DETERMINATION OF THE ANTIMICROBIAL PROPERTIES OF *WITHANIA SOMNIFERA* AND *XYSMALOBIUM UNDULATUM* PLANT TINCTURES IN TERMS OF THE DISC DIFFUSION ASSAY AND THE AGAR DILUTION SENSITIVITY TEST

BY

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I, Farhad Essop Motara do declare that this mini-dissertation represents my own work in both conception and execution.

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DEDICATION

To my beloved family, who through their infinite love, support and sacrifice, make the completion of this study all the more meaningful.

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ABSTRACT

This study was designed to investigate the antimicrobial efficacy of *Withania somnifera* and *Xysmalobium undulatum* plant tinctures in 62% ethanol in inhibiting the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

The study consisted of two parts. In part one, the Disc Diffusion Assay was utilized whilst in part two the Agar Dilution Method for antimicrobial susceptibility testing was utilized.

The negative control used throughout the study was 62% ethanol. Two positive controls, gentamicin and ciprofloxacin, were also included to account for plate-to-plate variations in the sensitivity of the bacteria to the antimicrobial substances. In both parts of the study, each of the test substances, negative and positive controls were tested in each of six dilutions i.e. a neat, 1:2, 1:4, 1:8, 1:16 and 1:32 dilution.

The sample size throughout part one and two of the study was five.

PART ONE: DISC DIFFUSION ASSAY

Ninety plates of cation adjusted Mueller-Hinton Agar were prepared. Of these, 30 plates each were inoculated with *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Five dry discs were placed equidistantly apart on the surface of each plate. Each disc was previously impregnated with one of the test and control

substances in appropriate dilution. Plates were incubated at 35ºC for eighteen hours after which results were recorded.

The Statistical Package for Social Sciences (SPSS) Version 9 was used for data capturing, while the Mann-Whitney U Test was performed in order to compare the mean inhibition zones between test and control substances.

The results of this part of the study revealed that both test substances were ineffective in inhibiting the *in vitro* growth of *Escherichia coli* and *Pseudomonas aeruginosa.* However, the neat concentration of *Withania somnifera* tincture in 62% ethanol was effective against *Staphylococcus aureus* (P = 0,003), whilst the 1:2 dilution of *Xysmalobium undulatum* tincture also proved effective in inhibiting the *in vitro* growth of *Staphylococcus aureus* (P = 0,004).

PART TWO: AGAR DILUTION SENSITIVITY TEST

For this part of the study 5ml of each test substance, negative and positive control was incorporated into 20ml of cation adjusted Mueller-Hinton Agar. Once again each substance was tested in each of six dilutions (neat, 1:2, 1:4, 1:8, 1:16 and 1:32). With a sample size of five, this resulted in a total of one hundred and fifty plates being prepared.

A Cathra Replicator was used to inoculate thirty-six isolates simultaneously on each plate. Of these, twelve isolates were of *Escherichia coli*, twelve of *Pseudomonas aeruginosa* and twelve of *Staphylococcus aureus.*

Following inoculation, plates were incubated at 35ºC for eighteen hours after which results were recorded.

Part two of the study revealed that neither *Withania somnifera* tincture nor *Xysmalobium undulatum* tincture were effective in inhibiting the *in vitro* growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The negative control (62% ethanol) produced exactly the same results as the two tinctures

CONTENTS

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER SIX

LIST OF TABLES

DEFINITIONS

FACULTATIVE ANAEROBE

An organism that does not require oxygen for it's metabolism and is capable of growth in the absence of oxygen (Talaro and Talaro, 1996: 205).

PATHOGENICITY

The ability of an infectious agent to produce disease in a susceptible host (Evans, 1991: 5).

INFECTION

The deposition, colonization and multiplication of a microorganism in a host (e.g. human or animal) usually accompanied by an immune response with or without clinical illness (Evans, 1991: 5).

NOSOCOMIAL INFECTION

An infection that develops only after entering a hospital or medical institution and that is not present or incubating at the time of admission or the residual of an infection acquired during a previous admission (Evans, 1991: 5).

ANTIBIOTIC

Refers to a substance produced by a microorganism, or to a similar substance (wholly or partly produced by chemical synthesis), which in low concentrations inhibits the growth of other microorganisms (Russel, 1987: 101).

STERILISATION

A process whereby all living cells, viable spores, viruses and viroids are either destroyed or removed from an object or habitat (Harley, Klein and Prescott, 1999: 137).

MINIMUM INHIBITORY CONCENTRATION

The lowest concentration of a drug that prevents the growth of a particular pathogen (Harley, Klein and Prescott, 1999: 68).

TINCTURE

Spiritous preparations (made with pure or diluted alcohol) employed so that a herb will more readily impart its active principles when prepared as a tincture (Lucas, 1991: xvi).

DISINFECTANTS

Chemical agents that kill microbial cells and are used on inanimate objects or certain instruments (Slack and Snyder, 1978: 438).

ANTISEPTIC

Chemical agents that inhibit growth of pathogenic bacteria and are used on tissue to prevent sepsis or infection (Slack and Snyder, 1978: 438).

CHAPTER ONE

1.0 INTRODUCTION

1.1 MOTIVATION FOR THIS STUDY

The discovery of antibiotics as potentially life saving drugs led to the belief that the scourge of infectious diseases would be gone forever and that humankind could live in a virtually infection-free world. Today, over fifty years later, the "truth" is quite different.

The development of bacterial resistance has meant that antibiotics are now being rendered useless by the very bacteria they were meant to destroy. In addition to this the search for newer drugs has been significantly slowed as pharmaceutical companies are not only finding it increasingly difficult to keep up with the pace at which bacterial resistance renders them useless but are also finding it more difficult to get approval for newer drugs (McKenna, 1997: 1-32).

In his book titled Alternatives to Antibiotics, McKenna (1997:32) points out that we may have lost sight of the fact that nature has it's own methods of fighting back i.e. in producing multi-resistant strains of bacteria and that ironically it is to nature and natural medicine that we must look to for a way out of this predicament.

In 1978, the World Health Assembly called on all governments of the world to give high priority to the incorporation of traditional medicines into national drug policies and legislation. In South Africa it has been estimated that at least 2500 species of plants are commonly used as medicines, however a close examination of the South African records of the folk-usage of medicinal plants has revealed that a great deal of information has been inadequately recorded, and that considerable effort is needed to gather information in sufficient detail to be of real scientific value (Gericke, 1996: 37-39). It is in this regard that this study has been undertaken.

1.2 THE STATEMENT OF THE PROBLEM

The purpose of this study was to investigate the efficacy of the antimicrobial properties of *Withania somnifera* and *Xysmalobium undulatum* plant tinctures against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*, using two separate methodologies i.e. the Disc Diffusion Assay and the Agar Dilution Sensitivity Test, and to measure the respective minimum inhibitory concentrations of both plant tinctures in order to determine their value in antimicrobial applications.

1.3 THE STATEMENT OF THE SUBPROBLEM

1.3.1 SUBPROBLEM ONE

To compare the efficacy of *Withania somnifera* tincture in 62% ethanol to the 62% ethanol control against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* in terms of the sizes of the zones of inhibition, using the Disc Diffusion Assay.

1.3.2 SUBPROBLEM TWO

To compare the efficacy of *Xysmalobium undulatum* tincture in 62% ethanol to the 62% ethanol control against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* in terms of the sizes of the zones of inhibition, using the Disc Diffusion Assay.

1.3.3 SUBPROBLEM THREE

To compare the efficacy of *Withania somnifera* tincture in 62% ethanol to the 62% ethanol control in inhibiting the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* using the Agar Dilution Sensitivity Test.

1.3.4 SUBPROBLEM FOUR

To compare the efficacy of *Xysmalobium undulatum* tincture in 62% ethanol to the 62% ethanol control in inhibiting the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* using the Agar Dilution Sensitivity Test.

1.3.5 SUBPROBLEM FIVE

To compare the efficacy of *Withania somnifera* tincture in 62% ethanol to the 62% ethanol control in inhibiting the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* in terms of the minimum inhibitory concentration using the Disc Diffusion Assay.

1.3.6 SUBPROBLEM SIX

To compare the efficacy of *Xysmalobium undulatum* tincture in 62% ethanol to the 62% ethanol control in inhibiting the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* in terms of the minimum inhibitory concentration using the Disc Diffusion Assay.

1.3.7 SUBPROBLEM SEVEN

To compare the efficacy of *Withania somnifera* tincture in 62% ethanol to the 62% ethanol control in inhibiting the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* in terms of the minimum inhibitory concentration using the Agar Dilution Sensitivity Test.

1.3.8 SUBPROBLEM EIGHT

To compare the efficacy of *Xysmalobium undulatum* tincture in 62% ethanol to the 62% ethanol control in inhibiting the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* in terms of the minimum inhibitory concentration using the Agar Dilution Sensitivity Test.

1.3.9 SUBPROBLEM NINE

To compare the efficacy of *Withania somnifera* tincture in 62% ethanol to *Xysmalobium undulatum* tincture in 62% ethanol against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* in terms of the diameter of zones of inhibition using the Disc Diffusion Assay.

1.3.10 SUBPROBLEM TEN

To compare the efficacy of *Withania somnifera* tincture in 62% ethanol to *Xysmalobium undulatum* tincture in 62% ethanol in inhibiting the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* using the Agar Dilution Sensitivity Test.

1.4 HYPOTHESIS

1.4.1 HYPOTHESIS ONE

Withania somnifera tincture in 62% ethanol will have no significant antibacterial effects on *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

1.4.2 HYPOTHESIS TWO

Xysmalobium undulatum tincture in 62% ethanol will have no significant antibacterial effects on *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

1.5 DELIMITATIONS

***** The study was limited to only three species of bacteria viz. *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

* The study was limited to a specific species of *Withania* species viz. *Withania somnifera.*

* The study was limited to a specific species of *Xysmalobium* species viz. *Xysmalobium undulatum*.

* Only *Withania somnifera* tincture produced in 62% ethanol was used.

* Only *Xysmalobium undulatum* tincture produced in 62% ethanol was used.

* Only Mueller-Hinton agar was used as a growth media.

* The incubation temperature of cell growth was 37°C.

* This was an *in vitro* study.

1.6 ASSUMPTIONS

1.6.1 THE FIRST ASSUMPTION

All cultures of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were grown under optimal conditions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE PLANTS

2.1.1 INTRODUCTION

South Africa has been blessed with both, a rich biocultural diversity as well as an enormous herbal medicine tradition probably dating back to palaeolithic times (Gericke, 1996: 37).

Sadly, the study and documentation of African medicinal plants has been greatly neglected when compared to other traditional societies such as the Indian and Chinese. The fact that Africa has the highest rate of deforestation in the world only highlights the urgency needed for the further study of African medicinal plants (Iwu, 1993: 2-3).

2.1.2 *XYSMALOBIUM UNDULATUM*

2.1.2.1 HABITAT AND DESCRIPTION

Xysmalobium undulatum (common name: iSHONGWE) is a perennial herb that grows throughout the grassland regions of South Africa as well as in Namibia, Lesotho and Botswana (Felhaber, 1997: 220).

It grows to an approximate height of one meter, with robust erect flowering stems. The roots consist of a branched, fleshy root system. Leaves are characteristically large, hairy, arranged in opposite pairs and when broken, exude a milky latex.

Flowers are small, yellowish-brown, bell-shaped and arranged in clusters along the stems. Numerous fluffy seeds are contained in large hairy capsules (Gericke, van Oudtshoorn and van Wyk, 1997: 278).

2.1.2.2 PLANT PARTS USED

The roots represent the main parts used. They are light brown on the outside, white inside and have a peculiar sweet almost nauseating smell (Gericke, van Oudtshoorn and van Wyk, 1997: 278).

2.1.2.3 TRADITIONAL MEDICINAL APPLICATIONS

The root of this plant represents one of the most important and widely used medicinal plants of South Africa (Gericke, van Oudtshoorn and van Wyk, 1997: 278).

In his book titled NaturAfrica - the herbalist handbook, Pujol (1993: 77-78) lists some of the known traditional medicinal uses:

* treatment of skin diseases;
* infections of the urinary tract and treatment of haematuria due to kidney and bladder infections;
* diuretic;

* treatment of gonorrhoea;
* fever and coughing due to colds and influenza;
* treatment of infected sores and wounds with the potential for developing into gangrene;
* the root is ground to a powder and inhaled as snuff to alleviate headaches and sinusitis.

Watt and Breyer-Brandwijk (1962: 138-141) have also listed other traditional uses of the plant in their book titled "The Medicinal and Poisonous Plants of Southern and Eastern Africa," these include:-

* using a root decoction or the powdered root alone as a remedy for dysentery;
* as a purgative;
* chewing a piece of the root as an antidote to food poisoning;
* as an emetic by the Zulu and as a snake-bite remedy;
* the Xhosa use an infusion or a decoction of the root to treat colic and abdominal troubles.

Neither Pujol (1993) nor Watt and Breyer-Brandwijk (1962) cite clinical trials which could back up the claims they list.

2.1.2.4 CONSTITUENTS

Breyer-Brandwijk (1962:139) isolated two glucosides from an alcoholic extract of the root. The action of the first, *xysmalobin* a pure crystalline product was investigated by Watt who found that it produces a digitalis-like action on the heart as well as smooth muscle contraction of the uterus, intestine, bladder and especially the bronchi (Breyer-Brandwijk and Watt, 1962: 139).

The second glucoside, having a similar action to *xysmalobin*, but being a lot more toxic, was discovered in the residues remaining following crystallization of *xysmalobin* and represents the main toxic principle of the root. In addition the root was found to contain a large amount of gum, no active volatile oils, no alkaloids, no tannins and only a small amount of acid saponins (Breyer-Brandwijk and Watt, 1962: 139).

2.1.3 *WITHANIA SOMNIFERA*

2.1.3.1 HABITAT AND DESCRIPTION

Withania somnifera (common name: ubuVIMBO) is indigenous to South Africa and is distributed mainly in drier tropical regions. The plant is also widely distributed across Africa, Asia and even Southern Europe (Iwu, 1993: 259).

It is a perennial undershrub with erect, densely velvety stems and leaves. The leaves are pale green, have an oblong shape and are covered with short, dense hairs especially when young. Flowers are small, white or yellowish in colour and occur in short clusters, followed by

small, round, orange-red berries with a diameter of about 8mm (Gericke, van Oudtshoorn and van Wyk, 1997: 274).

2.1.3.2 PLANT PARTS USED

Mainly the leaves and root bark are used (Gericke, van Oudtshoorn and van Wyk, 1997: 274).

2.1.3.3 TRADITIONAL MEDICINAL APPLICATIONS

Breyer-Brandwijk and Watt (1962: 1010-1011) have listed some of the medicinal applications as follows:-

* the Southern Sotho use a decoction of the root to treat colds and chills;
* the Zulu use the decorticated root as an enema to treat feverish infants;
* the Sotho use it to tone up the uterus in females that habitually miscarriage;
* the Xhosa use a decoction of the root bark to treat asthma and other chest complaints;
* the Masai use the leaf juice for conjunctivitis;
* the Xhosa make an ointment by boiling the leaf in fat and use it to treat wounds and sores;
* in Southern and Eastern Africa the leaf is used for treating nausea and rheumatism;

2.1.3.4 CONSTITUENTS

The plant contains alkaloids (eg. withanine) and a group of phytosteroids called withanolides. The plant also contains tannin, fatty acids and volatile oil (Iwu, 1993: 259-260). Compounds

found in the roots include a large variety of steroidal lactones, the most well-known being withaferine A (Cunningham, *et al*. 1996: 273).

2.1.3.5 ANTIMICROBIAL ACTIVITY

According to Breyer Brandwijk and Watt (1962: 1012), both the leaf and root have shown marked antibiotic activity against *Staphylococcus aureus*. Unfortunately, the methodology used to determine such results were not stated by these investigators. In a later study withaferine, a constituent of *Withania somnifera*, was shown to be antibiotic towards Gram-positive organisms, but again no information regarding the methodology used to determine these findings was furnished (Cunningham, *et al*. 1996: 273). A study conducted by Dümmer (2003), to determine the antimicrobial efficacy of *Withania somnifera* on *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* and *Bacillus cereus* using the Disc Diffusion Assay revealed that *Withania somnifera* tincture in 62% ethanol did have a significant antimicrobial effect on *Staphylococcus aureus*. However, inhibition of growth occurred in only three out of a total of fifteen repetitions (please see 2.3 : PREVIOUS RESEARCH).

Neutropenic mice infected with *Staphylococcus aureus* were pretreated with *Withania somniferum*, which resulted in a reduction in the mortality rate from 75% in the control group to 50%, unfortunately no information regarding the methodology used to determine these findings was furnished (Iwu, 1993: 260).

2.2 THE BACTERIA

Escherichia coli, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are three of the most common pyogenic bacteria (Mackie and McCartney, 1989: 618).

2.2.1 *ESCHERICHIA COLI*

2.2.1.1 CLASSIFICATION

Escherichia coli is the most commonly encountered member of the family Enterobacteriaceae in the normal colonic flora and the most common cause of opportunistic infections (Sherris, 1984: 241).

All members of the family Enterobacteriaceae are facultative, all ferment glucose and reduce nitrates to nitrites and all are oxidase negative (Sherris, 1984: 239).

2.2.1.2 MORPHOLOGY AND IDENTIFICATION

Gram-negative, non-sporing bacilli with most strains being motile and generally possessing both sex pili and adhesive fimbriae (Mahon and Manuselis, 1995: 450). Because most strains rapidly ferment lactose, colonies grown on MacConkey media are smooth, glossy, translucent and are rose-pink in colour. Some strains grown on on blood agar result in colonies being surrounded by zones of haemolysis.

Colonies are smooth, circular, 1 – 1,5mm in diameter and yellow opaque if lactose fermenting (blue, if non-lactose fermenting) when grown on cystine-lactose-electrolyte deficient (CLED) medium (Mackie and McCartney, 1989: 437).

2.2.1.3 EPIDEMIOLOGY

Strains of *Escherichia coli* predominate among the aerobic commensal bacteria present in the healthy gut (Mackie and McCartney, 1989: 440).

2.2.1.4 *ESCHERICHIA COLI* INFECTIONS

Escherichia coli was initially considered a non-harmful member of the colon flora, but is now associated with a wide range of diseases and infections including meningial, gastrointestinal, urinary tract, wound and bacteremic infections in all age groups (Mahon and Manuselis, 1995: 452).

Gastrointestinal Infections:

Escherichia coli may cause several types of diarrheal illnesses. Five major categories of diarrheogenic *Escherichia coli* have been identified based on the clinical manifestations produced, epidemiology, virulence factors and O:H serotypes. These include:

enteropathogenic (EPEC)

enterotoxigenic (ETEC)

enteroinvasive (EIEC)

enterohaemorrhagic (EHEC) and

enteroadherent (EAEC)

(Mahon and Manuselis, 1995: 452-454).

Urinary Tract Infections:

Escherichia coli is known to be the most common cause of urinary tract and kidney infections, with strains arising from the fecal flora. Among the factors contributing to the virulence of urethrogenic *Escherichia coli* is the ability of the organism to adhere to the epithelial cells lining the urinary tract (Mahon and Manuselis, 1995: 455).

Septicaemia and Meningitis:

Escherichia coli remains one of the most common causes of meningitis and septicaemia in neonates. The newborn usually acquires the infection in the birth canal just before or during delivery. *Escherichia coli* bacterimia in adults result mainly from genitourinary tract infection or from a gastrointestinal source (Mahon and Manuselis, 1995: 455).

Other infections caused by *Escherichia coli* include peritonitis, cholecystitis, septic wounds and bedsores. They may also infect the lower respiratory passages or cause bacteraemia and endotoxic shock especially in surgical or debilitated patients (Mackie and McCartney, 1989: 440).

2.2.1.5 ANTIMICROBIAL SUSCEPTIBILITY

Within the community, *Escherichia coli* strains are commonly susceptible to all agents active against the Enterobacteriaceae. However, because of the frequent occurrence of R plasmids, strains acquired in hospitals may be resistant to any combination of potentially effective antimicrobics and therapy must therefore be guided by susceptibility testing (Sherris, 1984: 244).

2.2.2 *PSEUDOMONAS AERUGINOSA*

2.2.2.1 CLASSIFICATION

Pseudomonas aeruginosa is a classic opportunist pathogen belonging to the genus Pseudomonas (Mackie and McCartney, 1989: 491). Characteristics common to the genus are as follows:-

* motile with polar or non-polar tufts;
* oxidase and catalase positive;
* usually oxidizes carbohydrates;
* usually grow on MacConkey agar

  (Mahon and Manuselis, 1995: 519).

2.2.2.2 MORPHOLOGY AND IDENTIFICATION

Gram-negative, non-sporing rods, motile usually with a single polar flagellum. Fimbriae may be present. *Pseudomonas aeruginosa* like other members of the genus is extremely adaptable in nutritional terms.

Cultures produce a characteristic sweet, musty smell of amino acetophenone.

A strict aerobe, six colonial types are recognised following incubation on nutrient agar for 24 hours at 37ºC. These are as follows:-

* Type 1 (most common) - large colonies, low convex, rough in appearance and often oval;

*	Type 2	-	small, domed, smooth colonies described as coliform-like;
*	Type 3 and 4	-	small and appear rough and rugose respectively;
*	Type 5	-	mucoid, alginate – producing colonies that often merge following overnight incubation;
*	Type 6	-	smallest colony form that may appear slightly mucoid.

(Mackie and McCartney, 1989: 493).

2.2.2.3 EPIDEMIOLOGY

Pseudomonas aeruginosa flourishes as a saprophyte in warm moist situations in the human environment including sinks, drains, respirators, humidifiers and disinfectant solutions (Mackie and McCartney, 1989: 491).

The primary habitat is environmental i.e. they are found in water, soil and various types of vegetation throughout the world. Infection with *Pseudomonas aeruginosa* is one of the most important causes of invasive infection in compromised patients with serious underlying disease such as leukaemia, cystic fibrosis and extensive burns (Sherris, 1984: 266).

2.2.2.4 *PSEUDOMONAS AERUGINOSA* INFECTIONS

Clinical diseases documented to be caused by *Pseudomonas aeruginosa* include:

* wound infections;
* pulmonary disease, especially in patients with cystic fibrosis;
* nosocomial urinary tract infections;
* endocarditis;
* meningitis;
* bacteremia with ecthyma gangrenosum of the skin;
* otitis externa;
* necrotising skin rash (hot tub syndrome);
* accounts for 6,2% of all bacteremias and up to 74% of all nosocomial bacteremias (Mahon and Manuselis, 1995: 519).

2.2.2.5 ANTIMICROBIAL SENSITIVITY

Of all pathogenic bacteria, *Pseudomonas aeruginosa* is the organism most consistently resistant to antimicrobics. Because it is so regularly resistant to penicillin, ampicillin, cephalothin, tetracycline, chloramphenicol and sulfonamides it has become fruitless to even perform susceptibility tests with these agents (Sherris, 1984: 267). Antibiotics likely to be most effective include gentamicin, tobramycin, amikacin, carbenicillin, ticarcillin, azlocillin and piperacillin.

Clinical trials with ciprofloxacin suggests it may provide a major advance as the first highly active anti-pseudomonal effective by oral administration (Mackie and McCartney, 1989: 494-495).

2.2.3 *STAPHYLOCOCCUS AUREUS*

2.2.3.1 CLASSIFICATION

Staphylococcus aureus belongs to the genus Staphylococcus commonly called Staphylococci. Members of this genus are round Gram-positive cocci that can divide in any plane and tend to be arranged in grape-like clusters (Sherris, 1984: 150).

2.2.3.2 MORPHOLOGY AND IDENTIFICATION

Spherical cocci, 0,8 - 1µm in diameter, Gram-positive, non-sporing, non-motile and except for rare strains, non-capsulate (Mackie and McCartney, 1989: 305).

Staphylococcus aureus is a facultative anaerobe that grows at an optimum temperature of 37ºC and an optimum pH of 7,5.

On nutrient agar, following aerobic incubation for 24 hours at 37ºC, colonies are 1 – 3mm in diameter, have a smooth glistening surface, an entire edge and an opaque pigmented appearance. In most strains, pigmentation is golden with orange, yellow and cream varieties (Mackie and McCartney, 1989: 305-306).

On blood agar, colonies have the same appearance as on nutrient agar, but may be surrounded by a zone of ß-haemolysis. On MacConkey agar, colonies are small to medium in size and pink or pink-orange in colour (Mackie and McCartney, 1989: 305-306).

2.2.3.3 EPIDEMIOLOGY

The basic habitat of *Staphylococcus aureus* is the skin and the anterior nares. Roughly 30% of individuals in the community carry the organism in the anterior nares at any given time. Some individuals have extensive colonization of the perineum. Most *Staphylococcus aureus* infections acquired in the community are the result of autoinfections from the anterior nares, the skin or both (Sherris, 1984: 158).

2.2.3.4 *STAPHYLOCOCCUS AUREUS* INFECTIONS

As with most infections any event that compromises the hosts immune system encourages colonization and infection. *Staphylococcus aureus* infections usually occur as a result of previous skin injuries such as cuts, burns and surgical wounds. Infections caused by *Staphylococcus aureus* are classically suppurative and pyogenic. Common skin infections caused by *Staphylococcus aureus* are boils, carbuncles, furuncles, folliculitis and bullous impetigo (Mahon and Manuselis, 1995: 328-329).

Diseases caused by Staphylococcal toxins include scalded skin syndrome and toxic shock syndrome (Sherris, 1984: 154).

Enterotoxins produced by *Staphylococcus aureus* have been identified and associated with gastrointestinal upset. Such food poisoning occurs when an individual ingests food contaminated with enterotoxin-producing strains (Mahon and Manuselis, 1995: 329).

Other infections caused by *Staphylococcus aureus* include:-

* pneumonia (secondary to influenza A);
* staphylococcal bacteremia leading to secondary pneumonia and endocarditis;
* staphylococcal osteomyelitis secondary to bacteremia;
* septic arthritis, seen in children and in patients with a history of rheumatoid arthritis and IV drug abuse has also been attributed to *Staphylococcus aureus* (Mahon and Manuselis, 1995: 330).

2.2.3.5 ANTIMICROBIAL SENSITIVITY

More than 80% of clinical isolates of *Staphylococcus aureus* in Britain are of the type that form penicillinase and are resistant to benzylpenicillin, phenoxymethylpenicillin, ampicillin and amoxycillin and partially resistant to cephaloridine (Mackie and McCartney, 1989: 308-309).

A few of the penicillin-resistant strains are also resistant to methicillin. A suitable selection for sensitivity tests include; erythromycin, benzylpenicillin, methicillin, trimethoprim, augmentin and fusidic acid. Sensitive strains may be treated with gentamicin, vancomycin, tetracycline, neomycin, etc. (Mackie and McCartney, 1989: 308-309).

2.3 PREVIOUS RESEARCH

Dümmer (2003), conducted a study on the antimicrobial efficacy of *Withania somnifera* tincture in 62% ethanol on the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* and *Bacillus cereus* using the Disc Diffusion Assay. The study revealed that *Withania somnifera* tincture was ineffective against all the bacteria tested except *Staphylococcus aureus*. However, out of a total of fifteen repetitions only three produced an inhibitory effect on the growth of *Staphylococcus aureus* (P = 0,073) (Dümmer, 2003: 68). Furthermore, the specific strain of *Staphylococcus aureus* tested was not indicated in the study.

In a similar study, Invernizzi (2002), performed a Disc Diffusion Assay to evaluate the antimicrobial efficacy of *Tulbagia violacea* herbal tincture in 30% ethanol on the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* and *Bacillus cereus*. This study revealed that *Tulbagia violacea* herbal tincture was ineffective in inhibiting the *in vitro* growth of all the bacteria tested. In consequence, Invernizzi searched for possible flaws in the methodology used.

In his dissertation, Invernizzi (2002: 9-11) makes reference to the three principal methods used to evaluate the antimicrobial properties of natural products i.e. diffusion assays, dilution tests and bioautography tests. Arising from his research, Invernizzi recommends that dry discs and not wet discs (containing liquid samples) be used when testing for the antimicrobial properties of ethanol extracts as this technique will nullify the superfluous antimicrobial

effect of the ethanol on the bacteria (Invernizzi, 2002: 79). Invernizzi, also recommends that dilution tests be performed as an alternative to the wet disc method (Invernizzi, 2002: 87).

It is arising from these findings that this research has utilized the dry disc and dilution methodologies. This research is therefore an extension of Invernizzi's research.

CHAPTER THREE

3.0 METHODOLOGY

3.1 THE DATA

This research involves both primary and secondary data, the nature of which is as follows:

3.1.1 THE PRIMARY DATA

3.1.1.1 DISC DIFFUSION METHOD : ZONES OF INHIBITION

The sensitivity of each bacteria to each of the test substances, negative and positive controls was determined by measuring the size of the zones of inhibition around each respective disc.

a) The results of the experiment determining the antimicrobial effect of a neat concentration of *Withania somnifera* tincture in 62% ethanol in comparison to

   i) 62% ethanol only

   ii) ciprofloxacin 42μg/ml and

   iii) gentamicin 60μg/ml.

b) The results of the experiment determining the antimicrobial effect of a 1:2 dilution of *Withania somnifera* tincture in 62% ethanol in comparison to

   i) a 1:2 dilution of 62% ethanol only

   ii) ciprofloxacin 21μg/ml and

   iii) gentamicin 30μg/ml

c) The results of the experiment determining the antimicrobial effect of a 1:4 dilution of *Withania somnifera* tincture in 62% ethanol in comparison to

i) a 1:4 dilution of 62% ethanol only

ii) ciprofloxacin 10,5μg/ml and

iii) gentamicin 15μg/ml

d) The results of the experiment determining the antimicrobial effect of a 1:8 dilution of *Withania somnifera* tincture in 62% ethanol in comparison to

i) a 1:8 dilution of 62% ethanol only

ii) ciprofloxacin 5,25μg/ml and

iii) gentamicin 7,5μg/ml

e) The results of the experiment determining the antimicrobial effect of a 1:16 dilution of *Withania somnifera* tincture in 62% ethanol in comparison to

i) a 1:16 dilution of 62% ethanol only

ii) ciprofloxacin 2,625μg/ml and

iii) gentamicin 3,75μg/ml

f) The results of the experiment determining the antimicrobial effect of a 1:32 dilution of *Withania somnifera* tincture in 62% ethanol in comparison to

i) a 1:32 dilution of 62% ethanol only

ii) ciprofloxacin 1,3125μg/ml and

iii) gentamicin 1,875μg/ml

g) The results of the experiment determining the antimicrobial effect of a neat concentration of *Xysmalobium undulatum* tincture in 62% ethanol in comparison to

i) 62% ethanol only

ii) ciprofloxacin 42μg/ml and

iii) gentamicin 60μg/ml

h) The results of the experiment determining the antimicrobial effect of a 1:2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol in comparison to

i) a 1:2 dilution of 62% ethanol only

ii) ciprofloxacin 21μg/ml and

iii) gentamicin 30μg/ml

i) The results of the experiment determining the antimicrobial effect of a 1:4 dilution of *Xysmalobium undulatum* tincture in 62% ethanol in comparison to

i) a 1:4 dilution of 62% ethanol only

ii) ciprofloxacin 10,5μg/ml and

iii) gentamicin 15μg/ml

j) The results of the experiment determining the antimicrobial effect of a 1:8 dilution of *Xysmalobium undulatum* tincture in 62% ethanol in comparison to

i) a 1:8 dilution of 62% ethanol only

ii) ciprofloxacin 5,25μg/ml and

iii) gentamicin 7,5μg/ml

k) The results of the experiment determining the antimicrobial effect of a 1:16 dilution of *Xysmalobium undulatum* tincture in 62% ethanol in comparison to

i) a 1:16 dilution of 62% ethanol only

ii) ciprofloxacin 2,625μg/ml and

iii) gentamicin 3,75μg/ml

l) The results of the experiment determining the antimicrobial effect of a 1:32 dilution of *Xysmalobium undulatum* tincture in 62% ethanol in comparison to

i) a 1:32 dilution of 62% ethanol only

ii) ciprofloxacin 1,3125μg/ml and

iii) gentamicin 1,875μg/ml

3.1.1.2 AGAR DILUTION METHOD

The sensitivity of each bacteria to each of the test substances, negative and positive controls when incorporated into the Mueller-Hinton agar media, in each of the six concentrations, was assessed by the incubated innoculum yielding only one of two outcomes i.e. either 'growth' or 'growth inhibition'. No gradations as to the extent of the 'growth' or 'growth inhibition' was made.

a) The results of the experiment determining the antimicrobial effect of a neat concentration, a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of *Withania somnifera* tincture in 62% ethanol.

b) The results of the experiment determining the antimicrobial effect of a neat concentration, a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of *Xysmalobium undulatum* tincture in 62% ethanol.

c) The results of the experiment determining the antimicrobial effect of a neat concentration, a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of 62% ethanol only.

d) The results of the experiment determining the antimicrobial effect of a neat concentration, a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of 42μg/ml of ciprofloxacin.

e) The results of the experiment determining the antimicrobial effect of a neat concentration, a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of 60μg/ml of gentamicin.

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 conducted by the researcher at the Department of Microbiology, Nelson R. Mandela School of Medicine, Durban, was used.

3.3 MATERIALS AND METHODS

3.3.1 THE BACTERIA

The bacteria *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from the pure culture collection at the Department of Microbiology at the Nelson R. Mandela School of Medicine, Durban. Although reference bacterial strains are recommended for antimicrobial susceptibility testing (see 6.2.1) owing to budgetary and other constraints this study was performed using bacterial strains with the following American Type Culture Collection numbers:-

Escherichia coli	**25924**
Pseudomonas aeruginosa	**27852**
Staphylococcus aureus	**29212**

3.3.2 THE TEST SUBSTANCES

The two test substances, *Withania somnifera* and *Xysmalobium undulatum* plant tinctures in 62% ethanol were obtained from Parceval (Pty) Ltd, Wellington, South Africa. The specifications of the two plant tinctures were as follows (Feiter and van Breda, 2003):-

Withania somnifera

Batch No.	002
Method of Preparation	HAB 4a (British Homoepathic Association, 1993:34)
Appearance	Pale yellow-brown clear liquid with no visible impurities or sedimentation
Odour and taste	Earthy odour with a slightly bitter aromatic taste

Specific gravity	0,90178967
Final alcohol	62%
Total solids	1,7%

Xysmalobium undulatum

Batch No.	9108
Method of Preparation	HAB 3a (British Homoepathic Association, 1993: 32 - 33)
Appearance	Light orange yellow liquid
Odour and taste	Aromatic odour and intense bitter taste
Specific gravity	0,8967
Final alcohol	62%
Total solids	4,4%

3.3.3 THE CONTROLS

The negative control, 62% ethanol, was obtained from Parceval (Pty) Ltd.

The two positive controls, gentamicin and ciprofloxacin, were included in order to account for plate to plate variations thereby ensuring that results were relative to each other by the application of ratios (Dümmer, 2003: 33). Both antibiotics were prepared from their respective antibiotic stock solutions, which were obtained from the Department of Microbiology, Nelson R. Mandela School of Medicine.

3.3.4 THE MEDIA

The media used throughout the study was cation-adjusted (calcium and magnesium divalent cations) Mueller-Hinton agar (Allen *et al*., 1992:623). The Mueller-Hinton agar was B.B.L. manufactured and the approximate formula per litre of purified water was as follows:-

Beef extract	2,0g
Acid hydrolysate of casein	17,5g
Starch	1,5g
Agar	17,0g

3.3.5 PART ONE

The Disc Diffusion Method for Antimicrobial Sensitivity Testing [modified Kirby-Bauer Antimicrobial Sensitivity Test (Mackie and McCartney, 1989: 162-163)].

3.3.5.1 PREPARATION OF CONCENTRATIONS

Each test substance, negative and positive controls were tested in six different concentrations i.e. neat, 1:2, 1:4, 1:8, 1:16 and 1:32. These different concentrations were prepared as follows (Pillay, 2003):-

* 1ml of test substance was transferred into a sterile screw-capped test tube marked "neat", using a micro-pipette with a disposable tip.
* 1ml of distilled water was then transferred into each of five additional screw-capped tubes labelled 2, 3, 4, and 5, also using a micro-pipette.
* A further 1ml of test substance was transferred into test tube 2 and was labelled 1:2.

* 1ml of this solution was transferred into test tube 3, which was marked 1:4. 1ml of the solution from test tube 3 was then transferred into test tube 4 and labeled 1:8.

This procedure of serial dilution was repeated until a dilution of 1:32 was achieved, with each test tube being labelled appropriately.

The concentrations of the antibiotic stock solutions, representing the neat concentrations, were as follows:-

42μg/ml for ciprofloxacin and 60μg/ml for gentamicin. With each serial dilution, the concentration for each antibiotic was halved i.e. 1:2 dilution represented 21μg/ml for ciprofloxacin and 30μg/ml for gentamicin and so on.

3.3.5.2 PREPARATION OF THE DISCS

Once the different preparations were prepared, 50μl of each substance, in each concentration was dropped onto a sterile autoclaved, assay disc of 6mm diameter (manufactured by Schleicher and Schuell) using a micro-pipette so that the discs were adequately impregnated. Fifteen discs were prepared for each test substance, in each concentration. Discs, which contained the same substance in the same concentration, were prepared in separate petri dishes, which were then sealed and stored overnight in a refrigerator.

3.3.5.3 PREPARATION OF THE GROWTH MEDIUM

Ninety 90mm petri dishes containing the Mueller-Hinton agar were then prepared as follows:-

* For each litre required (two litres were prepared), 38g of the Mueller-Hinton agar was weighed and added to a sterile 3000ml conical flask.
* To this, 1000ml of distilled water was added and mixed.
* The flask was then sealed and autoclaved at 121°C, with 15 pound pressure for fifteen minutes.
* The liquid agar was then allowed to cool to a temperature of 48 - 50°C.
* 25ml of agar was dispensed into each of the ninety petri dishes via an autoclaved dispensing machine.
* The depth of the agar medium in each plate was 4mm (Baron *et al*., 1995: 1338).
* The plates were then allowed to set and were stored overnight at 4°C in a refrigerator (Baron *et al*., 1995: 1338).

Just before use, the plates were removed from the refrigerator and placed in an incubator (35°C), with lids ajar for 10 – 30 minutes until no visible moisture was present on the surface of the agar (Baron *et al*., 1995: 1338).

3.3.5.4 PREPARATION OF THE INOCULUM

The procedure for the preparation of the inoculum was the same for each type of bacteria used in this study.

* Using a wire loop, the tops of four similar-appearing well isolated colonies, were touched and then transferred to a tube containing 5ml B.B.L. manufactured Mueller-Hinton broth (Allen, *et al*., 1992:659).

* Each tube was sealed, vortexed (to ensure adequate mixture) and then incubated at 35°C for 15-20 minutes (Baron *et al*., 1996: 1330).

* Thereafter the turbidity of each broth was adjusted to a McFarland 0,5 Standard i.e. 10^8 colony forming units per ml (Allen *et al*., 1992: 35).

  The McFarland 0,5 Standard was prepared as follows:-

  0,5ml Barium chloride was added to 99,5ml of sulphuric acid to form a barium sulphate precipitate. 6ml of this solution was transferred to a screw-capped tube that was tightly sealed (Allen *et al*., 1992: 624). The turbidity of each broth was then compared to the tube containing the McFarlands 0,5 against a Wickerham card (Allen *et al*., 1992: 624).

3.3.5.5 INOCULATION OF PLATES

Within 15 minutes of adjusting the turbidity of the inoculum suspension to the McFarland 0,5 Standard, a sterile cotton swab was dipped into the suspension and then rotated several times with firm pressure on the inside of the tube wall to remove excess fluid (Allen *et al*., 1992: 659).

Having been brought to room temperature, the dried surface of the Mueller-Hinton agar plate was inoculated by streaking the swab three times over the entire agar surface, rotating the plate approximately 60º to ensure an even distribution of the inoculum. The lids were then replaced (Allen *et al*., 1992: 659).

3.3.5.6 PLACEMENT OF THE PREPARED DISCS

Five minutes was allowed for the surface of the agar plates to dry before the discs were added (Allen *et al*., 1992: 659).

Thirty plates were inoculated with each bacteria. The sample size was 5, thus 5 plates were replicated with each containing 5 discs impregnated with each of the 5 test substances, all in the same concentration i.e. neat, 1:2, etc.

Disc one contained plant tincture one (*Withania somnifera*).

Disc two contained plant tincture two (*Xysmalobium undulatum*).

Disc three contained control one (62% ethanol).

Disc four contained control two (ciprofloxacin).

Disc five contained control three (gentamicin).

The under surface of each plate was labelled so as to identify the type of bacteria being tested, as well as the concentrations of all the discs. To save time a code was used to distinguish what each disc contains as follows:-

T1	-	represents plant tincture one	:	*Withania somnifera*
T2	-	represents plant tincture two	:	*Xysmalobium undulatum*
C1	-	represents control one	:	62% ethanol
C2	-	represents control two	:	ciprofloxacin
C3	-	represents control three	:	gentamicin

The discs, corresponding to the labelling of the under surface of the petri dish, were placed on the surface using sterile forceps, and gently pressed down onto the agar (Baron *et al*., 1995:1338).

Discs were evenly distributed on the surface, with at least 24mm (center to center) between them (Baron *et al*., 1995: 1338).

3.3.5.7 INCUBATION OF PLATES

Within fifteen minutes after the discs were placed, the plates were inverted and incubated at 35ºC (Baron *et al*., 1995:1338).

3.3.5.8 MEASURING OF RESULTS

Each plate was examined following incubation for eighteen hours (Allen *et al*., 1992:659). A millimeter ruler was used to measure the zone of complete inhibition around each disc to within the nearest millimeter. The diameter of the disc was included in this measurement (Allen *et al*., 1992: 659). All measurements were made with the unaided eye while viewing the back of the petri dish with reflected light against a black, non-reflecting surface (Allen *et al*., 1995: 659).

3.3.6 PART TWO

Agar Dilution Susceptibility Testing (Allen *et al*., 1992: 630 – 631).

The agar dilution testing method is a well standardized, reliable susceptibility testing technique that may be used as a reference for evaluating the accuracy of other testing systems (Baron *et al*., 1995: 1331). In this procedure, the two test substances, and the positive and negative controls are incorporated into the media in varied concentrations (Bryant *et al*., 1983: 433). Test organisms are then inoculated and the culture appropriately incubated. At

the minimal concentration of the antimicrobial in which bacterial growth fails to appear, the organism has found its minimum inhibitory concentration (Bryant *et al*., 1983: 433).

An added advantage of the Agar Dilution test is that several different organisms can be tested simultaneously on the same plate (Baron *et al*., 1995: 1331). Thus, in this part of the study the sample size was also 5, but each plate was inoculated 12 times with each bacteria. A total of 150 plates were prepared, i.e. 30 plates for each test substance, positive and negative controls. Of these 30 plates, 5 were made for each concentration i.e. neat, 1:2, 1:4, 1:8, 1:16 and 1:32.

3.3.6.1 PREPARATION OF PLATES

The preparation of the different concentrations of the various test substances were exactly the same as in Part One of the study (see 3.3.5.1), except the volumes were greater. A volume of 25ml were prepared for each test substance in the same concentration i.e. 5ml for each plate (Pillay, 2003). Once prepared, each screw-capped tube (25ml tubes) was sealed and stored until incorporation into the agar.

Mueller-Hinton agar is the recommended medium for testing commonly encountered aerobic and facultatively anaerobic bacteria (Baron *et al*., 1995: 1329). B.B.L. manufactured Mueller-Hinton agar was again used as the medium, and was prepared in exactly the same way as in Part One of the study (see 3.3.5.4).

The under surface of each plate was labelled as to identify which substances was to be tested and the concentration of the test substance eg. T1 – 1:2, represented test substance *Withania*

somnifera in a 1:2 dilution. Plates containing drug-free agar were prepared for growth controls (Baron *et al*., 1995: 1329).

Once the agar cooled to a temperature of between 48-50ºC, 20ml of the agar was dispensed via an automated dispensing machine into each tube containing the test substance (Pillay, 2003). The screw-capped tube was sealed and gently inverted to allow for mixing. The cap was removed and poured into the appropriately marked petri dish. The plates were allowed to set, thereafter they were stored overnight in a refrigerator at 4ºC (Baron *et al*., 1995: 1329).

3.3.6.2 PREPARATION OF THE INOCULUM

The recommended inoculam for agar dilution is 10^4 colony forming units per spot (Baron *et al*., 1995: 1330).

Five colonies were picked from pure cultured plats on agar based medium and inoculated into 4 – 5ml of B.B.L. manufactured Mueller-Hinton broth. Broths were incubated at 35ºC until turbid. As in Part One of the study, the turbidity was adjusted to match that of a 0,5ml McFarland Standard (Baron *et al*., 1995: 1330).

By use of sterile Mueller-Hinton agar, a 1:10 dilution of the suspension was made to give an adjusted concentration of 10^7 colony forming units per millimeter (Baron *et al*., 1995: 1330).

3.3.6.3 INOCULATION OF PLATES

A Cathra Replicator (similar to the Steers Replicator) was used to inoculate each plate, once the surface of the agar plates had dried. The device consists of a head that is fitted with 37 flat-surfaced inoculating pins and a counterpart consisting of an aluminium seed plate containing 37 wells, and delivers a consistent volume of inoculum to the agar surface (Baron, *et al*, 1995: 1330).

The first well was filled with india ink and was used as a marker. The second well was filled with the *Staphylococcus aureus* inoculam, the third with *Escherichia coli* and the fourth with *Pseudomonas aeruginosa.* The remainder of the wells were filled in the same sequence i.e. the fifth contained *Staphylococcus aureus,* the sixth *Escherichia coli,* the seventh *Pseudomonas aeruginosa* and so on. Using this replicating device allowed 0,001 to 0,002ml of the 10^7 CFU/ml suspension to be delivered to the agar surface, resulting in the final desired inoculam approximately 10^4 CFU per spot. Thus, each plate was inoculated with 12 isolates of each bacteria, including the marker.

To check for the viability of each test isolate and as an added check for purity, control plates not containing any test substance, were inoculated last (Baron *et al*., 1995: 1330).

Inoculated plates were allowed to stand until inocula were completely absorbed by the medium, and were then inverted and incubated in air at 35ºC for 18 hours before being read (Baron *et al*., 1995: 1330).

3.3.6.4 MEASURING OF RESULTS

Micro-organisms that are sensitive to the concentration of the test substance contained in any given agar plate do not produce a circle of growth at the inoculam site, whereas those that were resistant appear as circular colonies. Thus, one obtains only a positive or negative result, with no gradations (Allen *et al*., 1995: 1330).

Each plate was examined for any growth over a device (the same size as the plate), which had been labelled 1 to 37. The marker (india ink) was placed over the area labelled 1, thus allowing easy identification of any growth.

Example: Growth over area corresponding to the numbers 2, 5, 8, 11, 14, 17, 20, 23, etc. represented the growth of *Staphylococcus aureus*.

All results were recorded on sheets numbered 1 to 37. The record sheet also included the name of the test substance as well as its concentration.

3.4 DATA ANALYSIS AND STATISTICAL PROCEDURES

3.4.1 SAMPLE SIZE OF THE STUDY

The sample size throughout Part One (Disc Diffusion Method) and Part Two (Agar Dilution Method) of this study was five i.e. each sensitivity test was repeated five times, yielding five sets of data.

3.4.2 STATISTICAL PACKAGE

The Statistical Package for Social Sciences (SPSS) Version 9 was used data capturing and analysis.

3.4.3 SUBPROBLEM ONE

3.4.3.1 PROCEDURE 1.1

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the average diameter of the zones of inhibition of the two independent samples.

* Hypothesis testing

  The null hypothesis H_o states that there is no difference in diameter of the zone of inhibition between the *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control with respect to the variable comparison at the $\alpha = 0,05$ level of significance. The alternative hypothesis H_1, states that there is a difference at the same level of significance.

  H_o: $M_1 = M_2$

  $H_{1:}$ $M_1 = M_2$

* Decision rule

  At $\alpha = 0,05$ level of significance, the null hypothesis is rejected if $P < \alpha$, where P is the observed significance level or probability value. Otherwise, the null hypothesis is accepted at the same level of significance.

  Reject H_o if $P < \alpha$

  Accept H_o if $P \geq \alpha$

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

3.4.3.2 PROCEDURE 1.2

Intergroup comparison between the inhibitory effects of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameters of the zones of inhibition.

The Mann-Whitney U-test was used to compare the average diameter of the zones of inhibition of the two independent samples.

* Hypothesis testing (As per procedure 1.1)
* Decision rule (As per procedure 1.1)

3.4.3.3 PROCEDURE 1.3

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameters of the zones of inhibition.

The Mann-Whitney U-test was used to compare the average diameter of the zones of inhibition of the two independent samples.

* Hypothesis testing (As per procedure 1.1)
* Decision rule (As per procedure 1.1)

3.4.4 SUBPROBLEM TWO

3.4.4.1 PROCEDURE 1.4

Intergroup comparison between the inhibitory effect of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameters of the zones of inhibition.

The Mann-Whitney U-test was used to compare the average diameter of the zones of inhibition of the two independent samples.

* Hypothesis testing (As per procedure 1.1)
* Decision rule (As per procedure 1.1)

3.4.4.2 PROCEDURE 1.5

Intergroup comparison between the inhibitory effect of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameters of the zones of inhibition.

The Mann-Whitney U-test was used to compare the average diameter of the zones of inhibition of the two independent samples.

* Hypothesis testing (As per procedure 1.1)
* Decision rule (As per procedure 1.1)

3.4.4.3 PROCEDURE 1.6

Intergroup comparison between the inhibitory effect of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameters of the zones of inhibition.

The Mann-Whitney U-test was used to compare the average diameter of the zones of inhibition of the two independent samples.

* Hypothesis testing (As per procedure 1.1)
* Decision rule (As per procedure 1.1)

3.4.5 SUBPROBLEM THREE

3.4.5.1 PROCEDURE 1.7

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Escherichia coli* in terms of the Agar Dilution Method.

The results obtained from the Agar Dilution Method yields one of two possible outcomes i.e. growth or growth inhibition. This non-analytical data was visually summarised in the form of a table (Table 7, Appendix A).

3.4.5.2 PROCEDURE 1.8

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method.

The results obtained from the Agar Dilution Method yields one of two possible outcomes i.e. growth or growth inhibition. This non-analytical data was represented in the form of a table (Table 8, Appendix A).

3.4.5.3 PROCEDURE 1.9

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the Agar Dilution Method.

The results obtained from the Agar Dilution Method yields one of two possible outcomes i.e. growth or growth inhibition. This non-analytical data was represented in the form of a table (Table 9, Appendix A).

3.4.6. SUBPROBLEM FOUR

3.4.6.1 PROCEDURE 1.10

Intergroup comparison between the inhibitory effect of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control only on the growth of *Escherichia coli* in terms of the Agar Dilution Method.

The results obtained from the Agar Dilution Method yields one of two possible outcomes i.e. growth or growth inhibition. This non-analytical data was represented in the form of a table (Table 10, Appendix A).

3.4.6.2 PROCEDURE 1.11

Intergroup comparison between the inhibitory effect of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method.

The results obtained from the Agar Dilution Method yields one of two possible outcomes i.e. growth or growth inhibition. This non-analytical data was represented in the form of a table (Table 11, Appendix A).

3.4.6.3 PROCEDURE 1.12

Intergroup comparison between the inhibitory effect of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the Agar Dilution Method.

The results obtained from the Agar Dilution Method yields one of two possible outcomes i.e. growth or growth inhibition. This non-analytical data was represented in the form of a table (Table 12, Appendix A).

3.4.7 SUBPROBLEM FIVE

In order to determine and compare the minimum inhibitory concentration of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus,* both the tincture and the control were diluted to a 1:2, 1:4, 1:8, 1:16 and 1:32 concentration. The minimum concentration of each substance that inhibits all growth is regarded as the minimum inhibitory concentration of that substance.

The Mann-Whitney U-test was used to compare the two independent samples in each case for PROCEDURES 1.13 to 1.27.

3.4.7.1 PROCEDURE 1.13

Intergroup comparison between the inhibitory effect of a 1:2 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

3.4.7.2 PROCEDURE 1.14

Intergroup comparison between the inhibitory effect of a 1:2 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

3.4.7.3 PROCEDURE 1.15

Intergroup comparison between the inhibitory effect of a 1:2 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

3.4.7.4 PROCEDURE 1.16

Intergroup comparison between the inhibitory effect of a 1:4 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:4 dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

3.4.7.5 PROCEDURE 1.17

Intergroup comparison between the inhibitory effect of a 1:4 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:4 dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

3.4.7.6 PROCEDURE 1.18

Intergroup comparison between the inhibitory effect of a 1:4 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:4 dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

3.4.7.7 PROCEDURE 1.19

Intergroup comparison between the inhibitory effect of a 1:8 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:8 dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

3.4.7.8 PROCEDURE 1.20

Intergroup comparison between the inhibitory effect of a 1:8 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:8 dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

3.4.7.9 PROCEDURE 1.21

Intergroup comparison between the inhibitory effect of a 1:8 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:8 dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

3.4.7.10 PROCEDURE 1.22

Intergroup comparison between the inhibitory effect of a 1:16 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:16 dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

3.4.7.11 PROCEDURE 1.23

Intergroup comparison between the inhibitory effect of a 1:16 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:16 dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

3.4.7.12 PROCEDURE 1.24

Intergroup comparison between the inhibitory effect of a 1:16 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:16 dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

3.4.7.13 PROCEDURE 1.25

Intergroup comparison between the inhibitory effect of a 1:32 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:32 dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

3.4.7.14 PROCEDURE 1.26

Intergroup comparison between the inhibitory effect of a 1:32 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:32 dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

3.4.7.15 PROCEDURE 1.27

Intergroup comparison between the inhibitory effect of a 1:32 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:32 dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

3.4.8 SUBPROBLEM SIX

In order to determine and compare the minimum inhibitory concentration of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus,* both the tincture and the control were diluted to a 1:2, 1:4, 1:8, 1:16 and 1:32 concentration. The minimum concentration of each substance that inhibits all growth is regarded as the minimum inhibitory concentration of that substance.

The Mann-Whitney U-test was used to compare the two independent samples in each case for PROCEDURES 1.28 to 1.42.

3.4.8.1 PROCEDURE 1.28

Intergroup comparison between the inhibitory effect of a 1:2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

3.4.8.2 PROCEDURE 1.29

Intergroup comparison between the inhibitory effect of a 1:2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

3.4.8.3 PROCEDURE 1.30

Intergroup comparison between the inhibitory effect of a 1:2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

3.4.8.4 PROCEDURE 1.31

Intergroup comparison between the inhibitory effect of a 1:4 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:4 dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

3.4.8.5 PROCEDURE 1.32

Intergroup comparison between the inhibitory effect of a 1:4 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:4 dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

3.4.8.6 PROCEDURE 1.33

Intergroup comparison between the inhibitory effect of a 1:4 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:4 dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

3.4.8.7 PROCEDURE 1.34

Intergroup comparison between the inhibitory effect of a 1:8 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:8 dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

3.4.8.8 PROCEDURE 1.35

Intergroup comparison between the inhibitory effect of a 1:8 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:8 dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

3.4.8.9 PROCEDURE 1.36

Intergroup comparison between the inhibitory effect of a 1:8 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:8 dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

3.4.8.10 PROCEDURE 1.37

Intergroup comparison between the inhibitory effect of a 1:16 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:16 dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

3.4.8.11 PROCEDURE 1.38

Intergroup comparison between the inhibitory effect of a 1:16 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:16 dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

3.4.8.12 PROCEDURE 1.39

Intergroup comparison between the inhibitory effect of a 1:16 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:16 dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

3.4.8.13 PROCEDURE 1.40

Intergroup comparison between the inhibitory effect of a 1:32 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:32 dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

3.4.8.14 PROCEDURE 1.41

Intergroup comparison between the inhibitory effect of a 1:32 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:32 dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

3.4.8.15 PROCEDURE 1.42

Intergroup comparison between the inhibitory effect of a 1:32 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:32 dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

3.4.9 SUBPROBLEM SEVEN

In order to determine and compare the minimum inhibitory concentration of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* both the tincture and the control were diluted to a 1:2, 1:4, 1:8, 1:16 and 1:32 concentration.

Using the Agar Dilution method (where each test substance is incorporated into the Mueller-Hinton agar medium), the minimum concentration of the substance in the growth media at which "no growth" occurs is regarded as the minimum inhibitory concentration.

Since no gradations as to the extent of the growth or growth inhibition were made, the non-analytical data was expressed in the form of tables (see Tables 19, 20 and 21, Appendix A for raw data).

3.4.9.1 PROCEDURE 1.43

Intergroup comparison between the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of *Withania somnifera* tincture in 62% ethanol and a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution respectively of the 62% ethanol control on the growth of *Escherichia coli* in terms of the Agar Dilution Method.

3.4.9.2 PROCEDURE 1.44

Intergroup comparison between the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of *Withania somnifera* tincture in 62% ethanol and a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution respectively of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method.

3.4.9.3 PROCEUDRE 1.45

Intergroup comparison between the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of *Withania somnifera* tincture in 62% ethanol and a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution

respectively of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the Agar Dilution Method.

3.4.10 SUBPROBLEM EIGHT

In order to determine and compare the minimum inhibitory concentration of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* both the tincture and the control were diluted to a 1:2, 1:4, 1:8, 1:16 and 1:32 concentration.

Using the Agar Dilution method (where each test substance is incorporated into the Mueller-Hinton agar medium), the minimum concentration of the substance in the growth media at which "no growth" occurs is regarded as the minimum inhibitory concentration.

Since, no gradation as to the extent of the growth or growth inhibition was made, the non-analytical data was expressed in the form of tables (see Tables, 22, 23 and 24, Appendix A for raw data).

3.4.10.1 PROCEDURE 1.46

Intergroup comparison between the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution respectively of the 62% ethanol control on the growth of *Escherichia coli* in terms of the Agar Dilution Method.

3.4.10.2 PROCEDURE 1.47

Intergroup comparison between the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution respectively of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method.

3.4.10.3 PROCEDURE 1.48

Intergroup comparison between the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and a 1:2, 1:4, 1:8, 1:16 and 1:32 dilution respectively of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the Agar Dilution Method.

3.4.11 SUBPROBLEM NINE

3.4.11.1 PROCEDURE 1.49

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol on the growth of *Escherichia coli* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the average diameters of the zones of inhibition of the two independent samples

* Hypothesis testing (As per procedure 1.1.)
* Decision rule (As per procedure 1.1.)

3.4.11.2 PROCEDURE 1.50

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol on the growth of *Pseudomonas aeruginosa* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the average diameters of the zones of inhibition of the two independent samples

* Hypothesis testing (As per procedure 1.1.)
* Decision rule (As per procedure 1.1.)

3.4.11.3 PROCEDURE 1.51

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol on the growth of *Staphylococcus aureus* in terms of the average diameter of the zones of inhibition.

The Mann-Whitney U-test was used to compare the average diameters of the zones of inhibition of the two independent samples

* Hypothesis testing (As per procedure 1.1.)
* Decision rule (As per procedure 1.1.)

3.4.12 SUBPROBLEM TEN

3.4.12.1 PROCEDURE 1.52

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol on the growth of *Escherichia coli* in terms of the Agar Dilution Method.

Results obtained from the Agar Dilution Method yields only one of two possible outcomes i.e. growth or growth inhibition. This non-analytical data was visually summarised in the form of a table (see Table 28, Appendix A for raw data).

3.4.12.2 PROCEDURE 1.53.

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol on the growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method.

Results obtained form the Agar Dilution Method yields only one of two possible outcomes i.e. growth or growth inhibition. This non-analytical data was visually summarised in the form of a table (see Table 29, Appendix A for raw data).

3.4.12.3 PROCEDURE 1.54

Intergroup comparison between the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol on the growth of *Staphylococcus aureus* in terms of the Agar Dilution Method.

Results obtained form the Agar Dilution Method yields only one of two possible outcomes i.e. growth or growth inhibition. This non-analytical data was visually summarised in the form of a table (see Table 30, Appendix A for raw data).

CHAPTER FOUR

4.0 RESULTS

4.1 SUBPROBLEM ONE

4.1.1 PROCEDURE 1.1

Withania somnifera tincture in 62% ethanol and the 62% ethanol control were both ineffective in inhibiting the *in vitro* growth of *Escherichia coli.* Neither sample produced any zone of inhibition in all five repetitions (see Table 1, Appendix A for raw data).

According to the Mann-Whitney U-test, $P = 1$. Therefore $P > \alpha$, where $\alpha = 0,05$. The null hypothesis (H_o) is thus accepted i.e. there is no difference between the two samples in comparison.

4.1.2 PROCEDURE 1.2

Withania somnifera tincture in 62% ethanol and the 62% ethanol control were both ineffective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa.* Neither sample produced any zone of inhibition in all five repetitions (see Table 2, Appendix A for raw data).

According to the Mann-Whitney U-test, $P = 1$. Therefore $P > \alpha$, where $\alpha = 0,05$. The null hypothesis (H_o) is thus accepted i.e. there is no difference between the two samples in comparison.

4.1.3 PROCEDURE 1.3

Withania somnifera tincture in 62% ethanol did have an antimicrobial effect on the *in vitro* growth of *Staphylococcus aureus*, whereas the 62% ethanol control had none (see Table 3, Appendix A for raw data).

According to the Mann-Whitney U-test, P = 0,003. Therefore P < α, where α = 0,05. The null hypothesis (H_o) is thus rejected i.e. there is a statistically significant difference between the two samples in comparison (see Table 4.1 below).

Table 4.1:
Statistical analysis of *Withania somnifera* tincture in 62% ethanol compared to the 62% ethanol control against *Staphylococcus aureus* according to the Mann-Whitney Test.

	Reading
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-3.000
Asymp. Sig	.003

4.2 SUBPROBLEM TWO

4.2.1 PROCEDURE 1.4

Xysmalobium undulatum tincture in 62% ethanol and the 62% ethanol control were both ineffective in inhibiting the *in vitro* growth of *Escherichia coli*. Neither sample produced any zone of inhibition in all five repetitions (see Table 4, Appendix A for raw data).

According to the Mann-Whitney U-test, P = 1. Therefore P > α, where α = 0,05. The null hypothesis (H_o) is thus accepted i.e. there is no difference between the two samples in comparison.

4.2.2 PROCEDURE 1.5

Xysmalobium undulatum tincture in 62% ethanol and the 62% ethanol control were both ineffective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa*. Neither sample produced any zone of inhibition in all five repetitions (see Table 5, Appendix A for raw data).

According to the Mann-Whitney U-test, P = 1. Therefore P > α, where α = 0,05. The null hypothesis (H_o) is thus accepted i.e. there is no difference between the two samples in comparison.

4.2.3 PROCEDURE 1.6

Xysmalobium undulatum tincture in 62% ethanol and the 62% ethanol control were both ineffective in inhibiting the *in vitro* growth of *Staphylococcus aureus*. Neither sample

produced any zone of inhibition in all five repetitions (see Table 6, Appendix A for raw data).

According to the Mann-Whitney U-test, P = 1. Therefore P > α, where α = 0,05. The null hypothesis (H_o) is thus accepted i.e. there is no difference between the two samples in comparison.

4.3 SUBPROBLEM THREE

4.3.1 PROCEDURE 1.7

Withania somnifera tincture in 62% ethanol and the 62% ethanol control, when incorporated into their respective growth media, were both effective in inhibiting the *in vitro* growth of *Escherichia coli* in terms of the Agar Dilution Method, in that no bacterial growth was recorded in any of the repetitions (see Table 7, Appendix A for raw data).

4.3.2 PROCEDURE 1.8

Withania somnifera tincture in 62% ethanol and the 62% ethanol control, when incorporated into their respective growth media, were both effective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method, in that no bacterial growth was recorded in any of the repetitions (see Table 8, Appendix A for raw data).

4.3.3 PROCEDURE 1.9

Withania somnifera tincture in 62% ethanol and the 62% ethanol control, when incorporated into their respective growth media, were both effective in inhibiting the *in vitro* growth of *Staphylococcus aureus* in terms of the Agar Dilution Method, in that no bacterial growth was recorded in any of the repetitions (see Table 9, Appendix A for raw data).

4.4 SUBPROBLEM FOUR

4.4.1 PROCEDURE 1.10

Xysmalobium undulatum tincture in 62% ethanol and the 62% ethanol control, when incorporated into their respective growth media, were both effective in inhibiting the *in vitro* growth of *Escherichia coli* in terms of the Agar Dilution Method, in that no bacterial growth was recorded in any of the repetitions (see Table 10, Appendix A for raw data).

4.4.2 PROCEDURE 1.11

Xysmalobium undulatum tincture in 62% ethanol and the 62% ethanol control, when incorporated into their respective growth media, were both effective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method, in that no bacterial growth was recorded in any of the repetitions (see Table 11, Appendix A for raw data).

4.4.3 PROCEDURE 1.12

Xysmalobium undulatum tincture in 62% ethanol and the 62% ethanol control, when incorporated into their respective growth media, were both effective in inhibiting the *in vitro* growth of *Staphylococcus aureus* in terms of the Agar Dilution Method, in that no bacterial growth was recorded in any of the repetitions (see Table 12, Appendix A for raw data).

4.5 SUBPROBLEM FIVE

4.5.1 PROCEDURE 1.13 TO 1.27

The 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Withania somnifera* tincture in 62% ethanol and the corresponding dilutions of the 62% ethanol control were both ineffective in inhibiting the *in vitro* growth of *Esherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* No sample produced a zone of inhibition (see Tables 13, 14 and 15, Appendix A for raw data). In all cases, the Mann-Whitney U-test resulted in, $P = 1$. Therefore $P > \alpha$, where $\alpha = 0{,}05$. Thus, the null hypothesis (H_o) was accepted in all cases.

4.6 SUBPROBLEM SIX

4.6.1 PROCEDURE 1.28

The 1:2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol were both ineffective in inhibiting the *in vitro* growth of *Escherichia coli.* Neither sample produced any zone of inhibition in all five repetitions (see Table 16, Appendix A for raw data).

According to the Mann-Whitney U-test, P = 1. Therefore P > α, where α = 0,05. The null hypothesis (H_o) is thus accepted i.e. there is no difference between the two samples in comparison.

4.6.2 PROCEDURE 1.29

The 1:2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol were both ineffective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa*. Neither sample produced any zone of inhibition in all five repetitions (see Table 17, Appendix A for raw data).

According to the Mann-Whitney U-test, P = 1. Therefore P > α, where α = 0,05. The null hypothesis (H_o) is thus accepted i.e. there is no difference between the two samples in comparison.

4.6.3 PROCEDURE 1.30

The 1:2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol did have an effect on the *in vitro* growth of *Staphylococcus aureus*, whereas the 1:2 dilution of the 62% ethanol had none (see Table 18, Appendix A for raw data) in terms of the Disc Diffusion Method.

According to the Mann-Whitney U-test, P = 0,004. Therefore P > α, where α = 0,05. The null hypothesis (Ho) is thus rejected i.e. there is a statistically significant difference between the two samples in comparison (see Table 4.2 below)

Table 4.2:
Statistical analysis of the 1:2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol compared to the 1:2 dilution of the 62% ethanol control against *Staphylococcus aureus* according to the Mann-Whitney Test.

	Reading
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.887
Asymp. Sig	.004

4.6.4 PROCEDURES 1.31 TO 1.42

All further dilutions of both the test substance, *Xysmalobium undulatum* tincture in 62% ethanol, and the 62% ethanol control, were ineffective in inhibiting the *in vitro* growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in terms of the Disc Diffusion Method (see Tables 16, 17 and 18, Appendix A for raw data).

In all cases, according to the Mann-Whitney U-test, the observed significance level (P) was greater than α, where $\alpha = 0{,}05$ for each of the respective samples compared. Thus, the null hypothesis H_o was accepted in all cases.

4.7 SUBPROBLEM SEVEN

4.7.1 PROCEDURE 1.43

The 1: 2 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control were both ineffective in inhibiting the *in vitro* growth of *Escherichia coli* in terms of the Agar Dilution Method (Table 19, Appendix A).

All further dilutions of both the test substance, *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control, were ineffective in inhibiting the *in vitro* growth of *Escherichia coli* in terms of the Agar Dilution Method (Table 19, Appendix A).

4.7.2 PROCEDURE 1.44

The 1: 2 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control were both effective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method (Table 20, Appendix A).

All further dilutions of both the test substance, *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control, were ineffective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method (Table 20, Appendix A).

4.7.3 PROCEDURE 1.45

The 1: 2 dilution of *Withania somnifera* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control were both ineffective in inhibiting the *in vitro* growth of *Staphylococcus aureus* in terms of the Agar Dilution Method (Table 21, Appendix A).

All further dilutions of both the test substance, *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control, were ineffective in inhibiting the *in vitro* growth of *Staphylococcus aureus* in terms of the Agar Dilution Method (Table 21, Appendix A).

4.8 SUBPROBLEM EIGHT

4.8.1 PROCEDURE 1.46

The 1: 2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control were both ineffective in inhibiting the *in vitro* growth of *Escherichia coli* in terms of the Agar Dilution Method (Table 22, Appendix A).

All further dilutions of both the test substance, *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control, were ineffective in inhibiting the *in vitro* growth of *Escherichia coli* in terms of the Agar Dilution Method (Table 22, Appendix A).

4.8.2 PROCEDURE 1.47

The 1: 2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control were both effective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method (Table 23, Appendix A).

All further dilutions of both the test substance, *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control, were ineffective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method (Table 23, Appendix A).

4.8.3 PROCEDURE 1.48

The 1: 2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol and the 1:2 dilution of the 62% ethanol control were both ineffective in inhibiting the *in vitro* growth of *Staphylococcus aureus* in terms of the Agar Dilution Method (Table 24, Appendix A).

All further dilutions of both the test substance, *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control, were ineffective in inhibiting the *in vitro* growth of *Staphylococcus aureus* in terms of the Agar Dilution Method (Table 24, Appendix A).

4.9 SUBPROBLEM NINE

4.9.1 PROCEDURE 1.49

Withania somnifera tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol were both ineffective in inhibiting the *in vitro* growth of *Escherichia coli*. Neither sample produced any zone of inhibition in all five repetitions (see Table 25, Appendix A for raw data).

According to the Mann-Whitney U-test, $P = 1$, therefore $P > \alpha$. The null hypothesis (H_o) is thus accepted i.e. there is no difference between the two samples in comparison.

4.9.2 PROCEDURE 1.50

Withania somnifera tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol were both ineffective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa*. Neither sample produced any zone of inhibition in all five repetitions (see Table 26, Appendix A for raw data).

According to the Mann-Whitney U-test, P = 1, therefore P > α. The null hypothesis (H_o) is thus accepted i.e. there is no difference between the two samples in comparison.

4.9.3 PROCEDURE 1.51

Withania somnifera tincture in 62% ethanol did have an antimicrobial effect on the *in vitro* growth of *Staphylococcus aureus,* whereas the *Xysmalobium undulatum* tincture in 62% ethanol had none in terms of the Disc Diffusion Method. (Table 27, Appendix A).

According to the Mann-Whitney U-test, P = 0,003. Therefore P < α, where α = 0,5. The null hypothesis (H_o) is thus rejected i.e. there is a statistically significant difference between the two samples in comparison (see Table 4.3 below).

Table 4.3:
Statistical analysis of *Withania somnifera* tincture in 62% ethanol in compared to the *Xysmalobium undulatum* tincture in 62% ethanol against *Staphylococcus aureus* according to the Mann-Whitney Test.

	Reading
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-3.000
Asymp. Sig	.003

4.10 SUBPROBLEM TEN

4.10.1 PROCEDURE 1.52

Withania somnifera tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol when incorporated into their respective growth media, were both effective in inhibiting the *in vitro* growth of *Escherichia coli* in terms of the Agar Dilution Method, in that no bacterial growth was recorded in any of the repetitions (see Table 28, Appendix A for raw data).

4.10.2 PROCEDURE 1.53

Withania somnifera tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol when incorporated into their respective growth media, were both effective in inhibiting the *in vitro* growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method, in that no bacterial growth was recorded in any of the repetitions (see Table 29, Appendix A for raw data).

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4.10.3 PROCEDURE1.54

Withania somnifera tincture in 62% ethanol and *Xysmalobium undulatum* tincture in 62% ethanol when incorporated into their respective growth media, were both effective in inhibiting the *in vitro* growth of *Staphylococcus aureus* in terms of the Agar Dilution Method, in that no bacterial growth was recorded in any of the repetitions (see Table 30, Appendix A for raw data).

CHAPTER FIVE

5.0 DISCUSSION

5.1 PART ONE: DISC DIFFUSION METHOD

Withania somnifera tincture in 62% ethanol had no inhibitory effect on the *in vitro* growth of both *Escherichia coli* and *Pseudomonas aeruginosa.* However, the tincture did show an inhibitory effect on the growth of *Staphylococcus aureus*. Zones of inhibition measuring 5mm in diameter were achieved in each of the five trials resulting in a p-value of 0.003 for the five samples tested when compared to the negative control using the Mann-Whitney U-test.

These results support the findings of Watt and Breyer-Brandwijk (1962: 1012) regarding the leaf and root showing antibiotic activity against *Staphylococcus aureus.*

The results also support the findings of Cunningham, *et al* (1996: 273), where they found that withaferine, a constituent of *Withania somnifera,* has been shown to be antibiotic toward Gram-positive organisms.

In addition the results support a more recent study conducted by Dümmer (2003) who found that *Withania somnifera* tincture in 62% ethanol was effective in inhibiting the *in vitro* growth of *Staphylococcus aureus* in terms of the Disc Diffusion Assay (Dümmer, 2003: 68).

It is important to note, however, that the specific bacterial strains of *Staphylococcus aureus* used in the above studies were not indicated by the researchers. This would make direct comparisons to the above findings somewhat less reliable.

Xysmalobium undulatum tincture in 62% ethanol had no inhibitory effect on the *in vitro* growth of any of the three bacteria tested in a neat concentration. However, a 1:2 dilution of the tincture did produce an inhibitory effect on the *in vitro* growth of *Staphylococcus aureus* resulting in a p-value of 0.004 for the five samples when compared to the negative control using the Mann-Whitney U-test.

This unexpected finding could point to the possible disinfectant properties of *Xysmalobium undulatum*. It is well known that the effects of concentration or dilution of the active ingredients on the activity of a disinfectant are of paramount importance i.e. an exponential relationship exists between potency and concentration (Hugo and Russel, 1987: 257). In this regard, suspension tests and counting methods such as the Rideal-Walker Test and Viable Counting Tests could be employed in future research to evaluate the liquid disinfectant potential of *Xysmalobium undulatum* (Hugo and Russel, 1987: 261-266).

It is somewhat promising to find that both *Xysmalobium undulatum* and *Withania somnifera* tinctures were able to inhibit the growth of *Staphylococcus aureus* when one considers that *Staphylococcus aureus* is one of the most important micro-organisms responsible for nosocomial infections especially respiratory infections and wound and skin sepsis (Simpson, 1992: 782-783). The emergence of methicillin-resistant *Staphylococcus aureus* nosocomial

infections is even more troubling as these *Staphylococci* may cause lethal blood-poisoning infections in patients with injuries or weakened immunity (Postgate, 2000: 129-130).

5.2 PART TWO: AGAR DILUTION METHOD

The results of this part of the study show that neither *Withania somnifera* nor *Xysmalobium undulatum* tinctures in 62% ethanol were effective in inhibiting the *in vitro* growth of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* because the negative control (62% ethanol) produced exactly the same results as the two test substances.

These results are puzzling, considering that the Agar Dilution Testing Method is regarded as a well standardized, reliable susceptibility testing technique and furthermore may be used as a reference for the evaluation to the accuracy of other testing systems (Baron *et al*., 1995: 1331).

A possible flaw in this part of the study, resulting in a different set of results when compared to those obtained in the Disc Diffusion Assay, may in fact lie not in the Agar Dilution methodology itself but in the incorporation of the test substances in ethanolic form. Ethanol itself may mask the possible antimicrobial properties of the constituents of each respective test substance as ethanol is known to have a bactericidal action at concentrations between 60-95% (Scott and Gorman, 1987: 236). This bactericidal action of the ethanol appeared to have been eliminated when using the Disc Diffusion Assay. This was attributed to the evaporation of the ethanol during incubation of the plates.

In terms of the Agar Dilution Method, a possible means to overcome this, would be to dry each tincture and then store the remaining residue at -15ºC (Invernizzi, 2002: 85, citing Rios, Recio and Villar, 1988: 142). The residue can then be resuspended in sterile distilled water and be incorporated immediately into the appropriate media. In this way, the disinfectant and antimicrobial properties of the ethanol can be eliminated.

A worthwhile research would be to perform both Part One and Part Two of this study again and include a Part Three where, once again, the Agar Dilution Method is used with the exception that the test substances be incorporated in non-ethanolic forms. This would help to determine the possible role that the ethanol had on the results and possibly outline a much needed guideline for testing ethanolic extracts in the future.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The purpose of this study was to evaluate the efficacy of *Withania somnifera* and *Xysmalobium undulatum* tinctures in 62% ethanol in inhibiting the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* in terms of both the Disc Diffusion Assay and the Agar Dilution Method.

Both methodologies resulted in different findings, highlighting the importance of standardization required, especially when testing natural products and ethanolic extracts.

THE DISC DIFFUSION ASSAY

Withania somnifera tincture in 62% ethanol was effective in inhibiting the *in vitro* growth of *Staphylococcus aureus* but ineffective against *Escherichia coli* and *Pseudomonas aeruginosa.*

The Disc Diffusion Assay showed that no inhibitory effect was produced by a neat concentration of the *Xysmalobium undulatum* tincture in 62% ethanol on the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

The 1:2 dilution of *Xysmalobium undulatum* tincture in 62% ethanol was effective in inhibiting the *in vitro* growth of *Staphylococcus aureus* but ineffective against *Escherichia coli and Pseudomonas aeruginosa.*

THE AGAR DILUTION METHOD

This method showed that neither *Withania somnifera* nor *Xysmalobium undulatum* tincture in 62% ethanol were effective in inhibiting the *in vitro* growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

6.2 RECOMMENDATIONS

Owing to the different sets of results obtained from the two methodologies used the need for the standardization of antimicrobial susceptibility tests was highlighted. In this regard the following recommendations are made.

6.2.1 AMERICAN TYPE CULTURE COLLECTIONS (ATCC)

To ensure quality control and for purposes of standardization it is not only recommended that bacterial strains have identification numbers such as the American Type Culture Collection numbers but also that specific reference bacterial strains be used for antimicrobial testing. The reference strains for *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* are as follows:-

Escherichia coli - ATCC 25922 or ATCC 35218

Pseudomonas aeruginosa - ATCC 27853

Staphylococcus aureus - ATCC 29213

(Sahm and Washington II, 1991: 1113).

6.2.2 AGAR DILUTION TESTS

When conducting Agar Dilution Tests involving ethanolic extracts it is recommended that the extract be dried and that the residue be resuspended in sterile distilled H_2O so as to exclude the antimicrobial effect of the ethanol.

6.2.3 SUSPENSION TESTS AND COUNTING METHODS

These methods should be employed to evaluate the liquid disinfectant potential of *Xysmalobium undulatum.*

6.2.4 OTHER RECOMMENDATIONS

6.2.4.1 *IN VIVO* TESTING

Both *Withania somnifera* and *Xysmalobium undulatum* tinctures should be tested *in vivo* to establish any antibacterial effectiveness by means of controlled clinical trials.

6.2.4.2 A HOMOEOPATHIC PROVING

Homoeopathic provings should be conducted on both *Withania somnifera* and *Xysmalobium undulatum* in order to determine their possible homoeopathic applications.

6.2.4.3 REPETITION OF THIS RESEARCH

For purposes of establishing reliability of this research it is recommended that it be repeated.

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TABLE OF CONTENTS: APPENDIX A

APPENDIX A

TABLE 1: Data regarding the inhibitory effect of a neat concentration of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Escherichia coli* in terms of the diameter of the zones of inhibition

	Withania somnifera tincture in 62% ethanol	62% ethanol only	*ciprofloxacin 42µg/ml	*gentamicin 60µg/ml
Repetition one	0	0	38mm	23mm
Repetition two	0	0	38mm	23mm
Repetition three	0	0	38mm	23mm
Repetition four	0	0	38mm	23mm
Repetition five	0	0	38mm	23mm

* The zones of inhibition, produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 2: Data regarding the inhibitory effect of a neat concentration of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the diameter of the zones of inhibition

	Withania somnifera tincture in 62% ethanol	62% ethanol only	*ciprofloxacin 42µg/ml	*gentamicin 60µg/ml
Repetition one	0	0	38mm	35mm
Repetition two	0	0	38mm	35mm
Repetition three	0	0	38mm	35mm
Repetition four	0	0	38mm	35mm
Repetition five	0	0	38mm	35mm

* The zones of inhibition, produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 3: Data regarding the inhibitory effect of a neat concentration of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the diameter of the zones of inhibition

	Withania somnifera tincture in 62% ethanol	62% ethanol only	*ciprofloxacin 42µg/ml	*gentamicin 60µg/ml
Repetition one	11mm	0	30mm	35mm
Repetition two	11mm	0	30mm	35mm
Repetition three	11mm	0	30mm	35mm
Repetition four	11mm	0	30mm	35mm
Repetition five	11mm	0	30mm	35mm

* The zones of inhibition, produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 4: Data regarding the inhibitory effect of a neat concentration of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Escherichia coli* in terms of the diameter of the zones of inhibition

	Xysmalobium undulatum tincture in 62% ethanol	62% ethanol only	*ciprofloxacin 42µg/ml	*gentamicin 60µg/ml
Repetition one	0	0	38mm	23mm
Repetition two	0	0	38mm	23mm
Repetition three	0	0	38mm	23mm
Repetition four	0	0	38mm	23mm
Repetition five	0	0	38mm	23mm

* The zones of inhibition, produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 5: Data regarding the inhibitory effect of a neat concentration of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the diameter of the zones of inhibition

	Xysmalobium undulatum tincture in 62% ethanol	62% ethanol only	*ciprofloxacin 42µg/ml	*gentamicin 60µg/ml
Repetition one	0	0	38mm	35mm
Repetition two	0	0	38mm	35mm
Repetition three	0	0	38mm	35mm
Repetition four	0	0	38mm	35mm
Repetition five	0	0	38mm	35mm

* The zones of inhibition, produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 6: Data regarding the inhibitory effect of a neat concentration of *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the diameter of the zones of inhibition

	Xysmalobium undulatum tincture in 62% ethanol	62% ethanol only	*ciprofloxacin 42µg/ml	*gentamicin 60µg/ml
Repetition one	0	0	30mm	35mm
Repetition two	0	0	30mm	35mm
Repetition three	0	0	30mm	35mm
Repetition four	0	0	30mm	35mm
Repetition five	0	0	30mm	35mm

* The zones of inhibition, produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 7: Data regarding the inhibitory effect of a *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Escherichia coli* in terms of the Agar Dilution Method.

KEY: T1 - *Withania somnifera* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin (42µg/ml)
C3 - gentamicin (60µg/ml)
NG - No growth
G - Growth

	REPETITION ONE				**REPETITION TWO**				**REPETITION THREE**				**REPETITION FOUR**				**REPETITION FIVE**			
	T1	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**
Inoculate 1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

TABLE 8: Data regarding the inhibitory effect of a *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method.

KEY: T1 - *Withania somnifera* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin (42µg/ml)
C3 - gentamicin (60µg/ml)
NG - No growth
G - Growth

	REPETITION ONE				**REPETITION TWO**				**REPETITION THREE**				**REPETITION FOUR**				**REPETITION FIVE**			
	T1	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**
Inoculate 1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

TABLE 9: Data regarding the inhibitory effect of a *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the Agar Dilution Method.

KEY: T1 - *Withania somnifera* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin (42µg/ml)
C3 - gentamicin (60µg/ml)
NG - No growth
G - Growth

	REPETITION ONE				**REPETITION TWO**				**REPETITION THREE**				**REPETITION FOUR**				**REPETITION FIVE**			
	T1	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**	**T1**	**CI**	**C2**	**C3**
Inoculate 1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

TABLE 10: Data regarding the inhibitory effect of a *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Escherichia coli* in terms of the Agar Dilution Method.

KEY: T2 - *Xysmalobium undulatum* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin (42µg/ml)
C3 - gentamicin (60µg/ml)
NG - No growth
G - Growth

	REPETITION ONE				**REPETITION TWO**				**REPETITION THREE**				**REPETITION FOUR**				**REPETITION FIVE**			
	T2	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**
Inoculate 1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

TABLE 11: Data regarding the inhibitory effect of a *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method.

KEY: T2 - *Xysmalobium undulatum* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin (42µg/ml)
C3 - gentamicin (60µg/ml)
NG - No growth
G - Growth

	REPETITION ONE				**REPETITION TWO**				**REPETITION THREE**				**REPETITION FOUR**				**REPETITION FIVE**			
	T2	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**
Inoculate 1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

TABLE 12: Data regarding the inhibitory effect of a *Xysmalobium undulatum* tincture in 62% ethanol and the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the Agar Dilution Method.

KEY: T1 - *Xysmalobium undulatum* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin (42µg/ml)
C3 - gentamicin (60µg/ml)
NG - No growth
G - Growth

	REPETITION ONE				**REPETITION TWO**				**REPETITION THREE**				**REPETITION FOUR**				**REPETITION FIVE**			
	T2	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**	**T2**	**CI**	**C2**	**C3**
Inoculate 1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

TABLE 13: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Withania somnifera* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the diameter of the zones of inhibition.

	Withania somnifera tincture in 62% ethanol	62% ethanol	*ciprofloxacin	*gentamicin
	1:2 dilution	1:2 dilution	21µg/ml	30µg/ml
Repetition 1	0	0	35mm	25mm
Repetition 2	0	0	35mm	25mm
Repetition 3	0	0	35mm	25mm
Repetition 4	0	0	35mm	25mm
Repetition 5	0	0	35mm	25mm
	1:4 dilution	1:4 dilution	10,5µg/ml	15µg/ml
Repetition 1	0	0	25mm	30mm
Repetition 2	0	0	25mm	30mm
Repetition 3	0	0	25mm	30mm
Repetition 4	0	0	25mm	30mm
Repetition 5	0	0	25mm	30mm
	1:8 dilution	1:8 dilution	5.25µg/ml	7,5µg/ml
Repetition 1	0	0	23mm	27mm
Repetition 2	0	0	23mm	27mm
Repetition 3	0	0	23mm	27mm
Repetition 4	0	0	23mm	27mm
Repetition 5	0	0	23mm	27mm
	1:16 dilution	1:16 dilution	2,625µg/ml	3,75µg/ml
Repetition 1	0	0	20mm	25mm
Repetition 2	0	0	20mm	25mm
Repetition 3	0	0	20mm	25mm
Repetition 4	0	0	20mm	25mm
Repetition 5	0	0	20mm	25mm
	1:32 dilution	1:32 dilution	1,3125µg/ml	1,875µg/ml
Repetition 1	0	0	16mm	23mm
Repetition 2	0	0	16mm	23mm
Repetition 3	0	0	16mm	23mm
Repetition 4	0	0	16mm	23mm
Repetition 5	0	0	16mm	23mm

* The zones of inhibition, produced by the two positive controls ciprofloxacin and gentamicin

TABLE 14: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Withania somnifera* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the diameter of the zones of inhibition.

	Withania somnifera in 62% ethanol	62% ethanol control	* ciprofloxacin	* gentamicin
	1:2 dilution	1:2 dilution	21µg/ml	30µg/ml
Repetition 1	0	0	35mm	31mm
Repetition 2	0	0	35mm	31mm
Repetition 3	0	0	35mm	31mm
Repetition 4	0	0	35mm	31mm
Repetition 5	0	0	35mm	31mm
	1:4 dilution	1:4 dilution	10,5µg/ml	15µg/ml
Repetition 1	0	0	31mm	28mm
Repetition 2	0	0	31mm	28mm
Repetition 3	0	0	31mm	28mm
Repetition 4	0	0	31mm	28mm
Repetition 5	0	0	31mm	28mm
	1:8 dilution	1:8 dilution	5.25µg/ml	7,5µg/ml
Repetition 1	0	0	28mm	25mm
Repetition 2	0	0	28mm	25mm
Repetition 3	0	0	28mm	25mm
Repetition 4	0	0	28mm	25mm
Repetition 5	0	0	28mm	25mm
	1:16 dilution	1:16 dilution	2,625µg/ml	3,75µg/ml
Repetition 1	0	0	22mm	23mm
Repetition 2	0	0	22mm	23mm
Repetition 3	0	0	22mm	23mm
Repetition 4	0	0	22mm	23mm
Repetition 5	0	0	22mm	23mm
	1:32 dilution	1:32 dilution	1,3125µg/ml	1,875µg/ml
Repetition 1	0	0	16mm	18mm
Repetition 2	0	0	16mm	18mm
Repetition 3	0	0	16mm	18mm
Repetition 4	0	0	16mm	18mm
Repetition 5	0	0	16mm	18mm

* Zones of inhibition produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 15: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Withania somnifera* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Staphylococcus aurues* in terms of the diameter of the zones of inhibition.

	Withania somnifera in 62% ethanol	62% ethanol control	* ciprofloxacin	* gentamicin
	1:2 dilution	1:2 dilution	21µg/ml	30µg/ml
Repetition 1	0	0	29mm	31mm
Repetition 2	0	0	29mm	31mm
Repetition 3	0	0	29mm	31mm
Repetition 4	0	0	29mm	31mm
Repetition 5	0	0	29mm	31mm
	1:4 dilution	1:4 dilution	10,5µg/ml	15µg/ml
Repetition 1	0	0	25mm	30mm
Repetition 2	0	0	25mm	30mm
Repetition 3	0	0	25mm	30mm
Repetition 4	0	0	25mm	30mm
Repetition 5	0	0	25mm	30mm
	1:8 dilution	1:8 dilution	5.25µg/ml	7,5µg/ml
Repetition 1	0	0	23mm	27mm
Repetition 2	0	0	23mm	27mm
Repetition 3	0	0	23mm	27mm
Repetition 4	0	0	23mm	27mm
Repetition 5	0	0	23mm	27mm
	1:16 dilution	1:16 dilution	2,625µg/ml	3,75µg/ml
Repetition 1	0	0	20mm	25mm
Repetition 2	0	0	20mm	25mm
Repetition 3	0	0	20mm	25mm
Repetition 4	0	0	20mm	25mm
Repetition 5	0	0	20mm	25mm
	1:32 dilution	1:32 dilution	1,3125µg/ml	1,875µg/ml
Repetition 1	0	0	16mm	23mm
Repetition 2	0	0	16mm	23mm
Repetition 3	0	0	16mm	23mm
Repetition 4	0	0	16mm	23mm
Repetition 5	0	0	16mm	23mm

* Zones of inhibition produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 16: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Xysmalobium undulatum* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the diameter of the zones of inhibition.

	Xysmalobium undulatum in 62% ethanol	62% ethanol control	* ciprofloxacin	* gentamicin
	1:2 dilution	1:2 dilution	21µg/ml	30µg/ml
Repetition 1	0	0	35mm	25mm
Repetition 2	0	0	35mm	25mm
Repetition 3	0	0	35mm	25mm
Repetition 4	0	0	35mm	25mm
Repetition 5	0	0	35mm	25mm
	1:4 dilution	1:4 dilution	10,5µg/ml	15µg/ml
Repetition 1	0	0	35mm	20mm
Repetition 2	0	0	35mm	20mm
Repetition 3	0	0	35mm	20mm
Repetition 4	0	0	35mm	20mm
Repetition 5	0	0	35mm	20mm
	1:8 dilution	1:8 dilution	5.25µg/ml	7,5µg/ml
Repetition 1	0	0	30mm	16mm
Repetition 2	0	0	30mm	16mm
Repetition 3	0	0	30mm	16mm
Repetition 4	0	0	30mm	16mm
Repetition 5	0	0	30mm	16mm
	1:16 dilution	1:16 dilution	2,625µg/ml	3,75µg/ml
Repetition 1	0	0	30mm	16mm
Repetition 2	0	0	30mm	16mm
Repetition 3	0	0	30mm	16mm
Repetition 4	0	0	30mm	16mm
Repetition 5	0	0	30mm	16mm
	1:32 dilution	1:32 dilution	1,3125µg/ml	1,875µg/ml
Repetition 1	0	0	28mm	12mm
Repetition 2	0	0	28mm	12mm
Repetition 3	0	0	28mm	12mm
Repetition 4	0	0	28mm	12mm
Repetition 5	0	0	28mm	12mm

* Zones of inhibition produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 17: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Xysmalobium undulatum* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the diameter of the zones of inhibition.

	Xysmalobium undulatum in 62% ethanol	62% ethanol control	* ciprofloxacin	* gentamicin
	1:2 dilution	1:2 dilution	21µg/ml	30µg/ml
Repetition 1	0	0	35mm	31mm
Repetition 2	0	0	35mm	31mm
Repetition 3	0	0	35mm	31mm
Repetition 4	0	0	35mm	31mm
Repetition 5	0	0	35mm	31mm
	1:4 dilution	1:4 dilution	10,5µg/ml	15µg/ml
Repetition 1	0	0	31mm	28mm
Repetition 2	0	0	31mm	28mm
Repetition 3	0	0	31mm	28mm
Repetition 4	0	0	31mm	28mm
Repetition 5	0	0	31mm	28mm
	1:8 dilution	1:8 dilution	5.25µg/ml	7,5µg/ml
Repetition 1	0	0	28mm	25mm
Repetition 2	0	0	28mm	25mm
Repetition 3	0	0	28mm	25mm
Repetition 4	0	0	28mm	25mm
Repetition 5	0	0	28mm	25mm
	1:16 dilution	1:16 dilution	2,625µg/ml	3,75µg/ml
Repetition 1	0	0	22mm	23mm
Repetition 2	0	0	22mm	23mm
Repetition 3	0	0	22mm	23mm
Repetition 4	0	0	22mm	23mm
Repetition 5	0	0	22mm	23mm
	1:32 dilution	1:32 dilution	1,3125µg/ml	1,875µg/ml
Repetition 1	0	0	16mm	18mm
Repetition 2	0	0	16mm	18mm
Repetition 3	0	0	16mm	18mm
Repetition 4	0	0	16mm	18mm
Repetition 5	0	0	16mm	18mm

* Zones of inhibition produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 18: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Xysmalobium undulatum* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the diameter of the zones of inhibition.

	Xysmalobium undulatum in 62% ethanol	62% ethanol control	* ciprofloxacin	* gentamicin
	1:2 dilution	1:2 dilution	21µg/ml	30µg/ml
Repetition 1	0	0	29mm	31mm
Repetition 2	0	0	29mm	31mm
Repetition 3	0	0	29mm	31mm
Repetition 4	0	0	29mm	31mm
Repetition 5	0	0	29mm	31mm
	1:4 dilution	1:4 dilution	10,5µg/ml	15µg/ml
Repetition 1	0	0	25mm	30mm
Repetition 2	0	0	25mm	30mm
Repetition 3	0	0	25mm	30mm
Repetition 4	0	0	25mm	30mm
Repetition 5	0	0	25mm	30mm
	1:8 dilution	1:8 dilution	5.25µg/ml	7,5µg/ml
Repetition 1	0	0	23mm	27mm
Repetition 2	0	0	23mm	27mm
Repetition 3	0	0	23mm	27mm
Repetition 4	0	0	23mm	27mm
Repetition 5	0	0	23mm	27mm
	1:16 dilution	1:16 dilution	2,625µg/ml	3,75µg/ml
Repetition 1	0	0	20mm	25mm
Repetition 2	0	0	20mm	25mm
Repetition 3	0	0	20mm	25mm
Repetition 4	0	0	20mm	25mm
Repetition 5	0	0	20mm	25mm
	1:32 dilution	1:32 dilution	1,3125µg/ml	1,875µg/ml
Repetition 1	0	0	16mm	23mm
Repetition 2	0	0	16mm	23mm
Repetition 3	0	0	16mm	23mm
Repetition 4	0	0	16mm	23mm
Repetition 5	0	0	16mm	23mm

* Zones of inhibition produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 19: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Withania somnifera* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the Agar Dilution Method.

KEY: T1 - *Withania somnifera* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin
C3 - gentamicin
NG - No growth
G - Growth

	REPETITION 1				REPETITION 2				REPETITION 3				REPETITION 4				REPETITION 5			
1:2	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:4	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

	REPETITION 1				REPETITION 2				REPETITION 3				REPETITION 4				REPETITION 5			
1:8	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:16	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:32	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

TABLE 20: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Withania somnifera* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method.

KEY: T1 - *Withania somnifera* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin
C3 - gentamicin
NG - No growth
G - Growth

	REPETITION 1				**REPETITION 2**				**REPETITION 3**				**REPETITION 4**				**REPETITION 5**			
1:2	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
1:4	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

	REPETITION 1				REPETITION 2				REPETITION 3				REPETITION 4				REPETITION 5			
1:8	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:16	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:32	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

TABLE 21: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Withania somnifera* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the Agar Dilution Method.

KEY: T1 - *Withania somnifera* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin
C3 - gentamicin
NG - No growth
G - Growth

	REPETITION 1				**REPETITION 2**				**REPETITION 3**				**REPETITION 4**				**REPETITION 5**			
1:2	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
1:4	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

	REPETITION 1				REPETITION 2				REPETITION 3				REPETITION 4				REPETITION 5			
1:8	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:16	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:32	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**	**T1**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

TABLE 22: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Xysmalobium undulatum* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Escherichia coli* in terms of the Agar Dilution Method.

KEY: T2 - *Xysmalobium undulatum* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin
C3 - gentamicin
NG - No growth
G - Growth

	REPETITION 1				**REPETITION 2**				**REPETITION 3**				**REPETITION 4**				**REPETITION 5**			
1:2	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:4	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

	REPETITION 1				REPETITION 2				REPETITION 3				REPETITION 4				REPETITION 5			
1:8	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:16	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:32	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

TABLE 23: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Xysmalobium undulatum* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method.

KEY: T2 - *Xysmalobium undulatum* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin
C3 - gentamicin
NG - No growth
G - Growth

	REPETITION 1				**REPETITION 2**				**REPETITION 3**				**REPETITION 4**				**REPETITION 5**			
1:2	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**
Inoculate 1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
1:4	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

	REPETITION 1				REPETITION 2				REPETITION 3				REPETITION 4				REPETITION 5			
1:8	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:16	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:32	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

TABLE 24: Data regarding the inhibitory effect of a 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of *Xysmalobium undulatum* tincture in 62% ethanol and the corresponding dilution of the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the Agar Dilution Method.

KEY: T2 - *Xysmalobium undulatum* tincture in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin
C3 - gentamicin
NG - No growth
G - Growth

	REPETITION 1				**REPETITION 2**				**REPETITION 3**				**REPETITION 4**				**REPETITION 5**			
1:2	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**
Inoculate 1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
1:4	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**	**T2**	**C1**	**C2**	**C3**
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

	REPETITION 1				REPETITION 2				REPETITION 3				REPETITION 4				REPETITION 5			
1:8	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:16	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
1:32	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3	T2	C1	C2	C3
Inoculate 1	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
2	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
3	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
4	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
5	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
6	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
7	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
8	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
9	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
10	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
11	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG
12	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG	G	G	NG	NG

TABLE 25: Data regarding the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and the *Xysmalobium undulatum* tincture in 62% ethanol on the growth of *Escherichia coli* in terms of the diameter of the zones of inhibition

	Withania somnifera tincture in 62% ethanol	*Xysmalobium undulatum in 62%* ethanol	*ciprofloxacin 42µg/ml	*gentamicin 60µg/ml
Repetition 1	0	0	38mm	23mm
Repetition 2	0	0	38mm	23mm
Repetition 3	0	0	38mm	23mm
Repetition 4	0	0	38mm	23mm
Repetition 5	0	0	38mm	23mm

* The zones of inhibition, produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 26: Data regarding the inhibitory effect of a neat concentration of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Pseudomonas aeruginosa* in terms of the diameter of the zones of inhibition

	Withania somnifera tincture in 62% ethanol	*Xysmalobium undulatum in 62%* ethanol	*ciprofloxacin 42µg/ml	*gentamicin 60µg/ml
Repetition 1	0	0	38mm	35mm
Repetition 2	0	0	38mm	35mm
Repetition 3	0	0	38mm	35mm
Repetition 4	0	0	38mm	35mm
Repetition 5	0	0	38mm	35mm

* The zones of inhibition, produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 27: Data regarding the inhibitory effect of a neat concentration of *Withania somnifera* tincture in 62% ethanol and the 62% ethanol control on the growth of *Staphylococcus aureus* in terms of the diameter of the zones of inhibition

	Withania somnifera tincture in 62% ethanol	*Xysmalobium undulatum in 62%* ethanol	*ciprofloxacin 42µg/ml	*gentamicin 60µg/ml
Repetition 1	11mm	0	30mm	35mm
Repetition 2	11mm	0	30mm	35mm
Repetition 3	11mm	0	30mm	35mm
Repetition 4	11mm	0	30mm	35mm
Repetition 5	11mm	0	30mm	35mm

* The zones of inhibition, produced by the two positive controls ciprofloxacin and gentamicin.

TABLE 28: Data regarding the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and *Xysmalobium undulatum* in 62% ethanol on the growth of *Escherichia coli* in terms of the Agar Dilution Method.

KEY: T1 - *Withania somnifera* tincture in 62% ethanol
T2 - *Xysmalobium undulatum* in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin (21µg/ml)
C3 - gentamicin (30µg/ml)
NG - No growth
G - Growth

	REPETITION 1					**REPETITION 2**					**REPETITION 3**					**REPETITION 4**					**REPETITION 5**				
	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3
1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

TABLE 29: Data regarding the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and *Xysmalobium undulatum* in 62% ethanol on the growth of *Pseudomonas aeruginosa* in terms of the Agar Dilution Method.

KEY: T1 - *Withania somnifera* tincture in 62% ethanol
T2 - *Xysmalobium undulatum* in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin (21µg/ml)
C3 - gentamicin (30µg/ml)
NG - No growth
G - Growth

	REPETITION 1					**REPETITION 2**					**REPETITION 3**					**REPETITION 4**					**REPETITION 5**				
	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3
1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

TABLE 30: Data regarding the inhibitory effect of *Withania somnifera* tincture in 62% ethanol and *Xysmalobium undulatum* in 62% ethanol on the growth of *Staphylococcus aureus* in terms of the Agar Dilution Method.

KEY: T1 - *Withania somnifera* tincture in 62% ethanol
T2 - *Xysmalobium undulatum* in 62% ethanol
C1 - 62 % ethanol control
C2 - ciprofloxacin (21µg/ml)
C3 - gentamicin (30µg/ml)
NG - No growth
G - Growth

	REPETITION 1					**REPETITION 2**					**REPETITION 3**					**REPETITION 4**					**REPETITION 5**				
	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3	T1	T2	C1	C2	C3
1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG