JOURNAL OF PURE AND APPLIED MICROBIOLOGY, December 2012. Vol. **6**(4), p. 2017-2024

Antimicrobial Activity of Stem Bark of *Phyllanthus acidus* (L) Skeels and Root Bark of *Croton caudatus* Geiseler

Satya B. Paul and S. Saraju Devi*

Department of Chemistry, Assam University, Silchar - 788 010, India.

(Received: 08 April 2012; accepted: 07 May 2012)

The present study was conducted to evaluate the antibacterial and antifunal activities of methanol extract of root bark of *Croton caudatus* Geiseler and stem bark of *Phyllantus acidus* (L.) Skeels at different concentration by using agar disc diffusion method. The antibacterial activities were tested against some bacterial strains (*Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* and *Bascillus subtilis)* and the antifungal activities were tested against some fungal strains such as *Candida albicans, Tricophyton mentagrophytes, Trichosporan beigelli* and *Microsporum gypsum* respectively. The maximum activities were observed against all the tested organisms except *Bascillus subtilis, Pseudomonas aeruginosa* (bacterial strains) and *Microsporum gypsum* (fungal strains). The minimum inhibitory concentration (MIC) also determined by using tube dilution technoque. The MIC values for methanol extract of stem bark of *Phyllantus acidus* (L.) Skeels ranged from 3.78-500mg/ml and the lowest MIC value (3.78mg/ml) was recorded against *Staphylococcus aureus.*The MIC value of root bark of *Croton caudatus* Geiseler ranged from 31.25-500mg/ml and the lowest MIC value (31.25mg/ml) was recorded against *Klebsiella pneumonia.*

Key words: Antimicrobial activities, *Phyllantus acidus* (L.) Skeels, *Croton caudatus* Geiseler, Methanol extract, Minimum inhibitory concentration (MIC).

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. (Piddock and Wise, 1989; Singh *et al*., 1992; Mulligen *et al*., 1993; Davis, 1994; Robin *et al*., 1998).However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immune compromised, AIDS and cancer patients (Diamond, 1993).

The Otacheite gooseberry, *Phyllantus acidus*(L.)Skeels also called Malay gooseberry, Tahitian gooseberry, Country gooseberry, Star gooseberry, West India gooseberry, simply gooseberry tree, is one of the trees with edible small yellow berries fruits in the *Phyllanthaceae* family.Exhaustive literature survey showed that the plant is a good remedy for different types of ailments like emetic and purgative (Lemmens *et al*.,1999), hypertension and respiratory(Sausa *et al.,*2007), hepatoprotective (Lee *et al*., 2006), anti-diabetics. (Banik *et al*., 2010.),antinociceptive (Catapan *et al*., 2000).poisoning,coughs ,asthma and bronchitis ,poulticing and soles cathartic (Caius *et al.,* 2003), rehabilitation (Vongvanich *et*

* To whom all correspondence should be addressed.
E-mail: sarojmem@gmail.com

al., 2000), addiction (Mahidol *et al*., 2002),liver tonic, laxative, urticaria, eruptions and bronchial catarrh (Prasad D,1986). Sciatica, lumbago or rheumatism, sudorific and gonorrhea, skin disorders (Morton *et al*., 1987) .

Croton caudatus Geiseler belonging to the family of euphorbiaceae is a straggling shrub or woody climber. Most of the plant parts are used in traditional system of medicine. It forms an important Dai medicine in China. According to Kirtikar and Basu (1935), Chopra *et al*., (1956) and Caius (2003), the leaves are applied as a poultice to sprains. Burkill and Haniff (2002) reported that the leaves may be used for poulticing during fevers. Burkill and Haniff continue that a decoction of the root causes purging and so it is administered for constipation; and as purging may help fevers, it is used for them also. Colds are similarly treated. The plant has curative medicinal qualities for cancer, diabetes, malaria and indigestion etc. Leaves are claimed to have anticancer properties and people of Manipur region used to take juice of leaves (groups.yahoo.com, 458). In China, the stem and leaves which have been used for the treatment of malaria, ardent fever, convulsions, rheumatic and numbness (Jaingsu, 1975).

To overcome these problems many workers 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 & methanol extracts of stem bark of *Phyllantus acidus*(L.) Skeels.

MATERIALS AND METHODS

Preparation of the plant extract

The root bark of *Croton caudatus* Geisel and stem bark of *Phyllantus acidus*(L.) Skeels were subjected to shade drying for four weeks, removed and placed in a separate conical flask and extracted with soxhlet apparatus by using methanol solvent.

Making up extract solution

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.

Preparation of paper discs

The paper discs were prepared with the What-man No1. filter paper (5mm), 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 for1hr.The respective solvents are used as control.

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 from Defence Research Laboratory, Tezpur.

Known antibiotic discs activity against test organisms

For the antibacterial activity of some known antibiotics viz., Chloramphenicol 10µg/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 were used for the study of antifungal activity.

Muller Hinton Agar media was used for the 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 MIC determination of *Klebsiella pneumonia, Pseudomona aeruginosa, Escherichia coli* and *Bacillus subtilis* respectively.

Determination of antimicrobial activities of the plant 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 (5 mm 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 18 h 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.

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.

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 $p<0.001$

RESULTS AND DISCUSSIONS

Antibacterial activity of *Phyllantus acidus*(L.) Skeels

The highest activity of the plant extract was 10.5mm diameter of zone of inhibition found against *Klebsiella pneumonia* at the concentration of 10%(10^3µg/disc) followed by 10 mm diameter zone of inhibition found against *Escherichia coli* at the same concentration. On the other hand, the lowest activity of the plant extract was 6mm diameter of zone of inhibition observed against *Escherichia coli* at the concentration of 0.5 %(10^3µg/disc) (Table 1).

Antibacterial activity of *Croton caudatus* Geiseler

The highest activity of the plant extract was 19.5mm diameter of zone of inhibition found against *Klebsiella pneumonia* at the concentration of 10%(10^3µg/disc) followed by 19 mm diameter zone of inhibition at the concentration of 5%(µg/disc). On the other hand, the lowest activity of the plant extract was 6mm diameter of zone of inhibition observed against *Escherichia coli* at the

Table 1. Zone of inhibition shown by the stem bark extract of *Phyllantus acidus* (L.) Skeels against four pathogenic bacteria and compared the zone of inhibition with the standard antibiotic discs

Name of micro organisms	Zone of inhibition of extracts 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 (10^3µg/disc) M±S.E	S (10^3µg/disc) M±S.E	C (10^3µg/disc) M±S.E	NX disc) M±S.E
E. coli	6±0.33	6.5±0.33	7±0.33	10±0.33	6.3±0	10.2±0.88	15.0±1.2	8.33±0.33
S. aureus	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
K. pneumonia	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*
B. subtilis	-	-	-	-	7.5±1	10±1.5	9.7±0.33	7.0±0.33

Key:-T-Tetracycline, S- Streptomycin, C-Chloramphenicol, NX-Norfloxacin,

Note: The control disc used for solvent had no zone of inhibition, so there data was omitted from the above data. Data are represented in the form of mean of three tests±SEM of the standard group.n=3, *P<0.001as the plant extracts at different concentrations compared with the standard antibiotic discs by using Student's *t-test*.

concentration of 0.5%(10^3µg/disc).The plant extract were found to be inactive against *Pseudomonas aeruginosa* but almost moderately active against *Staphylococcus aureus* (Table 2).

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

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 s*taphylococcus aureus* (Table 3).

Minimum Inhibitory Concentration of *Croton caudatus* Geiseler

The MIC value for methanol extract of root bark of *Croton caudatus* Geiseler ranged from 31.25-500mg/ml. The lowest MIC value (31.25mg/ml) was recorded against *Klebsiella pneumonia* (Table 4).

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%,

Table 2. Zone of inhibition shown by the root bark extract of *Croton caudatus* Geiseler against four pathogenic bacteria and compared the zone of inhibition with the standard antibiotic discs

Name of micro organisms	Zone of inhibition of extracts 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 (10^3µg/disc) M±S.E	S (10^3µg/disc) M±S.E	C (10^3µg/disc) M±S.E	NX 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
S. aureus	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
K. pneunmonia	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*
P. aeruginosa	-	-	-	-	7.33±1.0	15.33±1.4	13±1.0	6±0

Key: T-Tetracycline, S- Streptomycin, C-Chloramphenicol, NX-Norfloxacin,
Note: The control disc used for solvent had no zone of inhibition, so there data was omitted from the above data. Data are represented in the form of mean of three tests±S.E of the standard group.n=3,*P<0.001as the plant extracts at different concentrations compared with the standard antibiotic discs by using Student's t-test.

Table 3. MIC of stem bark extracts of *Phyllantus acidus* (L.)Skeels againstfour pathogenic bacteria

Marked No. of the test tubes	Nutrient broth medium (ml)added	Diluted solution (µg/ml)	Inoculums added (µl)	Bacterial growth observed against			
				E. coli	*S. aureus*	*K. pneumonia*	*B. subtilis*
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_{MC}	1	500	10	-	-	-	-
T_{MI}	1	0	10	+	+	+	+
T_{M}	1	0	10	-	-	-	-

'+' Indicates 'growth' '-' Indicates 'no growth',

1%,5% and 10% of 10^3µg/disc against four pathogenic fungi (Table 5).

The highest activity was 14.66mm diameter of zone of inhibition observed against *Candida albicans* at the concentration of 10%(10^3µg/disc) followed by 13.5mm diameter of zone of inhibition observed against *Trichosporan beigelli* at the same concentration.

On the other hand, the lowest activity was 7mm diameter of zone of inhibition found against *Tricophyton mentagrophytes* 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*. Overall, the methanol extract of *Phyllantus acidus* (L.) Skeel stem bark showed significant activity against all the tested pathogenic fungi except *Microsporum gypsum*.

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 (Table 6).

Table 4. MIC of stem bark extracts of *Phyllantus acidus* (L.)Skeels againstfour pathogenic bacteria

Marked No. of the test tubes	Nutrient broth medium (ml)added	Diluted solution (µg/ml)	Inoculums added (µl)	Bacterial growth observed against			
				E.coli	*S. aureus*	*K. pneumonia*	*B. subtilis*
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_{MC}	1	500	10	-	-	-	-
T_{MI}	1	0	10	+	+	+	+
T_M	1	0	10	-	-	-	-

'+' indicates growth,'_'indicates no growth

Table 5. Antifungal activity of methanol extract of stem bark of *Phyllantus acidus* (L.) Skeels against four fungal strains and compared the zone of inhibition with the standard antibiotic discs

Name of micro organisms	Zone of inhibition of extracts 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 (10^3µg/ disc) M±S.E	Sc (10^3µg/ disc) M±S.E	Fu disc) M±S.E
C. albicans	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
T. mentagrophytes	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
T. beigelli	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*
M. gypsum	-	-	-	-	9.5±0.35	10±3.3	15±0.33	9±0.15

Key:-NF-Norfloxacin, AM- Amikacin, S-Sparfloxacin, Fu-Fluconazole

Note: The control disc used for solvent had no zone of inhibition, so there data was omitted from the above data. Data are represented in the form of mean of three tests±S.E of the standard group. n=3.*P<0.001as the plant extracts at different concentrations compared with the standard antibiotic discs by using Student's *t-test*

Table 6. Antifungal activities of methanol extract of root bark of *Croton caudatus* Geiseler against four fungal strains and compared the zone of inhibition with the standard antibiotic discs

Name of micro organisms	Zone of inhibition of extracts 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 (10^3µg/ disc) M±S.E	Sc (10^3µg/ disc) M±S.E	Fu disc) M±S.E
C. albicans	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
T. mentagrophytes	6.66 ± 0.35*	7.5±.53	8±0.32	8.7±0.35	0.33±2.1	.66±0.85*	12±1.5	8.66±0.35
T. beigelli	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
M. gypsum	-	-	-	-	8.4±1.5	16.3±1	14±1.0	7±0

Key:-NF-Nitrofurantoin,AM-Amikacin,Sc-Sparfloxacin,Fu-Fluconazole.
Note: The control disc used for solvent had no zone of inhibition, so there data was omitted from the above data.Data are 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 *t-test.*

The highest activity was 25mm diameter of zone of inhibition observed against *Tricophyton beigelli* at the concentration of 10 %(10^3µg/disc) followed by 20mm diameter of zone of inhibition observed against at the same species at the concentration of 5 %(10^3µg/disc).

On the other hand, the lowest activity was 6mm diameter of zone of inhibition 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*.

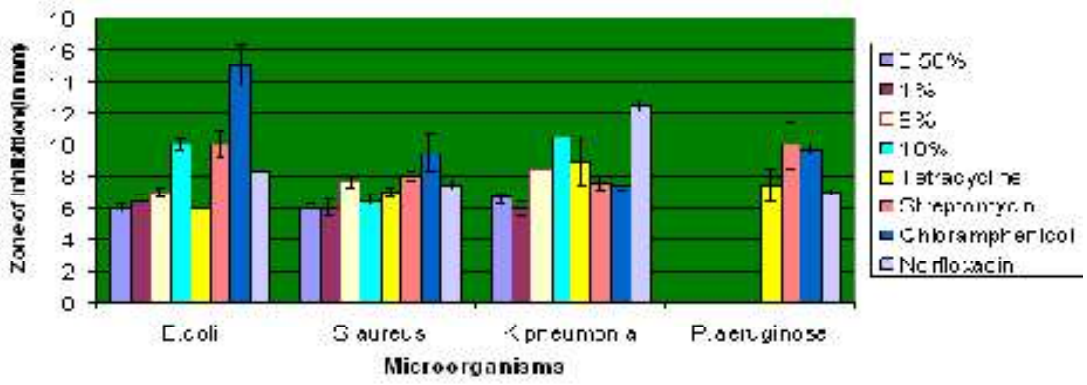

Fig. 1. Antibacterial activity of stem bark extract of *Phyllantus acidus* (L.) Skeels at different concentrations against four bacterial strains and standard antibiotic discs

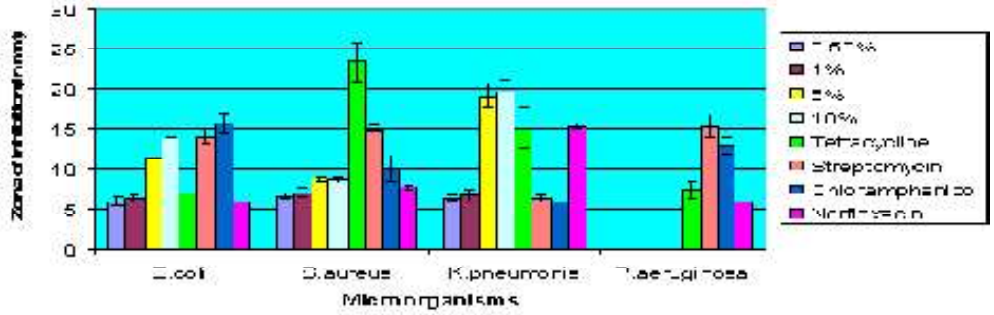

Fig. 2. Antibacterial activity of root bark extract of *croton caudatus* Geiseler at different concentrations against four bacterial strains and standard antibiotic discs

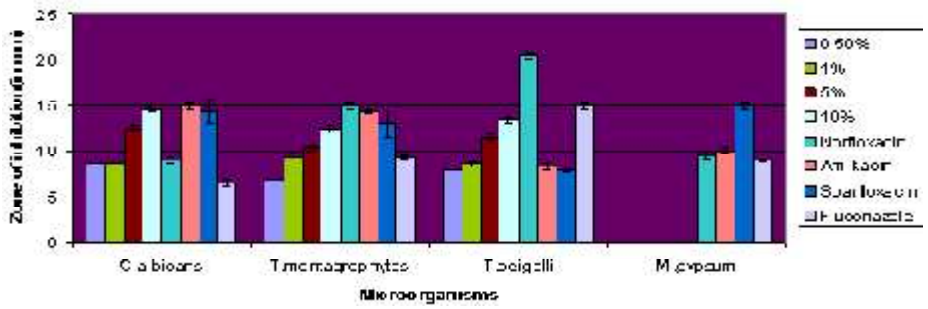

Fig. 3. Antifungal activity of stem bark extract of *Phyllantus acidus* (L.) Skeels extractat at different concentrations against the four fungal strains and standard antibiotic discs

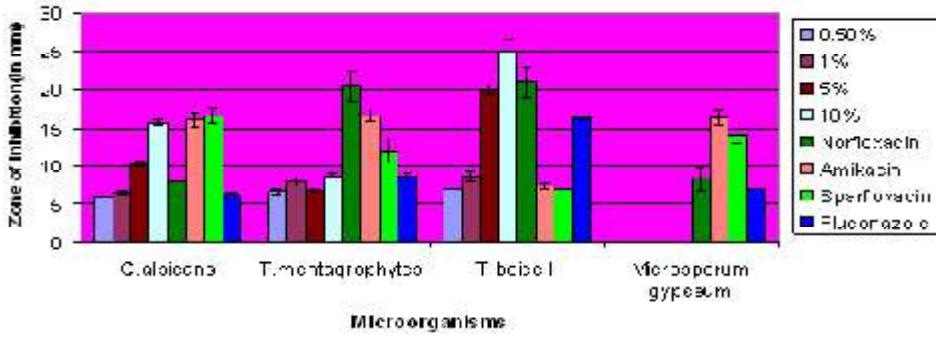

Fig. 4. Antifungal activity of root bark extract *Croton caudatus* Geiseler extract at different concentrations against the four fungal strains and standard antibiotic discs

Overall, the methanols extract of *Phyllantus acidus* (L.) Skeel stem bark showed significant activity against all the tested pathogenic fungi except *Microsporum gypsum.*

From the above discussions, it can be concluded that both the methanol extract of stem bark of *Phyllantus acidus* (L.) Skeels and root bark of *Croton caudatus* Geiseler demonstrates a strong activity against both bacteria and fungi. This investigation can be used in the folk medicine and source of antimicrobial substances for possible treatment of many diseases including bacterial and fungal infections. However, to know the extract mechanism of action, further studies with purified fractions/ bioactive compounds are warranted.

ACKNOWLEDGMENTS

The authors are thankful to the Principal of Silchar Medical College & Hospital, India for providing laboratory support and also thank Head of the department of Microbiology, Dr.Atanu Chakraborty and Dr.Samrat Biswas (Demonstrator) for technical help.

REFERENCES

1. Piddock, K.J.V., Wise, R.Mechanisms of resistance toquinolones and clinical Perspective.*Journal of Antimicrobial Chemotherapy,* 1989; **23:**475–483.
2. Singh, M., Chaudhry, M.A., Yadava, J.N.S., Sanyal, S.CThe spectrum of antibiotic resistance in human and veterinary isolates of *Escherichia coli* collected from 1984–1986.in Northern India. *Journal of Antimicrobial Chemotherapy*, 1992; **29**:159–168.
3. Mulligen, M.E., Murry-Leisure, K.A., Ribner, B.S., Standiford, H.C., John, J.F., Karvick, J.A., Kauffman, C.A., Yu, V.L.Methicillin resistant *Staphylococcus aureus. American Journal of Medicine*.1993; **94**: 313–328.
4. Davis, J.Inactivation of antibiotic and the dissemination of resistance genes. *Science,* 1994; **264**:375–382.
5. Robin, E.H., Anril, W., Alexander, M., Loeto, M., Keith, K.Nasopharyngeal carriage and antimicrobial resistance in isolates of *Streptococcus pneumoniae* and *Heamophilus influenzae* Type b in children under 5 years of age in Botswana. *International Journal of*

Infectious Diseases.1998; **3** (1*)*: 18–25.

6. Diamond, R.DThe growing problem of mycoses inpatients infested with human Immune deficiency virus. *Review of Infectious Diseases,* 1993;**13**: 480–486.
7. Lemmens,R.H., Bunyapraphatsara, M.J. Padua de,L.SPlant Resources of South- East Asia No12 (1).Medicinal and Poisonous Plants 1.Prosea Foundation,Bogor, Indonesia. 1999; pp386-387.
8. Sousa,M., Ousingsawat, J., Seitz, R., Puntheeranurak, S., Regalado, A.,Schmidt, A., Grego, T., Jansakul , C.,Amaral, M.D ., Schreiber, R.,Karl,. K.K .An Extract From the Medicinal Plant *Phyllanthus acidus* and Its Isolated Compounds Induce Airway Secretion:A Potential Treatment for *Cystric Fibrosis.Mol.Pharmacol.* 2007; **7**(1):336-337.
9. Lee,C.,Peng,Y.,Cheng,W.H.,Cheng, H.Y.,Lai, F.N.M.T.,and Chiu, T. *Hhepatoprotective Effect of Phyllanthus in Taiwan on Acute Liver Damage Induced by Carbon Tetrachloride.Am J.Chin.Med.,*2006;**30**(3):471-482.
10. Banik,G.,Bawari,M.,Dutta Choudhury, M.Choudhury, S., and Sharma, G.DSome Anti-Diabetic Plants of Southern Assam.Assam University Journal of Science&Technology: *Biological and Environmental Sciences*. 2010; **5** (1):114-119.
11. Catapan,E., M.Otuki, A.M.Viana, R.A.Yunes, L.F.Bresciani, J.Ferreira, A.R.Santos, J.B.Calixto and Cechinel-Filho,V.Pharmacological Activity and Chemical Composition of Callus Cultures Extracts from Selected Species of *Phyllanthus. Pharmazie*. 2000; **55**(12):945-946.
12. Caius, J.F.The *Medicinal and Poisonous Plants of India*. Scientific Publishers, Jodhpur- India. 2003; p528.
13. Vongvanich, N., Kittakoop, P., Kramyu, J., Tanticharoen, M., The btaranonth, Y. Phyllanthusols A and B, Cytotoxic Norbisabolane Glycosides from *Phyllanthus acidus Skeels.J.Org.Chem.,* 2000; **65**:5420-5423.
14. Mahidol, C., Prawat, H., Prachyawarakorn, V., Ruchirawat, S. Investigation of Some Bioactive *Thai Medicinal Plants Phytochemistry Reviews*. 2002; **1:** 278-297.
15. Prasad, D.Edible fruits and vegetables of the English speaking Caribbean. Caribbean Food and Nutrition Institute.Kingston, Jamaica.1986.
16. Morton,J.,Julia ,F., Morton, Miami, F.L. Otaheite Gooseberry.In:Fruits of warm climates. 1987; p 217–219.
17. Kirtikar, K.R., Basu, B.D. Indian Medicinal Plants,4 vols.Bisen Singh, Mahendra Pal Singh, Dehra Dun,India (Reprinted).1935.
18. Chopra, R.N., Nayer, S.L. Chopra, I.C..Glossary of Indian Medicinal Plants. CSIR, New Delhi (India).1956.
19. Burkill, I.H.,Mohammed Haniff,.A Botanical and Linguistic Update of Malay Village. 2002.
20. http//groups.yahoo.com/group/thangkhal/message/458.
21. Jiangsu New Medical College. A Dictionary of Traditional ChineseMedicine, 1stedn.*Shanghai Science and Technology: Shanghai.* 1975. p746.
22. Akinpelu, D.A.,Onakoya, T.M. Antimicrobial activities of medicinal plants used in folklore remedies in south-western, *African J.Biotech*. 2006;**5**(11):225.
23. Khan, A., Rahman, M., Islam, S. Antibacterial,antifungalandCytotoxic activities of Tuberous Roots of *Amorphophallus campanulatus Turk.J.Biol*. 2007;**31** :167-172.
24. Dash,S.L., Nath, K.and Bhise, SAntioxidant and antimicrobial activities of *Heracleum nepalense* D. *Don root. Trop. J. Pharm. Res*. 2005; **4**: 341-347.
25. Washington, J.A., Wood, G.L. Antimicrobial susceptibilitytests.Dilution and Disc Diffusion methods.In: *Manual of Clinical Microbiology*. 1995; 1327-1331.