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EC MICROBIOLOGY
Research Article

Assessment of Phytochemical Content and Antibacterial Activity of *Plumbago auriculata* against *E. coli* Species Isolated from Water Sources in Mthata Region Eastern Cape South Africa

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Received: December 30, 2016; **Published:** January 16, 2017

Introduction

The abundant use of anti-infective agents resulted in emergence of drug resistant bacteria, fungi and viruses. To overcome the increasing resistance of pathogenic microbes, a variety of medicinal plants worldwide have been screened for their antimicrobial properties. The impetus for this research area is to find new and effective antimicrobial agents with novel modes of actions. Essential oils derived from aromatic medicinal plants have been reported to exhibit exceptionally good antimicrobial effect against bacteria, yeast, filamentous fungi and viruses [3,4,8]. Medicinal plants used in traditional medicine to treat infectious diseases seem to be an abundant source of new bioactive secondary metabolites. Medicinal plants are known to contain one or more substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs [4,5]. The classes are as follows Plant derived anti diabetic, antidiarrhoeal, wound healing, and antibacterial chemotherapeutic agents. More than hundreds of plants worldwide are used in traditional medicine as treatments for bacterial infection [1,3,4,8].

Objective

To determine the antibacterial activity of *Plumbago auriculata* on *E. coli* isolates from water and stool samples.

To check for the phytochemical components and their link to antimicrobial activity.

Materials and Methods

The roots of *Plumbago auriculata* were collected and ground into fine powder, water, ethanol, methanol and chloroform were used to extract the active components. Antibacterial assays were done using kerbybaur disc diffusion method.

Phytochemical analysis was done to check for the active components and toxicity testing was done using acute toxicity testing with mice.

Results

About Hundred and fifty *E. coli* were isolated from water and patient stool samples. Antibiotic sensitivity patterns and β-lactamase production was tested on these strains. Molecular characterisation was done on the β-lactamase producing strains (Table 1-4).

Antimicrobial Agents	Water isolates	Stool isolates
	N = 62	N = 30
Ampicillin	36	5
Gentamicin	62	30
Ciprofloxacin	62	30
Imipenem	62	30
Nalidixic acid	38	10
Sulfametoxazol-trimetoprim	33	20
Tetracycline	27	15
Ciphalothin	51	20
Amikacin	62	10
Aztreonam	62	30
Cefotaxime	62	30

Table 1: *Comparison of Susceptibility profiles of E. coli from water and stool.*

Antimicrobials in mg/L						
Isolates	**Amp**	**CET**	**CTX**	**ATM**	**NAL**	**TC**
E. coli 1w	32	64	128	8	≥ 32	≥ 16
E. coli 2w	32	64	64	8	≥ 32	≥ 16
E. coli 3h	64	64	64	8	≥ 32	≥ 16
E. coli 4w	64	64	64	8	64	≥ 16
E. coli 5w	≥ 32	64	32	4	16	8
E. coli 6h	128	128	128	16	64	64
E. coli 7h	≥ 32	64	< 64	4	16	8
E. coli 8h	64	128	128	16	≥ 32	32
E. coli 9h	64	128	128	16	≥ 32	32
E. coli 10w	> 32	64	64	4	16	8
E. coli ATCC25922	> 32	64	64	4	≥ 32	≥ 16

Table 2: *Minimal inhibitory concentrations in TSB of selected antimicrobials against E. coli isolates.*

W: Water; h: Human Amp: Ampicillin; CET: Cephalothin; CTX: Cefotaxime; NAL: Nalidixicacid; TC: Tetracycline; ATM: Aztreonam

Water sources	β Lactamase positive *E. coli*	β Lactamase negative *E. coli*
Stool sources	30%	70%
Water sources	60%	40%

Table 3: *ESβL/β Lactamase of bacterial isolates from human and environmental sources and antibiograms.*

E. coli isolates	Number of tested isolates	PCR products				
		CTX-M	*SHV*	*TEM*	*OXA*	*negative*
Water isolates	65	18	18	12	0	17
Clinical Isolates	35	11	4	4	0	16
Total isolates	100	29	22	16	0	33

Table 4: *Distribution of TEM, SHV, OXA and CTX-M ESBL types among 100 isolates of E. coli.*

Ten organisms which presented with the CTX resistant genes were then used to screen for the activity of the *Plumbago auriculata* plant extract.

The extracts were from Ethanol, water, chloroform and acetone.

Zones of inhibition are as presented in Table 5.

Pathogens	Zone of inhibition in mm					
	Extracts Conc. in µg	C	A	E	AQ	Amikacin 30 µg
E. coli patient	20	nil	nil	4 ± 0.02	nil	15
	40	nil	nil	7 ± 0.01	nil	
	60	nil	nil	8 ± 0.02	nil	
	80	nil	nil	11 ± 0.01	3 ± 0.01	
	100	nil	nil	13 ± 0.02	4 ± 0.01	
E.coli water	20	1 ± 0.02	2 ± 0.02	4 ± 0.01	nil	20
	40	2 ± 0.01	4 ± 0.01	6 ± 0.01	nil	
	60	4 ± 0.02	5 ± 0.01	7 ± 0.02	1 ± 0.01	
	80	6 ± 0.02	7 ± 0.02	13 ± 0.01	4 ± 0.01	
	100	8 ± 0.01	10 ± 0.01	16 ± 0.01	6 ± 0.01	
E.coli control strain	20	4 ± 0.02	4 ± 0.01	10 ± 0.02	nil	11
	40	6 ± 0.01	6 ± 0.02	13 ± 0.01	nil	
	60	7 ± 0.02	7 ± 0.01	14 ± 0.01	3 ± 0.01	
	80	8 ± 0.01	9 ± 0.01	16 ± 0.02	4 ± 0.01	
	100	9 ± 0.02	12 ± 0.02	18 ± 0.01	6 ± 0.01	

Table 5: *Antibacterial activity of Plumbago auriculata.*

C: Chloroform; A: Acetone; E: Ethanol and AQ: Water

The Ethanolic extracts demonstrated maximum zone of inhibition (18mm). The extract was active against all the examined strains of *E. coli*. The significant antibacterial activity was observed more in ethanolic extacts compared to other tested extracts of *P. auriculata* Ethanolic (100%), acetone (48%), Chloroform (34%),and water (31%).

The MIC for the plant was 20mg/L as shown in Table 6.

	MIC assay		
Plant extraction			**MIC**
	E. coli stool strain	*E. coli* water strain	*E. coli* control
P. auriculata (water)	-	10^{-2} (20mg/ml)	10mg/ml
P. auriculata (acetone)	-	-	-
P. auriculata (Ethanol)	10^{-2} (20 mg/ml)	10^{-2} (20mg/ml)	10mg/ml
Ciprofloxacin disc/soln	0.005 mg/ml	0.005mg/ml	0.005mg/ml
Negative control disc/distilled water	-	-	-

Table 6: *Plant MIC assay.*

Phytochemical analysis showed that the plant had alkaloids, quinones, tannins, antocyanins, phenols, sterols and flavonoids in both Methanol and water extracts. Cardiac glycosides and saponins were absent.

Each phytochemical was determined using the analytical methods above (Table 7).

Phytochemical	Extract tested	Result
Saponins	Water	Absent
	Methanol	Absent
Alkaloids	Ethanol	Present
	Methanol	Present
Quinones	Ethanol	Present
	Methanol	Present
Cardiac Glycosides	Water	Absent
	Ethanol	Absent
	Methanol	Absent
Tannins	Water	Present
	Methanol	Present
Anthocyanins	Water	Present
	Methanol	Present
Phenols	Ethanol	Present
	Methanol	Present
Sterols	Ethanol	Present
	Methanol	Present
Flavanoids	Ethanol	Present
	Methanol	Present

Table 7: *Qualitative analysis Results.*

Toxicity

The extracts showed toxicity from a concentration of 50 µg/ml using the acute toxicity testing were mice were fed with concentration of the boiled plant from 10 µg/ml to 250 µg/ml number of mice dying per group of ten were counted as shown in Table 8.

Concentration of extract µg/ml	% cytotoxicity of *P. auriculata*	Number of Mice killed
10	0	0
50	12	1
100	18	2
150	25	3
200	45	5
250	52	6

Table 8: *Toxicity for Roots water extracts of Plumbago auriculata.*

Discussion and Conclusion

The plant *P. auriculata* presented with characteristics of antimicrobial activity as the active components included phenolic acids , sterols, to mention a few, confirming that it can be used as an alternative treatment although under strict monitoring as it also showed toxicity activities at higher concentrations [1,2,6,7].

There is need to work in collaboration with herbalist so that we can help patients by monitoring them to avoid toxicity side effects from the herbs they will be using.

Isolation of the active components is also crucial to enable us to analyse the component individually for activity and toxicity.

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Volume 5 Issue 2 January 2017
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