

SCREENING OF SOME BACTERICIDAL
HERBAL DRUGS IN SOUTH OF BAUCHI

BY

AYA, ANNE JUMMAI

DECEMBER, 1996

CH
A
1996

SCREENING OF SOME BACTERICIDAL HERBAL DRUGS IN
SOUTH OF BAUCHI STATE.

BY
AYA, ANNE JUMMAL.

A DISSERTATION SUBMITTED IN PARTIAL
FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF
THE DEGREE OF BACHELOR OF TECHNOLOGY (B. TECH)
APPLIED MICROBIOLOGY OF
ABUBAKAR TAFAWA BALEWA UNIVERSITY, BAUCHI.

* DECEMBER, 1996.

0334

ABUBAKAR TAFAWA BALEWA
UNIVERSITY LIBRARY
BAUCHI

CERTIFICATION.

This is to certify that AYA ANNE JUMMAI carried out this research in the Biological Science Programme of the School of Science and Science Education, of Abubakar Tafawa Balewa University Bauchi, Nigeria in partial fulfilment of the award of Bachelor of Technology (B. Tech) Applied Microbiology.

R. J. A. AUDU
(SUPERVISOR)

DR. W. A. ISTIFANUS
(Co-ordinator, Biological Science Programme)

EXTERNAL EXAMINER

ii

DEDICATION

To Baba, Momma, Bobai, Didi and Tisan.

ACKNOWLEDGEMENT

It has been a long journey but I thank God its over.

My sincere gratitude goes to all those who made this a reality.

Special thanks go to Dr. James Audu, Yinka Elutade, Mr. Kela, Mr. Dabri, Mallam Farouq, Momoh, Usman, Babayo, Zamani, Ahmed, Chindo and the ATBU honourable class of '96 for making the load a lot easier by rendering their unreserved assistance at one point or the other.

To Mallama and family, Aunty Aisha, Titi and Asabe, Zainab, Amina and Meg. Nasiru and Mohammed, Thank you for providing for me a home away from home.

The Dowyas, Yakubu and Ibrahim thanks for caring so much.

I pray God will continue to keep and Bless us all. Amen.

→ Please make sentences

TABLE OF CONTENTS

Certification	i
Dedication	ii
Acknowledgement	iii
Table of Contents	iv
List of Tables	v
List of Figure	vi
Abstract	vii

Chapter One

Introduction	1
--------------------	---

Chapter Two

Literature Review	3
-------------------------	---

Chapter Three

Materials and Methods	7
3.1 Collection of samples	7
3.2 Cultivation of Bacteria for Bioassay	8
3.3 Processing of Plant Methods	8
3.4 Anti microbial screening test	9
3.5 Determination of M.I.C	9
3.6 Determination of pH	10

Chapter Four

Results	11
-------------------	----

Chapter Five

Discussion	12
----------------------	----

Chapter Six

Conclusion	14
----------------------	----

Table 1	7
-------------------	---

Table 2	10
-------------------	----

Table 3	15
-------------------	----

Table 4	18
-------------------	----

Table 5	20
-------------------	----

Table 6	21
-------------------	----

Fig 1 and 2	22
-----------------------	----

Fig 3 and 4	23
-----------------------	----

Fig 5	24
-----------------	----

Fig 6	25
-----------------	----

Fig 7	26
-----------------	----

Fig 8	27
-----------------	----

Fig 9	28
-----------------	----

Fig 10	29
------------------	----

References	30
----------------------	----

ABSTRACT

Anogiesus leiocarpus, Guiera senegalensis and calotropis procera are medical herbs used in south of Bauchi State. These herbs were tested on some bacteria namely E.coli, P.aeruginosa, Klebsiella spp, S. aureus, B.cereus, and B. substills.

Anogiesus leiocarpus was found to be the most effective of the herbs used, in exhibited anti-bacterial activity against any of the organism tested.

Anogiesus leiocarpus inhibited bacterial growth where both cloxacillin and ampicillin (250mg) were ineffective.

Anogiesus leiocarpus leaves had a pH of 4.60 while the extract from the stem-bark had pH of 4.87 and a minimum inhibitory concentration of between 20% to 80%.

CHAPTER ONE

INTRODUCTION

The practise of herbal treatment has been known as far back as the history of man, not only in Africa, people in other parts of the world used medical herbs for the treatment of ailments before the advent of orthodox medicine.

In China, traditional treatment dates back to between 2730 and 3000 B. C, when Chaulmoogra oil which was obtained from species of Hydrocarpus was used in treating Leprosy cases.

Herbal treatment by traditional healers is still enjoying increased attendance due to the following reasons; (i) In accessibility to modern medicine, (ii) The exorbitant cost of modern drugs where available, (iii) Long term familiarity with traditional treatment and (iv) Insufficient or ill - trained orthodox medical personnel.

Many international organizations such as the World Health Organization (W. H. O) and the United Nations Educational, Scientific and Cultural Organization (U.N.E.S.C.O) to mention a few which are actively involved in the field of traditional medicine and medical plants have made resolutions on the subject for implementation by member states.

A great number of these resolutions recognize the fact that many herbal drugs used by traditional medical practioners for treatments, can be used for the manufacture of modern drugs, which could lead to an increase in the availability of drugs in hospitals and clinics. These International organizations have recommended that serious attention should be given to the study of plants of medicinal value, so as to determine their active components as well as their efficacy (Sofowora (1984).

Medicinal plants have been used by traditional healers in the South of Bauchi State for many years in treating different types of ailments but their active ingredients are yet to be determined and similarly, the toxic effects of many of them on man are not known. It has been reported that Lippia rugosa which is commonly used by traditional practitioners for treating hypertension may be toxic to man. When given in a high dose, it was observed to result in the swelling of the patient's big toe, after an hour of its administration, the toe remained swollen for three days. (Adu, 1989).

It is against this background that this study is being undertaken to; (i) Screen some herbal plants and evaluate their effects on some bacterial organisms, (ii) To determine whether or not the medicinal herbs used by traditional medical practitioners are effective for the treatment of other diseases as well, this makes detailed pharmacological and other academic studies possible.

(iii) To determine the approximate concentration at which the extracts of these herbal plants are bactericidal and (iv) To compare the potency of these plant extracts with standard antibiotics.

It is relevant to mention here that herbal practice should be allowed to complement orthodox medicine, as is already practised in some Asian countries like China and Indonesia. There should be no rivalry and no looking down on one by the other.

CHAPTER TWO

LITERATURE REVIEW

Herbal medicine is nature's gift to man for treating many different ailments. Since creation, man has been dependent on plants for food, drinks, shelter, clothing, equipment, dental care and medicine.

In some countries, until the discovery of bacteria and the advent of modern medicine, many communicable diseases were attributed to supernatural causes and regarded as evidence of the displeasure of ancestral gods and evil spirits or to black magic.

Many less sophisticated communities still hold to such beliefs - R.H. Bannerman (1979) is reported to have observed that this kind of belief is still to some extent held even by the industrialized countries. Some plants reported to have been used in such cases include, Ficus - Platyphylla (Gamji = Hausa), which is used for treating people who are disturbed by "evil spirits", the bark (stem) and roots are used, (Audu 1989). Tacazzea barkeria was reported as palliative in treating mental cases in Bauchi State (Audu 1993).

Although it is not known exactly when the first man practised herbalism in Africa, a number of theories have been brought forward by scholars and traditional medical practitioners alike in stating the origin of this practise. But, early man could have gained some knowledge by watching the effects of various plants on domestic animals when the plants were eaten by them.

Even today, herbalists try out new remedies on domestic animals especially when testing for toxicity, and on themselves or their relations. Such tests prove to the patient that the preparation is harmless and sometimes also confirm that the dosage prescribed is

justifiable. Such information on African medicinal and toxic plants has been passed orally from generation to generation and even today, ^{here} are many herbal cures which have not been written down.

For centuries, dropsy (Oedema) was effectively treated in Britain with a decoction consisting of a mixture of some twenty plants. It was not until 1785, however, that examination of the portin by William Withering resulted, in the discovery that the ony active ingredient was in the leaves of Digitalis Purpurea (Taylor, 1965), Le Strange, 1977). Two of the active constituents isolated from these leaves are Digoxin and Digitoxin, both of which are now official drugs in the British pharmacopoeia and other pharmacopoeia.

Quinine is obtained from the bark of Cinchona spp and is the most effective antimalarial till date, though synthetic versions have been developed in which the activity of quinine was retained but the toxicity reduced. Since it was discovered that the drug produces undesirable side effects such as impaired hearing on prolonged use (Trease and Evans,

1978). Reserpine from the roots of Rauwolfia spp has been shown to be responsible for its sedative properties. The roots of Rauwolfia SPP have been used as sedative on mentally disturbed patients by traditional medical practitioners in Nigeria and in many other Arican countries, for centuries. (Dalziel, 1956)

Reserpine also exhibits antihypertensive properties and is used in modern medical practise to treat certain cases of mild hypertension as well as cases of anxiety. (Watt and Breyer - Bradwijk, 1962, Kokwaro, 1976). The efficacy of herbs has been confirmed in different disease conditions all over the world. Herbs have succeeded where conventional or synthetic medicine has failed, especially in chronic diseases. Apart from

this, it is important to mention the little or no side effects of these herbs.

In the treatment of hypertension, for instance, the herb (e.g. Mistletoe) is used first, to lower the blood pressure, then to clean the arteries or the veins until they reach their maximum elastic point, suddenly burst and cause a vascular accident causing stroke and eventually death if proper care is not taken. A boiled decoction of Allium ascolonicum (Alubosa elewe = Yoruba) Ocimum basilicum (eferin were = Yoruba) and Kalanchoe crenato (Odundun = Yoruba) has also been reported to be more effective against migraine attacks than the orthodox drugs presently in use. (Kafaru, 1983).

Fransworth et al (1985) reported that at least one hundred and nineteen distinct chemical substances have been derived from plants and these compounds are as good as some of the orthodox drugs currently used in several countries of the world. It was also reported that out of these one hundred and nineteen compounds used as drugs derived from plants, eighty - eight were discovered as a result of studies to isolate the active antimicrobial chemical compounds.

Though there is a wide range of antibiotics currently available for the treatment of bacterial infections, there are still challenges to be faced in microbial chemotherapy, this is because the development of resistance to some chemotherapeutic agents is becoming an increasingly pressing problem. This brings about the need to search for more herbal drugs that may be used as antibiotics as advocated by the World Health Organization (W. H.O, 1978).

Some local herbs have been documented in literature to have both antibacterial and antifungal properties. (Gellerman and Schlenk, 1965) reported that Ancardium occidentale which has moderate bactericidal activity against Staphylococcus aureus and Bacillus subtilis

has also been found to be very effective against the bacterium Mycobacterium Smegmatis.

Allium sativum and Allium cepa, commonly known as garlic and onion respectively have been reported to have antibacterial and antifungal properties. They are said to be useful in the treatment of fungal infections of the skin as well as staphylococcal infections of the alimentary tract (Vohora et al, 1973). (George and Pondalia, 1949) demonstrated that aqueous extracts of the leaves and roots of Mormodica charantia have appreciable antibacterial activity against Escherichia coli and Staphylococcus aureus.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Samples

The leaves, stem - bark and roots of three different medicinal plants were collected from the South of Bauchi Local Government Area (Yelwa, Bayara, Birshin Fulani and the Junction of Liman Katagum and Dass).

These three medicinal plants had previously identified by Dalziel (1937) and their uses in traditional medicine were revealed to Kela (1988) and (Audu, 1989) by several herbalists in Bauchi state.

TABLE 1 : PLANTS SELECTED FOR SCREENING

PLANT	FAMILY	HAUSA NAME	PARTS TESTED	MEDICINAL USES	LOCALITY WHERE COLLECTED
<u>Anogeisus</u> <u>Leiocarpus</u>	Combret- aceae.	Marke	leaves and stem bark.	used in the treatment of abdominal pain, Jaundice, anaemia as an aphrodisiac.	Birshin - Fulani.
<u>Guiera</u> <u>Senega-</u> <u>lensis.</u>	Combret- aceae.	Sabara	Leaves and stem bark.	used in the treatment of abdominal discomfort and gastroentis.	The Junction of Liman Katagum and Dass.
<u>Calotropis</u> <u>Procera</u>	Asclepia- dacea.	Tumfafiya	Leaves, stem bark and roots.	used to stop vomit, dysentry and diarrhoea.	Bayara

3.2 ~~COLLECTION~~ CULTIVATION OF BACTERIA FOR BIOASSAY

Six different species of bacteria were collected from the Specialist Hospital Bauchi. The bacteria collected included three gram negative species namely; Escherichia coli, Pseudomonas aeruginosa, and Klebsiella spp and three gram positive species namely; Staphylococcus aureus, Bacillus cereus and Bacillus Subtilis. The bacteria were collected on sterile agar slants in Mc Cartney bottles and then a loopful of each of the bacteria isolates was transferred into Peptone water in Mc Cartney bottles and stored in the refrigerator at 40c until required.

These species of bacteria were chosen because they are indicator bacteria in most infections and can be easily cultivated in the laboratory.

3.3 PROCESSING OF PLANT MATERIAL

The plant parts collected were dried under shade before pounding to powder with a wooden mortar and pestle. A sieve (2mm, mesh) was then used to obtain fine powder. The crude extracts of the plant powder were made, adopting the methods of Akinyanju et al (1986) and Kela et al (1989).

Chloroform, Diethyl ether, cold water and hot water were used for the solvent extraction. The powdered samples were percolated with the solvents in the ratio of 1:5 weight per volume (w/v) and allowed to stand for 24 hours at ambient temperature as done by Ibrahim et al (1984).

The powdered samples percolated with diethyl ether were however allowed to stand at 4^oc in a refrigerator for 24 hours due to the volatile nature of the solvent. The plant extracts were then filtered with filter paper (Whatman No. 13) and the filtrates were concentrated using a rotary evaporator. ~~.....~~

3.4 ANTIMICROBIAL SCREENING TEST.

The bacterial suspensions cultured in Peptone water were removed from the refrigerator and 0.2 ml of each of the bacterial suspensions was then mixed with 10 ml of Nutrient agar in sterile petri dishes and allowed to solidify. Wells of 6mm in diameter were punched in the solidified agar media using a sterile stainless steel borer. Each well was then filled with 0.1 ml of the different plant extract.

The Petri dishes were then incubated at 37 oc for 24 hours. Each combination of bacteria strains and plant extract was carried out in duplicates. 8 wells were made in each plate of solidified agar, one well for each of the 7 plant extracts and the 8th well filled with the control liquid (chloroform, diethyl ether, hot water, cold water).

Two antibiotics (cloxacillin and ampicillin) were used for the treated control and the zones of inhibition were measured using a vernier calipers as described by Brass et al (1979).

3.5 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION OF EXTRACTS (MIC).

The tube (broth) dilution method described by Onawunmi et al (1989), was used to determine the approximate minimum inhibitory concentration (mic). The stock solution of the plant extracts were used to obtain various concentrations of the extracts in replicate tubes of Peptone water and the bacteria. After mixing, 2ml of the inoculum consisting of 10^7 organisms /ml dilution was added to the tubes of media containing different dilutions of the extracts in Peptone water. The tubes were then incubated at 37 oc for 24 hours. The bioassay for the tube (broth) dilution was carried out as shown in table 2.

TABLE 2 : DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

Concentration of Extract in %.	Bacteria (ml)	Extract (ml)	Peptone H ₂ O (ml)	Total volumn
Control	2	-	2	4
10	2	0.2	1.8	4
20	2	0.4	1.6	4
40	2	0.8	1.2	4
60	2	1.2	0.8	4
80	2	1.6	0.4	4
100	2	2	-	4

The lowest concentration of the extract at which no growth was observed was recorded as the minimum inhibitory concentration and this value remained constant on futher incubation. 20g of each of the powdered plant parts was placed into 80ml of distilled water in a beaker and this was allowed to stand on the laboratory bench for 24 hours after which it was filtered. The filtrate of each extract was taken as 100% concentration of the extract.

3.6 DETERMINATION OF ph OF PLANT EXTRACTS

The ph of each plant extract was determined using a Jenway 3020 ph meter. This was done by calibrating the glass electrode using ph buffers 4, 7 and 9 and the electrode was immersed into the extract without it touching the bottom of the beaker. The ph was read after 30 seconds (Iwuofor et al).

TABLE 5 : HYDROGEN ION CONCENTRATION (ph) THE PLANT EXTRACTS.

PLANT	PLANT PART	ph.
Anogeisus	Leaves	4.66
Leiocarpus	Stem - bark	4.87
Guiera	Leaves	4.18
Senegalensis	Stem - bark	4.60
Calotropis	Leaves	6.92
Procera	Stem - bark	6.10
	Roots.	6.07

CHAPTER FOUR

RESULTS

The cold water extracts of the plants produced cleaner colour intensity than the other extracts. Table 3 shows the preliminary antimicrobial screening of the plant extracts on each of the test organisms.

The chloroform extracts were generally very effective against most of the bacteria tested. Chloroform extract of Anogiesus Leiocarpus leaves was very potent on all the bacteria tested, where as that of Calotropis Procera roots was not potent on any of the bacteria test. Generally, all the plant extracts of Calotropis procera roots were not potent on any of the bacteria tested.

Anogiesus Leiocarpus leaves were the most effective genrally against all the test organisms, while the root extracts of Calotropis Procera were the least effective. The results of the detailed screening of the bactericidal activity are seen in table 4. The zones of inhibition of the untreatedd control using chloroform extract alone and that using diethyl ether alone were obtained and subtracted from each zone of inhibition obtained after extraction with the solvent.

The chloroform extract of Anogeisus Leiocarpus leaves had the highest activity against Staphylococcus aureus and the hot water extract of Guiera Senegalensis showed the lowest zone of inhibition on the growth of Bacillus Cereus.

Table 5 reveals that Calotropis Procera is the most Alkaline plant and Guiera Senegalensis the most acidic. The lowest concentration of extract at which no growth was observed was between 20% and 80% as shown on table 6.

CHAPTER 5

DISCUSSION

Antibacterial zones of inhibition were observed and recorded for all the 3 plants used except Calotropis procera root which recorded no inhibition on any of the test organisms. This could be attributed to the fact that the plant powder required a greater volume of water to percolate.

Both the pure chloroform and the Diethyl Ether solvents used as control had inhibitory action on all the bacteria tested, though more with chloroform. This implies that the active ingredients responsible for the inhibitory effects was easily extracted with chloroform.

In most cases, the cold water extracts were even more effective than the Diethyl Ether extracts. This is easily noticed in fig 7. where only the chloroform and the water extracts of Anogiesus leiocarpus leaves and Guiera senegalensis stem-bark showed antibacterial activity against Klebsiella spp.

It is interesting to note that the cold water extracts some the plant parts were more effective against some the bacteria tested than the chloroform extracts like ; the cold water extracts of Calotropis procera stem-bark against Bacillus cereus and that of Guiera senegalensis against Escherichia coli the local traditional use of these plants mainly requires cold water as the extracting solvent after which the concoction or decoction is taken orally. (Soofowora 1984, Adu 1989)

The Diethyl Ether plant extracts of Guiera senegalensis leaves had no effect at all against any of the bacteria tested. This could be as the result of the fact that the active ingredients of Guiera senegalensis leaves are not easily extracted with Diethyl Ether.

The inhibitory action of the surface sterilization effect of the solvent or the enhancing activity of the combined effects of the solvent and the active ingredients .

Hot water was the most ineffective solvent. the hot water denatures or destroys the active ingredients of the plants parts so this could explain the low activity level.

Anogiesus Leiocarpus extracts generally had a greater antibacterial activity of all the bacteria tested. The effect of leaves is more than that of the stem-bark. The difference might be due to the presence of bactericidal agents in the plant in difference concentration in difference parts of the plant (Obiora 1986).

The commercially prepared ampicillin (250mg) which was used for the treated control was found to inhibit only the growth of E.coli whereas the cloxacillin (250mg) inhibited the growth of only Bacillus cereus and Bacillus subtilis.

The ph tabs(5) shows that the most effective plant is not too acidic neither is it too alkaline.

The minimum inhibitory concentration generally was between 20% and 80%.

TABLE 3:

PRELIMINARY ANTIMICROBIAL SCREENING OF PLANT EXTRACTS ON EACH ORGANISM

PLANTS & PARTS	TYPES OF SOLVENTS	<u>E. coli</u>	<u>P. aeruginosa</u>	<u>Klebsiella spp</u>	<u>S. aureus</u>	<u>B. cereus</u>	<u>B. subtilis</u>
<u>Antocissus leiocarpus</u> (1) Leaves	Chloroform	++	++	++	++	++	++
	Diethyl ether						
	Cold water	+	++	-	++	+	++
	Hot water	++	++	+	++	++	++
		+	+	-	+	+	+
(2) Stem-bark	Chloroform	++	++	-	++	++	++
	Diethyl Ether	-	+	-	++	+	++
	Cold water	+	++	-	++	++	++
	Hot water	-	+	-	+	+	+

(6) Stem-bark	Chloroform	-	-	-	-	-	+
	Diethyl Ether	-	-	-	-	-	-
	Cold water	-	-	-	-	+	+
	Hot water	-	-	-	-	-	+
(7) Roots	Chloroform	-	-	-	-	-	-
	Diethyl Ether	-	-	-	-	-	-
	Cold water	-	-	-	-	-	-
	Hot water	-	-	-	-	-	-

15) : - = not potent on bacteria indicated.

+ = potent on bacteria indicated.

++ = very potent on bacteria indicated.

TABLE 1
 PLANT EXTRACTS.
 ZONES OF INHABITATION OF BACTERIAL ACTIVITY OF

PLANT & PART	TYPE OF SOLVENT	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella spp</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>
<i>Anacardium occidentale</i>							
1. Leaves	Chloroform	11.60	14.95	12.05	20.05	11.85	16.20
	Diethyl ether	8.05	10.30	-	14.50	4.60	12.50
	Cold water	10.30	12.20	8.60	16.08	10.05	14.30
	Hot water	7.20	8.05	-	8.02	9.10	8.03
Control	(Diethyl ether alone)	-	-	-	-	3.30	5.20
Control	(Chloroform alone)	-	-	-	-	3.75	4.75
2. Stem-bark	Chloroform	17.85	12.80	-	19.70	7.65	12.25
	Diethyl ether	-	6.90	-	16.65	8.50	11.75
	Cold water	8.02	10.05	-	16.90	12.07	11.05
	Hot water	-	5.02	-	9.10	6.20	5.30
Control	(Diethyl ether alone)	-	-	-	-	3.30	5.20
Control	(Chloroform alone)	-	-	-	-	3.75	4.75
<i>Guiera senegalensis</i>							
3. Leaves	Chloroform	8.25	10.60	-	16.25	3.80	5.55
	Diethyl	-	-	-	-	-	-
	Cold water	9.20	8.50	-	7.75	2.02	5.75
	Hot water	7.50	6.30	-	5.05	2.00	3.20
Control	(Diethyl ether alone)	-	-	-	-	3.30	5.20
Control	Chloroform alone)	-	-	-	-	3.75	4.75
4. Stem-bark	Chloroform	-	10.15	5.90	13.35	-	4.35
	Diethyl ether	-	4.60	-	13.50	3.90	-
	Cold water	5.40	7.30	6.25	9.03	5.20	5.00
	Hot water	-	3.45	-	8.45	2.15	-
Control	(Diethyl ether alone)	-	-	-	-	3.30	5.20
Control	(Chloroform alone)	-	-	-	-	3.75	4.75

4. Stem bark	Chloroform	-	10.15	5.90	13.35	-	4.35
	Diethyl ether	-	4.60	-	13.50	3.90	-
	Cold water	5.40	7.30	6.25	9.03	5.20	5.00
	Hot water	-	3.45	-	8.45	2.15	-
	(Diethyl ether alone)	-	-	-	-	3.30	5.20
Control	Chloroform alone	-	-	-	-	3.75	4.75
5. Leaves <i>Cordia pavetta</i>	Chloroform	-	8.70	-	-	-	-
	Diethyl ether	-	4.20	-	-	2.50	-
	Cold water	-	7.50	-	-	3.50	2.50
	Hot water	-	3.80	-	-	-	-
	(Diethyl ether alone)	-	-	-	-	3.30	5.20
Control	(Chloroform alone)	-	-	-	-	3.75	4.75
5. Stem-bark	Chloroform	-	-	-	-	-	6.25
	Diethyl ether	-	-	-	-	-	-
	Cold water	-	-	-	-	5.20	4.50
	Hot water	-	-	-	-	-	3.40
	(Diethyl ether alone)	-	-	-	-	3.30	5.20
Control	(Chloroform alone)	-	-	-	-	3.75	4.75

TABLE 6 : THE MINIMUM INHIBITORY CONCENTRATIONS OF THE PLANT EXTRACTS ON THE BACTERIA TESTED IN (%).

PLANT & PARTS	Escherichia Coli	Pseudomonas Aeruginosa	Klebsiella spp	Staphylococcus Aureus	Bacillus Cereus	Bacillus Subtilis
<hr/>						
Anogeisus Leiocarpus						
Leaves	80	80	20	60	20	60
Stem - bark	80	80	-	60	60	60
Guiera Senegalensis						
Leaves	60	80	-	40	60	40
Stem - bark	80	80	20	40	40	40
Calotropis Procera						
Leaves	-	60	-	-	80	60
Stem - bark	-	-	-	-	20	40
<hr/>						

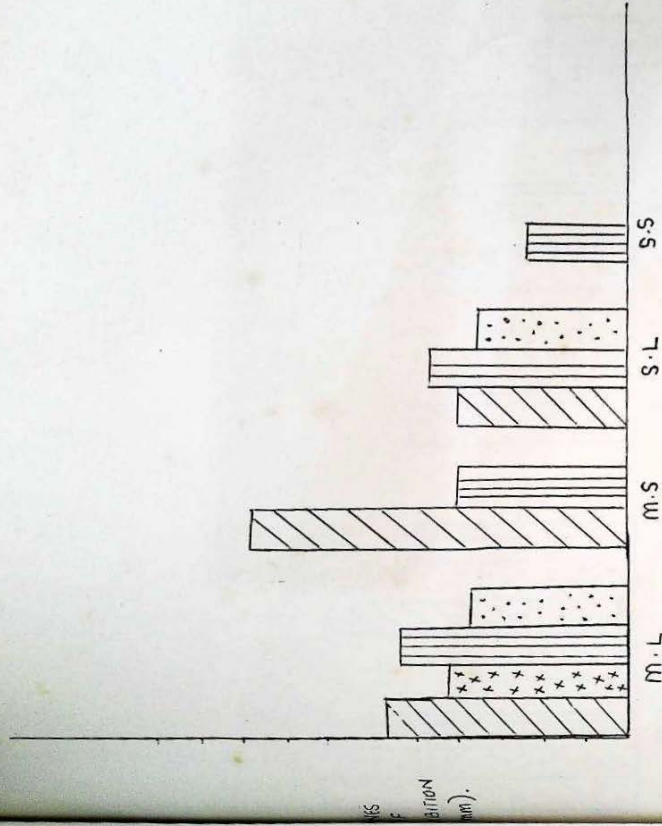


FIG 5: THE EFFECT OF THE PLANT EXTRACTS ON Escherichia coli.

KEY



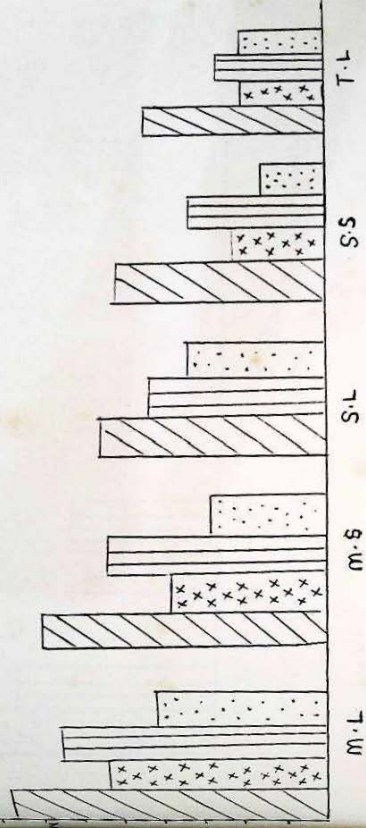
CHLOROFORM.

DIETHYL ETHER.

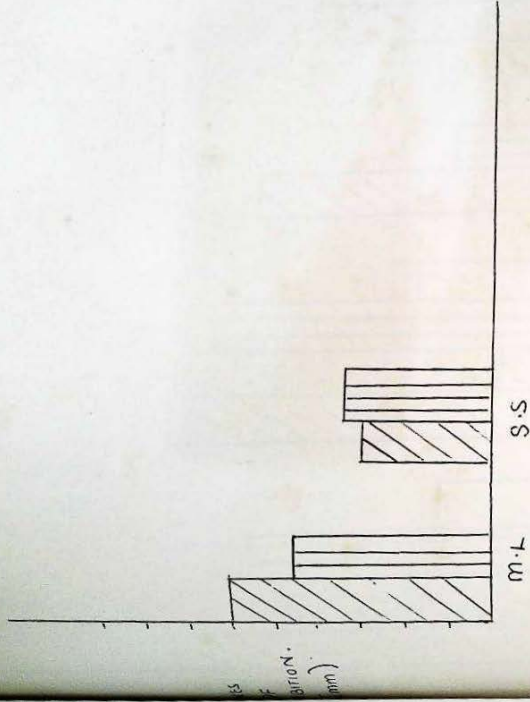
COLD WATER.

HOT WATER.

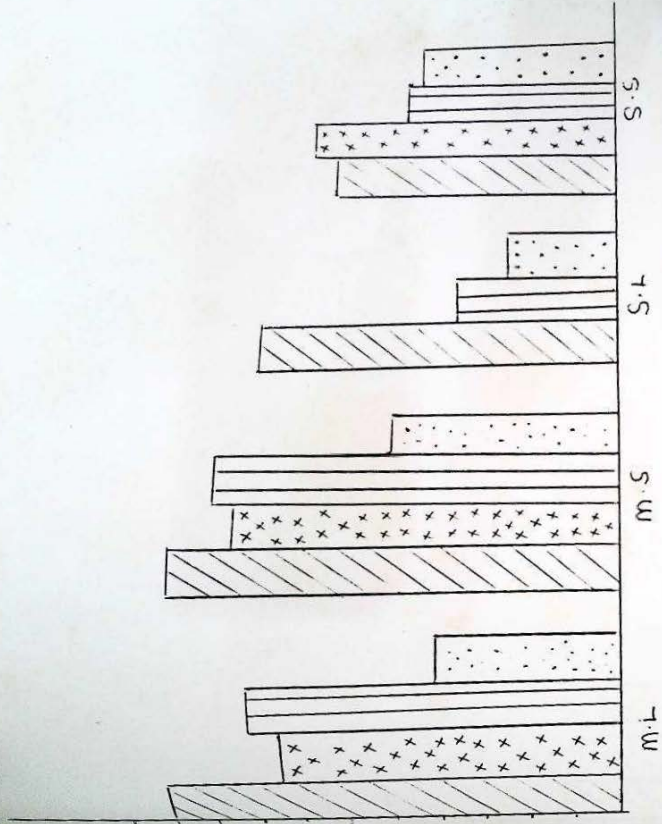
M.L = Anogiesus leiocarpus leaves.
 M.S = Anogiesus leiocarpus stem-bark.
 S.L = Guiera senegalensis leaves.
 S.S = Guiera senegalensis stem-bark.
 T.L = Calotropis procera leaves.
 T.S = Calotropis procera stem-bark.



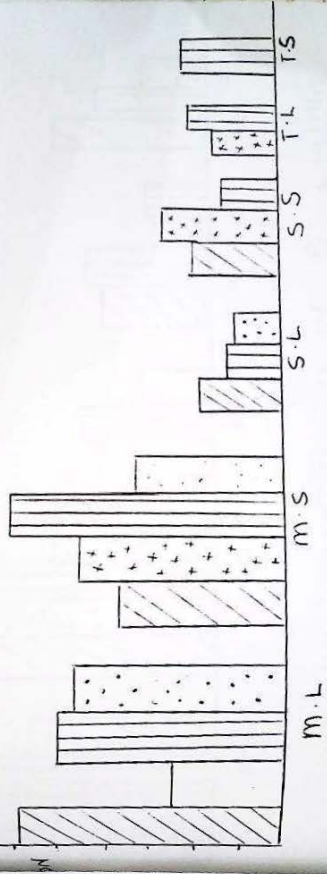
6. THE EFFECT OF THE PLANT EXTRACTS ON Pseudomonas deroginosa.



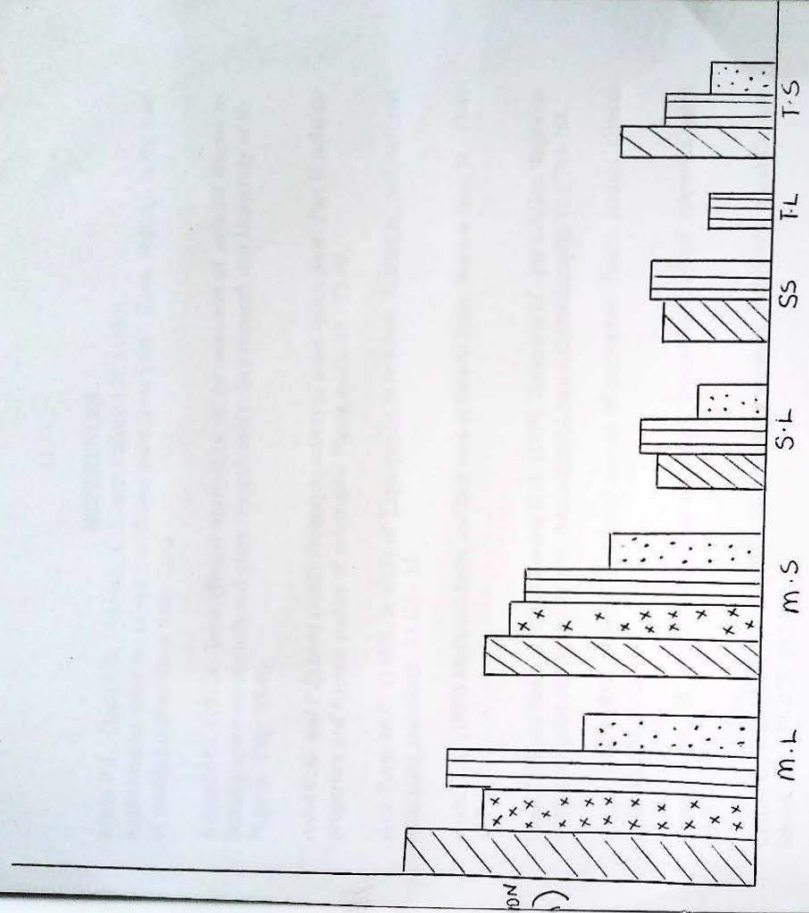
1. THE EFFECT OF THE PLANT EXTRACTS ON Klebsiella spp.



THE EFFECT OF THE PLANT EXTRACTS ON S. aureus



THE EFFECT OF THE PLANT EXTRACTS ON B. cereus.



: THE EFFECT OF THE PLANT EXTRACTS ON B. subtilis

REFERENCES

- Alade P.J., Dada J.D., Ahmed, A.A. and Yadock I.D. (1980). Antimicrobial activities of some medicinal plant from Soba - Zaria, Nigeria. In the state of medicinal plant from Soba Zaria.
- Ampofo O. (1977). Some clinical observation of the treatment of selected disease by herbal preparative in medicinal plant research today. Dry research unit University of Ife, Nigeria. Page 35-45.
- Athal C.K. and P.D Sethi (1962) Proteolytic activity of some Indian plant. Part II isolation properties and Kinetic studies of calotropin. *Planta media* 10 : 77-90.
- Atta Pattu M.C. (1989). A study of Tinea capitis in Srilanka. Journal of medicinal and veterinary mycology. 27 (1) - 32.
- Audu, J.A. (1989) Medicinal herbs and their uses in Bauchi State, Nigeria field 54 : (3-4) 157-162.
- Brass C. Shain house, J.Z. and Stevens D.A. (1979) Variability of agar dilution. Replicate method of yeast susceptibility test. Antimicrobial Agent chemotherapy 15 : 763-768.
- Casal, J.B. (1979). Tablets sensitivity testing of pathogenic fungi. Journal clinical pathology 32 : 719-722.
- Cheesbrough, M. (1984). ^{Medicinal} ~~Medicinal~~-laboratory manual for tropical countries vol.II pp.372-385.
- Collins C.H. and Lyne, P.M. (1985). Microbiology methods. Bulter Worth and Co. Ltd. 5th ed. PP.123-127, 107-181.
- Cooke, R.C. (1977) Fungi, man and his environment 1st ed. Page 49.
- Dalziel, T.M. (1937). The useful plant of West Africa crown Agent for the colonies London.
- Dalziel T.M. (1956). The useful plant for West Tropical Africa. Crown Agent London P.612.
- Dikshit A., Dubey, N.K. Trippalhi, R.D., Tripathi, N.W. and Dixit, S.N. (1982). Control of fungal deterioration of some species during storage by essential oils of Cedrus deodara. Test of Agrochemical and cultivars Annals of Applied Biology 100 supplement 3, 56-57.

- Dubel, N.K., Kishore N., Tripathi, R.D. and Dixit, S.N. (1982). Fungitoxicity of essential oil of Citrus medica agents storage fungi. Test of Agrochemical and cultivars Annal of Applied Biology 100 suppliment 3, 58-59.
- Farnsworth N.R., Akenele O., Bingel, A.S. Sojaifo, D.D. and Gno, 2 (1985). Medical plant in therapy. Bulletin WHO. 63(6), 165-981.
- Gbile, O.Z. (1985). Ethnobotany, Toxomy and conservation of medicinalplants in the state of medicinal plant research in Nigeria edited by Abayomi Sofowora.
- Georges, M. and Pandalaki, K.N. (1949) investigation of plant antibiotics. Further research for antibiotic substances in India medicinal plant, India journal of medica research. pp.169-181.
- Hammerman, K.J., Powell, K.E. and Tosh F.C. (1974). The incidence of hospitalized cases of systemic mycotic infection sabouraudis.
- Holt R.J. (1975). Laboratory test of antifungal agent. Journal of clinical pathology. 28. 767-774.
- Hugo, W.B. and Russell A.D. (1983). Pharmaceutical Microbiology (3rd ed) Blackwell scientific publication oxford London, Edinburgh, Boston, Melbourne page 81, 201.
- Iwu M.M. and court, W.E. (1977) planta Med 32. 88.
- J.C. H. Uphof, Von J. Cramer 91968)
A dictionary of Economic plant 3301 lettre vorlang.
- Khan M.R.G., Ndaaha M.H.S. Nkunya, H. Weaver and Sawbrey (1978). The antigenococci activity of some medicinal plants. Pakistan journal of science Ind. Res. Vol 2(51) pp.1.
- Kokwaro J.O., (1967). Medicinal plant for East Africa publication by East Africa literature Bureau, Kampala, Nairobi, Danes-salami.
- Lewis, W.H. and Elvin-Lewis, M.O.F. (1977). Medical Botany. John Wiley and Sons Inc. 346, 352-354.
- Malcolm S.A. and Sofowora E.A (1969). Antimicrobial activities of selected Nigerian folk remedies and their constituent plants. Antimicrobial property of Balanities lioydia. 32, 512.

Obini, C.N. (1990). Antifungal activities of some in local herbs on dermatophyte unpublished.

Ogunlana E.O. and Ramstad E. (1975). Investigation into the antibacterial activities of local plant, *plant medica* pp.27, 354.

Oliver, B. (1960). Medicinal plant in Nigeria college of Art, Science and technology Ibadan, Nigeria. pp.425.

Omotoye O. (1984). Taxonomy of West Africa flowering plant pp.94-98.

Sheklokoo W.D. (1988). Human skin and fungal MW publisher Moscow.

Sofowora, A. (1976). Africa medicinal plant proceeding a conference.

Sofowora, A. (1982). Medicinal plant and tradition in Africa. John Wiley and sons.

Sofowora, A. (1984). Medicinal plant and traditional medicine in Africa.

Sofowora, A. (1986). The state of medicinal plant research in Nigeria.

Some medicinal forest plant of Africa and Latin America. Food and Agric organization of United nation, Rome (1986). pp.103.

Traditional medical therapy. A critical Appraisal published by natural science and technology development Agency. pp.71.

Watt, J.M. and Brayer - Brand-Wiz M.G. (1962).

The medicinal and poisonous plant of South and Eastern Africa E & S Livingstone Edinburge. 1457p.

West African Botany F.R. Irvine Oxford University Press, London.

