CHAPTER 6

EVALUATION OF ANTIMICROBIAL (ANTIBACTERIAL AND ANTIFUNGAL) ACTIVITIES OF STEM BARK OF *PHYLLANTUS ACIDUS* (L.)SKEELS AND ROOT BARK OF *CROTON CAUDATUS* GEISELER

6.1: Introduction

"Let food be your medicine and let medicine be your food" was the advice of the father of medicine, Hippocrates, over two millennia ago ***(Wang et al., 2002***). Herbal and Natural products of folk medicine have been used for centuries in many parts of the world. Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine **(*Ang-Lee et al., 2001, Goldman, 2001).***

In India and China, herbal medicines are still used, and developed countries have rediscovered many of these traditional medicines as cheap source of complex bioactive compounds ***(Phillipson, 1994)***. The frequency of life-threatening infections caused by pathogenic microorganisms and the rapid development of multi-resistant bacterial and fungal strains of clinically important pathogens fetches the interest of scientist to develop newer broad spectrum antimicrobial agents **(*Weisser et al., 1966*).** The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity.

Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties. Plant-derived medicines have been part of traditional healthcare in most parts

of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases **(*Chariandy et al., 1999*).**

Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is the need to study of folklore uses of medicinal plants and to see how these can be utilized for better and inexpensive healthcare. But, the investigation on folklore medicine leading to the discovery of new drugs remains incomplete, if the information is not processed clinically, pharmacologically in the laboratory.

To overcome these problems many works have been done which aim at knowing the different antimicrobial constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs ***(Akinpelu et al., 2006).***

Considering the above mentioned facts, an attempt has been made for determine the antimicrobial activities of methanol extracts of root bark of *Croton caudatus* Geiseler and stem bark of *Phyllantus acidus* (L.)Skeels.

6.2: Materials and Methods

Detail of the methodology in this regard is as follows:

6.2.1 Preparation of the plant extract:

The root bark of *Croton caudatus* Geiseler and stem bark of *Phyllantus acidus* (L.)Skeels were clearly washed with water, shade dried (air dried) and powdered. The powdered dried materials (300g) were defatted with petroleum ether. After washing with petroleum ether, the residue was extracted exhaustively with 200ml distilled

methanol by using soxhlet apparatus. The extract was filtered through cotton followed by vacuum suction. The solvent was then removed by distilling at 20-30^{0}C to give a reddish brown sticky and a blakish brown semi-solid mass.

6.2.2 Antimicrobial activity tests:

6.2.2(a): Making up extract solution

Approximately 2g of the respective dried extracts were weighed and transferred to a 20 ml volumetric flask. The respective solvent methanol was then added to make up the 20 ml solution and get 10% solution. The solution is serially diluted to get 5%, 1% and 0.5% respectively.

For minimum inhibitory concentration (MIC), for *Phyllantus acidus* (L.)Skeels, 2mg of the extract was dissolved in 2ml distill water and for *Croton caudatus* Geiseler, 2mg of the extract was dissolved in 2ml DMSO. Thus a solution with concentration of 1mg per ml was obtained.

6.2.2(b) Preparation of paper discs:

The paper discs were prepared with the What-man No1. filter paper (5mm, diameter), then immersed in the filtered plant extract and kept for 24hours.These were then taken out, dried at room temperature and finally sterilized by keeping under ultraviolet (UV) radiation for 1hr. The paper discs with the respective solvents used in the extracts were used as negative control.

6.2.2(c) Preparation of media:

i) **Sterilization of glassware:** Glassware's were washed with cleansing solution of chromic acid (Potassium dichromate 100g, sulphuric acid 500ml, water 1000ml). Properly cleaned and dried

glassware were sterilized by keeping them in hot air oven at 160^0c for 80 minutes.

The Petridish, pipettes were wrapped in brown paper before sterilization and conical flasks and test tubes etc were plugged by cotton wool before heating. Forceps and inoculating loops are sterilized by holding in the flame until it becomes red hot (inside the laminar air flow).

(ii) **Preparation of Muller Hinton HiVeg Agar media**:

Formula	Gram/Liter
Ingredients	----
HiVeg extract	2.0
HiVeg hydrolysate	17.50
Strach	1.50
Agar	17.0
Final P^H (at 25^0C)	7.3±0.2

Muller Hinton HiVeg Agar media (Hi-media Pvt. Ltd.) was used for this. Muller Hinton HiVeg Agar (38.0gms) was added to 1000ml of distilled water in a conical flask. Heated to boiling to dissolve the medium completely and was sterilized by autoclaving at 15lbs pressure (121^0C) for15 minutes. The sterilized medium was then poured to petridishes and allowed to cool.

(iii) **Sabouraud Dextrose Agar (Sabour aud Glucose Agar):**

Formula	Gram/Litre
Dextrose	40.0
Agar	15.0
Pancreatic digest of casein	5.0
Peptic digest of animal tissue	5.0
Final pH at 25^0C	0.0±0.2

65.00gm Sabouraud Dextrose Agar (From Sisco research laboratories Pvt. Ltd.) powders was added to distilled water and the volume was made upto to 1.0 liter and mixed thoroughly. It was gently heated to boiling and then autoclaved at 15 psi pressure at 121^0C for 15 minutes and finally dispense into sterile Petri plates.

iv) **Nutrient Broth Media:**

Ingredients	Grams/litre
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Final pH (at 25^0C)	7.4±0.2

13gms of nutrient Broth media (Hi-media Pvt. Ltd) in 1000ml distilled water was mixed thoroughly and gently heated to boiling and autoclave at 15psi pressure at 121^0C for 15 minutes.

v) **Brain Heart Infusion Broth:**

Ingredients	Grams/litre
Nutrient substrate (Brain extract,Heart extract &Pepton)	27.5g
D(+)glucose	2.0g
Sodium chloride	5.0g
Di-sodium hydrogen phosphate	2.5g
Final pH (at 25^0C)	7.4

37.0gms of Brain Heart Infusion Broth (Tulip Diagnostic Pvt. Ltd) in 1000ml distill water was mixed thoroughly and gently heated to boiling and autoclaved at 15 psi pressure at 121^0 C for 15 minutes.

Of the above prepared media, Muller Hinton Agar media was used for antibacterial activity study. Sabouraud Dextrose Agar media

was used for antifungal activity study, Brain Heart Infusion broth for MIC determination of *Staphylococcus aureus* and Nutrient Broth media for the MIC determination of *Klebsiella pneumonia, Pseudomona aeruginosa, Escherichia coli* and *Bascillus subtilis* respectively.

vi) **Organisms Collection:**

For the determination of Antimicrobial activity, the following pathogenic strains were used.

Bacterial strains	Fungal strains
Staphylococcus aureus	*Candida albicans*
Klebsiella pneumonia	*Tricophyton mentagrophytes*
Pseudomonas aeruginosa	*Tricophyton beigelli*
Escherichia coli	*Microsporum gypsum*
Bascillus subtilis	----------

The bacterial strains were collected from the Microbiology Laboratory of Silchar Medical College & Hospital and fungal strains were from Defence Research Laboratory, Tezpur.

vii) **Known antibiotic (standard) discs activity against test organisms:**

For the antibacterial activity of some known antibiotics viz., Chloramphenicol10µg/disc (Ch), Tetracycline 30µg/disc (T), Streptomycin 10µg/disc(S), and Norfloxacin 10µg/disc (NX) from Hi-media were used. Nitrofurantoin300µg/disc (NF), Amikacin 10µg/disc (AM), Sparfloxacin 5µg/disc (Sc), Fluconazole 10µg/disc (Fu) also from Hi-media was used for the determination of antifungal activity.

6.2.3 Determination of antimicrobial activities of the extracts:

Antimicrobial (Antibacterial and Antifungal) screening is generally performed by disc diffusion method **(*Khan et al., 2007,***

Dash et al., 2005) which is a qualitative to semi quantitative test. Briefly 20ml quantities of nutrient agar were plated in petri dish with 0.1 ml of a 10^{-2} dilution of each microbial culture. Filter paper discs (5mm in diameter) impregnated with various concentrations of plant extracts were placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism seeded plates. Blank disc impregnated with solvent methanol followed by drying off was used as negative control. The activity was determined after 18hrs of incubation at 37°C. The diameters of zone of inhibition produced by the extract were then compared with the standard antibiotic. Each sample was used in triplicate for the determination of antimicrobial activity.

6.2.4 Determination of Minimum Inhibitory Concentration (MIC):

Minimum inhibitory concentration is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. In the present study, MIC was determined using "Serial tube dilution technique**"** (***Washington and Wood, 1995*****).** In this technique the tubes of broth medium, containing graded doses of extract are inoculated with the test organisms at 37^0C for 18hrs. After suitable incubation, growth will occur in those tubes where the concentration of extract is below the inhibitory level and the culture will become turbid (cloudy). Therefore, growth will not occur above the inhibitory level and the tube will remain clear.

Procedures:

1. Twelve test tubes were taken, nine of which were marked 1, 2, 3, 4, 5, 6, 7, 8, 9, and the rest three were assigned as T_M (medium), T_{ME} (medium + extract) and T_{MI} (medium + inoculum).

2. 1 ml of nutrient broth medium was poured to each of the 12 test tubes.
3. The test tubes were cotton plugged and sterilized in an autoclave for 15 lbs/sq. inch pressure.
4. After cooling 1 ml of the sample solution was added to the 1^{st} test tube and mixed well and then 1ml of this content was transferred to the 2^{nd} test tube.
5. The content of the second test tube was mixed well and again 1 ml of this mixture was transferred to the 3^{rd} test tube. This process of serial dilution was continued up to the 9^{th} test tube.
6. 10 µl of properly diluted inoculum was added to each of 9 test tubes and mixed well.
7. To the control test tube T_{MC}, 1 ml of the sample was added, mixed well and 1 ml of this mixed content was discarded to check the clarity of the medium in presence of diluted solution of the compound.
8. 10µl of the inoculum was added to the control test tube T_{MI}, observed the growth of the organism in the medium used. However, Brain Heart Infusion broth was used for *Streptococcus aureus.*
9. The control test tube T_M, containing medium only was used to confirm the sterility of the medium.
10. All the test tubes were incubated at 37^0C for 18hrs.

6.2.5 Statistical analysis:

Tests and analyses were run in triplicates. Mean value ± SEM of triplicates were calculated. Statistical analysis was performed using Student's *t*-test. The values were considered significant when $p<0.001$

6.3: Results and Discussions

6.3.1Antibacterial activity:

6.3.1(a) Antibacterial activity of *Phyllantus acidus* (L.)Skeels:

The results representing antibacterial activity of methanol extract of stem bark of *Phyllantus acidus* (L.)Skeels were presented in **Table and Fig.6.3.1(a).**

Table 6.3.1(a): Zone of inhibition shown by the stem bark extract of *Phyllantus acidus* (L.)Skeels against four pathogenic bacteria and the zone of inhibition with the standard antibiotic discs.

	Zone of inhibition of extracts in mm				Zone of inhibition of standard in mm			
Name of micro organisms	0.5% (10^3µg/ disc) M±S.E	1% (10^3µg/ disc) M±S.E	5% (10^3µg/ disc) M±S.E	10% (10^3µg/ disc) M±S.E	T (30µg/ disc) M±S.E	S (10µg/ disc) M±S.E	C (10µg/ disc) M±S.E	NX (10µg/ disc) M±S.E
E.coli	6±0.33	6.5±0.33	7±0.33	10±0.33	6.3±0	10.2±0.8	15.0±1.2	8.33±0.33
Sa	6.5±0.33	7±0.57	7.66±0.33	8.5±0.33	7±0.33	8±0.33	9.5±1.2	7.5±0.33
Kp	6.6±0.33*	6.9±0.5	8.5±0.33	10.5±0.33	9±1.5	7.6±0.5	7.5±0.33	12.5±0.33*
Bs	-------	------	---------	----------	7.5±1	10±1.5	9.7±0.33	7.0±0.33

Key:-T-Tetracycline, S-Streptomycin, C-Chloramphenicol, NX-Norfloxacin, *Sa-Staphylococcus aureus, Kp-Klebsiella pneumonia*, *Bs -Bascillus subtillis, E-coli- Escherichia coli*.

The increase in zone of inhibition (ZOI) was concomitant with increase in the concentration of the extract [Table 6.3.1(a) and 6.3.1(b)] which is in the line with findings of ***Usman and Osuji, 2007.***

The control disc used for solvent had no ZOI, so their data were not shown in the table. The other data were represented in the form of mean of three tests ± SEM of the standard group, n=3, *P<0.001 as the

statistically significant for the plant extracts at different concentrations compared with the standard antibiotic discs by using Student's *t-test*.

The highest activity of the plant extract was 10.5mm diameter of ZOI [Fig.6.3.1(a) and white arrow, Fig.6.3.1(b)I] found against *Klebsiella pneumonia* at the concentration of 10% (10^3µg/disc) to some extent comparable with Norfloxacin [standard antibiotic as shown in Table and Fig.6.3.1(a); Fig.6.3.1(b)I followed by 10 mm diameter zone of inhibition [Fig.6.3.1(a) and white arrow, Fig.6.3.1(b)III] found against *Escherichia coli* at the same concentration which is comparable to Streptomycin (standard antibiotic) as shown Table and Fig.6.3.1(a). On the other hand, the lowest activity of the plant extract was 6mm diameter of ZOI [Fig.6.3.1(a) and black arrow, Fig.6.3.1(b)III] observed against *Escherichia coli* at the concentration of 0.5 % (10^3µg/disc) but almost moderately active against *Staphylococcus aureus* of about 8.5mm [Fig.6.3.1(a) and black arrow, Fig.6.3.1(b)II].

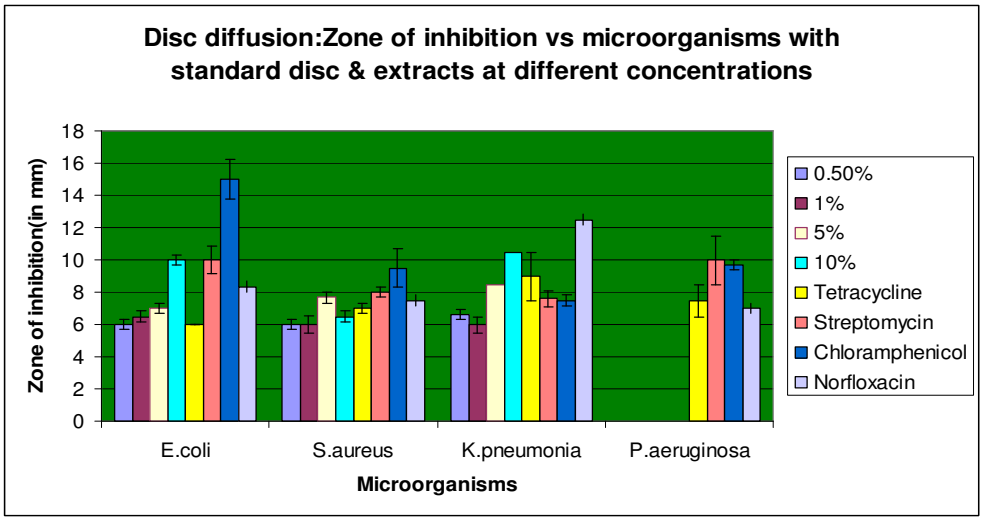
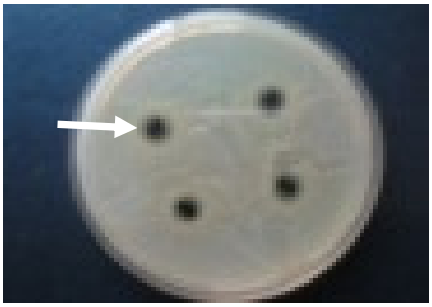


Fig 6.3.1(a): Graph showing the antibacterial activity of stem bark extract of *Phyllantus acidus* (L.)Skeels at different concentrations against four bacterial strains and standard antibiotic discs.



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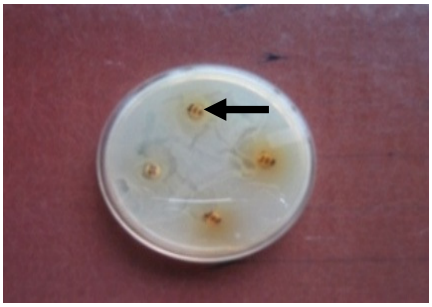

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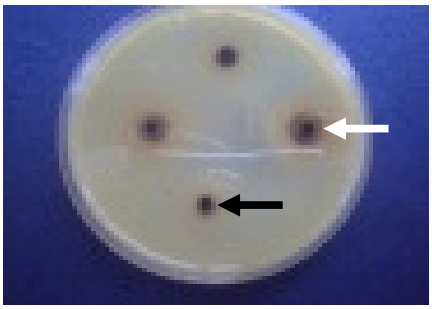


(III)

(IV)

Fig.6.3.1(b): Antibacterial activity: Zone of inhibiion shown by the stem bark extract of *Phyllanthus acidus* (L)Skeel against (I) *Klebsiella pneumonia* (II) *Staphylococcus aureus* (III) *E-coli* (IV) Zone of inhibition shown by the standard Norfloxacin (12.5 mm) against *Klebsiella pneumonia.*

6.3.2 Antibacterial activity of *Croton caudatus* Geiseler:

The antibacterial activity of root bark of *Croton caudatus* Geiseler presented in Table 6.3.2(a) and Fig.6.3.2(b).

Table 6.3.2(a): Zone of inhibition shown by the root bark extract of *Croton caudatus* Geiseler against four pathogenic bacteria and the zone of inhibition with the standard antibiotic discs.

Name of micro-organisms	Zone of inhibition of extract in mm				Zone of inhibition of standard in mm			
	0.5% (10^3µg/ disc) M±S.E	1% (10^3µg/ disc) M±S.E	5% (10^3µg/ disc) M±S.E	10% (10^3µg/ disc) M±S.E	T (30µg/ disc) M±S.E	S (10µg/ disc) M±S.E	C (10µg/ disc) M±S.E	NX (10µg/ disc) M±S.E
E. coli	6±0.57	6.33±0.33*	11.33±0.33	13.66±0.33	7±0	14±0.88*	15.66±1.20	6±0
Sa	6.5±0.33	7±0.57	8.66±0.33	8.65±0.33	23.33±2.6	14.66±0.8	10±1.7	7.66±0.3
Kp	6.4 ±0.33*	6.66±0.66*	19.0±.57	19.5±1.52	15±2.5	6.33±0.33	6±0	5.33±0.3*
Pa	--------	--------	---------	---------	7.33±1.0	15.33±1.4	13±1.0	6±0

Key: T-Tetracycline, S-Streptomycin, C-Chloramphenicol, NX-Norflofoxacin, *Sa-Staphylococcus aureus*, *Kp-Klebsiella pneumonia*, *Pa-Pseudomonas aeruginosa, E-coli- Escherichia coli*.

. The increase in zone of inhibition (ZOI) was concomitant with increase in the concentration of the extract [Table 6.3.2(a) and Fig.6.3.2(b)] which is in the line with the findings of ***Usman and Osuji, 2007.***

The control disc used for solvent had no ZOI, so no data were shown. Data were represented in the form of mean of three tests ± S.E of the standard group, n=3,*P<0.001as the statically significant value for the plant extracts at different concentrations compared with the standard antibiotic discs by using Student's *t-test*.

The highest activity of the plant extract was 19.5mm diameter of ZOI found against *Klebsiella pneumonia* [Fig.6.3.2(b) and white arrow, Fig.6.3.2(c)I] at the concentration of 10% (10^3µg/disc) followed by 19 mm diameter ZOI at the concentration of 5% (µg/disc)[Fig.6.3.2(b) and black arrow, Fig.6.3.2(c)I] of the same species. The highest activities were shown at a concentration of 10%

and 5% root extract [Table 6.3.2(a) and Fig.6.3.2(b)]. Their antibacterial activities were noteworthy even when compared with the activities of all the standard antibiotics used in this investigation. On the other hand, in the case of *E. coli* the highest activity of the root extract was 13.66mm [Fig.6.3.2(b) and black arrow, Fig.6.3.2(c)III] at the concentration of 10% (10^3µg/disc) comparable to Streptomycin [Table 6.3.2(a) and Fig.6.3.2(b)]. The lowest activity of the plant extract was 6mm diameter of ZOI observed against *Escherichia coli* [Fig.6.3.2(b) and white arrow, Fig.6.3.2(c)III] at the concentration of 0.5% (10^3µg/disc). The plant extract were found to be inactive against *Pseudomonas aeruginosa* [Table 6.3.2(a) and Fig.6.3.2(b)] but almost moderately active against *Staphylococcus aureus* of about 8.65mm [Fig.6.3.2(b) and white arrow, Fig.6.3.2(c)II] at the concentration of 10%(10^3µg/disc).

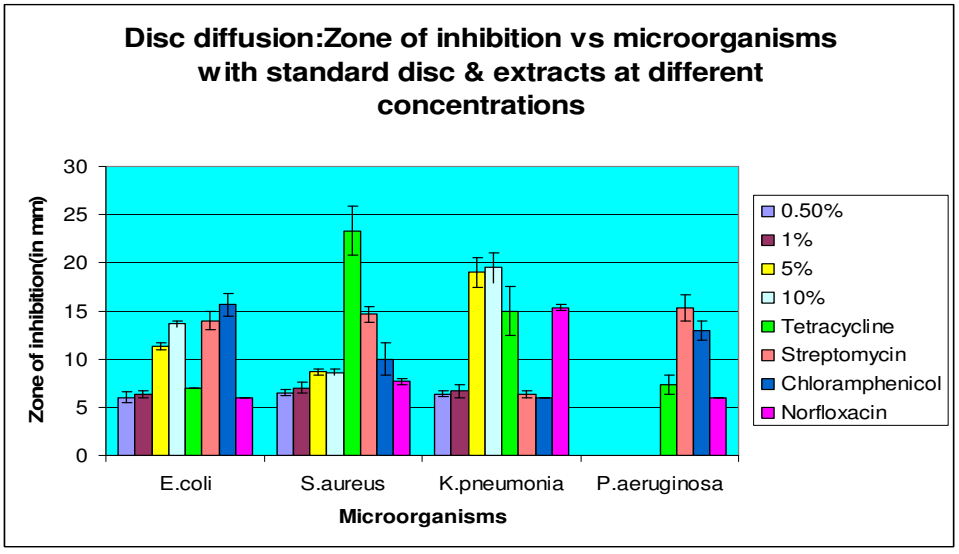
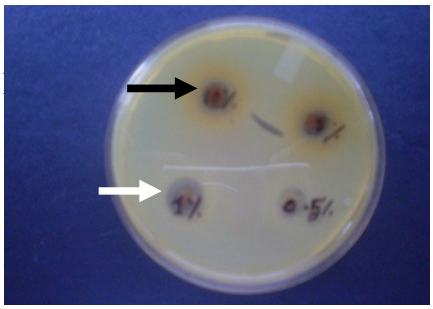
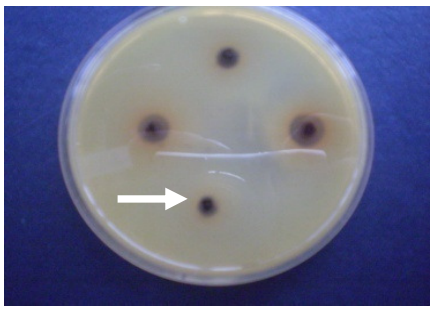


Fig.6.3.2(b): Graph showing the antibacterial activity of root bark extract of *croton caudatus* Geiseler at different concentrations against four bacterial strains and standard antibiotic discs.



(I)



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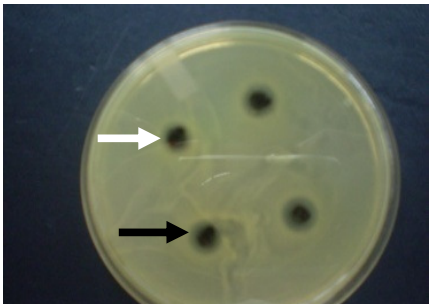

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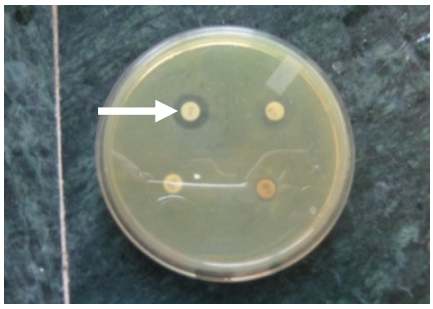


(IV)

Fig.6.3.2(c) Antibacterial activity: Zone of inhibition shown by root bark extract of *Croton caudatus* Geiseler against (I) *Klebsiella pneumonia* (II) *Staphylococcus aureus* (III) *E-coli* (IV) by the standard disc Tetracycline of 23.33mm [white arrow IV]against *Staphylococcus aureus*.

6.3.3 Antifungal Activity:

6.3.3(a) Antifungal activity of *Phyllantus acidus* (L.)Skeels:

The antifungal activities of methanol extract of stem bark of *Phyllantus acidus* (L.)Skeels were determined at the concentration of 0.5%, 1%, 5% and 10% of 10^3µg/disc against four pathogenic fungi shown in **Table 6.3.3(a) and Fig.6.3.3(b).**

Table 6.3.3(a): Antifungal activity of methanol extract of stem bark of *Phyllantus acidus* (L.)Skeels against four fungal strains and the zone of inhibition with the standard antibiotic discs.

	Zone of inhibition of extracts in mm				Zone of inhibition of standard in mm			
Name of micro - organisms	0.5% (10^3µg/ disc) M±S.E	1% (10^3µg/ disc) M±S.E	5% (10^3µg/ disc) M±S.E	10% (10^3µg/ disc) M±S.E	NF (300µg/ disc) M±S.E	AM (10µg/ disc) M±S.E	SC (5µg/ disc) M±S.E	FU (10µg/ disc) M±S.E
Ca	8.5±0	8.66±0.35*	12.33±0.35	14.66±0.35	9±0.33	15±0.35*	14.35±1.3	6.5±0.33
Tm	7±0	9.33±0.35	10.3±3.55	12.35±0.33	15±0.33	14.3±0.33	13±1.5	9.35±0.23
Tb	8±0	8.5±0.33*	11.5±0.35	13.5±0.33	20.5±0.33	8.3±0.35	8±0.30	15±0.35*
Mg	--------	-------	----------	----------	9.5±0.35	10±3.3	15±0.33	9±0.15

Key:-NF-Norfloxacin, AM- Amikacin, S-Sparfloxacin, Fu-Fluconazole *Ca-Candida albicans*, *Tm-Tricophyton mentagrophytes*, Tb-*Trichosporan beigelli*, Mg-*Microsporum gypsum*.

The increase in zone of inhibition (ZOI) was concomitant with increase in the concentration of the extract [Table 6.3.3(a) and Fig.6.3.3(b)] which is in the line with findings ***Usman and Osuji, 2007.***

The control disc used for solvent had no ZOI, so their data were not shown. Data were represented in the form of mean of three tests ± S.E of the standard group, n=3.*P<0.001 as the plant extracts at different concentrations compared with the standard antibiotic discs by using Student's *t-test.*

The highest activity was 14.66mm diameter of ZOI [Table 6.3.3(a) and Fig.6.3.3(b)] observed against *Candida albicans* [black arrow, Fig.6.3.3(c)V] at the concentration of 10% (10^3µg/disc) followed by 13.5mm diameter of ZOI observed against *Trichosporan*

beigelli [Fig.6.3.3(b) and white arrow, Fig.6.3.3(c)IV] at the same concentration.

The ZOI found against *Candida albicans* at the concentrations of 10% and 5% were comparable with the ZOI observed in case of reference antibiotics [Table 6.3.3(a) and Fig.6.3.3(b) except Amikacin] and in the case of *Trichosporan beigelli,* the antifungal activities were better when compared with Amikacin and Sparfloxacin.

On the other hand, the lowest activity was 7mm diameter of ZOI found against *Tricophyton mentagrophytes* [Fig.6.3.3(b) and white arrow, Fig.6.3.3(c)VI] at the concentration of 0.5% (10^3µg/disc) but show moderate activity as the concentration increases. The plant extract were found to be inactive against *Microsporum gypsum* [Fig.6.3.3(b) and black & white arrow, Fig.6.3.3(c)III]. Overall, the methanol extracts of *Phyllantus acidus* (L.)Skeels stem bark showed significant activity against all the tested pathogenic fungi except *Microsporum gypsum.*

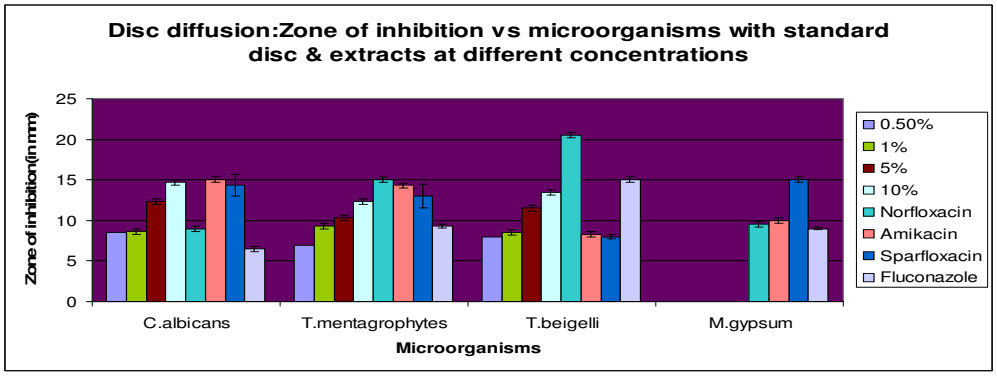
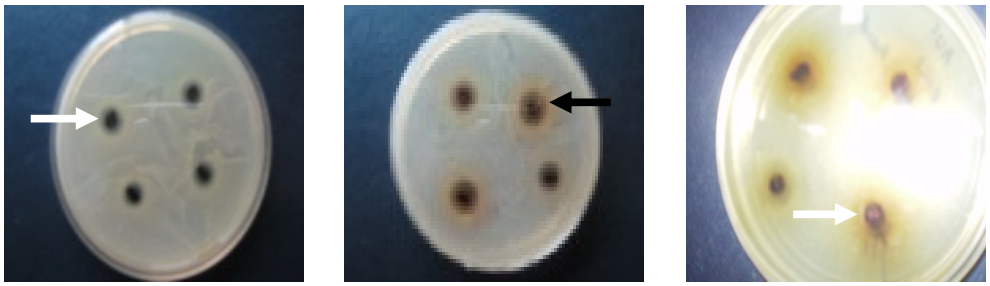


Fig.6.3.3(b): Graph showing the antifungal activity of stem bark extract of *Phyllantus acidus* (L.)Skeels extract at different concentrations against the four fungal strains and standard antibiotic discs.

(I) (II) (III)



(IV) (V) (VI)

Fig.6.3.3(c): Antifungal activity: Zone of inhibition shown by standard disc against (I) *Tricophyton beigelli* (II) *Tricophyton mentagrophytes* (III) *Microsporum gypsum* does not show any zone of inhibition (IV) zone of inhibition shown by the stem bark extract of *Phyllanthus acidus* (L.)Skeels against *Tricophyton beigelli* (V) Candida albicans (VI) *Tricophyton mentagrophytes.*

6.3.4 Antifungal activity of *Croton caudatus* Geiseler:

The antifungal activities of methanol extract of root bark of *Croton caudatus* Geiseler were determined at the concentration of 0.5%, 1%, 5% and 10% of 10^3µg/disc against four pathogenic fungi as shown in **Table 6.3.4(a) and Fig.6.3.4(b).**

Table 6.3.4(a): Antifungal activities of methanol extract of root bark of *Croton caudatus* Geiseler against four fungal strains and the zone of inhibition with the standard antibiotic discs.

Name of micro-organism	Zone of inhibition of extract in mm				Zone of inhibition of standard in mm			
	0.5% (10^3µg/ disc) M±S.E	1% (10^3µg/ disc) M±S.E	5% (10^3µg/ disc) M±S.E	10% (10^3µg/ disc) M±S.E	NF (10^3µg/ disc) M±S.E	AM (300µg/ disc) M±S.E	Sc (5µg/ disc) M±S.E	Fu (10µg/ disc) M±S.E
Ca	6.±0*	6.5 ±0.25*	10.33±0.35	15.66±0.37	8±0	16±0.85*	16.66±1.0*	6.1±0.5
Tm	6.66 ±	7.5±.53	8±0.32	8.7±0.35	20.33±2.1	16.66±0.85*	12±1.5	8.66±0.35
Tb	7±0	8.66±0.65	20±0.55	25±1.5	21±2.0	7.35±0.35	7±0	16.33±0.3
Mg	--------	--------	---------	---------	8.4±1.5	16.3±1	14±1.0	7±0

Key:-NF-Nitrofurantoin, AM-Amikacin, Sc-Sparfloxacin, Fu-Fluconazole. *Ca-Candida albicans*, *Tm-Tricophyton mentagrophytes*, Tb-*Trichosporan beigelli*, Mg-*Microsporum gypsum*.

The increase in zone of inhibition (ZOI) was concomitant with the increase in the concentration of the extract [Table 6.3.4(a) and Fig.6.3.4(b)] which is in the line with the findings of ***Usman and Osuji***, ***2007.***

The control disc used for solvent had no ZOI, so their data were not shown. Data were represented in the form of mean of three tests ± S.E of the standard group, n=3, *P<0.001 as statistically significant value for the plant extracts at different concentrations compared with the standard antibiotic discs by using student *t-test.*

The highest activity was 25mm diameter of ZOI observed against *Tricophyton beigelli* [Fig.6.3.4(b) and white arrow, Fig.6.3.4(c)II] at the concentration of 10% (10^3µg/disc) followed by 20mm diameter of zone of inhibition observed against [Fig.6.3.4(b) and black arrow, Fig.6.3.4(c)II] of the same species at the concentration of 5% (10^3µg/disc).

The fact to be noted was that the highest activity at the concentration of 10% and 5% of the extract against *Tricophyton beigelli* were the exhibited activities [Table 6.3.4(a); Fig.6.3.4 (b) and black and white arrow, Fig.6.3.4(c) II] and found to be admirably higher than that of all the reference antibiotics. Further ZOI observed against *Candida albicans* by the extract at 10% and 5% concentration were comparable to Amikacin and Sparfloxacin and the activities at the same concentration were much better than Nitrofurantion and Flucolazone (aforesaid Table and Figures).

On the other hand, the lowest activity was 6mm diameter [Fig.6.3.4(b) and white arrow, Fig.6.3.4(c)VI] of ZOI found against *Candida albicans* at the concentration of 0.5% (10^3µg/disc) but show moderate activity as the concentration increases. The plant extract were found to be inactive against *Microsporum gypsum* [Fig.6.3.4(b) and black and white arrow, Fig.6.3.4(c) III] . Overall, the methanols extract of *Croton caudatus* Geiseler root bark showed significant activity against all the tested pathogenic fungi except *Microsporum gypsum*.

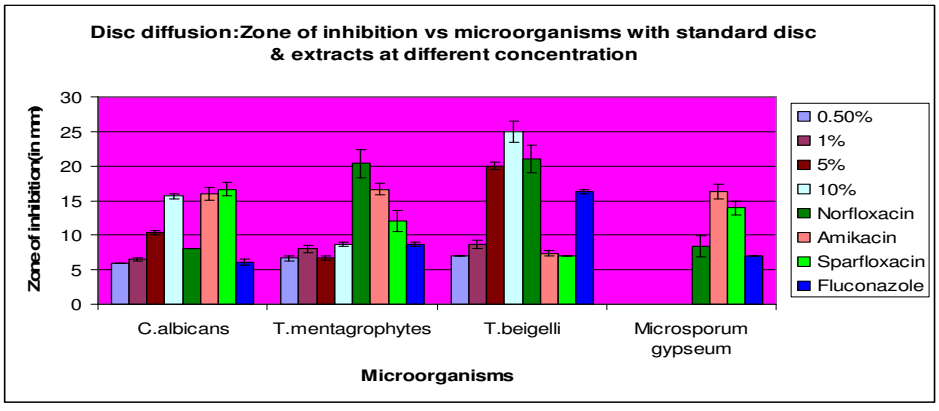


Fig 6.3.4(b): Graph showing the antifungal activity of root bark extract of *Croton caudatus Geiseler* extract at different concentrations against the four fungal strains and standard antibiotic discs.

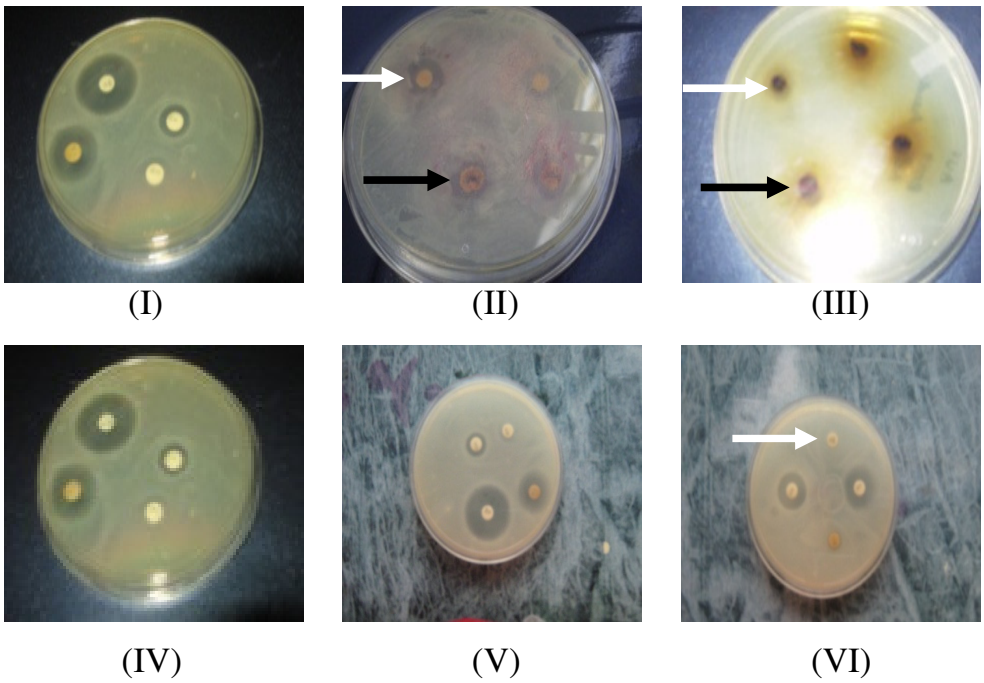


Fig. 6.3.4(c) Antifungal activity: Zone of inhibition shown by the root bark extract of *Croton caudatus* Geiseler against (I) Standard disc against *Microsporum gypsum*. (II) *Tricophyton beigelli* (III) *Microsporum gypsum*. (IV) Standard disc against *Tricophyton beigelli* (V) Standard disc against *Candida albicans* (VI) *Candida albicans.*

The preliminary qualitative screening of phytochemicals indicated the presence of alkaloids, flavonoids, glycosides, saponins, tannins, carbohydrades, triterpenoids etc. in the plant extracts**.** However, carbohydrades were not present in *Phyllantus acidus* (L.)Skeels.

The phytochemicals such as alkaloids, saponins, tannins and flavonoids are known to possess curative activity against various pathogens. Therefore, the presence of these chemicals could suggest the use of traditional treatment several types of illness (***Hassan et al., 2004 and Usman and Osuji, 2007***). The broad antibacterial and

antifungal activities of this extrat may be due to the presence of plant secondary metabolites available in the extract.

Micro-organism have been developing resistance to many antibiotics due to indiscriminate use of antimicrobial drugs. In addition, antibiotics are sometimes associated with the adverse effects. So, there is a need for alternative antimicrobial drugs for the treatment of infectious diseases,. Therefore, search for the lead from the locally available medicinal plants adorned with antimicrobial properties are to be carried out. The medicinal plants represent the rich source from which the noval antimicrobial and antifungal chemotherapeutic agents may be obtained ***(Rates, 2001).*** In this investigation, the methanolic extracts of both the plants showed antimicrobial activities.

The presence of tannins in the plant extracts may form irreversible complexes with proline rich proteins inhibiting the cellwall protein synthesis of microbes. This would explain the mechanisms of antimicrobial activity and the activity might be due to the individual susceptibility of the organisms to the plant extract also ***(Audu et al., 2000)***.

Plants can produce antifungal compound to protect themselves from biotic attack that would be essential for fungal resistance ***(Wojtaszek, 1997).***

Our results suggest a potential importance for use of active constituents from the plants as lead to develop new drug for treatment of antifungal and antibacterial infections.

However, further work is necessary to knit logic for explanations of the discussed facts.

6.3.5 Minimum Inhibitory Concentration (MIC) measurement:

6.3.5(a) Minimum Inhibitory Concentration of *Phyllantus acidus* (L.)Skeels:

The minimum inhibitory concentration (MIC) values of the extract against tested bacteria were shown in **Table 6.3.5(a)**. The MIC value for methanol extract of stem bark of *Phyllantus acidus* (L.)Skeels ranged from 3.78-500mg/ml. The lowest MIC value (3.78mg/ml) was recorded against *Staphylococcus aureus*.

Table 6.3.5(a): MIC of stem bark extracts of *Phyllantus acidus* (L.)Skeels against four pathogenic bacteria.

Marked No. of the test tubes	Nutrient broth medium added (ml)	Diluted solution (μg/ml)	Inoculums added (μl)	Bacterial growth observed against			
				E.coli	*Sa*	*Kp*	*Bs*
1	1	500	10	--	--	--	--
2		250	10	--	--	--	+
3	1	125	10	+	--	--	+
4	1	62.5	10	+	--	+	+
5	1	31.25	10	+	--	+	+
6	1	15.12	10	+	--	+	+
7	1	7.56	10	+	--	+	+
8	1	3.78	10	+	--	+	+
9	1	1.88	10	+	+	+	+
T_{ME}	1	500	10	--	--	--	--
T_{MI}	1	0	10	+	+	+	+
T_M	1	0	10	--	--	--	--

Key: '+' Indicates 'growth' '--' Indicates 'no growth', *Sa-Staphylococcus aureus, Kp-Klebsiella pneumonia, Bs-Bascillus subtilis.*

T_{MI} - Test tube containing media and innoculum.

T_M - Test tube containing media only.

T_{ME} - Test tube containing extract and media.

6.3.5(b) Minimum Inhibitory Concentration of *Croton caudatus* Geiseler:

The minimum inhibitory concentration (MIC) values of the extract against tested bacteria were shown in **Table 6.3.5(b)**. The MIC value for methanol extract of root bark of *Croton caudatus* Geiseler ranged from 31.25-250mg/ml. The lowest MIC value (31.25mg/ml) was recorded against *Klebsiella pneumonia*.

Table 6.3.5(b) MIC of root bark extract of *Croton caudatus* Geisler against four pathogenic bacteria.

Marked No. of the test tubes	Nutrient broth medium added (ml)	Diluted solution (μg/ml)	Inoculums added (μl)	Bacterial growth observed against			
				E.coli	*Sa*	*Kp*	*Bs*
1	1	500	10	–	–	–	–
2	1	250	10	–	–	–	–
3	1	125	10	–	–	–	+
4	1	62.5	10	+	+	–	+
5	1	31.25	10	+	+	–	+
6	1	15.12	10	+	+	+	+
7	1	7.56	10	+	+	+	+
8	1	3.78	10	+	+	+	+
9	1	1.88	10	+	+	+	+
T_{ME}	1	500	10	–	–	–	–
T_{MI}	1	0	10	+	+	+	+
T_M	1	0	10	–	–	–	–

Key:‘ +’ indicates growth,‘_’indicates no growth ,*Sa-Staphylococcus aureus*,*Kp-Klebsiella pneumonia*, *Bs-Bascillus subtilis.*

T_{MI} - Test tube containing media and innoculum.

T_M - Test tube containing media only.

T_{ME} - Test tube containing extract and media.

Fig.6.3.5(c): Photographs of the Minimum Inhibitory Concentration (MIC) assay of the plant materials of stem bark extract of *Phyllanthus acidus* (L)Skeels and root bark extract of *Croton caudatus* Geiseler.

6.4: Conclusion

It was concluded that both the methanolic extract of stem bark of *Phyllantus acidus* (L.)Skeels and root bark of *Croton caudatus* Geiseler demonstrates a strong activity against bacteria and fungi. The results of this investigation can be used in designing the folk medicines, suitable lead and finally drugs for possible treatment of many diseases including bacterial and fungal infections. However, to know the extact mechanism of action, isolation of more bioactive compounds are warranted.

6.5: Bibliography

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