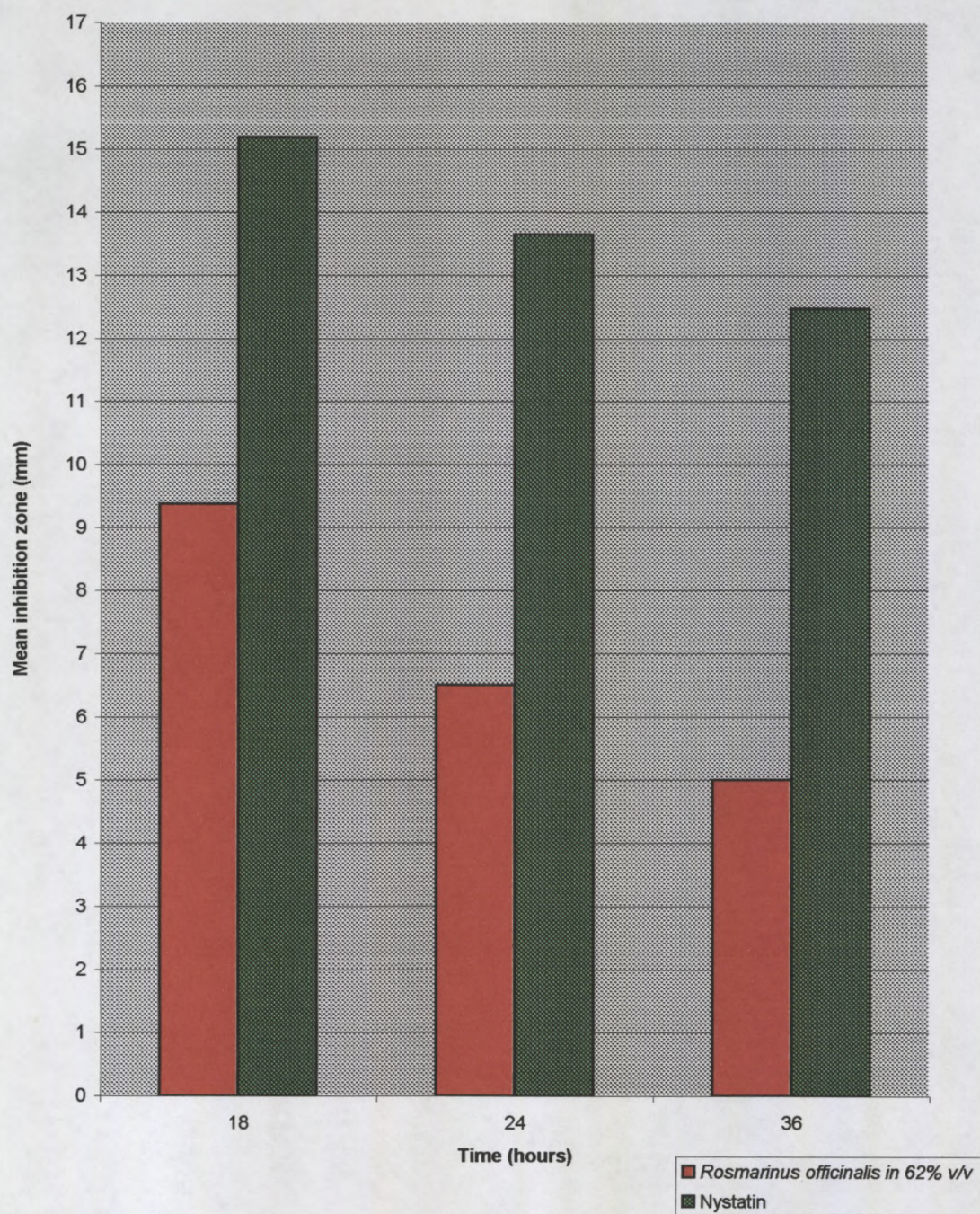


Figure 4.10 Bar chart comparing the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and nystatin with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.11 The inter-group comparison between *Salvia officinalis* in 62% v/v ethanol and nystatin with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney-U test was used to compare *Salvia officinalis* in 62% v/v ethanol and nystatin.

Table 4.22 Inter-group comparison between *Salvia officinalis* in 62% v/v ethanol and nystatin.

| TIME | P-VALUE |
|----------|---------|
| 18 hours | .000 |
| 24 hours | .000 |
| 36 hours | .000 |

CONCLUSION: At 18 hours, $P=.000$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. There is a difference in the diameters of the zones of inhibition between *Salvia officinalis* in 62% v/v ethanol and nystatin.

At 24 hours, $P=.000$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition between *Salvia officinalis* in 62% v/v ethanol and nystatin.

At 36 hours, $P=.000$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition between *Salvia officinalis* in 62% v/v ethanol and nystatin.

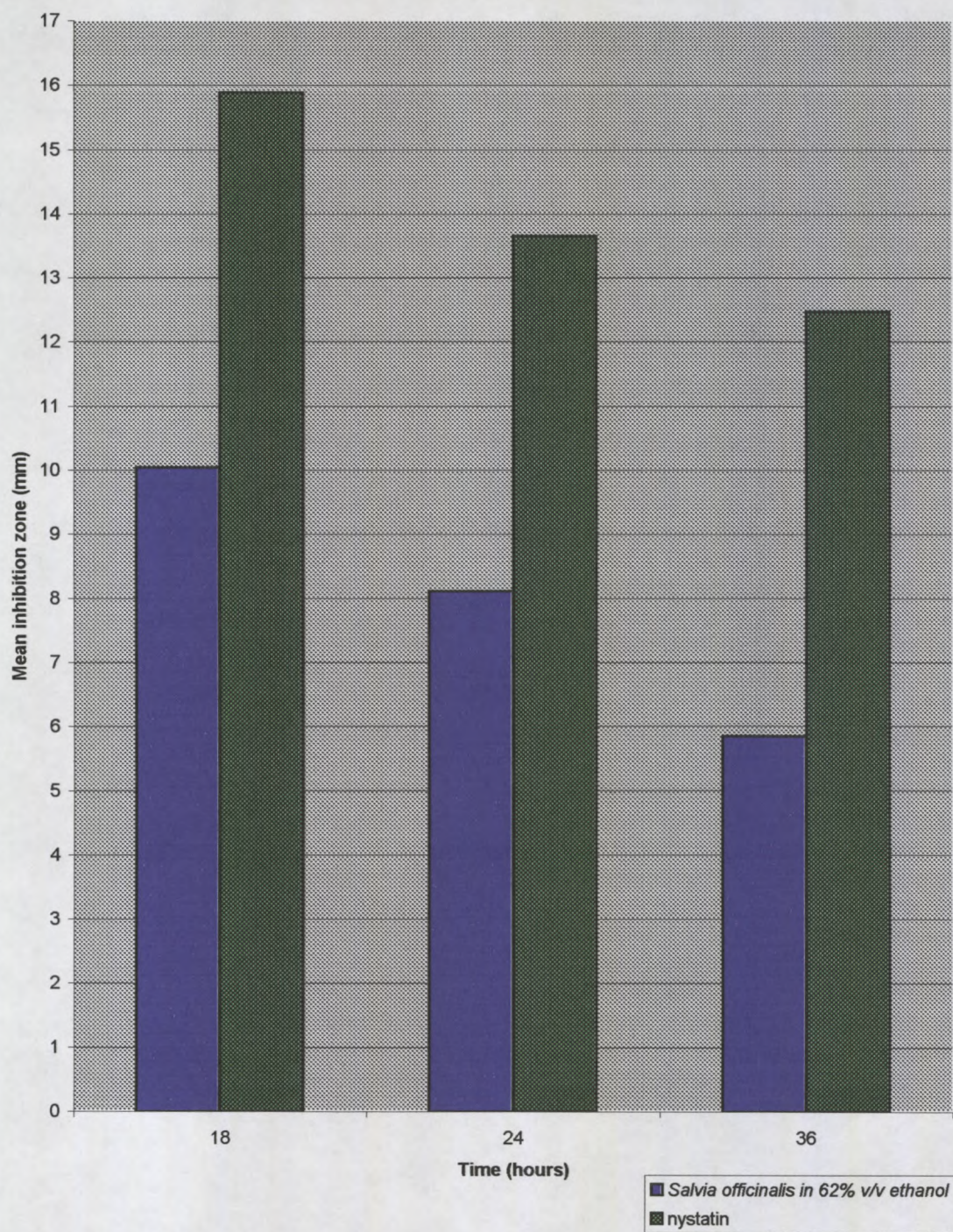


Figure 4.11 Bar chart comparing the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and nystatin with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.12 The inter-group comparison between *Thymus vulgaris* in 43% v/v ethanol and nystatin with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney-U test was used to compare *Thymus vulgaris* in 43% v/v ethanol and nystatin.

Table 4.23 Intergroup comparison between *Thymus vulgaris* in 43% v/v ethanol and nystatin.

| TIME | P-VALUE |
|----------|---------|
| 18 hours | .000 |
| 24 hours | .000 |
| 36 hours | .000 |

CONCLUSION: At 18 hours, $P=.000$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition of *Thymus vulgaris* in 43% v/v ethanol and nystatin.

At 24 hours, $P=.000$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition between *Thymus vulgaris* in 43% v/v ethanol and nystatin.

At 36 hours, $P=.000$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition between *Thymus vulgaris* in 43% v/v ethanol and nystatin.

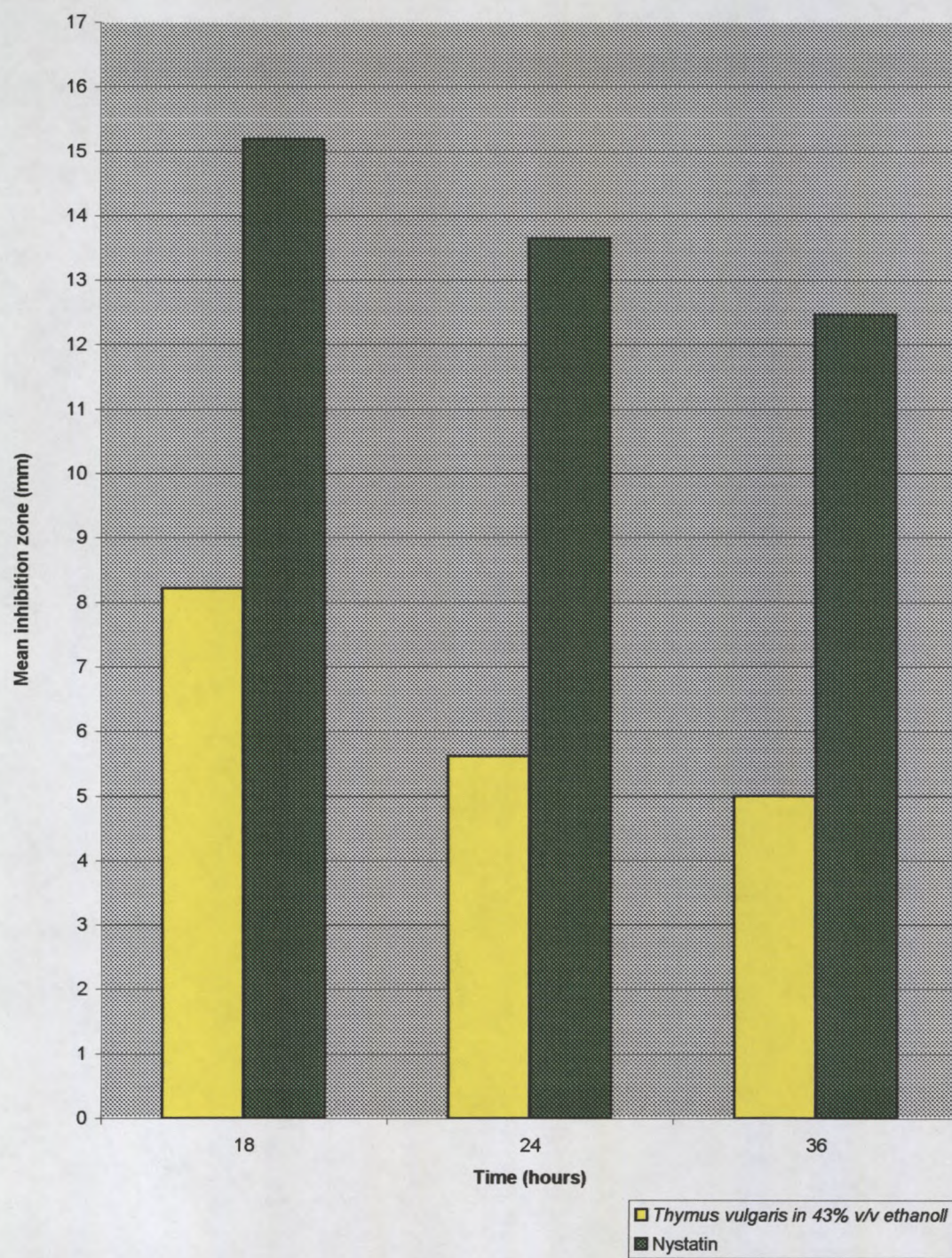


Figure 4.12 Bar chart comparing the diameters of the zones of inhibition of *Thymus vulgaris* in 43% v/v ethanol and nystatin with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.13 The inter-group comparison of *Rosmarinus officinalis* in 62% v/v ethanol and *Salvia officinalis* in 62% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney-U test was used to compare *Rosmarinus officinalis* in 62% v/v ethanol and *Salvia officinalis* in 62% v/v ethanol.

Table 4.24 Inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol and *Salvia officinalis* in 62% v/v ethanol.

| TIME | P-VALUE |
|----------|---------|
| 18 hours | 0.040 |
| 24 hours | 0.020 |
| 36 hours | 0.017 |

CONCLUSION: At 18 hours, $P=0.040$, therefore $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Salvia officinalis* in 62% v/v ethanol.

At 24 hours, $P=0.020$, therefore $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Salvia officinalis* in 62% v/v ethanol.

At 36 hours, $P=0.017$, therefore $P < \alpha$. Therefore the null hypothesis was rejected. There is a difference in the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% ethanol and *Salvia officinalis* in 62% v/v ethanol.

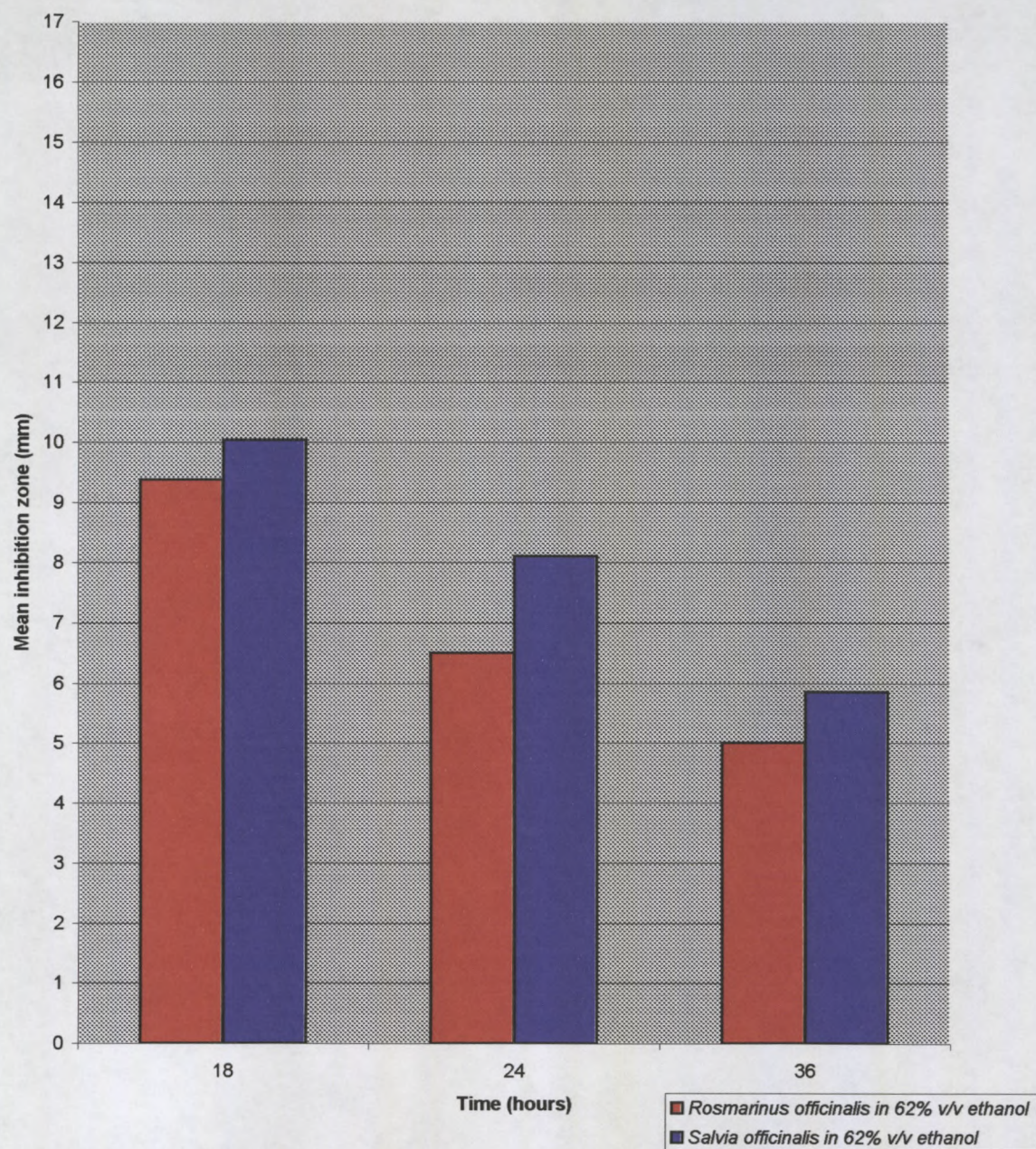


Figure 4.13 Bar chart comparing the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Salvia officinalis* in 62% v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.14 The inter-group comparison of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney-U test was used to compare *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol.

Table 4.25 Inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol.

| TIME | P-VALUE |
|----------|---------|
| 18 hours | 0.001 |
| 24 hours | 0.155 |
| 36 hours | 1.00 |

CONCLUSION: At 18 hours, $P=0.001$, therefore $P < \alpha$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol.

At 24 hours, $P=0.155$, therefore $P > \alpha$. Therefore, the null hypothesis was accepted. There was no difference in the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v and *Thymus vulgaris* in 43% v/v ethanol.

At 36 hours, $P=1.00$, therefore $P > \alpha$. Therefore, the null hypothesis was accepted. There was no difference in the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol.

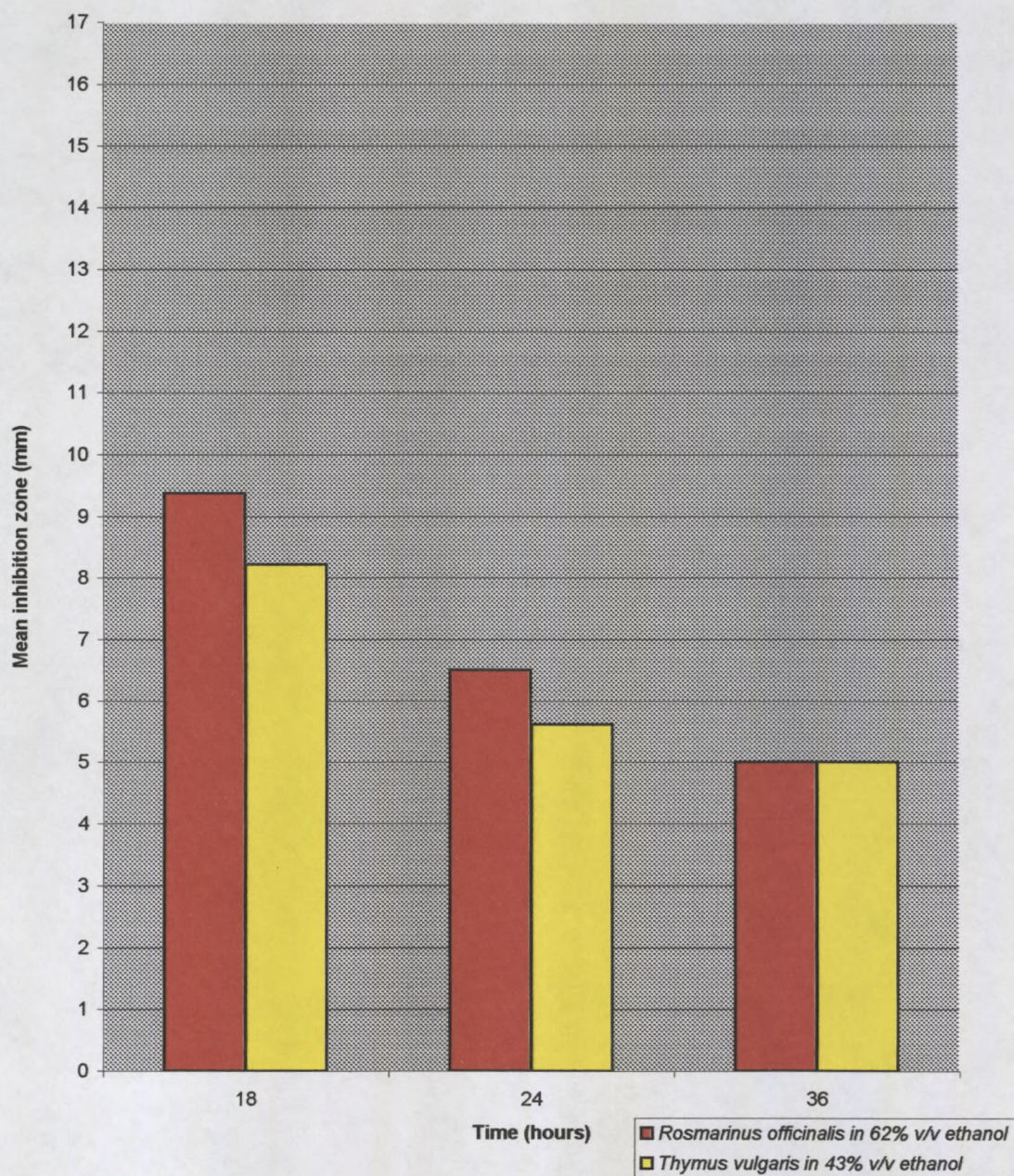


Figure 4.14 Bar chart comparing the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.15 The inter-group comparison of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Mann-Whitney-U test was used to compare *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol.

Table 4.26 Inter-group comparison between *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol.

| TIME | P-VALUE |
|----------|---------|
| 18 hours | .000 |
| 24 hours | .000 |
| 36 hours | 0.017 |

CONCLUSION: At 18 hours, $P=.000$, therefore $P<a$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol.

At 24 hours, $P=.000$, therefore $P<a$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol.

At 36 hours, $P=0.017$, therefore $P<a$. Therefore the null hypothesis was rejected. There was a difference in the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol.

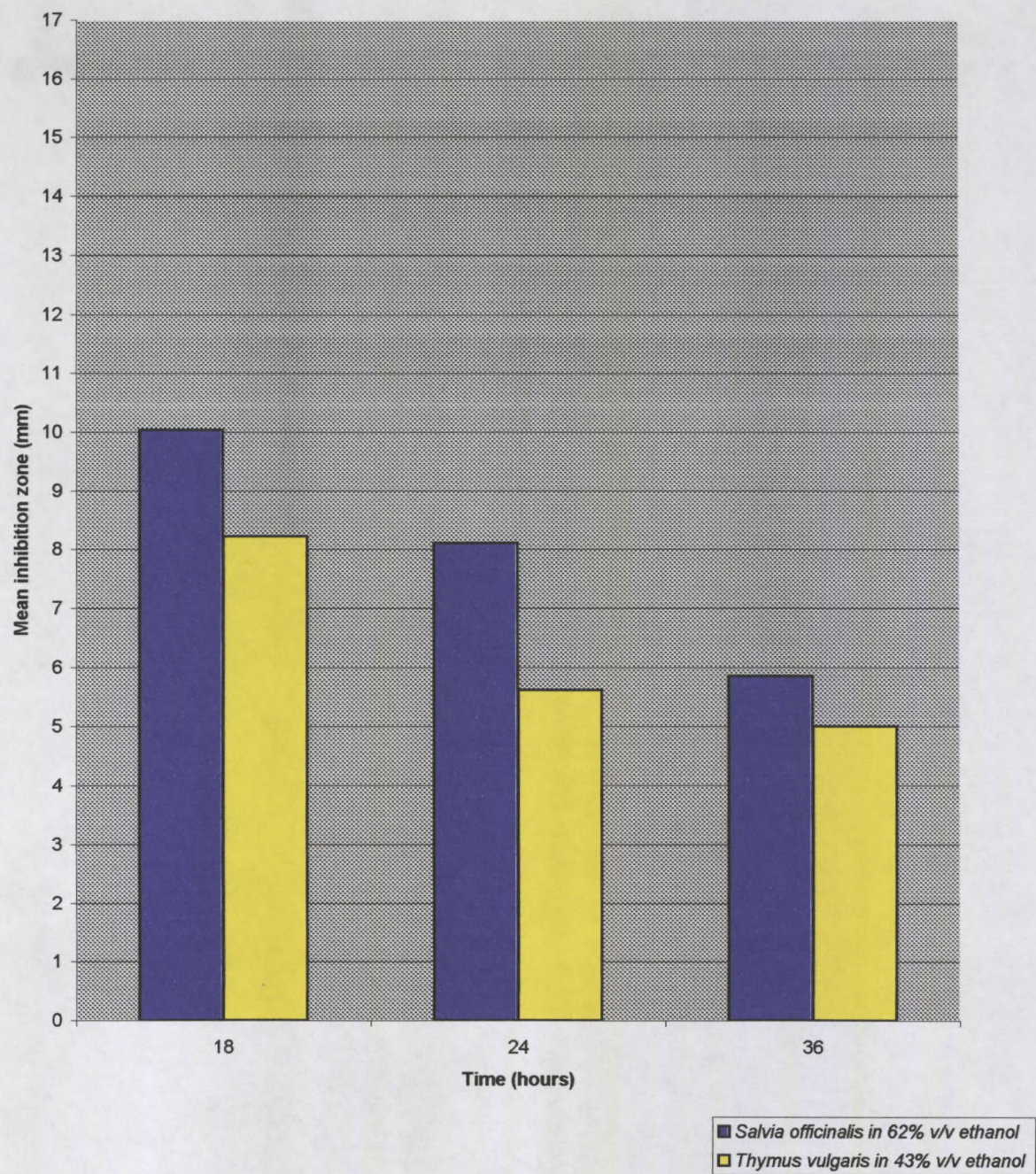


Figure 4.15 Bar chart comparing the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to the observations at 18 hours, 24 hours and 36 hours.

4.3.16 The inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 18 hours, 24 hours and 36 hours

The Kruskal-Wallis test was used to compare the three herbs to each other.

Table 4.27 Inter-group comparison between *Rosmarinus officinalis* in 62% v/v ethanol, *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol.

| TIME | P-VALUE |
|----------|---------|
| 18 hours | .000 |
| 24 hours | .000 |
| 36 hours | 0.004 |

4.3.16.1 Observations at 18 hours

At 18 hours, $P=.000$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. Therefore, there was a difference in diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol, *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol. Since the null hypothesis was rejected, the Dunn Procedure was used to identify the groups that differed.

Table 4.28 Mean values and ranks at 18 hours.

| SAMPLE NO. | ROSMARINUS OFFICINALIS IN 62% V/V ETHANOL (R ₁) | SALVIA OFFICINALIS IN 62% V/V ETHANOL (R ₂) | THYMUS VULGARIS IN 43% V/V ETHANOL (R ₃) |
|------------|---|--|---|
| 1 | 8.38 [9] | 8.63 [11.5] | 7.67 [5] |
| 2 | 9.67 [30.5] | 8.75 [14.5] | 5 [1] |
| 3 | 9 [19.5] | 9.38 [26] | 7.63 [3.5] |
| 4 | 9.34 [24] | 11.17 [44] | 8.7 [13] |
| 5 | 9.38 [26] | 10.25 [39] | 7.63 [3.5] |
| 6 | 8 [7] | 9.88 [35] | 7.25 [2] |
| 7 | 9 [19.5] | 9.13 [22] | 8 [7] |
| 8 | 9.13 [22] | 10 [36] | 8.63 [11.5] |
| 9 | 9.13 [22] | 9.67 [30.5] | 8 [7] |
| 10 | 9.63 [29] | 10.33 [41] | 8.88 [17] |
| 11 | 10.75 [43] | 12.5 [45] | 8.88 [17] |
| 12 | 9.75 [32.5] | 10.25 [39] | 8.88 [17] |
| 13 | 9.38 [26] | 9.83 [34] | 8.5 [10] |
| 14 | 9.75 [32.5] | 10.17 [37] | 9.5 [28] |
| 15 | 10.25 [39] | 10.63 [42] | 8.75 [14.5] |

$$\text{If } |\bar{R}_i - \bar{R}_j| \leq \frac{z\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

then the difference $|\bar{R}_i - \bar{R}_j|$ was not declared significant at the α level.

$R_1=381.5$ (sum total of ranks for *Rosmarinus officinalis* in 62% v/v ethanol)

$R_2=496.5$ (sum total of ranks for *Salvia officinalis* in 62% v/v ethanol)

$R_3=157$ (sum total of ranks for *Thymus vulgaris* in 43% v/v ethanol)

$\bar{R}_1=25.43$

$$\bar{R}_2=33.1$$

$$\bar{R}_3=10.47$$

$$k=3$$

$$N=45$$

$$Z=1.96$$

$$A=0.15$$

$$\sum(t^3-t)=5(3^3-3) + 4(2^3-2)$$

$$=120 + 24$$

$$=144$$

$$\text{if } |\bar{R}_2 - \bar{R}_1| \leq \frac{z\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

$$|33.1 - 25.43| \leq \frac{1.96\sqrt{3[45(45^2-1) - (144)]}}{\sqrt{6 \cdot 45(45-1)}}$$

$$|7.67| \leq \frac{1.96\sqrt{3[91080 - 144]}}{\sqrt{11880}}$$

$$|7.67| \leq 1.96\sqrt{22.96}$$

$$|7.67| \leq 9.39$$

The difference between \bar{R}_2 and \bar{R}_1 was not significant. There was not a difference between the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Salvia officinalis* in 62% v/v ethanol with regard to observations at 18 hours.

$$\text{if } |\bar{R}_2 - \bar{R}_3| \leq \frac{z\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

$$|33.1 - 10.47| \leq \frac{1.96\sqrt{3[45(45^2-1) - (144)]}}{\sqrt{6 \cdot 45(45-1)}}$$

$$|22.63| \leq \frac{1.96\sqrt{3[91080 - 144]}}{\sqrt{11880}}$$

$$|22.63| \leq 1.96\sqrt{22.96}$$

$$|22.63| \leq 9.39$$

The difference between \bar{R}_2 and \bar{R}_3 was significant. There was a difference between the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 18 hours.

$$\text{if } |\bar{R}_1 - \bar{R}_3| \leq \frac{z\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

$$|25.43 - 10.47| \leq \frac{1.96\sqrt{3[45(45^2-1) - (144)]}}{\sqrt{6 \cdot 45(45-1)}}$$

$$|14.96| \leq \frac{1.96\sqrt{3[91080 - 144]}}{\sqrt{11880}}$$

$$|14.96| \leq 1.96\sqrt{22.96}$$

$$|14.96| \leq 9.39$$

The difference between \bar{R}_1 and \bar{R}_3 was significant. There was a difference between the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62%

v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 18 hours.

4.3.16.2 Observations at 24 hours

At 24 hours, $P=.000$, therefore. $P < \alpha$. Therefore the null hypothesis was rejected. Therefore, there was a difference in diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol, *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol. Since the null hypothesis was rejected, the Dunn Procedure will have to be used to identify the groups that differed.

Table 4.29 Mean values and ranks at 24 hours.

| SAMPLE NO. | ROSMARINUS OFFICINALIS IN 62% V/V ETHANOL (R_1) | SALVIA OFFICINALIS IN 62% V/V ETHANOL (R_2) | THYMUS VULGARIS IN 43% V/V ETHANOL (R_3) |
|------------|---|--|---|
| 1 | 6 [16.5] | 5 [7.5] | 5 [7.5] |
| 2 | 5 [7.5] | 8.67 [41] | 5 [7.5] |
| 3 | 5 [7.5] | 7.67 [26] | 5 [7.5] |
| 4 | 5 [7.5] | 9.75 [45] | 5 [7.5] |
| 5 | 8.63 [40] | 8.88 [42] | 5 [7.5] |
| 6 | 5 [7.5] | 8.33 [39] | 6 [16.5] |
| 7 | 5 [7.5] | 7.66 [24] | 5 [7.5] |
| 8 | 5 [7.5] | 7.83 [29.5] | 6 [16.5] |
| 9 | 5 [7.5] | 8.13 [35] | 6 [16.5] |
| 10 | 7.67 [26] | 7.83 [29.5] | 6.25 [19] |
| 11 | 8.17 [37] | 9.67 [44] | 8 [33] |
| 12 | 7.83 [29.5] | 7.67 [26] | 7 [22] |
| 13 | 8.17 [37] | 7.5 [23] | 6.66 [20] |
| 14 | 7.83 [29.5] | 9 [43] | 8 [33] |
| 15 | 8.17 [37] | 8 [33] | 6.83 [21] |

$$\text{if } |\bar{R}_2 - \bar{R}_1| \leq \frac{z\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

then the difference $|\bar{R}_i - \bar{R}_j|$ was declared significant at the α level.

$R_1=305$ (sum total of ranks for *Rosmarinus officinalis* in 62% v/v ethanol)

$R_2=487.5$ (sum total of ranks for *Salvia officinalis* in 62% v/v ethanol)

$R_3=242.5$ (sum total of ranks for *Thymus vulgaris* in 43% v/v ethanol)

$$\bar{R}_1=20.33$$

$$\bar{R}_2=32.5$$

$$\bar{R}_3=16.16$$

$$k=3$$

$$N=45$$

$$Z=1.96$$

$$\sum(t^3 - t) = (14^3 - 14) + 2(4^3 - 4) + 3(3^3 - 3)$$

$$= 2730 + 120 + 72$$

$$= 2922$$

$$\text{if } |\bar{R}_2 - \bar{R}_1| \leq \frac{z\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

$$|32.5 - 20.33| \leq \frac{1.96\sqrt{3[45(45^2-1) - (2922)]}}{\sqrt{6 \cdot 45(45-1)}}$$

$$|12.17| \leq \frac{1.96\sqrt{3[91080 - 2922]}}{\sqrt{11880}}$$

$$|12.17| \leq 1.96\sqrt{22.26}$$

$$|12.17| \leq 9.24$$

The difference between \bar{R}_2 and \bar{R}_1 was significant. There was a difference in the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Rosmarinus officinalis* in 62% v/v ethanol with regard to observations at 24 hours.

$$\text{if } |\bar{R}_2 - \bar{R}_3| \leq z \frac{\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

$$|32.5 - 16.16| \leq 1.96 \frac{\sqrt{3[45(45^2-1) - (2922)]}}{\sqrt{6 \cdot 45(45-1)}}$$

$$|16.34| \leq 1.96 \frac{\sqrt{3[91080 - 2922]}}{\sqrt{11880}}$$

$$|16.34| \leq 1.96 \sqrt{22.26}$$

$$|16.34| \leq 9.24$$

The difference between \bar{R}_2 and \bar{R}_3 was significant. There was a difference in the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 24 hours.

$$\text{if } |\bar{R}_1 - \bar{R}_3| \leq z \frac{\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

$$|20.33 - 16.16| \leq 1.96 \frac{\sqrt{3[45(45^2-1) - (2922)]}}{\sqrt{6 \cdot 45(45-1)}}$$

$$|4.17| \leq 1.96 \frac{\sqrt{3[91080 - 2922]}}{\sqrt{11880}}$$

$$|4.17| \leq 1.96 \sqrt{22.26}$$

$$|4.17| \leq 9.24$$

The difference between \bar{R}_1 and \bar{R}_3 was not significant. There was no difference in the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 24 hours.

4.3.16.3 Observations at 36 hours

At 36 hours, $P=0.004$, therefore, $P < \alpha$. Therefore the null hypothesis was rejected. Therefore, there was a difference in diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol, *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol. Since the null hypothesis was rejected, the Dunn Procedure was used to identify the groups that differed.

Table 4.30 Mean values and ranks at 36 hours.

| SAMPLE NO. | ROSMARINUS OFFICINALIS IN 62% V/V ETHANOL (R ₁) | SALVIA OFFICINALIS IN 62% V/V ETHANOL (R ₂) | THYMUS VULGARIS IN 43% V/V ETHANOL (R ₃) |
|------------|---|---|--|
| 1 | 5 [20.5] | 5 [20.5] | 5 [20.5] |
| 2 | 5 [20.5] | 5 [20.5] | 5 [20.5] |
| 3 | 5 [20.5] | 5 [20.5] | 5 [20.5] |
| 4 | 5 [20.5] | 5 [20.5] | 5 [20.5] |
| 5 | 5 [20.5] | 5 [20.5] | 5 [20.5] |
| 6 | 5 [20.5] | 5 [20.5] | 5 [20.5] |
| 7 | 5 [20.5] | 5 [20.5] | 5 [20.5] |
| 8 | 5 [20.5] | 5 [20.5] | 5 [20.5] |
| 9 | 5 [20.5] | 5 [20.5] | 5 [20.5] |
| 10 | 5 [20.5] | 5 [20.5] | 5 [20.5] |
| 11 | 5 [20.5] | 8.5 [45] | 5 [20.5] |
| 12 | 5 [20.5] | 7.17 [42.5] | 5 [20.5] |
| 13 | 5 [20.5] | 7.17 [42.5] | 5 [20.5] |
| 14 | 5 [20.5] | 7.83 [44] | 5 [20.5] |
| 15 | 5 [20.5] | 7 [41] | 5 [20.5] |

$$\text{if } |\bar{R}_i - \bar{R}_j| \leq \frac{z\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

then the difference $|\bar{R}_i - \bar{R}_j|$ was not declared significant at the α level.

$R_1=307.5$ (sum total of ranks for *Rosmarinus officinalis* in 62% v/v ethanol)

$R_2=420$ (sum total of ranks for *Salvia officinalis* in 62% v/v ethanol)

$R_3=307.5$ (sum total of ranks for *Thymus vulgaris* in 43% v/v ethanol)

$$\bar{R}_1=20.5$$

$$\bar{R}_2=28$$

$$\bar{R}_3=20.5$$

$$k= 3$$

$$N=45$$

$$z=1.96$$

$$\sum(t^3 - t) = (40^3 - 40) + (2^3 - 2)$$

$$= 63960 + 6$$

$$= 63966$$

$$\text{if } |\bar{R}_2 - \bar{R}_1| \leq z \sqrt{\frac{k[N(N^2-1) - (\sum t^3 - \sum t)]}{\sqrt{6N(N-1)}}}$$

$$|28 - 20.5| \leq 1.96 \sqrt{\frac{3[45(45^2-1) - (63966)]}{\sqrt{6 \cdot 45(45-1)}}}$$

$$|7.5| \leq 1.96 \sqrt{\frac{3[91080 - 63966]}{\sqrt{11880}}}$$

$$|7.5| \leq 1.96 \sqrt{6.847}$$

$$|7.5| \leq 5.13$$

The difference between \bar{R}_2 and \bar{R}_1 was significant. There was a difference in the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Rosmarinus officinalis* in 62% v/v ethanol with regard to observations at 36 hours.

$$\text{if } |\bar{R}_2 - \bar{R}_3| \leq \frac{z\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

$$|28 - 20.5| \leq 1.96 \frac{\sqrt{3[45(45^2-1) - (63966)]}}{\sqrt{6 \cdot 45(45-1)}}$$

$$|7.5| \leq 1.96 \frac{\sqrt{3[91080 - 63966]}}{\sqrt{11880}}$$

$$|7.5| \leq 1.96\sqrt{6.847}$$

$$|7.5| \leq 5.13$$

The difference between \bar{R}_2 and \bar{R}_3 was significant. There was a difference in the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 36 hours.

$$\text{if } |\bar{R}_1 - \bar{R}_3| \leq \frac{z\sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)]}}{\sqrt{6N(N-1)}}$$

$$|20.5 - 20.5| \leq 1.96 \frac{\sqrt{3[45(45^2-1) - (63966)]}}{\sqrt{6 \cdot 45(45-1)}}$$

$$|0| \leq 1.96 \frac{\sqrt{3[91080 - 63966]}}{\sqrt{11880}}$$

$$|0| \leq 1.96\sqrt{6.847}$$

$$|0| \leq 5.13$$

The difference between \bar{R}_1 and \bar{R}_3 was not significant. There was no difference in the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to observations at 36 hours.

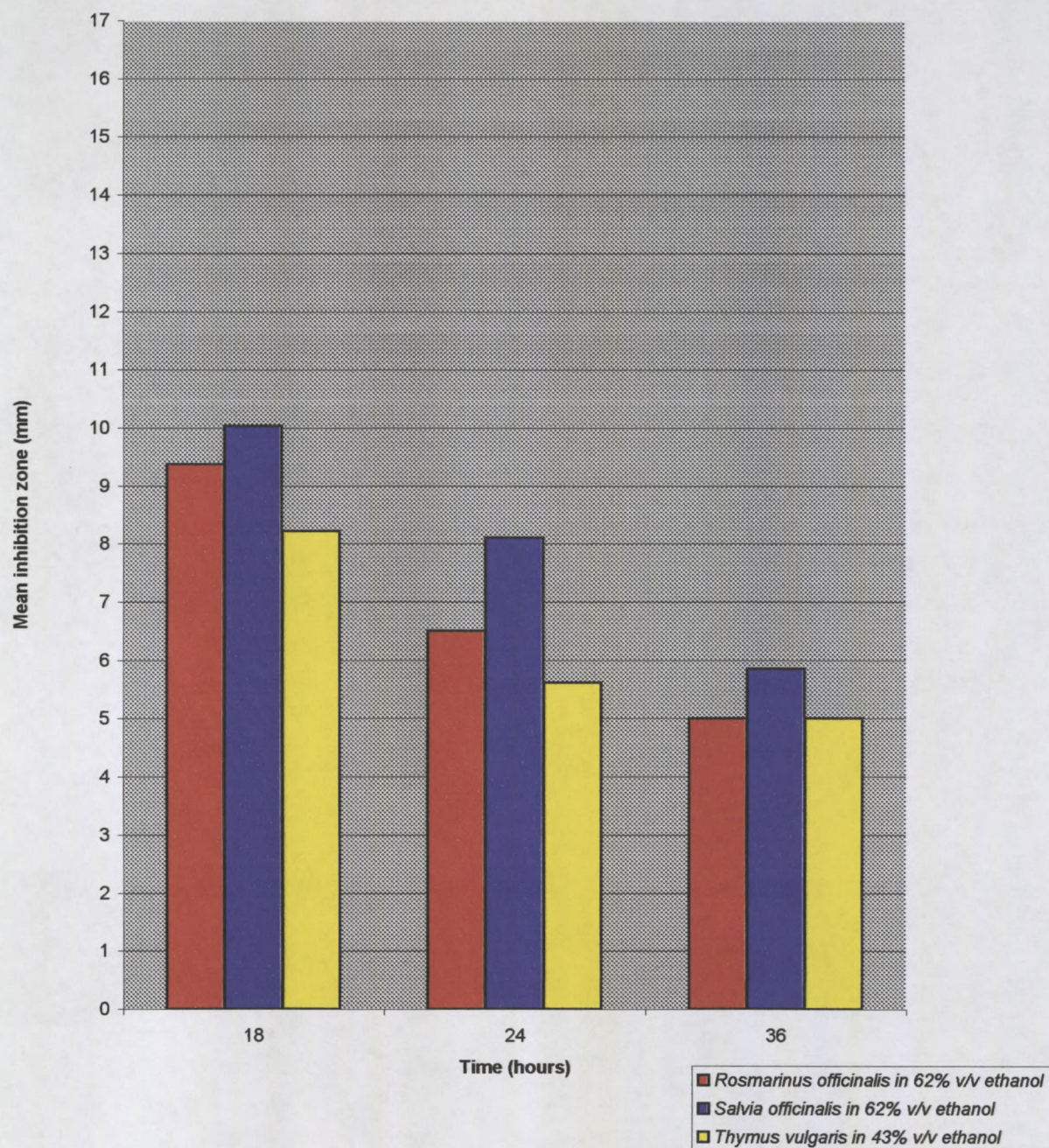


Figure 4.16 Bar chart comparing *Rosmarinus officinalis* in 62% v/v ethanol, *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol with regard to the observations at 18 hours, 24 hours, and 36 hours.

CHAPTER FIVE

DISCUSSION

5.1 THE FIRST OBJECTIVE

5.1.1 Observations at 18 hours

Rosmarinus officinalis in 62% v/v ethanol did produce a zone of inhibition at 18 hours; it did inhibit the *in vitro* growth of *Candida albicans*. However there was no significant difference between the diameters of *Rosmarinus officinalis* in 62% v/v ethanol and 62% v/v ethanol only ($P=0.520$). Therefore, at 18 hours *Rosmarinus officinalis* did not significantly inhibit the *in vitro* growth of *Candida albicans*.

5.1.2 Observations at 24 hours

The diameter of the zone of inhibition produced by *Rosmarinus officinalis* in 62% v/v ethanol decreased significantly in size from 18 hours to 24 hours, as did that of 62% v/v ethanol. *Rosmarinus officinalis* still did not inhibit the *in vitro* growth of *Candida albicans* after 24 hours. The zone size produced by *Rosmarinus officinalis* in 62% v/v ethanol decreased by a lesser degree than that of 62% v/v ethanol. At 24 hours, *Rosmarinus officinalis* in 62% v/v ethanol had a significantly ($P=0.001$) greater zone of inhibition than 62% v/v ethanol.

5.1.3 Observations at 36 hours

The diameter of the zone of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol decreased significantly from 24 hours to 36 hours whilst that of 62% v/v

ethanol remained the same. The diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and 62% v/v ethanol were not significantly different at 36 hours ($P=1.000$). They both had a mean zone size of five millimetres. As this was the diameter of the disc, it can thus be said that the organism grew back up to the edge of the disc, and therefore neither of these substances inhibited the *in vitro* growth of *Candida albicans* after 36 hours.

5.2 THE SECOND OBJECTIVE

5.2.1 Observations at 18 hours

Salvia officinalis in 62% v/v ethanol did produce a zone of inhibition at 18 hours; it did inhibit the *in vitro* growth of *Candida albicans*. However there was no significant difference between the diameters of *Salvia officinalis* in 62% v/v ethanol and 62% v/v ethanol only ($P=0.350$). Therefore, at 18 hours *Salvia officinalis* did not significantly inhibit the *in vitro* growth of *Candida albicans*.

5.2.2 Observations at 24 hours

The diameter of the zone of inhibition produced by *Salvia officinalis* in 62% v/v ethanol decreased significantly in size from 18 hours to 24 hours, as did that of 62% v/v ethanol ($P=.000$). *Salvia officinalis* still did not inhibit the *in vitro* growth of *Candida albicans* after 24 hours. The zone size produced by *Salvia officinalis* in 62% v/v ethanol decreased by a lesser degree than that of 62% v/v ethanol. At 24 hours, *Salvia officinalis* in 62% v/v ethanol had a significantly ($P=.000$) greater zone of inhibition than 62% v/v ethanol.

5.2.3 Observations at 36 hours

The zones of inhibition produced by *Salvia officinalis* in 62% v/v ethanol decreased significantly from 24 hours to 36 hours, whilst that of 62% v/v ethanol remained constant. At 36 hours, *Salvia officinalis* in 62% v/v ethanol had a significantly greater zone of inhibition than 62% v/v ethanol ($P=0.017$).

5.3 THE THIRD OBJECTIVE

5.3.1 Observations at 18 hours

Thymus vulgaris in 43% v/v ethanol did produce a zone of inhibition at 18 hours; it did inhibit the *in vitro* growth of *Candida albicans*. 43% v/v ethanol produced a zone diameter of 5 millimetres; this was the diameter of the disc. Therefore there was growth of *Candida albicans* up to the edge of the disc, it can thus be concluded that 43% v/v ethanol did not inhibit the *in vitro* growth of *Candida albicans*. There was a significant difference between the zone sizes of *Thymus vulgaris* in 43% v/v ethanol and 43% v/v ethanol only ($P=.000$). As 43% v/v ethanol only was the control, it can thus be said that *Thymus vulgaris* inhibited the *in vitro* growth of *Candida albicans*.

5.3.2 Observations at 24 hours

The diameter of the zone of inhibition of *Thymus vulgaris* in 43% v/v ethanol decreased significantly from 18 hours to 24 hours. The diameter of 43% v/v ethanol was still 5 millimetres. The difference between the zone sizes of *Thymus*

vulgaris in 43% v/v ethanol was significantly greater than that of 43% v/v ethanol only ($P=.000$). It can thus be concluded that *Thymus vulgaris* had an inhibitory effect on the *in vitro* growth of *Candida albicans* after 24 hours, although this effect was not as marked as at 18 hours.

5.3.3 Observations at 36 hours

The diameter of the zone of inhibition produced by *Thymus vulgaris* in 43% v/v ethanol did not decreased significantly in size from 24 hours to 36 hours. The zone diameter was 5 millimetres. The diameter of the zone of inhibition of 43% v/v ethanol only remained at 5 millimetres. The diameters of the zones of inhibition of *Thymus vulgaris* in 43% v/v ethanol and 43% v/v ethanol were not significantly different at 36 hours ($P=0.017$). They both had a mean zone size of 5 millimetres. As this was the diameter of the disc, it can thus be said that there was growth of *Candida albicans* up to the edge of the disc, and therefore, neither of these substances inhibited the *in vitro* growth of *Candida albicans* after 36 hours.

5.4 THE FOURTH OBJECTIVE

5.4.1 Comparison of *Rosmarinus officinalis* and nystatin

On observation at 18 hours ($P=.000$), 24 hours ($P=.000$) and 36 hours ($P=.000$), nystatin produced a significantly greater zone of inhibition than *Rosmarinus officinalis* in 62% v/v ethanol. It can therefore be concluded that nystatin had a

greater inhibitory effect on the *in vitro* growth of *Candida albicans* than *Rosmarinus officinalis* in 62% v/v ethanol.

5.4.2 Comparison of *Salvia officinalis* and nystatin

On observation at 18 hours (P=.000), 24 hours (P=.000) and 36 hours (P=.000), nystatin produced a significantly greater zone of inhibition than *Salvia officinalis* in 62% v/v ethanol. It can therefore be concluded that nystatin had a greater inhibitory effect on the *in vitro* growth of *Candida albicans* than *Salvia officinalis* in 62% v/v ethanol.

5.4.3 Comparison of *Thymus vulgaris* and nystatin

On observation at 18 hours (P=.000), 24 hours (P=.000) and 36 hours (P=.000), nystatin produced a significantly greater zone of inhibition than *Thymus vulgaris* in 43% v/v ethanol. It can therefore be concluded that nystatin had a greater inhibitory effect on the *in vitro* growth of *Candida albicans* than *Thymus vulgaris* in 43% v/v ethanol.

5.5 THE FIFTH OBJECTIVE

5.5.1 Comparison of *Rosmarinus officinalis* and *Salvia officinalis*

The results of this study demonstrate that *Rosmarinus officinalis* (see 5.1) and *Salvia officinalis* (see 5.2) did not significantly inhibit the *in vitro* growth of *Candida albicans*. Thus discussion of differences in zone sizes between these two herbs is superfluous.

5.5.2 Comparison of *Rosmarinus officinalis* and *Thymus vulgaris*

The results of this study indicate that *Rosmarinus officinalis* does not significantly inhibit the *in vitro* growth of *Candida albicans* (see 5.1), whereas *Thymus vulgaris* does (see 5.3). Thus discussion of differences in zone sizes between these two herbs is superfluous.

5.5.3 Comparison of *Salvia officinalis* and *Thymus vulgaris*

The results of this study indicate that *Salvia officinalis* does not significantly inhibit the *in vitro* growth of *Candida albicans* (see 5.2), whereas *Thymus vulgaris* does (see 5.3). Thus discussion of differences in zone sizes between these two herbs is superfluous.

5.5.4 Comparison of *Rosmarinus officinalis*, *Salvia officinalis* and *Thymus vulgaris*

5.5.4.1 Observations at 18 hours

According to the Kruskal-Wallis test, the difference between the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Salvia officinalis* in 62% v/v ethanol was not significant.

According to the Kruskal-Wallis test, the difference between the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol was significant.

According to the Kruskal-Wallis test, the difference between the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol was significant.

As compared to each other, *Salvia officinalis* in 62% v/v ethanol and *Rosmarinus officinalis* in 62% v/v ethanol produced equally greater diameters of zones of inhibition than *Thymus vulgaris* in 43% v/v ethanol. This was probably due to the effect of the higher alcoholic concentrations of *Salvia officinalis* in 62% v/v ethanol and *Rosmarinus officinalis* in 62% v/v ethanol, and not due to the effect of the herbs.

5.5.4.2 Observations at 24 hours

According to the Kruskal-Wallis test, the difference between the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Salvia officinalis* in 62% v/v ethanol was significant.

According to the Kruskal-Wallis test, the difference between the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol was not significant.

According to the Kruskal-Wallis test, the difference between the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol was significant.

As compared to each other, *Salvia officinalis* in 62% v/v ethanol produced a greater diameter of zone of inhibition than *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol. The diameters of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol were equally less than that of *Salvia officinalis* in 62% v/v ethanol.

5.5.4.3 Observations at 36 hours

According to the Kruskal-Wallis test, the difference between the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Salvia officinalis* in 62% v/v ethanol was significant.

According to the Kruskal-Wallis test, the difference between the diameters of the zones of inhibition of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol was not significant.

According to the Kruskal-Wallis test, the difference between the diameters of the zones of inhibition of *Salvia officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol was significant.

As compared to each other, *Salvia officinalis* in 62% v/v ethanol produced a significantly larger zone of inhibition than both *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol. The diameters of *Rosmarinus officinalis* in 62% v/v ethanol and *Thymus vulgaris* in 43% v/v ethanol were equally less than that of *Salvia officinalis* in 62% v/v ethanol.

5.6 SUMMARY

The only herb to demonstrate a significant inhibitory effect on the *in vitro* growth of *Candida albicans* was *Thymus vulgaris* as measured after 18 hours ($P=.000$) and 24 hours ($P=0.008$) of incubation. However, there was a significant difference between *Thymus vulgaris* and nystatin, with nystatin being more effective than *Thymus vulgaris* at 18 hours ($P=.000$) and at 24 hours ($P=.000$) when comparing zones diameters (*Thymus vulgaris* measured an average of 8.22mm at 18 hours and 5.62mm at 24 hours, whilst nystatin measured an average of 15.19mm at 18 hours and 13.65mm at 24 hours).

5.7 GENERAL DISCUSSION

It was seen as a general trend that the zone sizes of the herbs and their alcohol controls decreased from 18 hours to 24 hours and at times decreased from 24 hours to 36 hours. The reasons include the possible regrowth of *Candida albicans* as well as the possible evaporation of the test and control substances.

It was also seen that often the zone size of the herb in alcohol decreased by a lesser degree than the alcohol control. This could possibly have been due to the fact that the presence of the herb in the alcohol may have slowed down this evaporation, thus leaving a larger zone size. Another possible explanation for the differing zone sizes of the herbs and the alcohol is that some non-volatile component of the herb was maintaining an anti-microbial effect, or a component of the herb had an inhibitory effect on *Candida albicans* which was of a delayed and longer duration than alcohol.

It was observed that 43% v/v ethanol did not inhibit the growth of *Candida albicans* at all. The reason for this could be that alcohol is most anti-microbial between 50% and 70% (Ketchum, 1998:183). This could also possibly explain the inhibitory effect of 62% v/v ethanol on the *in vitro* growth of *Candida albicans*.

The findings with regard to nystatin were consistent with other studies.

According to Mcfadden (1995), the essential oils of *Rosmarinus officinalis* and *Thymus vulgaris* inhibit the *in vitro* growth of *Candida albicans*. This does not tally with the results obtained in this current study using tinctures, as only *Thymus vulgaris* was found to inhibit the *in vitro* growth of *Candida albicans*. The volatile oils of a plant are more concentrated in an essential oil extract compared to a tincture, infusion or decoction (Lawless, 1995: 24) and, if it is these volatile oils that are responsible for the hypothesised inhibitory effect of

these herbs on the *in vitro* growth of *Candida albicans*, then the experiment should be repeated using the essential oils of these herbs. The inhibitory effects of *Rosmarinus officinalis* and *Thymus vulgaris* (as essential oils) on the *in vitro* growth of *Candida albicans* has been established (McFadden, 1995; Panizzi *et al.*, 1993:167-170; Larrondo *et al.*, 1995:171-172; Mangena *et al.*, 1999:291-296).

The results of this study with regard to *Salvia officinalis* do not correlate with the related literature. According to WebMD Corporation (2001), *Salvia officinalis* is antifungal, but this study has demonstrated otherwise. The reasons for this are possibly due to the fact that *Salvia officinalis* is often administered as an oil and not as a tincture, as previously mentioned.

The results of this study with regard to *Thymus vulgaris* correlate with other studies and related literature. According to Chevallier (1996:142), *Thymus vulgaris* is useful in fungal infections including ringworm, athlete's foot and thrush. According to McFadden (1995), the essential oil of *Thymus vulgaris* inhibits the *in vitro* growth of *Candida albicans*.

A possible weakness in the methodology of this study was the times of checking the zones of inhibition. The *Candida albicans* was most visible at 18 hours (Duguid *et al.*, 1978: 544), hence the first checking time. In clinical practice herbal tinctures of *Rosmarinus officinalis*, *Salvia officinalis* and *Thymus vulgaris*

are applied three times daily (Hoffman, 1996:136, 138, 153). This study's method of infrequent measuring times could have meant that by the time the zone was measured, the effect of the herb had diminished. An alternative method should therefore be employed in further studies to overcome this. Perhaps, repipetting the test substances onto the discs at frequent intervals would conform to clinical practice protocol, and at the same time would rule out the evaporation effect.

The disc diffusion test was found useful but perhaps just as a screening test for antimicrobial sensitivity. Further, more specific investigations and methods are required to finally determine the *in vitro* antimicrobial efficacy of any substance.

Apart from varying the methodology as an *in vitro* study, the herbs could also be tested *in vivo* by means of a controlled clinical trial. These herbs may not have been effective *in vitro*, but perhaps when used *in vivo*, in clinical trials, they may have had more of an inhibitory effect on the growth of *Candida albicans*. It is possible that a remedy showing no inhibition *in vitro* could have a significant effect *in vivo* by, for example, modifying the adherence characteristics of the fungus to the host epithelium (McFadden, 1995). This could be an area for further studies.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The purpose of this study was to determine the efficacy of herbal extracts of certain *Labiatae* species (*Rosmarinus officinalis*, *Salvia officinalis* and *Thymus vulgaris*) in the inhibition of the *in vitro* growth of *Candida albicans* as compared to nystatin in terms of the disc diffusion test.

Rosmarinus officinalis proved to be ineffective in the inhibition of the *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

Salvia officinalis proved to be ineffective in the inhibition of the *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

Thymus vulgaris proved to be effective in the inhibition of the *in vitro* growth of *Candida albicans* in terms of the disc diffusion test. It was most effective at 18 hours and less effective at 24 hours, and ineffective at 36 hours of observation.

Nystatin proved to be more effective than any of the above herbs in inhibiting the *in vitro* growth of *Candida albicans* in terms of the disc diffusion test.

6.2 RECOMMENDATIONS

- 1) Compare tinctures to essential oils.
- 2) The herbs should be tested *in vivo* by means of a controlled clinical trial.
- 3) The herbs could be repipetted onto the discs three times a day as per common clinical protocol.
- 4) Use an alternative, more accurate methodology:
 - A suggested method is to determine the number of cells of *Candida albicans* after the addition of a test substance. This can be done by spectrophotometric analysis, where the turbidity or optical density is an index of growth; or by the serial dilution-agar plate method. The culture is diluted serially and aliquots of each dilution are pipetted onto plates. (Cappucino and Sherman, 1992:77.)
 - The serial dilution agar plate method involves the incorporation of the test substance into the agar and inhibition can be assessed by measuring the colony area from a single point inocula. This method would also have overcome the difficulties encountered by comparing herbs in different alcoholic concentrations. (McFadden, 1995.)

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APPENDIX A

| Tests & Control | Zone Diameter (mm) | | | | | | | | | | | | | | |
|-------------------------------|--------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | TEST 1 | | | TEST 2 | | | TEST 3 | | | TEST 4 | | | TEST 5 | | |
| | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs |
| 1) Rosmarinus officinalis 60% | 8.38 | 6 | 5 | 9.67 | 5 | 5 | 9 | 5 | 5 | 9.34 | 5 | 5 | 9.375 | 8.625 | 5 |
| 2) Salvia officinalis 60% | 8.63 | 5 | 5 | 8.75 | 8.666 | 5 | 9.375 | 7.666 | 5 | 11.17 | 9.75 | 5 | 10.25 | 8.875 | 5 |
| 3) Ethanol 60% | 7.67 | 5 | 5 | 5 | 5 | 5 | 7.625 | 5 | 5 | 8.17 | 5 | 5 | 7.625 | 5 | 5 |
| 4) Thymus Vulgaris 43% | 10 | 5 | 5 | 5 | 5 | 5 | 9.5 | 5 | 5 | 6.83 | 5 | 5 | 10.25 | 5 | 5 |
| 5) Ethanol 43% | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 6) Nystatin | 12.8 | 12.75 | 10.17 | 13.87 | 13.87 | 11.33 | 14.13 | 13.17 | 11.33 | 12.13 | 11.33 | 9.83 | 14.5 | 12.17 | 11 |

| | Zone Diameter (mm) | | | | | | | | | | | | | | |
|-------------------------------|--------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|--------|--------|
| | TEST 6 | | | TEST 7 | | | TEST 8 | | | TEST 9 | | | TEST 10 | | |
| | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs |
| 1) Rosmarinus officinalis 60% | 8 | 5 | 5 | 9 | 5 | 5 | 9.125 | 5 | 5 | 9.125 | 5 | 5 | 9.625 | 7.666 | 5 |
| 2) Salvia officinalis 60% | 9.88 | 8.33 | 5 | 9.125 | 7.66 | 5 | 10 | 7.833 | 5 | 9.625 | 8.125 | 5 | 10.33 | 7.833 | 5 |
| 3) Ethanol 60% | 7.25 | 6 | 5 | 8 | 5 | 5 | 8.625 | 6 | 5 | 8 | 6 | 5 | 8.875 | 6.25 | 5 |
| 4) Thymus Vulgaris 43% | 8.75 | 5 | 5 | 8.67 | 5 | 5 | 11.5 | 5 | 5 | 8.833 | 5 | 5 | 10 | 5 | 5 |
| 5) Ethanol 43% | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 6) Nystatin | 17 | 14.63 | 14 | 17.25 | 16.5 | 14.83 | 16.25 | 13.83 | 13.5 | 14.25 | 12.25 | 11.66 | 13.5 | 11.5 | 11.5 |

| | Zone Diameter (mm) | | | | | | | | | | | | | | |
|-------------------------------|--------------------|--------|--------|---------|--------|--------|---------|--------|--------|---------|--------|--------|---------|--------|--------|
| | TEST 11 | | | TEST 12 | | | TEST 13 | | | TEST 14 | | | TEST 15 | | |
| | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs | 18 hrs | 24 hrs | 36 hrs |
| 1) Rosmarinus officinalis 60% | 10.8 | 8.167 | 5 | 9.75 | 7.833 | 5 | 9.375 | 8.166 | 5 | 9.75 | 7.833 | 5 | 10.25 | 8.166 | 5 |
| 2) Salvia officinalis 60% | 12.5 | 9.667 | 8.5 | 10.25 | 7.666 | 7.167 | 9.833 | 7.5 | 7.166 | 10.17 | 9 | 7.833 | 10.63 | 8 | 7 |
| 3) Ethanol 60% | 8.88 | 8 | 5 | 8.875 | 7 | 5 | 8.5 | 6.667 | 5 | 9.5 | 8 | 5 | 8.75 | 6.833 | 5 |
| 4) Thymus Vulgaris 43% | 11 | 5 | 5 | 9.667 | 5 | 5 | 9.125 | 5 | 5 | 11.83 | 5 | 5 | 10.13 | 5 | 5 |
| 5) Ethanol 43% | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 6) Nystatin | 14.3 | 11.88 | 11.83 | 15.38 | 13 | 12.83 | 16.63 | 15.33 | 13.67 | 16.88 | 15.67 | 14.33 | 19 | 16.83 | 15.33 |