ANNEXURE: IV

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AN OVERVIEW ON *Cicca acida (Phyllanthus acidus)*

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Abstract

An attempt was made to explore the ethnobotanical, economical and biological importance of Cicca acida or Phyllanthus acidus. The plant is used for 28 types of remedies like cathartic, emetic, coughs, hypertension, asthma, skin diseases etc and as a food in the raw form. In India, it is found in different states including Maharastra, Assam, Manipur, Tamilnadu and South Indian states as home garden ornaments. The paper reviews the data related to scientific works carried out with the plant and listed the bioactive compounds isolated from the plant till date. Based on the review made, present paper highlights the need of future research with Cicca acida or Phyllanthus acidus so that more active principle for treating new ailments can be isolated and made available from the plant.

Key words: *Cicca acida*, Ethno medicinal, Hypertension, Ornament.

Introduction

Terrestrial plants especially higher plants have a long history of use in the treatment of human diseases. Several well-known species, including *Papaver somniferum* ,*Glycyrrhiza glabra* myrrh(Commiphora species),were referred to by the first known written record on clay tablets from Mesopotamia in 2600 BC and these plants are still in use today for the treatment of various diseases as ingredients of official drugs or herbal preparations used in system of traditional medicine (Newman et al., 2000). This study highlights the use of terrestrial medicinal plant in the traditional medical practices of the people especially those used in treatment of cathartic, emetic, coughs, hypertension, asthma and skin diseases etc.

The Otacheite gooseberry, *Phyllanthus acidus* Skeels (syns.*P.distichus* Muell. Arg.; *Cicca acida* Merr.; *C.disticha* L.), 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. Despite its name, the plant does not resemble the gooseberry except for the acidity of its fruits. This is a curious and ornamental shrub or tree, 61/2 to 30 ft (2-9 m) high, with spreading, dense, bushy crown of thickish, rough, main branches, in general aspect resembling the Bilimbi. At the branch tips are clusters of deciduous, greenish or pinkish branchlets 6 to 12 in (15-30 cm)long, bearing alternate, short-petioled, ovate or ovate-lanceolate pointed leaves 3/4 to 3 in (2-7.5 cm) long, thin green and smooth on the upper surface, blue-green with a bloom on the underside; altogether giving the impression of pinnate leaves with numerous leaflets. There are 2 tiny, pointed stipules at the base of each leaf. Small, male, female and some hermaphrodite,4-parted,rosy flowers, are borne together in little clusters arranged in panicles 2 to 5 in (5-12.5 cm) long, hanging directly from leafless lengths of the main branches and the upper trunk, and the fruits develop so densely that they form spectacular masses. The fruit is oblate with 6 to 8 ribs, 3/8 to 1 in (1-2.5 cm) wide, pale-yellow to nearly white when fully ripe, waxy, fleshy, crisp juicy and highly acidic. Tightly embedded in the center is a hard, ribbed stone containing 4 to 6 seeds(Morton et al., 1987).

The Botanical Classification of the Plant

Kingdom...Plantae
Division.....Mgnoliophyta
Class.....Mgnoliopsida
Order.....Mglgiphiales
Family.....Phyllanthaceae
Tribe.....Phyllanthaceae
Subtribe.....Flueggeinae
Genus.....?hyllanthus
Species.....*Phyllanthus acidus.*

Other scientific names are

Cicca disticha Linn.
Cicca acida Linn
*Averrhoa acida*Linn.
Cicca acidissima Blanco
Phyllanthus distichus Muell.-*Arg.*
Phyllanthus acidissimus Muell.-*Arg.*
Phyllanthus acidus Skeels

This species is believed to have originated in Madagascar and to have been carried to the East Indies. Quisumbing (Morton et al., 1987) says that it was introduce, into the Philippines in prehistoric times and is cultivated throughout those islands but not extensively. It is more commonly grown in Indonesia, South Vietnam and Laos, and frequently in northern Malaya, and in India in home gardens. The tree is a familiar one in villages and on farms in Guam, where the fruit is favored by children, and occurs in Hawaii and some other Pacific Islands. It was introduced into Jamaica from Timor in 1793 and has been casually spread throughout the Caribbean islands and to the Bahamas and Bermuda. It has long been naturalized in southern Mexico and the lowlands of Central America and is occasionally grown in Colombia, Venezuela, Surinam, Peru and Brazil. Formerly an escape from cultivation in South Florida, there are now only scattered specimens remaining here as curiosities. The tree prefers hot, humid tropical low land up to 1000m altitude (Morton et al.,1987).

Ethnomedicinal Utility of *Cicca acida (Phyllanthus acidus)*

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), psoriasis (Burkill et al., 2002),anti-diabetics. (Banik et al., 2010), antinociceptive (Catapan et al., 2000). poisoning (Caius et al., 2003), coughs (Caius et al., 2003), asthma and bronchitis (Caius et al., 2003), poulticing and soles (Caius et al., 2003), cathartic (Caius et al., 2003), rehabilitation (Vongvanich et al., 2000), addiction (Mahidol et al., 2002), liver tonic (Prasad D, 1986), laxative (Prasad D, 1986), urticaria (Prasad D, 1986), eruptions & bronchial catarrh (Prasad D, 1986). sciatica (Morton et al., 1987), lumbago or rheumatism (Morton et al., 1987), sudorific & gonorrhea, skin disorders (Morton et al., 1987).

Biological Utility of *Cicca acida (Phyllanthus acidus)*

The plant is considered as an ornamental plant. In Malaya, the ripe or unripe fruits of *Cicca acida* is cooked and served as a relish or made into a thick syrup or sweet preserve. It is also combined with other fruits in making chutney and jam because, it helps these products to "set". Often, the fruits are candied, or pickled in salt. In the Philippines, they are used to make vinegar. In Indonesia, the tart flesh is added to many dishes as a flavoring agent. The juice is used in cold drinks in the Philippines (Morton et al., 1987). Young leaves are cooked as a vegetable in Indonesia, Thailand and India (Prasad D, 1986). The root bark is used in India as tanning agent. (Rizk, 1987). The tree is used as fuel wood. The wood is fairly hard, strong, tough and durable if seasoned. The seasoned wood is used for making utensils and other small objects. (Mackeen et al.,1997), (Rizk,1987). The energy value of the edible portion of *Cicca acida* (per 100gm) (Table -1) (Morton et al., 1987).

Fig:1(A) *Cicca acida* fruits

Fig: 1(B) *Cicca acida* twig with leaves & fruits.

Moisture	91.9 g
Protein	0.155g
Fat	0.52 g
Fiber	0.8 g
Ash	0.51 g
Calcium	5.4 mg
Phosphorus	17.9 mg
Iron	3.25 mg
Carotene	0.019mg
Thiamine	0.025mg
Riboflavin	0.013mg
Niacin	0.292mg
Ascorbic Acid	4.6 mg

Table-1. Food Value Per 100 g of Edible Portion.

The Scientific Work carried with *Cicca acida (Phyllanthus acidus)*

1. Methanolic extracts of 79 Malaysian plants were assessed for antinematodal activity against *Bursaphelenchus xylophilus*. *Cicca acida* showed strong antinematodal activity. (Muhammad et al., 1997).
2. Removal of Brill Red 5B from an aqueous solution using *Cicca acida* biomass. (Karthik et al., 2009).
3. Effect of Auxin and Cytokinin on Phyllanthusol A Production by Callus Cultures of *Phyllanthus acidus* Skeels. (Duangporn et al., 2009)
4. Rat fed with the extracts from *P.acidus* showed a hepatoprotective effect against acute liver damage induced by carbon tetrachloride. (Lee et al., 2006).
5. Methanolic extracts of *P.acidus* possess strong antibacterial activity *in vitro*. (Melencez et al., 2006).
6. An extract from the medicinal plant Phyllanthus acidus and its isolated compounds induce airway chloride secretion: A potential treatment for cystic fibrosis (Sousa et al., 2007).
7. Selective Antimicrobial properties of *Phyllanthus acidus leaf* extract against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* using Stokes Disc diffusion, Well diffusion, Streak plate and a dilution method (Jagessar et al., 2008).
8. Antibacterial properties of tropical plants from Puerto Rico: In the study, *Phyllanthus acidus* or *Cicca acida* was one of the plants that showed the highest antibacterial activity against *E-coli* and *Staphylococcus aureus* (Melendez et al., 2006).

Compounds Isolated from *Cicca acida (Phyllanthus acidus)*

(1) Phyllanthusols A and B, Aglycon. Saccharide has been isolated from the MeOH extract of the roots of *Cicca acida*.

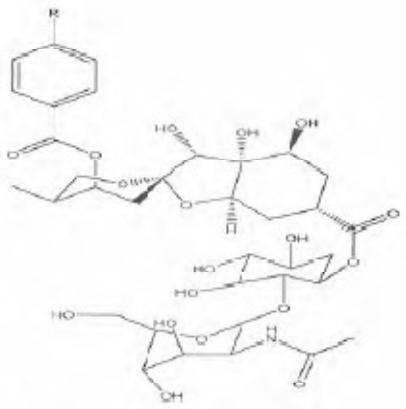

Fig: 2.- 1. Phyllanthusol A, R = OH.
2. Phyllanthusol B, R = H.

Phyllanthusol A and B has been isolated from *Cicca acida* (*Phyllanthus acidus*), has been proposed as possible antitumor agent (Mahidol et al., 2002). Phyllanthusol A and B has attracted considerable attention as it exhibits cytotoxicity against BC and KB cell lines in vitro.

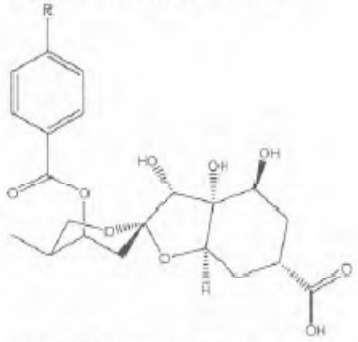

Fig. 3:-The norbisabolane aglycon
The norbisabolane skeleton is rare in nature

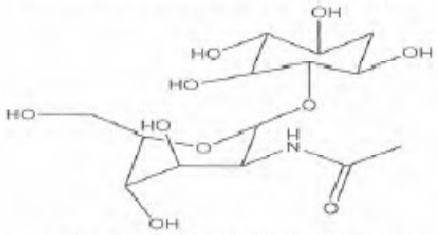

Fig. 4: - The Saccharide Structure
The norbisabolane aglycon and the Saccharide showed no cytotoxicity

Future Research Needs with *Cicca acida (Phyllanthus acidus)*

Some research work like antinociceptive, hypertension and respiratory ailments, antibacterial, hepatoprotective, antinematodal, cyctic fibrosis, antimicrobial activity of leaves and anticancer activity have been carried out with *Cicca acida*. The triterpenoids Phyllanthusols A and B,?-amyrin and Cytotoxic Norbisabolane glycosides have been isolated from *Cicca acida*. In traditional medicine, it is used in the treatment of different types of aliments but very few biological activities have been undertaken to prove the traditional claims. So, as far as isolation of bioactive principles is concerned, only four compounds have so far been isolated from the plant. Keeping the traditional potentials of the plant in mind following researches may be undertaken with *Cicca acida (Phyllanthus acidus)*.

1. Hepatoprotectivity and Neurotoxicity of the stem bark of the plant and isolation of active compounds from the plants.
2. The plant may be evaluated for its use in asthma and bronchitis, poulticing &soles and skin disorders.
3. Extractives from the plant may be evaluated for it's use in cathartic activity.
4. Antinociceptive and anti-diabetic activities of the plant by phytochemical and biological screening methods.
5. Laxative and urticaria activitities of the plant and its related compounds may be studied.
6. Sciatica and lumbago or rheumatism activities of the plant and its related compounds on the animals.
7. Sudorific & gonorrhea, eruptions & bronchial catarrh properties of the plant by observing chemical and biological entities of the plant.
8. Antimicrobial activities of the stem bark of the plant by phytochemical and biological Screening methods.

References

Burkill, I.H. and Mohammed H. (2002). A Botanical and Linguistic Update of Malaya Village.

Caius, J.F. (2003). The Medicinal and Poisonous Plants of India. Scientific Publishers, Jodhpur. (India).

Catapan, E., Otuki, M., Viana, A.M., Yunes, R.A., Bresciani, L.F., Ferreira, J., Santos, A.R., Calixto, J.B. and Cechinel-Filho, V. (2000). Pharmacological Activity and Chemical Composition of Callus Cultures Extracts from Selected Species of *Phyllanthus*. Pharmazie. 55(12):945-946.

Duangporn, P and Siripong, P. (2009). Effect of Auxin and Cytokinin on Phyllanthusol A Production by Callus Cultures of *Phyllanthus acidus* Skeels. *American-Eurasian J.Agric &Environ.Sci.* 5(2):258-263.

Banik, G., Bawari, M., Dutta Choudhury, M., Choudhury, S. and Sharma, G.D. (2010). Some Anti-Diabetic Plants of Southern Assam. *Assam University Journal of Science &Technology: Biological and Environmental Sciences*. 5(1): 114-119.

Jagessar, R.C., Marsa, A. and Gomes, G. (2008). Selective Antimicrobial properties of *Phyllanthus acidus leaf* extract against *Candida albicans, Escherichia coli* and *Staphylococcus aureus* using Stokes Disc diffusion, Well diffusion, Streak plate and a dilution method. *Nature and Science*. 6:23-38..

Karthik, V. V., Dharmendira, M. K. and Renganathan, S. (2009). Removal of Brill Red 5B from an aqueous solution using *Cicca acida* biomass. 87(4):945-946.

Lee,C., Peng, Y., Cheng, W.H., Cheng, H.Y., Lai, F.N.M.T. and Chiu, T.H. (2006). Hepatoprotective Effect of *Phyllanthus* in Taiwan on Acute Liver Damage Induced by Carbon Tetrachloride. *Am. J.Chin.Med*. 30(3):471-482.

Lemmens, R.H., Bunyapraphatsara, M.J. and Padua De, L.S. (1999). Plant Resources of South-East Asia No12(1). Medicinal and Poisonous Plants 1.Prosea Foundation, Bogor, Indonesia. pp.386-387.

Mahidol, C., Prawat, H., Prachyawarakorn, V. and Ruchirawat, S. (2002). Investigation of Some Bioactive Thai Medicinal Plants. *Phytochemistry Reviews*. 1:278-297.

Melendez, P.A. and Capriles, V.A. (2006). Antibacterial Properties of Tropical Plants from Puerto Rico. *Phytomedicine*. 13(4):272-6.

Morton, J., Julia, Morton, F., Miami, F.L. (1987). Otaheite Gooseberry. In:Fruits of warm climates.pp. 217–219.

Muhammad, M. Mackeen. (1997). Antinematodal activity of some Malaysian plant extracts against the pine wood nematode,Bursaphelenchus xylophilus. Pesticide Science. 51(2):165-170.

Newman, D.J., Cragg, G.M., Snader, K.M. (2000). The influence of natural products upon drug discovery. *Nat Prod Rep*. 17:215-234.

Prasad, D.(1986). Edible fruits and vegetables of the English speaking Caribbean.Caribbean Food and Nutrition Institute. Kingston, Jamaica.

Rizk, A.F.M. (1987). The chemical constituents and economic plants of the Euphorbiaceae. *Botanical Journal of the Linnean Society*. 94(1-2):293-326.

Sousa, M., Ousingsawat,J., Seitz, R., Puntheeranurak, S., Regalado, A., Schmidt, A., Grego, T., Jansakul, C., Amaral, M.D., Schreiber, R. and Karl, K.K. (2007). An Extract From the Medicinal Plant *Phyllanthus acidus* and Its Isolated Compounds Induce Airway Secretion:A Potential Treatment for Cystric Fibrosis. *Mol. Pharmacol*. 7 (1):336-337.

Vongvanich, N., Kittakoop, P., Kramyu, J., Tanticharoen, M. and Btaranonth, Y. (2000). Phyllanthusols A and B, Cytotoxic Norbisabolane Glycosides from *Phyllanthus acidus* Skeels. *J.Org.Chem*. 65:5420-5423.

Antimicrobial and Phytochemical Analysis of *Croton caudatus* Geiseler

S. B.Paul and S.Saraju Devi

Abstract

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. Hence, a study was conducted to determine the phytochemical, and antimicrobial activities of the methanolic extract of *Croton caudatus* Geiseler root bark against both bacteria and fungi using the disc diffusion method. The extract exhibited pronounced antibacterial activities against *Escherichia coli, Stapphylococcus aureus* and *Klebsiella pneumonia* but no significant effect on *Pseudomonas aeruginosa,* as compared to the standard discs.

The minimum inhibitory concentration (MIC) values of the extract against these bacteria ranged from 62.5 to 250µg/ml. The antifungal activities were found against *Candida albicans, Tricophyton mentagrophytes, Trichosporan beigelli* but has no effect on *Microsporum gypsum*. The phytochemical screening revealed the presence of saponins, steroids, flavonoids, resins, triterpenoids, and carbohydrates in varying concentration. This research work supports the local use of roots bark of the *croton caudatus* Geiseler for prophylatic and therapeutic purpose against bacterial and fungal infection.

Key words: *Croton caudatus* Geiseler, Phytochemical analysis,Antimicrobial activity, Saponins.

Introduction

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. 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 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.

Croton caudatus Geiseler belonging to the family of euphorbiaceae is a straggling shrub or woody climbe. Most of the plant parts are used in traditional system of medicin. 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. The plant has curative medicinal qualities for canecr, diabetes, malaria and indigestion etc. In China, the stem and leaves which have been used for the treatment of malaria, ardent fever, convulsions, rheumatic and numbness (Jaingsu,1975).Hence, the study has been made to investigate the phytochemical and antimicrobial screening of the *Croton caudatus* Geiseler root bark.

Materials and Methods

Collection of Plant Material

Plant materials were collected from Pochou village of Jiribam, Manipur. The plant was taxonomically identified and authenticated by the Botanical Survey of India, Howrah and the voucher specimen (CHN/I-I/(301) /2009/Tech.II/343) was retain in our laboratory for future references.

Preparation of Plant Material

The dried root barks were grind into a coarse powder. The powder was extracted with Methanol by using soxhlet apparatus and filtered through Whatman filter paper no 1 and dried to obtain the crude extracts which was used for further study.

Phytochemical Screening

The crude extracts of root bark thus obtained were subjected to preliminary phytochemical screening following the methodologies of Harbone (1998), Sofowora (1993) and Ngbege (2008).

Collection of Microbes

Antibacterial activity and minimum inhibitory concentration (MIC) were determined against *Stapphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia.* All the organisms were collected from Microbiology Laboratory of Silchar Medical College and Hospital. Antifungal screening was carried out against four fungi (*Candida albicans, Tricophyton mentagrophytes, Trichosporan beigelli and Microsporum Gypsum*).These organisms were collected from Microbiology Laboratory of Defence Research Laboratory, Tezpur.

Antimicrobial (Antibacterial and Antifuhgal) Screening

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 bacterial 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 antibacterial 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 et al, 1995).In this technique the tubes of broth medium, containing graded doses of extract are inoculated with the test organisms at 37°C 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.

Results and Discussion

Phytochemical of the Plant Extracts

Phytochemical analysis of methanolic extract reveals that Saponins, Steroids, Flavonoids, Triterpenoids, Resins and Carbohydrates are present. Table1. shows the phytochemical screening results of methanolic extracts of the plant *Croton caudatus* Geiseler used in this study.

Table1.Qualitative analysis of methanol extract of root bark of *Croton caudatus* Geisele.

Serial no	Chemical constituents	Methanol extract
1	Alkaloids	-ve
2	Tanins	-ve
3	Saponins	+ve
4	Steroids	+ve
5	Terpenoids	-ve
6	Flavonoids	+ve
7	Phlobatinins	-ve
8	Cardic glycosides	-ve
9	Triterpenoids	+ve
10	Anthraquinones	-ve
11	Amino acids	-ve
12	Acids	-ve
13	Resins	+ve
14	Sterols	-ve
15	Carbohydrates	+ve
16	Reducing sugar	-ve
17	Phenols	-ve

+ve indicates presence of chemical constituents and –ve indicates absence of chemical constituents

Antimicrobial (Antibacterial and Antifungal) Activity

The results representing antibacterial activity of methanol extract of root bark of *Croton caudatus* Geiseler is shown in Table 2. The highest activity of plant extract was 19.5mm diameter of zone of inhibition found against *Klebsiella pneumonia* at 10% of 10^3 µg/disc followed by 19 mm diameter of the zone of inhibition of the same species at the concentration of 5% of 10^3 µg/disc. On the left hand, the lowest activity of plant extract was 6.0mm diameter of zone inhibition observed against *E. coli* at 0.5% of 10^3µg/disc. In comparison with the standard antibiotics such as Tetracycline (30 µg/disc), Streptomycin (10 µg/disc),Chloramphenicol (10 µg/disc)and Norfloxacin(10 µg/disc),the methanolic extract of *Croton Caudatus* Geiseler root showed significant antibacterial activity at 10^3 µg/disc at different concentrations.

Table 2. Antibacterial activity of methanol extract of *Croton caudatus* Geiseler root bark using the disc diffusion method.

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 (10µg/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	21.33±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	15.33±0.3*
Pa	---------	---------	---------	---------	7.33±1.0	15.33±1.4	13±1.0	6±0

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

Sa-Staphylococcus aureus,Kp-Klebsiella pneumonia,Pa-Pseudomonas aeruginosa.

Note: The control disc used for solvent excised no zone of inhibition. 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*.

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 3).The highest activity was 25mm diameter of zone of inhibition observed against *Tricophyton beigelli* of 10% of 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% Of 10^3µg/disc but has no effect on *Microsporum gypsum*. Overall, the methanol extract of root bark of *Croton caudatus* Geisel showed significant activity against all the tested pathogenic fungi except *Microsporum gypsum*.

Table 3. Antifungal activity of methanol extract of root bark of *Croto* Geiseler

Name of micro-organism	Zone of inhibition of extract in mm				Zone of inhibition of standa		
	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/d 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
Tm	6.66 ±0.35*	8±.53*	6.66±0.32*	8.7±0.35	20.33±2.1	16.66±0.85*	12±1.5
Tb	7±0	8.66±0.65	20±0.55	25±1.5	21±2.0	7.35±0.35	7±0
Mg	-------	-------	-------	-------	8.4±1.5	16.3±1	14±1

Key:-NF-Nitrofurantoin,AM-Amikacin,Sc-Sparfloxacin,Fu-Fluconazole,*Ca-Candida Tricophyton mentagrophytes*, Tb-*Trichosporan beigelli*, Mg-*Microsporum gyps* control disc used for solvent had no zone of inhibition, so there data was omitted fr data.Data are represented in the form of mean of three tests±S.E of the standa *P<0.001 as the plant extracts at different concentrations compared with the stand discs by using student *t-test*.

Minimum Inhibitory Concentration (MIC) of *Croton Caudatus* Geisel

The minimum inhibitory concentration (MIC) values of the extract ag bacteria were shown in Table 4.The MIC value for methanol extract of root ba *caudatus* Geiseler ranged from 31.25-500mg/ml. The lowest MIC value (31.2 recorded against *Klebsiella pneumonia*.

Table 4. MIC determination of root bark of *Croton caudatus* Geisler a pathogenic bacteria.

Marked No. of the test tubes	Nutrient broth medium added (ml)	Diluted solution (µg/ml)	Inoculums added (µl)	Bacterial growth obs		
				E.coli	*Sa*	*Kp*
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			

Conclusion

It was concluded that the both the methanolic extract of root bark of *Croton caudatus* Geiseler demonstrates a strong activity against bacteria and fungi. This investigation can be used in the folk medicine and source of antibacterial 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.

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References

Akinpelu,D.A.,and Onakoya,T.M.(2006).Antimicrobial activities of medicinal plants used in folklore remedies in south-western,*African J.Biotech.* 5(11).225.

Ang-Lee MK, Moss J, Yuan CS.(2001).Herbal medicines and perioperative care.*JAMA11*:208-216.

Caius,J.F.(2003).The Medicinal and Poisonous Plants of India. Scientific Publishers, Jodhpur (India).

Chopra,R.N.,S.L.Nayer and I.C.Chopra,(1956).Glossary of Indian Medicinal Plants. CSIR, New Delhi (India).

Dash,S.,L.K.Nath and S.Bhise.(2005).Antioxidant and antimicrobial activities of *Heracleum nepalense* D.Don root.*Trop.J.Pharm.Res.*, 4:341-347.

Goldman.P.(2001).Herbal medicines today and the roots of modern pharmacology. *Ann Intern Med 135*:594-600.

Harborne, J.B.(1998).Phytochemical methods. Guide diabetes in rats, Biological Trace Element to modern techniques of plant analysis.3rd Edn Chapman and Hall Int.Ed., New York.

Jiangsu New Medical College.(1975).Dictionary of Traditional Chinese Medicine; Shanghai Science and Technology Press: Shanghai, China, p.447.

Khan.A.,M.Rahman and S.Islam.(2007).Antibacterial,antifungal and cytotoxic activities of Tuberous Roots of *Amorphophallus campanulatus Turk.J.Biol.*, 31:167-172.

Kirtikar, K.R.and B.D.Basu.(1935).Indian Medicinal Plants.4 vols. Bisen Singh, Mahendra Pal Singh, Dehra Dun, India.(Reprinted).

Ngbede,J., R.A.Yarkubu and D.A.Njam.(2008).Phytochemical screening for active compounds in *Conarium schweinfurthii* (Atile) leaves from Jos North, Plateau State Nigeria. Research Journal of Biological Science,3(9):1070-1078.

Phillipson, J.D.(1994).Natural products as drugs. *Trans Royal Soc Trop Med Hyg* 88:17-19.

Sofowora, A. (1993).Medicinal Plants and Traditional Medicine in Africa, John Wily and son Ltd. pp: 150-153.

Washington, J.A.,and Wood, G.L. (1995).Antimicrobial susceptibility tests.Dilution and Disc Diffusion methods.*In:Manual of Clinical Microbiology*.1327-1331..

Weisser, R.,Asscher, A.W.,Winpenny, J.(1966).*In vitro reversal of antibiotic resistance by DTA.Nature*; 219:1365-1366.