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Bactericidal action of *Croton macrostachyus* leaf extract against common human pathogenic bacteria

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Abstract
Bacterial ailment remains a serious health problem due to its development of resistance to available antibiotics and created the need for replacement. In Ethiopia, the plant *Croton macrostachyus* are used as a traditional medicine for infectious diseases, but reports on its possible bactericidal activity is deficient, therefore the present study aimed at evaluating the in vitro antibacterial activity of different extracts of *C. macrostachyus* leaves against six human pathogens of *Moraxella catarrhalis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus pneumonia* and *Neisseria meningitides.* The methanol extract was the best in terms of zone of inhibition as exhibited maximum of 12.3 mm diameter followed by chloroform (9.6 mm) and ethanol (7 mm) and clearly indicated that unique bactericidal activity. However, its efficacy will be evaluated in future. The present results give scientific evidence and support the traditional use of *C. macrostachyus* as a source for antibacterials eventually for unearthing new drugs.

Keywords: antibacterial activity, *Croton macrostachyus*, pathogens, medicinal plants, solvent extract

1. Introduction
The continuous evolution of bacterial resistant to currently available antibiotics has necessitated the search for novel and more effective antibacterials [1]. For a long time, medicinal plants and herbs were used intensively in folkloric medicine for treatment of various infectious and non-infectious diseases all over the world [2]. Efforts in this regard have focused on plants due to traditional practice by most of the world's population significantly, particularly in developing countries and in Africa [3]. For these reasons, medicinal plants are important substances recently for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. In Ethiopia, plants have been used habitually in the home to treat family sickness, and occasionally traditional healers may be consulted [4, 5]. Due to its long period of practice and existence, traditional medicine has become an integral part of the culture in Ethiopian people [6], however, recently been given importance for the ethnobotanical study on the use and knowledge of medicinal plants in Ethiopia [7, 8]. *Macrostachyus* one of the Croton species found in Ethiopia is used regularly for treatment of a number of health ailments including diabetes, malaria, typhoid, measles, stomach ache, ascariasis, abdominal pain, gonorrhea, wounds, ringworm infestation, hemorrhoids and more [9, 10, 11]. Recently, stem bark [12] and leaves [13, 14] extract of *Croton macrostachyus* shown antimicrobial activity, however, bactericidal effect of *C. macrostachyus* leaves was not studied in great deal though it has enormous potential. Therefore in this study aimed at evaluating the *in vitro* bactericidal activity of *C. macrostachyus* leaf extracts against few common human pathogenic bacteria eventually this result would give more knowledge and possible new drug for the importance.

2. Materials and Methods
2.1 Plant sample Collection
Plant leaves sample of *C. macrostachyus* was collected from Ambo, Addis Ababa and thoroughly washed in running tap water and air dried at room temperature in the shade for 5 days (Fig. 1), then powdered using a mixer grinder and stored in an air tight container at 4°C for further use.

Fig 1: Dried leaf samples of *C. macrostachyus*

2.2 Organic solvent extraction

Four different solvents (methanol, ethanol, chloroform and distilled water) were used to prepare leaf extract. Twenty gram of *C. macrostachyus* powder was mixed with 200 mL of each solvent separately and kept in an orbital shaker at 120 rpm for 48 h at room temperature. Then the extract was filtered using a Whatman No. 1 filter paper or centrifuged at 5000 rpm for 10 min and transferred to a petri-dish and allowed to evaporate at room temperature and weighed. Finally, all dried extract were dissolved in 50 mg/mL concentration of respective solvents.

2.3 Antimicrobial activity

The different solvent extracts were subjected to antibacterial assay by Kirby Bauer disc diffusion method using Muller Hinton agar plates against six human bacterial pathogens of *M. catarrhalis*, *S. pyogenes*, *P. aeruginosa*, *E. coli*, *S. pneumoniae and N. meningitides.* The pathogens were obtained from Armauer Hansen Research Institute (AHIR) Addis Ababa and maintained in LB broth at 37°C. The extract discs were prepared using sterile Whatmann No.1 filter paper (6 mm diameter) by soaking in the extract (5 min) and air dried. For positive control 10 mg penicillin, ampicillin and tetracycline (50 mg) disc was used. The 50 µL of 12 hrs old cultures was spreaded on Muller Hinton agar plates and placed extract disc and positive control disc and incubated for 24 hrs at 37°C, and measured zone of inhibition (ZoI) as in diameter.

3. Results and Discussions

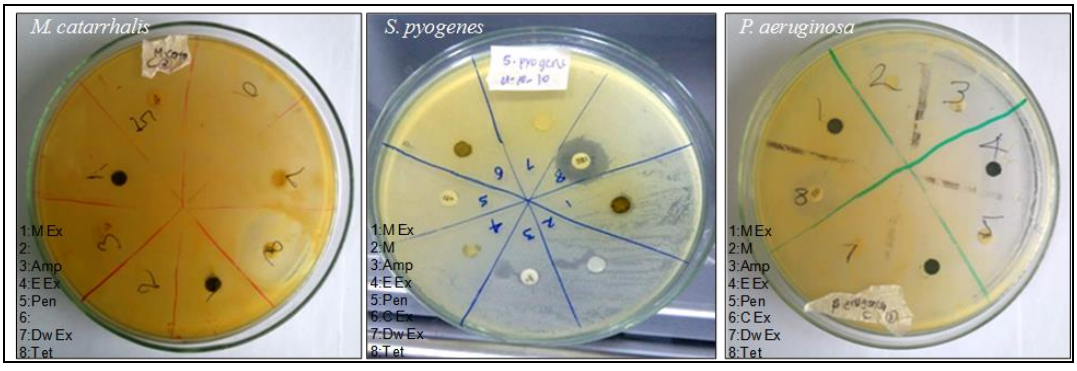
Ethnobotanical investigations have been found to offer important clues in the identification and development of traditionally used medicinal plants in to modern drugs [1]. Moreover, increasing resistance of clinically important bacteria to existing antibiotics is a major problem throughout the world [15]. Over the past 20 years, investigators from virtually every corner of the world have documented that increasing proportions of bacteria are resistant to penicillin and other antibiotics. Recently there has been a great deal of interest in searching for novel natural antibiotics [1] to restrain resistance and our studies have shown that organic solvent extract of *C. macrostachyus* can act as potential antibacterial agents that may be useful in the pharmaceutical industries.

The Table 1 and Fig. 1 shows the zone of inhibition of *C. macrostachyus* extract against few human pathogenic bacteria. The ZoI at maximum of 12.5 mm was observed in methanol extract of C. *macrostachyus* against *M. catarrhalis*, which is a Gram negative bacterium. While ethanol and chloroform extract observed maximum of 7 mm and 9.6 mm against *E. coli*, respectively, which also a Gram negative bacterium. Therefore, this plant having highest bactericidal activity against Gram negative pathogens, however it had shown some bactericidal against Gram positive bacteria of *S. pneumoniae* and *S. pyogenes* which was at maximum of 8.6 mm ZoI in chloroform and 10 mm in methanol extract, respectively. Interestingly, the methanol extract did not shown any inhibition against *N. meningitidis*, while ethanol at *M. catarrhalis* and chloroform at *P. aeruginosa* and *S. pyogenes.* There was no bactericidal activity was observed in distilled water extract against any of the pathogens, it indicated that the bactericidal compounds were soluble in organic solvents. The positive antibiotic control of tetracycline, ampicillin and penicillin expressed the bactericidal against all pathogens used as expected, however, the plant extract was reached maximum of 12.5 mm ZoI shown which was higher than the ampicillin (7.5 mm) against the same pathogen of *M. catarrhalis.*

Table 1: Zones of inhibition (diameter in mm) of different solvent extracts of *C. macrostachyus* against common human pathogens

Test substances	Zone of inhibition diameter (mm)					
	M. catarrhalis	***S. pyogenes***	***P. aeruginosa***	***E. coli***	***S. pneumoniae***	***N. meningitidis***
Methanol Extract*	12.5 ± 0.63	10 ± 0.5	4.5 ± 0.3	4.4 ± 0.2	3.3 ± 0.2	0
Ethanol Extract*	0	6 ± 0.25	5.5 ± 0.3	7 ± 0.3	4.3 ± 0.25	6 ± 0.25
Chloroform extract*	4.5 ± 0.2	0	0	9.6 ± 0.5	8.6 ± 0.5	3 ± 0.2
Distilled water extract*	0	0	0	0	0	0
Ampicillin (10 mg)	7.5	15	12.5	7	10.3	10
Penicillin (10 mg)	14	5	10	11.6	7.6	7.5
Tetracycline (50 mg)	16	14	12.5	15	17.3	16.5

*50 mg/mL concentration



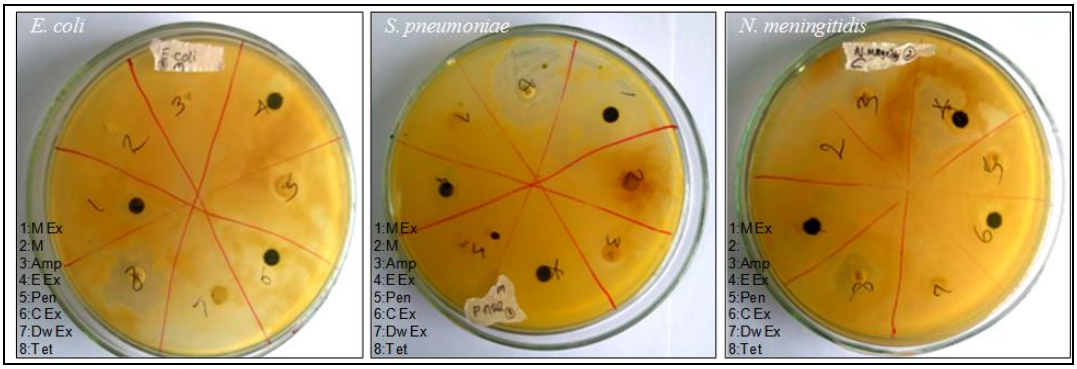


Fig 2: Muller Hinton agar plate showing the zone of inhibition of different solvent extract of *C. macrostachyus against* common bacterial human pathogens (1: methanol extract, 2: Methanol alone, 3: Ampicillin-10mg, 4: Ethanol extract, 5: Penicillin-10mg, 6: Chloroform extract, 7: Distilled water Extract, 8: Tetracycline-50mg).

Table 2: Comparative analysis of antibacterial action of *C. macrostachyus* plant extract

Plant part	Solvent extract	Maximum ZoI (□mm)	Bacteria	MIC (mg/mL)	Country	Ref
leaves	Methanol	12.5	*M. catarrhalis*	-	Ethiopia	This study
	Ethanol	7	*E. coli*	-		
	Chloroform	9.6	*E. coli*	-		
Leaves	dichlomethane/methanol (1:1)	10	*E. coli*	-	Ethiopia	[14]
		15	*S. aureus*	-		
		14	*P. aeruginosa*	-		
		19	*Salmonella*	-		
Stem bark	Butanol	13.7	*E. coli*	-	Kenya	[12]
	Ethyl acetate	16	*Salmonella typhi*	-		
	Methanol	14.9	*Klebsiella pneumoniae*	-		
	Methanol	10.8	*Enterobacter aerogenes*	-		
	Ethyl acetate	11.7	*Listeria monocytogenes*	-		
Leaves	Ethanol	13.3	*E. coli*	-	Ethiopia	[13]
	Chloroform	24.7	*K. pneumoniae*	-		
	Chloroform	30.7	*S. flexner*	-		
	Chloroform	24.3	*S. aureus*	-		
	Chloroform	25.3	*S. pneumonia*	-		
Mixed parts	70% Methanol	-	*Bacillus cereus*	15.6	Kenya	[16]
		-	*P. aeruginosa*	250		
		-	*E. coli*	250		
		-	*Micrococcus lutea*	ND		

ZoI;zone of inhibition, MIC; minimal inhibitory concentration, ND; not determined

The present study clearly indicated that methanol, ethanol, and chloroform extract of *C. macrostachyus* could be able to inhibit most tested bacteria and compared with previous studies (Table 2). However, the degree of their inhibition pattern was different. This may be because of the difference in bacterial strain and the kind of solvent used. Accordance to our study, Obey *et al*., [12] found that the stem bark extract of the *C. macrostachyus* shown the different bactericidal activity and stated ethyl acetate extract was better (16 mm ZoI) followed by methanol (14.9 mm ZoI) and butanol (13.7 mm ZoI). In an another study in Ethiopia Kibre *et al.*, [14] found leaf extract using dichlomethane/methanol (1:1) has shown bactericidal action against human pathogens of *E. coli, S. aureus, P. aeruginosa* and *Salmonella.* Wagate *et al.*, [16] observed 70% methanol extract of *C. macrostachyus* shown antibacterial effect against *Bacillus cereus* at 15.6 mg/mL minimal inhibitory concentration whereas *E. coli* and *P. aeruginosa* shown at 250 mg/mL. However in argument with our study, Sendeku *et al*., [13] noticed that chloroform leaf extract of *C. macrostachyus* shown more ZoI (32 mm) followed by methanol (24 mm) and ethanol (25 mm), this increased ZoI was due to high concentration of extract used in the previous study moreover time of sample collection, storage conditions and methods of analysis also could have influenced. Despite the existence of excess information regarding the prolonged and uneventful local use of *C. macrostachyus*, scientific evidences regarding their value are scarce [7, 8]. This study clearly indicated that the plant *C. macrostachyus* leaf solvent extract has ample potential to inhibit some common human pathogenic bacteria as it were seen from its strong inhibition against tested organism. Currently due to the emergence in antibiotic resistant infections, the search for new alternative drugs to treat infections is entirely necessary and in this regard *C. macrostachyus* can give an alternative source for design of novel drugs.

4. Conclusion

This study evidently concludes that the leaf extract of *C. macrostachyus* has ample potential to inhibit common human pathogenic bacteria at unique intensity upon different genus, however, the methanol extract was the best among other solvents. In that way, further investigations needed to find out the efficacy, active molecule and elucidate the mechanism of wide bactericidal action.

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