

# MEMORANDUM

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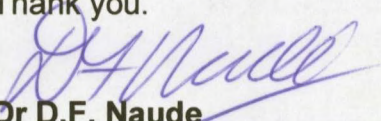
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**Dr D.F. Naude**  
**Research Co-ordinator**  
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**A CONTROLLED *IN VITRO* STUDY OF THE EFFECTIVENESS OF  
*WITHANIA SOMNIFERA* HERBAL TINCTURE AND  
HOMOEOPATHIC DILUTION (1X AND 6X) AGAINST SELECTED  
GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA**

**BY**

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

I, Karen Joanne Dümmer do declare that this mini-dissertation represents my  
own work in both conception and execution.

Signature of student

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Signature of Supervisor  
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08-04-03  
Date of signature

## DEDICATION

To my parents, who through their infinite  
love, are a pillar of strength and a  
constant inspiration in my life.

## ACKNOWLEDGMENTS

I wish to express my warm gratitude to the following people for their effort and time invested in the completion of this dissertation:

Dr Richard Steele for his unlimited enthusiasm and guidance as supervisor.

Mr Ron Gauldie for his technical supervision, fine attention to detail, knowledge and experience.

Mrs Reena Brijlal for her much appreciated advice and assistance.

Dr David Naudé for guidance through the field of research.

Mr Dillon Williams and Mr Jonathan Invernizzi with whom I am privileged to have shared this task.

Nigel, Jon, Edyne, Dillon, Nicky, Heather and Kimmy who have brightened my past six years in so many ways, thank you.

## ABSTRACT

The aim of this study was to establish the efficacy of *Withania somnifera* in tincture, 1X and 6X homoeopathic dilutions (in 62% v/v ethanol) as an antimicrobial agent against the *in vitro* growth of *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus*, as compared to 62% v/v ethanol only. The disc diffusion method was employed.

*W. somnifera* is indigenous to southern Africa and its use is well established amongst the traditional healers for many varied complaints. Infusions, decoctions and tinctures of the fresh and dry whole root are used. (Gericke and Van Wyk, 2000:150.)

For this study 20 plates of Mueller-Hinton agar were inoculated with each bacteria, resulting in a total of 100 plates. Four dry discs previously impregnated with the test substances and two antibiotic discs were equidistantly placed on each plate and incubated at 37°C. The vancomycin and gentamycin discs were included to account for plate-to-plate variations in the sensitivity of the bacteria to the antimicrobial substances. The plates were observed at 18, 24 and 48-hour intervals.

Statistical analysis was performed using the Friedman test to compare test and control substances at each observation interval. The Mann-Whitney-U test was used to compare the mean inhibition zones between test and control substances

at each observation interval and the Kruskal-Wallis test was used to compare the efficacy of tincture, 1X and 6X to each other at each observation interval. Analysis was calculated with the SPSS statistical package and all tests were performed at a 0.05 significance level.

The results of the study revealed all preparations of *Withania somnifera* in 62% v/v ethanol to be ineffective in inhibiting the *in vitro* growth of *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus*.

## CONTENTS

	Page
ACKNOWLEDGEMENTS	i
ABSTRACT	ii
CONTENTS	iv
LIST OF TABLES	xi
DEFINITION OF TERMS	xv

## CHAPTER ONE

1.1 Overview	1
1.2 Statement of Research Objectives	5
1.2.1 Objective one	5
1.2.2 Objective two	5
1.2.3 Objective three	5
1.2.4 Objective four	6
1.3 Delimitations	6

## CHAPTER TWO

2.1 Ethnobotany	7
2.2 Ethnopharmacology	7
2.3 Homoeopathy	9
2.4 Ethnopharmacology and Homoeopathy	11
2.5 <i>Withania somnifera</i> Dun.	11
2.5.1 Classification	11
2.5.2 Description	12
2.5.3 Flowering	12
2.5.4 Distribution	13
2.5.5 Habitat	13
2.5.6 Parts used and Constituents	13
2.5.7 Preparations	15
2.5.8 Traditional Uses	16

2.5.9 Other Clinical Applications	17
2.6 Bacteria	18
2.6.1 <i>Escherichia coli</i>	18
2.6.1.1 Classification	18
2.6.1.2 Morphology and Culture Identification	19
2.6.1.3 Epidemiology	19
2.6.1.4 Pathogenesis	19
2.6.1.5 Antimicrobial Sensitivity	20
2.6.2 <i>Pseudomonas aeruginosa</i>	21
2.6.2.1 Classification	21
2.6.2.2 Morphology and Culture Identification	21
2.6.2.3 Epidemiology	22
2.6.2.4 Pathogenesis	22
2.6.2.5 Antimicrobial Sensitivity	23
2.6.3 <i>Staphylococcus aureus</i>	23
2.6.3.1 Classification	23
2.6.3.2 Morphology and Culture Identification	24
2.6.3.3 Epidemiology	24
2.6.3.4 Pathogenesis	24
2.6.3.5 Antimicrobial Sensitivity	25
2.6.4 <i>Klebsiella pneumonia</i>	26
2.6.4.1 Classification	26
2.6.4.2 Morphology and Culture Identification	26
2.6.4.3 Epidemiology	26
2.6.4.4 Pathogenesis	26
2.6.4.5 Antimicrobial Sensitivity	27
2.6.5 <i>Bacillus cereus</i>	28
2.6.5.1 Classification	28
2.6.5.2 Morphology and Culture Identification	28
2.6.5.3 Epidemiology	28
2.6.5.4 Pathogenesis	28



2.6.5.5 Antimicrobial Sensitivity	29
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### CHAPTER THREE

3.1 Materials and Method	30
3.1.1 Preparation of the Media	30
3.1.2 Preparation of the Innoculum	31
3.1.3 Preparation of the Test Substances	31
3.1.3.1 Tincture methodology	31
3.1.3.1.1 Preparation of <i>W. somnifera</i> 1X	32
3.1.3.1.1 Preparation of <i>W. somnifera</i> 6X	32
3.1.4 Preparation of the Controls	32
3.1.4.1 Preparation of 62% v/v ethanol	32
3.1.4.2 Preparation of Vancomycin and Gentamycin Discs	33
3.1.5 Preparation of the Discs	33
3.1.6 Preparation of the Plates	34
3.1.7 Incubation of the Plates	35
3.1.8 Recording the Results	35
3.2 Data Analysis	35
3.2.1 Sample Size of the Study	35
3.2.2 Statistical Procedures	36
3.2.2.1 Intra-group Comparison of <i>W. somnifera</i> tincture in 62% v/v ethanol with regard to observations at 18, 24 and 48 hours	36
3.2.2.2 Intra-group Comparison of <i>W. somnifera</i> 1X in 62% v/v ethanol with regard to observations at 18, 24 and 48 hours	38
3.2.2.3 Intra-group Comparison of <i>W. somnifera</i> 6X in 62 % v/v ethanol with regard to observations at 18, 24 and 48 hours	38

3.2.2.4	Intra-group Comparison of 62% v/v ethanol with regard to observations at 18, 24 and 48 hours	39
3.2.2.5	Inter-group comparison between <i>W. somnifera</i> tincture in 62% v/v ethanol and 62% v/v ethanol with regard to observations at 18, 24 and 48 hours	39
3.2.2.6	Inter-group comparison between <i>W. somnifera</i> 1X in 62% v/v ethanol and 62% v/v ethanol with regard to observations at 18, 24 and 48 hours	40
3.2.2.7	Inter-group comparison between <i>W. somnifera</i> 6X in 62% v/v ethanol and 62% v/v ethanol with regard to observations at 18, 24 and 48 hours	40
3.2.2.8	Inter-group comparison between <i>W. somnifera</i> tincture in 62% v/v ethanol and <i>W. somnifera</i> 1X with regard to observations at 18, 24 and 48 hours	41
3.2.2.9	Inter-group comparison between <i>W. somnifera</i> tincture in 62% v/v ethanol and <i>W. somnifera</i> 6X with regard to observations at 18, 24 and 48 hours	41
3.2.2.10	Inter-group comparison between <i>W. somnifera</i> 1X in 62% v/v ethanol and <i>W. somnifera</i> 6X with regard to observations at 18, 24 and 48 hours	42
3.2.2.11	Inter-group comparison between <i>W. somnifera</i> tincture, <i>W. somnifera</i> 1X and <i>W. somnifera</i> 6X all in 62% v/v ethanol with regard to observations at 18, 24 and 48 hours	42
3.2.3	Statistical Package	44

## CHAPTER FOUR

4.1	The Data	45
4.1.1	The Primary Data	45
4.1.2	The secondary Data	45
4.2	Criteria Governing the Admissibility of Data	46

4.3	Intra-group comparisons at 18, 24 and 48 hour observation intervals	46
4.3.1	<i>Withania somnifera</i> tincture	46
4.3.1.1	<i>Bacillus cereus</i>	46
4.3.1.2	<i>Pseudomonas aeruginosa</i>	47
4.3.1.3	<i>Klebsiella pneumonia</i>	47
4.3.1.4	<i>Escherichia coli</i>	47
4.3.1.5	<i>Staphylococcus aureus</i>	48
4.3.2	<i>Withania somnifera</i> 1X	48
4.3.2.1	<i>Bacillus cereus</i>	48
4.3.2.2	<i>Pseudomonas aeruginosa</i>	49
4.3.2.3	<i>Klebsiella pneumonia</i>	49
4.3.2.4	<i>Escherichia coli</i>	50
4.3.2.5	<i>Staphylococcus aureus</i>	50
4.3.3	<i>Withania somnifera</i> 6X	51
4.3.3.1	<i>Bacillus cereus</i>	51
4.3.3.2	<i>Pseudomonas aeruginosa</i>	52
4.3.3.3	<i>Klebsiella pneumonia</i>	52
4.3.3.4	<i>Escherichia coli</i>	52
4.3.3.5	<i>Staphylococcus aureus</i>	52
4.3.4	62% v/v ethanol only	53
4.3.4.1	<i>Bacillus cereus</i>	53
4.3.4.2	<i>Pseudomonas aeruginosa</i>	53
4.3.4.3	<i>Klebsiella pneumonia</i>	54
4.3.4.4	<i>Escherichia coli</i>	54
4.3.4.5	<i>Staphylococcus aureus</i>	54
4.4	Inter-group comparisons at 18, 24 and 48 hour observation intervals	55
4.4.1	<i>Withania somnifera</i> tincture versus 62% v/v ethanol only	55
4.4.1.1	<i>Bacillus cereus</i>	55
4.4.1.2	<i>Pseudomonas aeruginosa</i>	55
4.4.1.3	<i>Klebsiella pneumonia</i>	56
4.4.1.4	<i>Escherichia coli</i>	56

4.4.1.5	<i>Staphylococcus aureus</i>	56
4.4.2	<i>Withania somnifera</i> 1X versus 62% v/v ethanol only	57
4.4.2.1	<i>Bacillus cereus</i>	57
4.4.2.2	<i>Pseudomonas aeruginosa</i>	57
4.4.2.3	<i>Klebsiella pneumonia</i>	57
4.4.2.4	<i>Escherichia coli</i>	58
4.4.2.5	<i>Staphylococcus aureus</i>	58
4.4.3	<i>Withania somnifera</i> 6X versus 62% v/v ethanol only	59
4.4.3.1	<i>Bacillus cereus</i>	59
4.4.3.2	<i>Pseudomonas aeruginosa</i>	59
4.4.3.3	<i>Klebsiella pneumonia</i>	59
4.4.3.4	<i>Escherichia coli</i>	60
4.4.3.5	<i>Staphylococcus aureus</i>	60
4.4.4	<i>Withania somnifera</i> tincture versus <i>Withania somnifera</i> 1X	60
4.4.4.1	<i>Bacillus cereus</i>	60
4.4.4.2	<i>Pseudomonas aeruginosa</i>	61
4.4.4.3	<i>Klebsiella pneumonia</i>	61
4.4.4.4	<i>Escherichia coli</i>	61
4.4.4.5	<i>Staphylococcus aureus</i>	61
4.4.5	<i>Withania somnifera</i> tincture versus <i>Withania somnifera</i> 6X	62
4.4.5.1	<i>Bacillus cereus</i>	62
4.4.5.2	<i>Pseudomonas aeruginosa</i>	63
4.4.5.3	<i>Klebsiella pneumonia</i>	63
4.4.5.4	<i>Escherichia coli</i>	63
4.4.5.5	<i>Staphylococcus aureus</i>	63
4.4.6	<i>Withania somnifera</i> 1X versus <i>Withania somnifera</i> 6X	64
4.4.6.1	<i>Bacillus cereus</i>	64
4.4.6.2	<i>Pseudomonas aeruginosa</i>	65
4.4.6.3	<i>Klebsiella pneumonia</i>	65
4.4.6.4	<i>Escherichia coli</i>	65
4.4.6.5	<i>Staphylococcus aureus</i>	65

4.4.7	<i>Withania somnifera</i> tincture versus <i>Withania somnifera</i> 1X versus <i>Withania somnifera</i> 6X	66
4.4.7.1	<i>Bacillus cereus</i>	66
4.4.7.2	<i>Pseudomonas aeruginosa</i>	66
4.4.7.3	<i>Klebsiella pneumonia</i>	66
4.4.7.4	<i>Escherichia coli</i>	67
4.4.7.5	<i>Staphylococcus aureus</i>	67
 <b>CHAPTER FIVE</b>		
	Discussion	68
 <b>CHAPTER SIX</b>		
6.1	Conclusion	71
6.2	Recommendations	71
6.2.1	Preparation of the Tincture	71
6.2.2	Experimental Procedure	72
6.2.3	Experimental Design	73
 <b>REFERENCES</b>		
		74
 <b>APPENDIX A</b>		
	Effect of test and control substances on <i>Bacillus cereus</i>	81
 <b>APPENDIX B</b>		
	Effect of test and control substances on <i>Pseudomonas aeruginosa</i>	82
 <b>APPENDIX C</b>		
	Effect of test and control substances on <i>Klebsiella pneumonia</i>	83
 <b>APPENDIX D</b>		
	Effect of test and control substances on <i>Escherichia coli</i>	84
 <b>APPENDIX E</b>		
	Effect of test and control substances on <i>Staphylococcus aureus</i>	85

## LIST OF TABLES

### TABLE 4.1

Page 46

Descriptive statistics for *Withania somnifera* tincture versus *Bacillus cereus*.

### TABLE 4.2

Page 46

Friedman's test statistics for *Withania somnifera* tincture versus *Bacillus cereus*.

### TABLE 4.3

Page 48

Descriptive statistics for *Withania somnifera* tincture versus *Staphylococcus aureus*.

### TABLE 4.4

Page 48

Friedman's test statistics for *Withania somnifera* tincture versus *Staphylococcus aureus*.

### TABLE 4.5

Page 49

Descriptive statistics for *Withania somnifera* 1X versus *Bacillus cereus*.

### TABLE 4.6

Page 49

Friedman's test statistics for *Withania somnifera* 1X versus *Bacillus cereus*.

**TABLE 4.7****Page 50**

Descriptive statistics for *Withania somnifera* 1X versus *Staphylococcus aureus*.

**TABLE 4.8****Page 50**

Friedman's test statistics for *Withania somnifera* 1X versus *Staphylococcus aureus*.

**TABLE 4.9****Page 51**

Descriptive statistics for *Withania somnifera* 6X versus *Bacillus cereus*.

**TABLE 4.10****Page 51**

Friedman's test statistics for *Withania somnifera* 6X versus *Bacillus cereus*.

**TABLE 4.11****Page 53**

Descriptive statistics for 62% v/v ethanol versus *Bacillus cereus*.

**TABLE 4.12****Page 53**

Friedman's test statistics for 62% v/v ethanol versus *Bacillus cereus*.

**TABLE 4.13****Page 55**

Inter-group comparison between *Withania somnifera* tincture and 62% v/v ethanol with regards to *Bacillus cereus*.

**TABLE 4.14****Page 56**

Inter-group comparison between *Withania somnifera* tincture and 62% v/v ethanol with regards to *Staphylococcus aureus*.

**TABLE 4.15****Page 57**

Inter-group comparison between *Withania somnifera* 1X and 62% v/v ethanol with regards to *Bacillus cereus*.

**TABLE 4.16****Page 58**

Inter-group comparison between *Withania somnifera* 1X and 62% v/v ethanol with regards to *Staphylococcus aureus*.

**TABLE 4.17****Page 59**

Inter-group comparison between *Withania somnifera* 6X and 62% v/v ethanol with regards to *Bacillus cereus*.

**TABLE 4.18****Page 60**

Inter-group comparison between *Withania somnifera* tincture and *Withania somnifera* 1X with regards to *Bacillus cereus*.

**TABLE 4.19****Page 62**

Inter-group comparison between *Withania somnifera* tincture and *Withania somnifera* 1X with regards to *Staphylococcus aureus*.



**TABLE 4.20****Page 62**

Inter-group comparison between *Withania somnifera* tincture and *Withania somnifera* 6X with regards to *Bacillus cereus*.

**TABLE 4.21****Page 64**

Inter-group comparison between *Withania somnifera* tincture and *Withania somnifera* 6X with regards to *Staphylococcus aureus*.

**TABLE 4.22****Page 64**

Inter-group comparison between *Withania somnifera* 1X and *Withania somnifera* 6X with regards to *Bacillus cereus*.

**TABLE 4.23****Page 66**

Inter-group comparison between *Withania somnifera* tincture, *Withania somnifera* 1X and *Withania somnifera* 6X with regards to *Bacillus cereus*.

**TABLE 4.24****Page 67**

Inter-group comparison between *Withania somnifera* tincture, *Withania somnifera* 1X and *Withania somnifera* 6X with regards to *Staphylococcus aureus*.

## DEFINITIONS

- **Adaptogen:** A chemical or substance, which increases the body's ability to resist stress and not suffer cumulative damage (Yasgur, 1998:4).
- **Alkaloid:** Any of various physiologically active nitrogen-containing organic bases derived usually from plants. They are generally bitter in taste, alkaline and unite with acids to form salts. (Yasgur, 1998:8.)
- **Anthelmintic:** Having the power to destroy or expel intestinal worms (Yasgur, 1998:16).
- **Anti-oxidant:** Something added to a product to prevent or delay its deterioration by the oxygen in air (Dorland, 1995:53).
- **Ayurvedic Medicine:** Originated in India. Maintains that medicinal plants growing in a country are the most effective for the health maintenance of the people in that country; it divides the medicinal effects of herbs, which it uses in ponderal doses, broadly speaking into three categories: (1) active creating energy (2) passive destroying or resisting energy (3) unifying and preserving energy. It addresses the mind, body, behavior and the environment in its approach to health recovery and maintenance. An examination of the phytopharmaka in the materia medica of Ayurveda reveals first of all that there are vast numbers of herbal drugs (used in ponderal doses), which are identical to those of the homoeopathic materia medica. In fact the preponderance of herbal drugs used by Ayurveda have also undergone homoeopathic provings and are known to produce pronounced reactions in patients when

administered in substantial doses. But an examination of homoeopathy's materia medica shows that a number of homoeopathic medicines started out as Ayurvedic herbal drugs, then after undergoing provings they were incorporated into homoeopathy's materia medica. (Gaier, 1991:48-49.)

- Decoction: The process of boiling down in order to concentrate a mixture (as apposed to infusion where the medicinal substance steeps in hot water or liquid) (Yasgur, 1998:63).
- Doctrine of Signatures: A postulate which says the external characteristics of a substance serve to indicate possible therapeutic effects (Yasgur, 1998:70).
- Emollient: An agent that softens or soothes the skin (Dorland, 1995:280).
- Iatrogenic: Relating to an abnormal state or condition produced by a doctor by his or her administration of poor treatment (either procedures or drugs)(Yasgur, 1998:121).
- Infusion: A medicinal brew. Prepared by plant material being steeped in freshly boiled water. (Gaier, 1991:285.)
- Ointments: Salve, unguentum, a semi-solid vehicle for medicinal substances intended for external application (Gaier, 1991:370).
- Potentisation: Dynamization, imparting (along serial dilutions) the pharmacological message of the original substance (i.e. creating a template of the active principle) by means of trituration or succussion. It describes the process of

modification of medicines as invented by Hahnemann. (Gaier, 1991:441.) It is a physical process through which latent curative powers of medicines are aroused into activity, though these may have been inevident in their crude states. By this process quantitative deconcentration of drug substance occurs as a qualitative increment takes place. (Gaier, 1991:444.)

- Poulitice: A moist, warm, pasty mass folded inside a thin cloth and laid on inflamed skin to reduce inflammation, draw out infection or act as a counter-irritant (Yasgur, 1998:198).
- Succus: A preparation obtained by expressing the juice of a plant and adding to it sufficient alcohol to preserve it (Yasgur, 1998:242).
- Tincture: It is the drug solution prepared in accordance with homoeopathic pharmacopoeial standards from the corresponding original succus or other soluble base constituent of the medicine (Gaier, 1991:354).
- Tonic: A remedy that enhances enfeebled functions and promotes well being (Gaier, 1991:556).

## CHAPTER ONE

### 1.1 OVERVIEW

Berkowitz (1995:797-804) states that the numbers of resistant strains of microbial pathogens are growing since penicillin resistant and multiresistant pneumococci caused a major problem in South African hospitals in 1977. He calls the emergence of drug resistant bacteria a medical catastrophe. It is critically important to develop new antimicrobial compounds before we enter a post-antibiotic era (Leggiadro, 1995:884). New compounds inhibiting microorganisms have been isolated from plants and some of these new antimicrobial compounds may inhibit bacteria by a different mechanism than the presently used antibiotics and hence have clinical value in the treatment of resistant microbial strains (Cox, 1994:25-41).

Developed and developing countries show a great interest in indigenous medicine, and many developing countries use traditional medicines at the primary health care level. Many currently used drugs are expensive or not readily available, and a major setback to their continued usage is the development of resistance. There is thus an urgent need for new, inexpensive drugs that will be able to act for longer periods before resistance sets in. (McGaw, Jager and Van Staden, 2000:247.)

The aim of this study was to establish the antibacterial efficacy of *Withania somnifera* (*W. somnifera*) against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* in order to establish its application as an antibacterial agent.

*W. somnifera* is indigenous to southern Africa, commonly known in English as winter cherry and is known by the Zulus as "ubuvimba" (Gericke, Van Oudtshoorn and Van Wyk, 2000:274). It is utilised in the treatment of fever, colds, asthma, general ill health and debility, syphilis, abdominal discomfort, diarrhoea, typhus, typhoid, proctitis, worms, cancer, candida, coughs, bronchitis, tuberculosis and as a sedative and hypnotic (Gericke, Van Oudtshoorn and Van Wyk, 2000:150; Iwu, 1993:259). The root of this plant is used to make ointments to treat various infected sores and abscesses. The root is made into a powder and then boiled with fat of the python snake. It is then applied directly on sores to aid prompt healing. (Pujol, 1990:78.)

*W. somnifera* is an important component of the Ayurvedic pharmacopoeia of India where it is known as "ashwaganda", and is regarded as the "Indian ginseng". *W. somnifera* is rapidly becoming known and appreciated in industrialised countries. Infusions, decoctions and tinctures of the fresh and dry whole root are used. (Gericke, Van Oudtshoorn and Van Wyk, 2000:150.)

The plant contains alkaloids (e.g. withaferine, withanine) and a distinctive group of phytosteroids called withanolides (Iwu, 1993:259). The activity of withanolides and other compounds have been the subject of studies, particularly the antibiotic and anti-inflammatory effects (Gericke, Van Oudtshoorn and Van Wyk, 2000:274). Withaferine has been shown to be antibiotic towards Gram-positive organisms and certain fungi (Hutchings, 1996:273). Iwu (1993:260) states that in his study of neutropaenic mice that were infected with *Staphylococcus aureus*, the mortality due to sepsis was reduced from 75% to 50% in the group that was pre-treated with *W. somnifera* root extract.

*Klebsiella pneumonia* is a respiratory pathogen that is also present in the respiratory tract and faeces of about 5% of normal individuals. It causes a small portion of bacterial pneumonias and can produce extensive haemorrhagic necrotising consolidation of the lung (Adelberg, *et al.*, 1989:208). In the early days of antibiotic usage, *Klebsiella pneumonia* was naturally resistant to the available antibiotics and with the passage of time they acquired resistance to the newly developed ones. The emergence of *Klebsiella pneumonia* as an important cause of infection in hospitals is undoubtedly related to the use of antibiotics. (Greenwood, Peutherer and Slack, 1992:338.)

*Staphylococcus aureus* is an opportunistic pathogen resulting in infection at compromised sites within an organism. Staphylococcal pneumonia is a recognised complication of influenza (Greenwood, Peutherer and Slack,

1992:204). In view of the rapid emergence of drug resistance among *staphylococci*, hospitals have sometimes restricted the use of antistaphylococcal drugs (Adelberg, *et al.*, 1989:191).

Most episodes of food poisoning caused by *Bacillus cereus* are due to preformed toxin (Greenwood, Peutherer and Slack, 1992:276). *Bacillus cereus* has also been associated with various opportunistic clinical infections (Adelberg *et al.*, 1989:175).

*Pseudomonas aeruginosa* can affect almost any external site or organ. In hospitalised patients, *pseudomonas* infections are more common, more severe and more varied. The lungs of children with cystic fibrosis are very susceptible to infection with *Pseudomonas aeruginosa*. The reason for its pre-eminent status as an opportunistic pathogen lie in its adaptability, its innate resistance to many antibiotics and disinfectants, its varied armoury of putative virulence factors, and in an increasing supply of patients compromised by age, underlying disease or immunosuppressive therapy. (Greenwood, Peutherer and Slack, 1992:345-346.)

*Escherichia coli* become pathogenic only when they reach tissues outside the intestinal tract, particularly the urinary and biliary tracts, lungs, peritoneum, and meninges, resulting in inflammation at these sites (Adelberg, *et al.*, 1989:207).



## **1.2 STATEMENT OF RESEARCH OBJECTIVES**

The purpose of this study was to determine the antibacterial effectiveness of *W. somnifera* in tincture and homoeopathic dilutions, all in 62% v/v ethanol, against selected Gram-positive and Gram-negative bacteria as measured by the disc diffusion test.

### **1.2.1 OBJECTIVE 1**

To determine the efficacy of *W. somnifera* tincture in 62% v/v ethanol as compared to 62% v/v ethanol (negative control) in the *in vitro* growth inhibition of *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* in terms of the disc diffusion test.

### **1.2.2 OBJECTIVE 2**

To determine the efficacy of *W. somnifera* 1X in 62% v/v ethanol as compared to 62% v/v ethanol (negative control) in the *in vitro* growth inhibition of *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* in terms of the disc diffusion test.

### **1.2.3 OBJECTIVE 3**

To determine the efficacy of *W. somnifera* 6X in 62% v/v ethanol as compared to 62% v/v ethanol (negative control) in the *in vitro* growth inhibition of

*Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* in terms of the disc diffusion test.

#### 1.2.4 OBJECTIVE 4

To determine the relative efficacy of *W. somnifera* tincture, 1X and 6X in the *in vitro* growth inhibition of *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* in terms of the disc diffusion test.

#### 1.3 DELIMITATIONS

- This study was limited to only five species of bacteria namely *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*.
- This study was limited to the species *Withania somnifera* Dun.
- Only the following growth media was used:  
Meuller-Hinton Agar.
- Only *Withania somnifera* in tincture, 1X and 6X homoeopathic potencies in 62% v/v ethanol were used as test substances.
- The incubation temperature for culture growth was 37°C.
- This was an *in-vitro* study.

## CHAPTER 2

### 2.1 ETHNOBOTANY

Due to the broad spectrum of subjects embraced by the term "ethnobotany" a standard definition has not yet been established. However, it is understood to encompass all studies that concern the mutual relationship between plants and traditional peoples. (Cotton, 1996:1.)

### 2.2 ETHNOPHARMACOLOGY

This branch of ethnobotany is defined as the scientific evaluation of traditional medicines (Cotton, 1996:10).

The African continent is a rich source of materials for the development of new therapeutic agents. Indeed, many modern pharmaceuticals and commonly used herbs owe their origin to Africa. There is therefore little doubt that a systematic ethnobotanical study of African plants is a viable approach for the development of new therapeutic agents. (Iwu, 1993:116-117.)

Developed and developing countries show a great interest in indigenous medicine, and many developing countries use traditional medicines at the primary health care level. Many currently used drugs are expensive or not readily

available, and a major setback to their continued usage is the development of resistance. There is an urgent need for new, inexpensive drugs that will be able to act for longer periods before resistance sets in. (McGaw, Jager and Van Staden, 2000:247.)

The true therapeutic potential of many medicinal plants is often unappreciated and is generally interpreted through the narrow window of known plant chemistry and pharmacology (Gericke, 2001:3).

The World Health Organisation estimates that 80% of the people living in developing countries almost exclusively use traditional medicine for their primary health care needs. Thus there is a need to study these plants for safety and efficacy. (Farnsworth, 1994:44.)

Southern Africa contains approximately 10% of the world's plant diversity, but relatively little chemical work has been done on plants from this region (Fourie *et al.*, 1992:191).

In the African system of medicine, healing is concerned with the utilization of human energy, the environment and the cosmic balance of natural forces as tools of healing. In the African world, the natural environment is a living entity, whose components are intrinsically bound to humans. Plants therefore play a participatory role in healing. Treatment therefore is not limited to the sterile use of

different leaves, roots, fruit, barks, grasses, and various objects like minerals or bones. If a sick person is given a leaf infusion to drink, he or she drinks it believing not only in the organic properties of the plant but also in the magical or spiritual force imbibed by nature in all living things and the role of spirits, ancestors and gods in the healing process. (Iwu, 1994:118.)

Iwu (1994:117) states that the search for new drugs from African plants has been limited, and has identified three of the main reasons. Firstly, the relationship between African communities and the environment, which portrays a highly spiritual and sacred bond, making it extremely difficult to separate the physical properties of medicinally used plants from their spiritual attributes. Secondly, is the dominant European influence on Africa where drugs are mainly isolated compounds from plants as compared to the traditional approach whereby whole plants or parts thereof are used. And thirdly is the fact that African remedies are not studied within the paradigm that they are utilised but according to Western scientific methods.

### **2.3 HOMOEOPATHY**

Homoeopathy is a system of medicine, which discerns that the body has within it a controlling and defending force and power. When this fighting power or resistance is disturbed illness occurs. The disease is thus not an affection of the parts but a disorder of the patient as a whole. (Sankaran, 1999:5-6.)

Homoeopathy is based on the principle that "like cures like". This means that a medicine capable of producing certain affects when taken by a healthy human being is capable of curing an illness that displays similar effects. (Sankaran, 1999:5-6.)

Homoeopathic medicine stimulates the natural healing power of the body resulting in the illness being driven outward and thrown off. Since one patient differs from another each requires a different medicine based on his or her individual and mental symptoms. Experiment led to the process of serial dilution and potentisation in the preparation of homoeopathic medicines. (Sankaran, 1999:5-6.)

Substances in their crude form e.g. salt have a limited medicinal application. However, when a substance is serially diluted it may become extremely active in the treatment of many conditions. Similarly gold, silver, charcoal and silica take on medicinal properties when they are diluted according to homoeopathic principles. The substance gains a more powerful action through the process of potentisation. (Kayne, 1997:27.)

## 2.4 ETHNOPHARMACOLOGY AND HOMOEOPATHY

The value of most of the herbal medicine administered by African traditional healers is based on the Doctrine of Signatures, a concept introduced by Paracelsus, giving rise to the ideas of similars in medicine. The idea of similars is a key element in homoeopathy, and is more clearly reflected in the thinking of the African herbalist than with any other population group in the world. The Zulu healers believe that all medicinal herbs are signposted by God, who created identical structure in humans and plants so that the sages could recognise them for the benefit of all humankind. An example of the Doctrine of Signatures is a milk producing plant being used for boosting milk production in a lactating mother. (Pujol, 1990:24.)

## 2.5 WITHANIA SOMNIFERA DUN.

### 2.5.1 CLASSIFICATION

*W. somnifera* falls under the Solanaceae family. Its common name in English is winter cherry but it is also known as poisonous gooseberry and Indian ginseng. (Gericke and Van Wyk, 2000:139.)

Other names include *ashwagandha* (Hindi), *ubuvimbha*, *ibuvimba*, *ubuvimbo*, *umaqhunsula* (Zulu), *ubuvuma* (Xhosa), *bofepha*, *moferangopa*, *mosala-marupi*

(Sotho), *geneesblaarbossie*, *bitterappelliefie*, *spanjoolbossie*, *stuipebossie*, *vermietbossie*, and *vuilsiektebossie* (Afrikaans), *vimhepe* (Swati) and *ol asajet* (Masai). (Iwu, 1993:259; Hutchings *et al.*, 1996:273; Gericke and Van Wyk, 2000:139.)

### 2.5.2 DESCRIPTION

*W. somnifera* can be identified as an erect much-branched perennial undershrub, growing up to 2m high with distinctively hairy stems and leaves. The pale green leaves are simple, about 10cm long, elliptic to broadly ovate-lanceolate, with entire or wavy margins. (Iwu, 1993:259.) The leaves are covered with short dense hairs, particularly when young. Small white or yellowish flowers are produced in short axillary clusters, followed by small, round, orange-red berries of about 8mm in diameter. The berries are completely enclosed in brown papery and bladdery structures, which are remains of the sepals. (Gericke, Van Oudtshoorn and Van Wyk, 2000: 274.)

### 2.5.3 FLOWERING

Mid to late summer (Blakley and Sturdivant, 1999:162).



#### 2.5.4 DISTRIBUTION

*W. somnifera* has a wide distribution in Africa, southern Europe and Asia. It is considered indigenous to South Africa (Gericke, Van Oudtshoorn and Van Wyk, 2000:274). It is Native from India to the Mediterranean and Africa (Blakley and Sturdivant, 1999:162).

### 2.5.5 HABITAT

Drier tropical regions (Iwu, 1993:259).

### 2.5.6 PARTS USED AND CONSTITUENTS

The leaves and roots are mainly used (Gericke, Van Oudtshoorn and Van Wyk, 2000:274), the berry to a lesser extent (Watt and Breyer-Brandwijk, 1962:1010-1011; Hutchings *et al.*, 1996:273). Constituents include:

## ➤ ROOTS

- ☐ 3-tigloyl-hydroxy-tropane
- ☐ anaferine
- ☐ anhygrine
- ☐ choline
- ☐ cuscohygrine
- ☐ glycowithanaloids: sitoindoside IX  
sitoinoside X

withasomnin

- isopelletierine
- pseudotrpanol
- tropanol
- withaferine
- withanolides
- withanol  $C_{25}H_{23}O_4OH$

➤ **LEAVES AND STEMS**

- ascorbic acid
- nicotine
- potassium nitrate
- pseudowithanine
- somniferine
- somniferinine
- somnine
- somnirol  $C_{32}H_{43}O_6OH$
- somnitol  $C_{33}H_{44}O_5(OH)_2$
- withaferine
- withanoloids
- withananine
- withananinine
- withanic acid  $C_{29}H_{45}O_6COOH$
- withanine  $C_{44}H_{80}O_{12}N_2CHCL_3$

(Buckingham and Buckingham, 1994:582.)

### 2.5.7 PREPARATIONS

#### ➤ **ROOTS**

- ❑ OINTMENT: Powdered roots mixed with the fat of a python or crocodile used as ointment (Pujol, 1990:78).
- ❑ TINCTURE
- ❑ DECOCTION
- ❑ INFUSION
- ❑ FRESH

(Watt and Breyer-Brandwijk, 1962:1010; Iwu, 1993:259; Gericke, Van Wyk and Van Oudtshoorn, 2000:274; Hutchings *et al.*, 1996:273.)

#### ➤ **LEAVES**

- ❑ POULTICE
- ❑ JUICE
- ❑ DECOCTION
- ❑ OINTMENTS
- ❑ EMOLLIENT
- ❑ FRESH: Bruised leaves with ground roots.

(Watt and Breyer-Brandwijk, 1962:1010; Hutchings *et al.*, 1996:273; Iwu, 1993:259; Gericke, Van Wyk and Van Oudtshoorn, 2000:274.)

#### ➤ **BERRIES**

- ❑ FRESH

(Watt and Breyer-Brandwijk, 1962:1010.)

#### 2.5.8 TRADITIONAL USES

##### □ SOTHO

*W. somnifera* is used as an anthelmintic, ritual plant against witchcraft (Iwu, 1993:259), for colds and chills, asthma, bed sores, to tone the uterus of female who habitually miscarries, or to remove contained conception products (Watt and Breyer-Brandwijk, 1962:1010).

##### □ ZULU

Gangrenous rectitis, syphilis, healing of sores (Watt and Breyer-Brandwijk, 1962:1010) and hyperpyrexia (Iwu, 1993:259).

##### □ XHOSA

Chest complaints, ringworm, syphilitic sores, black gall sickness (Watt and Breyer-Brandwijk, 1962:1010), anthrax infected meat and anthrax pustules (Iwu, 1993:259).

##### □ SWATI

Eruptive diseases and smallpox (Iwu, 1993:259).

##### □ MASAI

Pink eye and conjunctivitis (Watt and Breyer-Brandwijk, 1962:1011).

#### □ AYURVEDIC

Fatigue, fever, asthma, learning and memory enhancement, (Gericke and Van Wyk, 2000:150). Arthritis, stress, herbal tonic, health food (Ziauddin *et al.*, 1996:70), anxiety and depression (Bhattacharya, 2001:33). Non-specific host defence, hypnotic, emphysema, alcoholism and pulmonary tuberculosis (Iwu, 1993:260).

#### □ SOUTHERN AFRICA

Asthma, bronchial diseases, syphilis, antiseptic dressing for wounds (Iwu, 1993:259), chest complaints, haemorrhoids, diarrhoea and proctitis (Watt and Breyer-Brandwijk, 1962:1011)

#### □ TANZANIA

Sexual stimulant and abortifacient (Watt and Breyer-Brandwijk 1962:1011).

### 2.5.9 OTHER CLINICAL APPLICATIONS

Anti-inflammatory, antibiotic, sedative and hypnotic properties have been described. Although both immuno-suppressing and immuno-stimulating effects have been described for isolated withanolides, aqueous extracts of the actual roots have an immuno-stimulatory effect. Sitoindosides IX and X, two withanolide glycosides, have been reported as the constituents responsible for the immuno-stimulant activity of the plant. This activity has been identified as increasing

activity of the lysosomal enzymes secreted by macrophages, increased mobilization and activation of peritoneal macrophages and increased phagocytosis. These biological reactions have been attributed to sitoindosides IX and X. (Iwu, 1993:260.) Ziauddin *et al.* (1995:74) observed the improvement of marrow activity and hence leukocyte production along with increased agglutinin antibody titres and in complement fixing antibodies which supports the immunostimulatory activity of *W. somnifera*.

Katiyar (1997:45) documents its memory-enhancing, anti-oxidant, anti-inflammatory, anti-stress, anti-aging, anti-cancer, improvement of anxiety and mild to moderate depression and adaptogenic activity (Gericke and Van Wyk, 2000:150). Antibiotic, antiviral, sedative, hypnotic and marked antibiotic activity against *Staphylococcus aureus* has been reported by Watt and Breyer-Brandwijk (1962:1012). Other properties documented are antiseptic and antimutagenic properties (Evans, 1996:320).

## **2.6 BACTERIA**

### **2.6.1 ESCHERICHIA COLI**

#### **2.6.1.1 CLASSIFICATION**

*Escherichia coli* is a member of the *Enterobacteriaceae* family and genus *Escherichia* (Collee *et al.*, 1996:363).

#### 2.6.1.2 MORPHOLOGY AND CULTURE IDENTIFICATION

*Escherichia coli* are aerobic, Gram-negative bacilli, most strains being motile and some strains producing a polysaccharide capsule. It grows well on non-selective media, forming smooth, colourless colonies 2-3mm in diameter after 18hr growth on nutrient agar. (Greenwood, Peutherer and Slack, 1992:323.) The optimal temperature for growth is 36-37°C, though growth occurs over a fairly wide temperature range (18-44°C) (Collee *et al.*, 1996:364).

#### 2.6.1.3 EPIDEMIOLOGY

*Escherichia coli* predominate among the aerobic commensal flora present in the gut, especially the colon, of humans and animals (Greenwood, Peutherer and Slack, 1992:323).

#### 2.6.1.4 PATHOGENESIS

The species *Escherichia coli* encompasses a great variety of strains that include purely commensal organisms as well as those possessing combinations of virulence determinants that enable them to act as specific pathogens of the gut and of extra-intestinal sites, especially the urinary tract (Greenwood, Peutherer and Slack, 1992:323). *Escherichia coli* is commonly implicated in infections of

the urinary tract and is by far the most common cause of acute uncomplicated urinary tract infection (Greenwood, Peutherer and Slack, 1992:325).

From their normal site in the human body they are able to cause frequent opportunistic infections: they are often present in appendix abscesses, peritonitis, cholecystitis and septic wounds. They cause bacteraemia, endotoxic shock and occasionally meningitis in neonates. They are often present in the lower respiratory tract, especially in surgical or otherwise debilitated patients who are being treated with antibiotics to which they are resistant. (Collee *et al.*, 1996:364.)

#### 2.6.1.5 ANTIMICROBIAL SENSITIVITY

Antimicrobial agents used to treat urinary and other *Escherichia coli* infections include those with activity against Gram-negative organisms such as sulphonamides, trimethoprim, cotrimaxazole, nalidixic acid, nitrofurantoin, tetracycline, ampicillin, cephalosporins and aminoglycosides (Cheesbrough, 1984:255).



## 2.6.2 PSEUDOMONAS AERUGINOSA

### 2.6.2.1 CLASSIFICATION

*Pseudomonas aeruginosa* falls into the *Pseudomonadaceae* family and genus *Pseudomonas* (Cheesbrough, 1984:264).

### 2.6.2.2 MORPHOLOGY AND CULTURE IDENTIFICATION

*Pseudomonas aeruginosa* is an aerobic, Gram-negative bacillus, non-sporing, non-capsulate, and usually motile. The organism grows readily on a wide variety of culture media and emits a sweet grape-like odour that is easily recognised. Most strains of *Pseudomonas aeruginosa* produce diffusible pigments; typically, the colony and surrounding medium is greenish blue. (Greenwood, Peutherer and Slack, 1992:345.) Six differing colonial forms can be identified after 24 hours growth on nutrient agar. Type 1 is the most common presenting as large, low convex, rough in appearance and oval in shape. Type 2 colonies are small, smooth, domed and described as coliform-like. Types 3 and 4 small and described as rough and rugose respectively. Type 5 presents as a mucoid colony whilst Type 6 may also appear mucoid but is described as a dwarf colony. (Collee *et al.*, 1996: 414.)

#### 2.6.2.3 EPIDEMIOLOGY

*Pseudomonas aeruginosa* is physiologically very versatile and flourishes as a saprophyte in warm moist situations in the human environment, including sinks, drains, respirators, humidifiers and disinfectant solutions (Collee *et al.*, 1996: 414). The ability of the species to persist and multiply is of particular importance in cross-infection control. Healthy carriers of *Pseudomonas aeruginosa* usually harbour strains in the gastro-intestinal tract, but in the open community the carriage rate seldom exceeds 10%. In contrast acquisition of *Pseudomonas aeruginosa* in a hospital is rapid and up to 30% of patients may excrete the organisms within two days of admission. (Greenwood, Peutherer and Slack, 1992:348.) In hospitalised patients, pseudomonas infections are more common, more severe and more varied. The reason for its pre-eminent status as an opportunistic pathogen lies in its adaptability, its innate resistance to many antibiotics and disinfectants, its varied armoury of putative virulence factors, and in an increasing supply of patients compromised by age, underlying disease or immunosuppressive therapy (Greenwood, Peutherer and Slack, 1992:345-346).

#### 2.6.2.4 PATHOGENESIS

*Pseudomonas aeruginosa* is seldom encountered in healthy adults but in the last two decades the organism has become increasingly recognised as the aetiological agent in a variety of serious infections in hospitalised patients with

impaired immune defences. Colonisation is often iatrogenic and associated with poor instrumentation, e.g. catheterisation. (Collee *et al.*, 1996:414.) Some more common infections are otitis externa and otitis media in deep-sea divers, folliculitis (Jacuzzi rash) from unsanitised jacuzzis and eye infections from contaminated contact lenses or ophthalmic medicaments. Endocarditis and septicaemia caused by *Pseudomonas aeruginosa* is rare but carries a 70% mortality rate in compromised patients. The most significant pathogenic role of *Pseudomonas aeruginosa* at present is chronic debilitating pulmonary infections. (Collee *et al.*, 1996:414; Greenwood, Peutherer and Slack, 1992:345-348.)

#### 2.6.2.5 ANTIBICROBIAL SENSITIVITY

*Pseudomonas* species are resistant to most of the commonly used antibiotics. Those antimicrobial agents that usually show activity against *Pseudomonas* species include aminoglycosides, polymyxins, and some penicillins and cephalosporins. (Cheesbrough, 1984:265.)

#### 2.6.3 STAPHYLOCOCCUS AUREUS

##### 2.6.3.1 CLASSIFICATION

*Staphylococcus aureus* belongs to the genus *Staphylococci* and the family Micrococcaceae (Collee *et al.*, 1996:245).

#### 2.6.3.2 MORPHOLOGY AND CULTURE IDENTIFICATION

*Staphylococcus aureus* is a Gram-positive coccus, which is non-sporing, non-motile and usually non-capsulate. When grown on nutrient agar individual colonies are circular, 2-3cm in diameter with a smooth, shiny surface. (Greenwood, Peutherer and Slack, 1992:204.) They grow well aerobically and in a carbon dioxide enriched atmosphere. Most strains grow anaerobically but not as well. (Cheesbrough, 1984:226.) Colonies appear opaque and are frequently pigmented (Greenwood, Peutherer and Slack, 1992:204). The colours range from cream, through buff to gold (Collee *et al.*, 1996:246).

#### 2.6.3.3 EPIDEMIOLOGY

*Staphylococci* are widely distributed in the environment. They form part of the normal microbial flora of the skin, upper respiratory tract and intestinal tract. *Staphylococcus aureus* is carried in the nose of 40% or more of healthy people. (Cheesbrough, 1984:225.)

#### 2.6.3.4 PATHOGENESIS

*Staphylococcus aureus* is an opportunistic pathogen resulting in infection at compromised sites within an organism (Greenwood, Peutherer and Slack, 1992:204). Localised infections sometimes progress to systemic infections, while

"spontaneous" bacteraemia also occurs without an evident septic focus, particularly in patients with an underlying chronic disease e.g. diabetes mellitus. Some of the commoner infections caused by *Staphylococcus aureus* can be divided into pyogenic infections, disseminated infections and toxin-mediated illnesses. (Collee *et al.*, 1996:248.) *Staphylococcus aureus* causes abscesses, boils, styes, impetigo, secondary infection of bites, burns and wounds, conjunctivitis, septicaemia, endocarditis, osteomyelitis, pneumonia, empyema and mastitis. It is also the cause of food poisoning from enterotoxin B and scalded skin syndrome in young children due to the production of the toxin exfoliation. (Cheesbrough, 1984:226.)

#### 2.6.3.5 ANTIMICROBIAL SENSITIVITY

Antibiotics with sensitivity against *Staphylococcus aureus* include; penicillins, flucloxacillin, methicillin, cephalosporins, erythromycin, lincosamycin, clindamycin, fusidic acid and vancomycin. Many strains are penicillin resistant due to production of penicillinase. (Cheesbrough, 1984: 227.)

## 2.6.4 KLEBSIELLA PNEUMONIA

### 2.6.4.1 CLASSIFICATION

*Klebsiella pneumonia* belongs to the genus *Klebsiella*, family enterobacteriaceae (Greenwood, Peutherer and Slack, 1992:335).

### 2.6.4.2 MORPHOLOGY AND CULTURE IDENTIFICATION

*Klebsiella* are facultatively anaerobic, Gram-negative rods. They are non-motile but most strains have fimbriae and are usually capsulate. (Greenwood, Peutherer and Slack, 1992:335.) Colonies are identified as large, greyish-white and mucoid (Collee *et al.*, 1996:368).

### 2.6.4.3 EPIDEMIOLOGY

Commonly isolated from water and human and animal species (Collee *et al.*, 1996:369).

### 2.6.4.4 PATHOGENESIS

The main importance of *Klebsiella pneumonia* as human pathogens is in causing infections in hospital patients. Clinical sepsis develops in surgical wounds and in the urinary tract. Colonisation of the respiratory tract is very common in

hospitalised patients receiving antibiotics. Some debilitated patients develop bronchopneumonia in which *Klebsiella pneumonia* appears to be the primary infecting agent. (Greenwood, Peutherer and Slack, 1992:338.) Other infections caused by *Klebsiella pneumonia* are septicaemia, meningitis and peritonitis (Cheesbrough, 1984:263).

*Klebsiella pneumonia* is a respiratory pathogen that is also present in the respiratory tract and faeces of about 5% normal individuals. It causes a small portion of bacterial pneumonias and can produce extensive haemorrhagic necrotising consolidation of the lung. (Adelberg *et al.*, 1989:208.) In the early days of antibiotic usage, *Klebsiella pneumonia* was naturally resistant to the available antibiotics and with the passage of time they acquired resistance to the newly developed ones. The emergence of *Klebsiella pneumonia* as an important cause of infection in hospitals is undoubtedly related to the use of antibiotics. (Greenwood, Peutherer and Slack, 1992:338.)

#### 2.6.4.5 ANTIMICROBIAL SENSITIVITY

*Klebsiella pneumonia* shows wide variations in their sensitivity to antibiotics. Cephalosporins and aminoglycosides are used in the treatment of *Klebsiella pneumonia*. Most strains are resistant to ampicillin and some show multiple drug resistance. (Cheesbrough, 1984:263.)

## 2.6.5 BACILLUS CEREUS

### 2.6.5.1 CLASSIFICATION

*Bacillus cereus* belongs to the bacillus genus (Greenwood, Peutherer and Slack, 1992:276).

### 2.6.5.2 MORPHOLOGY AND CULTURE IDENTIFICATION

*Bacillus cereus* is a large Gram-positive, motile, non-capsulate bacillus. (Greenwood, Peutherer and Slack, 1992:276). It is a spore-forming organism with most strains being motile. On nutrient agar it forms large grey-white, granular colonies. (Collee *et al.*, 1996:322.)

### 2.6.5.3 EPIDEMIOLOGY

*Bacillus cereus* is saprophytic, frequenting soil, water and vegetation. The organism is widespread in the environment and found in most raw foods, especially cereals such as rice. (Greenwood, Peutherer and Slack, 1992:276.)

### 2.6.5.4 PATHOGENESIS

*Bacillus cereus* causes a wide variety of localised and generalised, mainly opportunistic infections. It has been found in a variety of infections, mostly in



injured, debilitated or immunosuppressed subjects. (Collee *et al.*, 1996:322.) Occasionally pneumonia and bronchopneumonia have been documented (Cheesbrough, 1984:234). Although most infections are in debilitated hosts, some severe infections follow entry into a lesion in an apparently normal host, showing that the organism has considerable pathogenicity. *Bacillus cereus* produces several toxins (Collee *et al.*, 1996:322) and is an important cause of toxin-mediated food poisoning of two distinct types; diarrhoeal and vomiting illnesses (Collee *et al.*, 1996:323).

#### 2.6.5.5 ANTIMICROBIAL SENSITIVITY

Effective antibiotics include penicillin, tetracycline, streptomycin and cotrimoxazole (Cheesbrough, 1984:235).

## CHAPTER THREE

### METHODOLOGY

#### 3.1 MATERIALS AND METHOD

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

##### 3.1.1 PREPARATION OF MEDIA

Mueller-Hinton Agar was the medium used in the experiments. It was prepared according to The Oxoid manual (1979), as follows:

- 35g of Mueller-Hinton agar powder was weighed out.
- The Mueller-Hinton agar powder was added to 1 litre of distilled water in a screw top flask.
- A magnetic stirrer was added to aid dissolution.
- The mixture was shaken until well mixed.
- The mixture was sterilized by autoclaving at 121°C for 15 minutes.
- The flask was allowed to cool, on a magnetic stirring machine. This ensured adequate mixing and prevented the mixture from solidifying.
- Once the flask had cooled enough to hold, the agar was poured into agar plates as follows:
  - The top of the flask was flamed with a bunsen burner before pouring each plate to prevent contamination.
  - Each plate was poured to a depth of approximately 5 millimetres.
  - A total of 100 plates was prepared.
  - The plates were stacked and allowed to cool and solidify.

- They were visually checked for contamination.

### 3.1.2 PREPARATION OF THE INNOCULUM

- Single colonies (from the Durban Institute of Technology stock cultures) of each bacterium to be tested were used to inoculate individual plates of Mueller-Hinton Agar.
- Plates were incubated at 37°C for 24 hours.
- Working with sterile technique, individual colonies were placed into 5ml of sterile saline solution.
- The solution was adjusted to McFarland's Equivalence Turbidity Standards 0,5, to ensure a standard concentration of bacteria.

### 3.1.3 PREPARATION OF TEST SUBSTANCES

#### 3.1.3.1 TINCTURE METHODOLOGY

The *W. somnifera* tincture in 62% v/v ethanol (Batch Number 03948, expiry date 04/2004) was produced by Parceval (Pty) Ltd., according to the German Homoeopathic Pharmacopoeia Method 3a (British Homoeopathic Association, 1985:17-18), using the roots of *W. somnifera*. According to Parceval (Feiter, 2003), The German Homoeopathic Pharmacopoeia is recognised as an international standard in the manufacture of plant extracts and dilutions, and they manufacture all their tinctures according to these standards. The German Homoeopathic Pharmacopoeia outlines the manufacture of homoeopathic products, however, the manufacturing method of a homoeopathic mother tincture is the same as for a herbal mother tincture. At 62% v/v ethanol a broad

spectrum of ingredients are extracted, both lipophilic and hydrophilic. This ensures a wide range of plant constituents to be extracted in the tincture.

#### 3.1.3.1.1 PREPARATION OF *W. SOMNIFERA* 1X

*W. somnifera* 1X in 62% v/v ethanol was prepared at the Durban Institute of Technology Homoeopathic Laboratory according to the German Homoeopathic Pharmacopoeia Method 3a (British Homoeopathic Association, 1985:18).

#### 3.1.3.1.2 PREPARATION OF *W. SOMNIFERA* 6X

*W. somnifera* 6X in 62% v/v ethanol was prepared at the Durban Institute of Technology Homoeopathic Laboratory according to the German Homoeopathic Pharmacopoeia Method 3a (British Homoeopathic Association, 1985:18).

#### 3.1.4 PREPARATION OF THE CONTROLS

##### 3.1.4.1 PREPARATION OF 62% V/V ETHANOL

Ethanol 99% (batch number 318/0/68) was purchased from Illovo Sugar Ltd. Parceval (Pty) Ltd. produces their tinctures using Illovo Sugar Ltd. ethanol. The percentage ethanol required, 62% v/v, was produced using distilled water and ethanol 99% according to the following formula:

$$\frac{\text{Volume of alcohol required} \times \text{percentage of alcohol required}}{\text{Percentage of source alcohol}} = \text{amount of 99\% required in producing 62\% alcohol.}$$

To produce 500ml of 62% ethanol:

$$\frac{500\text{ml of 62\%} \times 62\%}{99\%} = 313,13\text{ml of 99\% ethanol}$$

186,87ml distilled water was added to 313,13ml 99% ethanol to make up 500ml 62% ethanol.

#### 3.1.4.2 PREPARATION OF VANCOMYCIN AND GENTAMYCIN DISCS

Vancomycin (Batch number 261401, expiry 2005/01) and Gentamycin antibiotic discs (Batch number 263551, expiry 2005/02) were prepared by Oxoid limited. These discs served as a standard between the plates, ensuring results were relative to each other by the application of ratios.

#### 3.1.5 PREPARATION OF THE DISCS

- Round discs of 5mm diameter were punched from Whatman<sup>®</sup> No. 4 filter paper.
- Discs were placed into a Schott bottle and autoclaved at 121°C for 15 minutes, to ensure sterility.
- Using sterile forceps, discs were placed in sterile petri dishes, which were labelled for each test substance.
- Using a sterile micropipette 15ul of the relevant substance was pipetted onto each disc.

- Petri dishes were incubated at 37°C for 1 hour to dry the discs.
- The above two steps were repeated twice using 10ul and 5ul respectively.
- Discs were left overnight at room temperature to complete the drying process.

### 3.1.6 PREPARATION OF THE PLATES

- Each plate was labelled with the letter corresponding to the test culture:

A = *Escherichia coli*

B = *Staphylococcus aureus*

C = *Pseudomonas aeruginosa*

D = *Bacillus cereus*

E = *Klebsiella pneumonia*

- And with numbers corresponding to the test discs:

1 = *W. somnifera* tincture 62% v/v ethanol

2 = *W. somnifera* 1X 62% v/v ethanol

3 = *W. somnifera* 6X 62% v/v ethanol

4 = 62% v/v ethanol

5 = Gentamycin

6 = Vancomycin

- Plates were inoculated with 2 loopfuls of bacterial culture, using a 4mm sterile loop. Plates were inoculated in groups of five.
- A sterile glass spreader was used to spread the organisms evenly over the agar.

- The discs were placed, using sterile forceps, equidistant around the periphery of the plate according to the appropriate numbering.
- The above three steps were carried out 4 times for each test bacteria, resulting in a total of 20 plates per test bacteria.

### 3.1.7 INCUBATION OF THE PLATES

Plates were incubated at 37°C.

### 3.1.8 RECORDING OF THE RESULTS

Plates were examined at 18, 24 and 48 hours for the presence of growth inhibition, which was indicated by a clear zone surrounding each disc. The susceptibility of the test bacteria towards each test or control substance was determined by the size of this zone. The zone diameters were measured in millimetres using a ruler. The measurements were performed in triplicate. The average inhibition zone diameter was calculated and recorded on a table (see Appendix A-E).

## 3.2 DATA ANALYSIS

### 3.2.1 SAMPLE SIZE OF THE STUDY

Twenty plates were prepared for each bacteria. The sample size of the study was 15, allowing for an error margin of 5 plates per bacteria, which means each

test yielded 15 data sets. The efficacy of each test and control substance was tested against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus* 15 times each to make the study statistically viable.

### 3.2.2 STATISTICAL PROCEDURES

#### 3.2.2.1 Intra-group comparison of *W. somnifera* tincture in 62% v/v ethanol with regard to the observations at 18, 24 and 48 hours

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

##### (i) Hypothesis testing

The null hypothesis  $H_0$ , states that at 18, 24 and 48 hour observations there would be no change in diameter of the zone of inhibition at the  $\alpha = 0.05$  level of significance. The alternative hypothesis  $H_1$ , states that there would be a change in the diameter of the zone of inhibition at the same observation intervals and significance level.

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

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



(ii) Decision rule

At  $\alpha = 0.05$  level of significance,  $H_0$  is rejected if  $P < \alpha$ , where  $P$  is the observed significance level or probability value. If  $P > \alpha$ ,  $H_0$  is accepted at the same level of significance.

Reject  $H_0$  if  $P < \alpha$ .

Accept  $H_0$  if  $P \geq \alpha$ .

$P$  is the observed significance level or probability value.

If the null hypothesis  $H_0$  is rejected for Friedman's T test, then the Dunn Procedure will be used to determine which of the times are significantly different. Let  $R_j$  and  $R_j'$  be the  $j^{th}$  and  $j'^{th}$  time rank totals.

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

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

In the above formula:

$b$  = the number of blocks;

$k$  = the number of times;

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

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

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

(Daniel, 1999:702.)

3.2.2.2 Intra-group comparison of *W. somnifera* 1X in 62% v/v ethanol with regard to the observations at 18, 24 and 48 hours

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

(i) Hypothesis testing

As per 3.2.2.1.

(ii) Decision rule

As per 3.2.2.1.

3.2.2.3 Intra-group comparison of *W. somnifera* 6X in 62% v/v ethanol with regard to the observations at 18, 24 and 48 hours

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

(i) Hypothesis testing

As per 3.2.2.1.

(ii) Decision rule

As per 3.2.2.1.

#### 3.2.2.4 Intra-group comparison of 62% v/v ethanol with regard to the observations at 18, 24 and 48 hours

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

##### (i) Hypothesis testing

As per 3.2.2.1.

##### (ii) Decision rule

As per 3.2.2.1.

#### 3.2.2.5 Inter-group comparison between *W. somnifera* tincture in 62% v/v ethanol and 62% v/v ethanol with regard to observations at 18, 24 and 48 hours

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

##### (i) Hypothesis testing

The null hypothesis  $H_0$ , states that there would be no difference between the zonal inhibition values, with respect to the two test substances, at the  $\alpha = 0.05$  level of significance. The alternative hypothesis  $H_1$ , states that there would be a difference at the same level of significance.

$$H_0 : M_1 = M_2.$$

$$H_1 : M_1 \neq M_2.$$

(ii) Decision rule

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

Reject  $H_0$  if  $P < \alpha$ .

Accept  $H_0$  if  $P \geq \alpha$ .

$P$  is the observed significance level or probability value.

(Daniel, 1999:678-680.)

3.2.2.6 Inter-group comparison between *Withania somnifera* 1X in 62% v/v ethanol and 62% v/v ethanol with regard to observations 18, 24 and 48 hours

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

(i) Hypothesis testing

As per 3.2.2.5.

(ii) Decision rule

As per 3.2.2.5.

3.2.2.7 Inter-group comparison between *Withania somnifera* 6X in 62% v/v ethanol and 62% v/v ethanol with regard to observations at 18, 24 and 48 hours

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

(i) Hypothesis testing

As per 3.2.2.5.

(ii) Decision rule

As per 3.2.2.5.

3.2.2.8 Inter-group comparison between *Withania somnifera* tincture in 62% v/v ethanol and *Withania somnifera* 1X 62% v/v ethanol with regard to observations at 18, 24 and 48 hours

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

(i) Hypothesis testing

As per 3.2.2.5.

(ii) Decision rule

As per 3.2.2.5.

3.2.2.9 Inter-group comparison between *Withania somnifera* tincture in 62% v/v ethanol and *Withania somnifera* 6X 62% v/v ethanol with regard to observations at 18, 24 and 48 hours

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

(i) Hypothesis testing

As per 3.2.2.5.

(ii) Decision rule

As per 3.2.2.5.

3.2.2.10 Inter-group comparison between *Withania somnifera* 1X in 62% v/v ethanol and *Withania somnifera* 6X 62% v/v ethanol with regard to observations at 18, 24 and 48 hours

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

(i) Hypothesis testing

As per 3.2.2.5.

(ii) Decision rule

As per 3.2.2.5.

3.2.2.11 Inter-group comparison between *Withania somnifera* tincture, *Withania somnifera* 1X and *Withania somnifera* 6X all in 62% v/v ethanol with regard to observations at 18, 24 and 48 hours

The Kruskal-Wallis non-parametric Analysis of Variance by Ranks method was used to compare the diameters of the zones of inhibition of the herbs, to each other, with regard to observations at 18 hours, 24 hours and 48 hours.

(i) Hypothesis testing

In each test, the null hypothesis ( $H_0$ ) states that there would be no difference in diameter among the means of the herbs being compared to each other. The alternative hypothesis ( $H_0$ ) states that there would be a difference among the means.

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

$$H_1 : \mu_1 \neq \mu_2 \neq \mu_3$$

(ii) Decision rule

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

Reject  $H_0$  if  $P < \alpha$ .

Accept  $H_0$  if  $P \geq \alpha$ .

$P$  is the observed significance level or probability value.

The Dunn Procedure for use with the Kruskal-Wallis test:

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

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

Let  $\alpha$  be the experiment wise error rate. The values of  $\alpha$  are usually 0.15, 0.20, or 0.25, depending on the value of  $k$  (as  $k$  increases,  $\alpha$  increases).

If  $|R_i - R_j| > Z_{(1-\alpha/k(k-1))} \sqrt{N(N+1)/12} (\frac{1}{n_i} + \frac{1}{n_j})$ , then the difference  $|R_i - R_j|$  is declared significant at the  $\alpha$  level.

In the above formula:

$k$  = the number of samples;

$n$  = the number of observations in all samples combined;

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

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

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

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

(Fisher and Van Belle, 1993:430).

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

$$|R_i - R_j| \leq z \sqrt{k[N(N^2-1) - (\sum t^3 - \sum t)] / 6N(N-1)}$$

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

### 3.2.3 STATISTICAL PACKAGE

The statistical package for Social Sciences (SPSS) version 9 was used for data entry and analysis.



## CHAPTER FOUR

### RESULTS

#### 4.1 THE DATA

The research involved two types of data: primary and secondary. The nature of the data was as follows:

##### 4.1.1 THE PRIMARY DATA

- Results of the experiment determining the antimicrobial effects of *W. somnifera* tincture in 62% v/v ethanol on *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli*.
- Results of the experiment determining the antimicrobial effects of *W. somnifera* 1X in 62% v/v ethanol on *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli*.
- Results of the experiment determining the antimicrobial effects of *W. somnifera* 6X in 62% v/v ethanol on *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli*.
- Results of the experiment determining the antimicrobial effects of 62% v/v ethanol on *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli*.

##### 4.1.2 THE SECONDARY DATA

Research articles from journal publications, books and manuals.

## 4.2 CRITERIA GOVERNING THE ADMISSIBILITY OF DATA

Only data obtained from laboratory experiments carried out by the researcher at the Department of Biotechnology, Durban Institute of Technology was used.

## 4.3 INTRA-GROUP COMPARISONS AT 18, 24 AND 48 HOUR OBSERVATION INTERVALS

### 4.3.1 *Withania somnifera* tincture

#### 4.3.1.1 *Bacillus cereus*

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* tincture with regard to *Bacillus cereus* at 18, 24 and 48 hours (see Tables 4.1 and 4.2).

Table 4.1 Descriptive statistics for *W. somnifera* tincture versus *Bacillus cereus*.

TIME	N	MEAN	STD. DEVIATION	MINIMUM	MAXIMUM
18 hours	15	0	0	0	0
24 hours	15	0	0	0	0
48 hours	15	0	0	0	0

Table 4.2 Friedman's test statistics for *W. somnifera* tincture versus *Bacillus cereus*.

N	15
Chi-Square	0
df	2
Asymp. Sig.	1

CONCLUSION:  $P = 1$ , therefore  $P > \alpha$ . Therefore the null hypothesis was accepted.

#### 4.3.1.2 *Pseudomonas aeruginosa*

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* tincture with regard to *Pseudomonas aeruginosa* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.1.1.

#### 4.3.1.3 *Klebsiella pneumonia*

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* tincture with regard to *Klebsiella pneumonia* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.1.1.

#### 4.3.1.4 *Escherichia coli*

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* tincture with regard to *Escherichia coli* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.1.1.

#### 4.3.1.5 Staphylococcus aureus

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* tincture with regard to *Staphylococcus aureus* at 18, 24 and 48 hours (see Tables 4.3 and 4.4).

Table 4.3 Descriptive statistics for *W. somnifera* tincture versus *Staphylococcus aureus*.

TIME	N	MEAN	STD. DEVIATION	MINIMUM	MAXIMUM
18 hours	15	1,3333	2,7946	0	8
24 hours	15	1,3333	2,7946	0	8
48 hours	15	1,3333	2,7946	0	8

Table 4.4 Friedman's test statistics for *W. somnifera* tincture versus *Staphylococcus aureus*.

N	15
Chi-Square	1.000
df	2
Asymp. Sig.	1

CONCLUSION:  $P = 1$ , therefore  $P > \alpha$ . Therefore the null hypothesis was accepted.

#### 4.3.2 W. somnifera 1X

##### 4.3.2.1 Bacillus cereus

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* 1X with regard to *Bacillus cereus* at 18, 24 and 48 hours (see Tables 4.5 and 4.6).

Table 4.5 Descriptive statistics for *W. somnifera* 1X versus *Bacillus cereus*.

TIME	N	MEAN	STD. DEVIATION	MINIMUM	MAXIMUM
18 hours	15	0	0	0	0
24 hours	15	0	0	0	0
48 hours	15	0	0	0	0

Table 4.6 Friedman's test statistics for *W. somnifera* 1X versus *Bacillus cereus*.

N	15
Chi-Square	0
df	2
Asymp. Sig.	1

CONCLUSION:  $P = 1$ , therefore  $P > \alpha$ . Therefore the null hypothesis was accepted.

#### 4.3.2.2 *Pseudomonas aeruginosa*

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* 1X with regard to *Pseudomonas aeruginosa* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.2.1.

#### 4.3.2.3 *Klebsiella pneumonia*

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* 1X with regard to *Klebsiella pneumonia* at 18, 24 and

48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.2.1.

#### 4.3.2.4 Escherichia coli

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* 1X with regard to *Escherichia coli* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.2.1.

#### 4.3.2.5 Staphylococcus aureus

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* 1X with regard to *Staphylococcus aureus* at 18, 24 and 48 hours (see Tables 4.7 and 4.8).

Table 4.7 Descriptive statistics for *W. somnifera* 1X versus *Staphylococcus aureus*.

TIME	N	MEAN	STD. DEVIATION	MINIMUM	MAXIMUM
18 hours	15	0.9333	2.4631	0	7
24 hours	15	0.9333	2.4631	0	7
48 hours	15	0.9333	2.4631	0	7

Table 4.8 Friedman's test statistics for *W. somnifera* 1X versus *Staphylococcus aureus*.

N	15
Chi-Square	1.000
df	2
Asymp. Sig.	1

CONCLUSION:  $P = 1$ , therefore  $P > \alpha$ . Therefore the null hypothesis was accepted.

#### 4.3.3 W. somnifera 6X

##### 4.3.3.1 Bacillus cereus

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* 6X, with regard to *Bacillus cereus* at 18, 24 and 48 hours (see Tables 4.9 and 4.10).

Table 4.9 Descriptive statistics for *W. somnifera* 6X versus *Bacillus cereus*.

TIME	N	MEAN	STD. DEVIATION	MINIMUM	MAXIMUM
18 hours	15	0	0	0	0
24 hours	15	0	0	0	0
48 hours	15	0	0	0	0

Table 4.10 Friedman's test statistics for *W. somnifera* 6X versus *Bacillus cereus*.

N	15
Chi-Square	0
df	2
Asymp. Sig.	1

CONCLUSION:  $P = 1$ , therefore  $P > \alpha$ . Therefore the null hypothesis was accepted.

#### 4.3.3.2 Pseudomonas aeruginosa

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* 6X, with regard to *Pseudomonas aeruginosa* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.3.1.

#### 4.3.3.3 Klebsiella pneumonia

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* 6X, with regard to *Klebsiella pneumonia* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.3.1.

#### 4.3.3.4 Escherichia coli

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* 6X with regard to *Escherichia coli* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.3.1.

#### 4.3.3.5 Staphylococcus aureus

The Friedman's test was used to compare the diameters of the zones of inhibition of *W. somnifera* 6X with regard to *Staphylococcus aureus* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.3.1.



#### 4.3.4 Ethanol 62% v/v only

##### 4.3.4.1 *Bacillus cereus*

The Friedman's test was used to compare the diameters of the zones of inhibition of ethanol 62% v/v with regard to *Bacillus cereus* at 18, 24 and 48 hours (see Tables 4.11 and 4.12).

Table 4.11 Descriptive statistics for ethanol 62% v/v versus *Bacillus cereus*.

TIME	N	MEAN	STD. DEVIATION	MINIMUM	MAXIMUM
18 hours	15	0	0	0	0
24 hours	15	0	0	0	0
48 hours	15	0	0	0	0

Table 4.12 Friedman's test statistics for ethanol 62% v/v versus *Bacillus cereus*.

N	15
Chi-Square	0
df	2
Asymp. Sig.	1

CONCLUSION:  $P = 1$ , therefore  $P > \alpha$ . Therefore the null hypothesis was accepted.

##### 4.3.4.2 *Pseudomonas aeruginosa*

The Friedman's test was used to compare the diameters of the zones of inhibition of ethanol 62% v/v with regard to *Pseudomonas aeruginosa* at 18, 24

and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.4.1.

#### 4.3.4.3 Klebsiella pneumonia

The Friedman's test was used to compare the diameters of the zones of inhibition of ethanol 62% v/v with regard to *Klebsiella pneumonia* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.4.1.

#### 4.3.4.4 Escherichia coli

The Friedman's test was used to compare the diameters of the zones of inhibition of ethanol 62% v/v with regard to *Escherichia coli* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.4.1.

#### 4.3.4.5 Staphylococcus aureus

The Friedman's test was used to compare the diameters of the zones of inhibition of ethanol 62% v/v with regard to *Staphylococcus aureus* at 18, 24 and 48 hours. No zone of inhibition occurred. Therefore, results and conclusion as per 4.3.4.1.

#### 4.4 INTER-GROUP COMPARISONS AT 18, 24 AND 48 HOURS

##### 4.4.1 *W. somnifera* tincture versus 62% v/v ethanol only

###### 4.4.1.1 *Bacillus cereus*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture to 62%v/v ethanol (see Table 4.13).

Table 4.13 Inter-group comparison between *W. somnifera* tincture and 62% v/v ethanol with regard to *Bacillus cereus*.

TIME	P-VALUE
18 hours	1
24 hours	1
48 hours	1

CONCLUSION: At 18, 24 and 48 hours  $P = 1$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of inhibition of *W. somnifera* tincture and 62% v/v ethanol at a 0,05 level of significance.

###### 4.4.1.2 *Pseudomonas aeruginosa*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture to 62% v/v ethanol with regard to *Pseudomonas aeruginosa*.

Results and conclusion as per 4.4.1.1.

#### 4.4.1.3 Klebsiella pneumonia

The Mann-Whitney-U test was used to compare *W. somnifera* tincture to 62% v/v ethanol with regard to *Klebsiella pneumonia*.

Results and conclusion as per 4.4.1.1.

#### 4.4.1.4 Escherichia coli

The Mann-Whitney-U test was used to compare *W. somnifera* tincture to 62% v/v ethanol with regard to *Escherichia coli*.

Results and conclusion as per 4.4.1.1.

#### 4.4.1.5 Staphylococcus aureus

The Mann-Whitney-U test was used to compare *W. somnifera* tincture to 62% v/v ethanol with regard to *Staphylococcus aureus* (see Table 4.14).

Table 4.14 Inter-group comparison between *W. somnifera* tincture and 62% v/v ethanol with regard to *Staphylococcus aureus*.

TIME	P-VALUE
18 hours	0.073
24 hours	0.073
48 hours	0.073

CONCLUSION: At 18, 24 and 48 hours  $P = 0,073$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of inhibition of *W. somnifera* tincture and 62% v/v ethanol, with regard to *Staphylococcus aureus* at a 0,05 level of significance.

#### 4.4.2 W. somnifera 1X versus 62% v/v ethanol only

##### 4.4.2.1 Bacillus cereus

The Mann-Whitney-U test was used to compare *W. somnifera* 1X to 62% v/v ethanol (see Table 4.15).

Table 4.15 Inter-group comparison between *W. somnifera* 1X and 62% v/v ethanol with regard to *Bacillus cereus*.

TIME	P-VALUE
18 hours	1
24 hours	1
48 hours	1

CONCLUSION: At 18, 24 and 48 hours  $P = 1$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of inhibition of *W. somnifera* 1X and 62% v/v ethanol, with regard to *Bacillus cereus* at a 0,05 level of significance.

##### 4.4.2.2 Pseudomonas aeruginosa

The Mann-Whitney-U test was used to compare *W. somnifera* 1X to 62% v/v ethanol with regard to *Pseudomonas aeruginosa*.

Results and conclusion as per 4.4.2.1.

##### 4.4.2.3 Klebsiella pneumonia

The Mann-Whitney-U test was used to compare *W. somnifera* 1X to 62% v/v ethanol with regard to *Klebsiella pneumonia*.

Results and conclusion as per 4.4.2.1.

#### 4.4.2.4 Escherichia coli

The Mann-Whitney-U test was used to compare *W. somnifera* 1X to 62% v/v ethanol with regard to *Escherichia coli*.

Results and conclusion as per 4.4.2.1.

#### 4.4.2.5 Staphylococcus aureus

The Mann-Whitney-U test was used to compare *W. somnifera* 1X to 62% v/v ethanol with regard to *Staphylococcus aureus* (see Table 4.16).

Table 4.16 Inter-group comparison between *W. somnifera* 1X and 62% v/v ethanol with regard to *Staphylococcus aureus*.

TIME	P-VALUE
18 hours	0.15
24 hours	0.15
48 hours	0.15

CONCLUSION: At 18, 24 and 48 hours  $P = 0,073$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of inhibition of *W. somnifera* 1X and 62% v/v ethanol, with regard to *Staphylococcus aureus* at a 0,05 level of significance.

#### 4.4.3 W. somnifera 6X versus 62% v/v ethanol only

##### 4.4.3.1 Bacillus cereus

The Mann-Whitney-U test was used to compare *W. somnifera* 6X to 62% v/v ethanol (see Table 4.17).

Table 4.17 Inter-group comparison between *W. somnifera* 6X and 62% v/v ethanol with regard to *Bacillus cereus*.

TIME	P-VALUE
18 hours	1
24 hours	1
48 hours	1

CONCLUSION: At 18, 24 and 48 hours  $P = 1$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of inhibition of *W. somnifera* 6X and 62% v/v ethanol, with regard to *Bacillus cereus* at a 0,05 level of significance.

##### 4.4.3.2 Pseudomonas aeruginosa

The Mann-Whitney-U test was used to compare *W. somnifera* 6X to 62% v/v ethanol with regard to *Pseudomonas aeruginosa*.

Results and conclusion as per 4.4.3.1.

##### 4.4.3.3 Klebsiella pneumonia

The Mann-Whitney-U test was used to compare *W. somnifera* 6X to 62% v/v ethanol with regard to *Klebsiella pneumonia*.

Results and conclusion as per 4.4.3.1.

#### 4.4.3.4 Escherichia coli

The Mann-Whitney-U test was used to compare *W. somnifera* 6X to 62% v/v ethanol with regard to *Escherichia coli*.

Results and conclusion as per 4.4.3.1.

#### 4.4.3.5 Staphylococcus aureus

The Mann-Whitney-U test was used to compare *W. somnifera* 6X to 62% v/v ethanol with regard to *Staphylococcus aureus*.

Results and conclusion as per 4.4.3.1.

#### 4.4.4 *W. somnifera* tincture versus *W. somnifera* 1X

##### 4.4.4.1 Bacillus cereus

The Mann-Whitney-U test was used to compare *W. somnifera* tincture and *W. somnifera* 1X with regard to *Bacillus cereus* (see Table 4.18).

Table 4.18 Inter-group comparison between *W. somnifera* tincture and *W. somnifera* 1X with regard to *Bacillus cereus*.

TIME	P-VALUE
18 hours	1
24 hours	1
36 hours	1

CONCLUSION: At 18, 24 and 48 hours  $P = 1$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of



inhibition of *W. somnifera* tincture and *W. somnifera* 1X, with regard to *Bacillus cereus* at a 0,05 level of significance.

#### 4.4.4.2 *Pseudomonas aeruginosa*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture and *W. somnifera* 1X with regard to *Pseudomonas aeruginosa*.

Results and conclusion as per 4.4.4.1.

#### 4.4.4.3 *Klebsiella pneumonia*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture and *W. somnifera* 1X with regard to *Klebsiella pneumonia*.

Results and conclusion as per 4.4.4.1.

#### 4.4.4.4 *Escherichia coli*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture and *W. somnifera* 1X with regard to *Escherichia coli*.

Results and conclusion as per 4.4.4.1.

#### 4.4.4.5 *Staphylococcus aureus*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture and *W. somnifera* 1X with regard to *Staphylococcus aureus* (see Table 4.19).

Table 4.19 Inter-group comparison between *W. somnifera* tincture and *W. somnifera* 1X with regard to *Staphylococcus aureus*.

TIME	P-VALUE
18 hours	0.678
24 hours	0.678
48 hours	0.678

CONCLUSION: At 18, 24 and 48 hours  $P = 0,678$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of inhibition of *W. somnifera* tincture and *W. somnifera* 1X, with regard to *Staphylococcus aureus* at a 0,05 level of significance.

#### 4.4.5 *W. somnifera* tincture versus *W. somnifera* 6X

##### 4.4.5.1 *Bacillus cereus*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture and *W. somnifera* 6X with regard to *Bacillus cereus* (see Table 4.19).

Table 4.20 Inter-group comparison between *W. somnifera* tincture and *W. somnifera* 6X with regard to *Bacillus cereus*.

TIME	P-VALUE
18 hours	1
24 hours	1
36 hours	1

CONCLUSION: At 18, 24 and 48 hours  $P = 1$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of

inhibition of *W. somnifera* tincture and *W. somnifera* 6X, with regard to *Bacillus cereus* at a 0,05 level of significance.

#### 4.4.5.2 *Pseudomonas aeruginosa*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture and *W. somnifera* 6X with regard to *Pseudomonas aeruginosa*.

Results and conclusion as per 4.4.5.1.

#### 4.4.5.3 *Klebsiella pneumonia*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture and *W. somnifera* 6X with regard to *Klebsiella pneumonia*.

Results and conclusion as per 4.4.5.1.

#### 4.4.5.4 *Escherichia coli*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture and *W. somnifera* 6X with regard to *Escherichia coli*.

Results and conclusion as per 4.4.5.1.

#### 4.4.5.5 *Staphylococcus aureus*

The Mann-Whitney-U test was used to compare *W. somnifera* tincture and *W. somnifera* 6X with regard to *Staphylococcus aureus* (see Table 4.20).

Table 4.21 Inter-group comparison between *W. somnifera* tincture and *W. somnifera* 6X with regard to *Staphylococcus aureus*.

TIME	P-VALUE
18 hours	0.073
24 hours	0.073
48 hours	0.073

CONCLUSION: At 18, 24 and 48 hours  $P = 0.073$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of inhibition of *W. somnifera* 6X and *W. somnifera* tincture, with regard to *Staphylococcus aureus* at a 0,05 level of significance.

#### 4.4.6 *W. somnifera* 1X versus *W. somnifera* 6X

##### 4.4.6.1 *Bacillus cereus*

The Mann-Whitney-U test was used to compare *W. somnifera* 1X and *W. somnifera* 6X with regard to *Bacillus cereus* (see table 4.21).

Table 4.22 Inter-group comparison between *W. somnifera* 1X and *W. somnifera* 6X with regard to *Bacillus cereus*.

TIME	P-VALUE
18 hours	1
24 hours	1
48 hours	1

CONCLUSION: At 18, 24 and 48 hours  $P = 1$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of

inhibition of *W. somnifera* 1X and *W. somnifera* 6X, with regard to *Bacillus cereus* at a 0,05 level of significance.

#### 4.4.6.2 *Pseudomonas aeruginosa*

The Mann-Whitney-U test was used to compare *W. somnifera* 1X and *W. somnifera* 6X with regard to *Pseudomonas aeruginosa*.

Results and conclusion as per 4.4.6.1.

#### 4.4.6.3 *Klebsiella pneumonia*

The Mann-Whitney-U test was used to compare *W. somnifera* 1X and *W. somnifera* 6X with regard to *Klebsiella pneumonia*.

Results and conclusion as per 4.4.6.1.

#### 4.4.6.4 *Escherichia coli*

The Mann-Whitney-U test was used to compare *W. somnifera* 1X and *W. somnifera* 6X with regard to *Escherichia coli*.

Results and conclusion as per 4.4.6.1.

#### 4.4.6.5 *Staphylococcus aureus*

The Mann-Whitney-U test was used to compare *W. somnifera* 1X and *W. somnifera* 6X with regard to *Staphylococcus aureus*.

Results and conclusion as per 4.4.6.1.

#### 4.4.7 W. somnifera tincture versus W. somnifera 1X versus W. somnifera 6X

##### 4.4.7.1 Bacillus cereus

The Kruskal-Wallis test was used to compare the three test substances to each other (see Table 4.22).

Table 4.23 Inter-group comparison between *W. somnifera* tincture, 1X and 6X with regard to *Bacillus cereus*.

TIME	P-VALUE
18 hours	1
24 hours	1
48 hours	1

CONCLUSION: At 18, 24 and 48 hours  $P = 1$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of inhibition of *W. somnifera* tincture, *W. somnifera* 1X and *W. somnifera* 6X, with regard to *Bacillus cereus* at a 0,05 level of significance.

##### 4.4.7.2 Pseudomonas aeruginosa

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

Results and conclusion as per 4.4.7.1.

##### 4.4.7.3 Klebsiella pneumonia

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

Results and conclusion as per 4.4.7.1.

#### 4.4.7.4 Escherichia coli

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

Results and conclusion as per 4.4.7.1.

#### 4.4.7.5 Staphylococcus aureus

The Kruskal-Wallis test was used to compare the three test substances to each other (see Table 4.23).

Table 4.24 Inter-group comparison between *W. somnifera* tincture, 1X and 6X with regard to *Staphylococcus aureus*.

TIME	P-VALUE
18 hours	0.222
24 hours	0.222
48 hours	0.222

CONCLUSION: At 18, 24 and 48 hours  $P = 0.222$ , therefore  $P \geq \alpha$ . The null hypothesis was accepted. There was no difference in diameters of the zones of inhibition of *W. somnifera* tincture, *W. somnifera* 1X and *W. somnifera* 6X, with regard to *Staphylococcus aureus* at a 0,05 level of significance.

## CHAPTER 5

### DISCUSSION

The only tests which produced any results were *W. somnifera* in tincture ( $P = 0,073$ ) and *W. somnifera* in 1X dilution ( $P = 0,150$ ) against *Staphylococcus aureus*. However, these results were not significant.

The results of this study do not correlate with literature supporting its antibacterial nature (Hutchings, 1996:273; Iwu, 1993:260; Gericke, Van Oudtshoorn and Van Wyk, 2000:274).

One of the reasons for this difference could be that in traditional clinical practice it is used in its crude form as poultices which are applied externally to treat open cuts, wounds and abscesses (Gericke, Van Oudtshoorn and Van Wyk, 2000:274) and as a general antiseptic for wound dressing (Iwu, 1993:259). This supports the need for a clinical *in vitro* trial with the application of the plant as it is used in traditional medicine.

A possible reason for lack of results could be that *W. somnifera* acts in combating bacteria via bringing about a response in an organism and not by direct effect on bacteria. Immuno-stimulatory actions, for instance (see Iwu, 1993:260 and Ziauddin *et al.*, 1996:74) cannot be observed in an *in vitro* study of the type conducted here.



The preparation of the *W. somnifera* used in this study may not have been optimal for the following reasons:

- Plants are sensitive to local conditions and they do not always produce the same chemicals consistently (Prance, 1994:2);
- A preparation of the leaves may have demonstrated more antibacterial activity as both leaf and root exhibit antibiotic activity towards *Staphylococcus aureus* but the leaf is more active (Watt and Breyer-Brandwijk, 1962:1012; Mohan Das and Kurup, 1963:157);
- The use of ethanol as the extractant may not have been ideal, as acetone has been proven to be a more effective due to its volatility, miscibility with polar and non-polar solvents and its relatively low toxicity to possible test organisms (Eloff, 1998:1-8);
- The dilute nature of the substance to start with. In the GHP method employed, only 50% of the crude plant is represented. A method whereby a less diluted tincture is produced could be more effective in screening for antibiotic sensitivity of a plant. (Wat *et. al.*, 1980:297-290.)

Problems may have arisen in this research due to dissolution and diffusion of the test substances through the Mueller-Hinton Agar. The disc diffusion test may not adequately accommodate the dissolution and diffusion of hydrophobic substances present in the *W. somnifera* preparations. The root contains volatile oils and fatty acids both of which may contain antibiotic

properties and would therefore not be effective in this experimental design (Watt and Breyer-Brandwijk, 1962:1012). Lawless (1995:24) states that the volatile oils of a plant are more concentrated in an essential oil extract as compared to a tincture, infusion or decoction.

When it was evident by observation that the zones of inhibition were not significant, the researcher conducted a smaller test series (five plates of *Staphylococcus aureus*) using the wet disc method (where neutral discs are placed on the agar and then impregnated with the test substances), but the zones of inhibition were similar.

## CHAPTER SIX

### 6.1 CONCLUSION

The purpose of this study was to investigate the efficacy of *W. somnifera* in herbal tincture, 1X and 6X dilutions as an antibacterial agent against selected Gram-positive and Gram-negative bacteria, in terms of the disc diffusion test.

The results of the experiment showed that *W. somnifera* in herbal tincture, 1X and 6X dilutions was ineffective as an *in vitro* antimicrobial agent.

### 6.2 RECOMMENDATIONS

#### 6.2.1 PREPARATION OF THE TINCTURE

- (i) Plant identity must be verified before the tincture is made. Plants are often mistaken for a similar species when not examined correctly. Plant identity can be verified by a herbarium and a voucher specimen lodged there. (McGaw, Jager and Van Staden, 2000:248).
- (ii) Thin layer chromatography of the tincture plant to record the levels of plant constituents. These vary according to environmental conditions surrounding plant, which may affect its concentration of antibacterial substances.
- (iii) Harvesting and preparation of the plant prior to tincture manufacture should also be recorded to ensure the plant was handled in such a manner as to ensure maximum preservation of its constituents.

- (iv) A tincture of *W. somnifera* leaves instead of the roots, as the leaves have been more extensively used as a source of withaferine as compared to the roots. Withaferine has been shown to be antibiotic towards Gram-positive organisms (Hutchings, 1996:273).
- (v) A tincture of the whole plant, as all parts of the plant are used in clinical treatment.
- (vi) Acetone to be used as the extractant rather than ethanol due to its volatility, miscibility with polar and non-polar solvents and its relatively low toxicity to possible test organisms (Eloff, 1998:1-8).
- (vii) A preparation representing a higher percentage of the crude plant to be employed. For example, add 1g of crushed plant material to 5ml of ethanol and immerse the discs overnight. Air dry before placing on the inoculated plate. (Wat *et. al.*, 1980:297-290.)
- (viii) An essential oil extract of *W. somnifera* to provide a concentrated form of the volatile oils (Lawless, 1995:24).

#### 6.2.2 EXPERIMENTAL PROCEDURE

- (i) Store inoculated plates at a low temperature before incubation to allow the test substance to diffuse through the agar before the bacteria commence rapid growth at a warmer temperature. This increases the inhibition diameter. (Rios, Recio and Villar, 1988:128.)
- (ii) When inoculating discs, space them far apart in the petri dishes to ensure fluid does not coalesce between adjacent discs. The low viscosity of the 62%

v/v ethanol caused the fluid to form a large circle around each disc instead of a small bead on top of each disc.

(iii) Multiple impregnations of discs to ensure maximum absorption of each disc, for example 5 impregnations of 10ul each. Drying the discs in between each impregnation.

(iv) Re-pipetting of test substance onto wet discs to ensure multiple inoculations. This duplicates the clinical application of herbal treatments. Re-application also supports the usage of 1X and 6X dilutions as they are often prescribed more frequently than the higher homoeopathic dilutions. Re-application only applies to wet discs placed on the agar

### 6.2.3 EXPERIMENTAL DESIGN

(i) Use alternative diffusion methods:

- The hole or reservoir method whereby test substance is contained within a cylinder, which has been punched out of the agar.
- Dilution method whereby the test substance is homogenously dispersed in liquid media.

(ii) *In vivo* versus *in vitro*. Plant may show antibacterial properties due to altered functioning of the organism and not action of the herb on bacteria.

(iii) Clinical trials to be carried out.

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**APPENDIX A**  
**BACILLUS CEREUS**

ZONE OF INHIBITION (mm)																				
TEST 1					TEST 2				TEST 3				TEST 4				TEST 5			
18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	
TEST & CONTROL																				
1)W.somnifera tincture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2)W.somnifera 1X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3)W.somnifera 6X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4)Ethanol 62%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5)Gentamycin	25	25	25	24	24	24	24	24	24	24	25	25	25	25	24	24	24	24	24	
6)Vancomycin	16	16	16	15	15	15	14	14	14	14	15	15	15	14	14	14	14	14	14	
TEST 10																				
TEST 6					TEST 7				TEST 8				TEST 9				TEST 10			
18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	
TEST & CONTROL																				
1)W.somnifera tincture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2)W.somnifera 1X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3)W.somnifera 6X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4)Ethanol 62%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5)Gentamycin	25	25	25	25	25	25	26	26	26	26	25	25	25	25	24	24	24	24	24	
6)Vancomycin	16	16	16	16	16	16	16	16	16	16	16	15	15	15	15	15	15	15	15	
TEST 15																				
TEST 11					TEST 12				TEST 13				TEST 14				TEST 15			
18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	18hrs	24hrs	48hrs	
TEST & CONTROL																				
1)W.somnifera tincture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2)W.somnifera 1X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3)W.somnifera 6X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4)Ethanol 62%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5)Gentamycin	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	
6)Vancomycin	15	15	15	15	15	15	15	15	15	15	13	13	13	13	15	15	14	14	14	

**APPENDIX B**  
PSEUDOMONAS AERUGINOSA

		ZONE OF INHIBITION (mm)														
		TEST 1					TEST 2					TEST 3				
		18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs
TEST & CONTROL		18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs
1)W somnifera tincture		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2)W somnifera 1X		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3)W somnifera 6X		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4)Ethanol 62%		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5)Gentamycin		18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
6)Vancomycin		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TEST 6					TEST 7					TEST 8				
		18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs
TEST & CONTROL		18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs
1)W somnifera tincture		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2)W somnifera 1X		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3)W somnifera 6X		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4)Ethanol 62%		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5)Gentamycin		17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
6)Vancomycin		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TEST 11					TEST 12					TEST 13				
		18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs
TEST & CONTROL		18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs
1)W somnifera tincture		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2)W somnifera 1X		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3)W somnifera 6X		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4)Ethanol 62%		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5)Gentamycin		19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
6)Vancomycin		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

**APPENDIX C**  
**KLEBSIELLA PNEUMONIA**

ZONE OF INHIBITION (mm)																
	TEST 1				TEST 2				TEST 3				TEST 4			
	18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs	
TEST & CONTROL																
1)W. somnifera tincture	0	0	0		0	0	0		0	0	0		0	0	0	
2)W. somnifera 1X	0	0	0		0	0	0		0	0	0		0	0	0	
3)W. somnifera 6X	0	0	0		0	0	0		0	0	0		0	0	0	
4)Ethanol 62%	0	0	0		0	0	0		0	0	0		0	0	0	
5)Gentamycin	24	24	24		25	25	25		25	25	25		25	25	25	
6)Vancomycin	0	0	0		0	0	0		0	0	0		0	0	0	
	TEST 6				TEST 7				TEST 8				TEST 9			
	18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs	
TEST & CONTROL																
1)W. somnifera tincture	0	0	0		0	0	0		0	0	0		0	0	0	
2)W. somnifera 1X	0	0	0		0	0	0		0	0	0		0	0	0	
3)W. somnifera 6X	0	0	0		0	0	0		0	0	0		0	0	0	
4)Ethanol 62%	0	0	0		0	0	0		0	0	0		0	0	0	
5)Gentamycin	24	24	24		24	24	24		24	24	24		24	24	25	
6)Vancomycin	0	0	0		0	0	0		0	0	0		0	0	0	
	TEST 11				TEST 12				TEST 13				TEST 14			
	18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs	
TEST & CONTROL																
1)W. somnifera tincture	0	0	0		0	0	0		0	0	0		0	0	0	
2)W. somnifera 1X	0	0	0		0	0	0		0	0	0		0	0	0	
3)W. somnifera 6X	0	0	0		0	0	0		0	0	0		0	0	0	
4)Ethanol 62%	0	0	0		0	0	0		0	0	0		0	0	0	
5)Gentamycin	25	25	25		25	25	25		25	25	25		25	25	25	
6)Vancomycin	0	0	0		0	0	0		0	0	0		0	0	0	

**APPENDIX D**  
**ESCHERICHIA COLI**

ZONE OF INHIBITION (mm)																			
TEST 1				TEST 2				TEST 3				TEST 4				TEST 5			
18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs	18hrs	24hrs	48hrs		
TEST & CONTROL																			
1)W.somnifera tincture	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
2)W.somnifera 1X	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
3)W.somnifera 6X	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
4)Ethanol 62%	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
5)Gentamycin	21	21	21	22	22	23	23	22	23	23	23	22	22	22	21	21	21		
6)Vancomycin	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
TEST 6																			
TEST 7				TEST 8				TEST 9				TEST 10							
18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs	18hrs	24hrs	48hrs		
TEST & CONTROL																			
1)W.somnifera tincture	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
2)W.somnifera 1X	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
3)W.somnifera 6X	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
4)Ethanol 62%	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
5)Gentamycin	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22		
6)Vancomycin	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
TEST 11																			
TEST 12				TEST 13				TEST 14				TEST 15							
18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs		18hrs	24hrs	48hrs	18hrs	24hrs	48hrs		
TEST & CONTROL																			
1)W.somnifera tincture	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
2)W.somnifera 1X	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
3)W.somnifera 6X	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
4)Ethanol 62%	0		0	0	0	0		0	0	0		0	0	0	0	0	0		
5)Gentamycin	21	21	21	22	22	23	23	22	23	23	23	22	22	22	23	23	23		
6)Vancomycin	0		0	0	0	0		0	0	0		0	0	0	0	0	0		



**APPENDIX E**  
**STAPHYLOCOCCUS AUREUS**

ZONE OF INHIBITION (mm)																			
TEST 1					TEST 2					TEST 3					TEST 4				
TEST 1					TEST 2					TEST 3					TEST 4				
18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs
<b>TEST &amp; CONTROL</b>																			
1)W.somnifera tincture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6	6
2)W.somnifera 1X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	7	7
3)W.somnifera 6X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4)Ethanol 62%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5)Gentamycin	30	30	30	30	30	30	30	30	31	31	31	31	30	30	30	30	29	29	29
6)Vancomycin	20	20	20	21	21	21	21	21	20	20	20	20	20	20	20	20	20	20	20
<b>TEST 5</b>																			
TEST 6					TEST 7					TEST 8					TEST 9				
18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs
<b>TEST &amp; CONTROL</b>																			
1)W.somnifera tincture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2)W.somnifera 1X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3)W.somnifera 6X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4)Ethanol 62%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5)Gentamycin	29	29	29	29	29	29	29	29	30	30	30	30	32	32	32	32	29	29	29
6)Vancomycin	20	20	20	20	20	20	20	20	20	20	20	20	21	21	21	19	19	19	19
<b>TEST 10</b>																			
TEST 11					TEST 12					TEST 13					TEST 14				
18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs	18hrs	24hrs	48hrs	18hrs	24hrs
<b>TEST &amp; CONTROL</b>																			
1)W.somnifera tincture	0	0	0	0	6	6	6	8	8	8	8	8	8	8	0	0	0	0	0
2)W.somnifera 1X	0	0	0	0	7	7	7	7	7	7	7	7	7	7	0	0	0	0	0
3)W.somnifera 6X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4)Ethanol 62%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5)Gentamycin	33	33	33	33	28	28	28	28	29	29	29	29	30	30	30	30	30	30	30
6)Vancomycin	21	21	21	21	20	20	20	20	20	20	20	20	20	20	20	21	21	21	21