

Evaluation of antibacterial activities of *euphorbia heterophylla*

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Abstract: Preparations of the extract of *Euphorbia heterophylla* are used for the treatment of different infections by Nigerian traditional medicine practitioners. This work evaluated the antibacterial actions of aqueous leaf extract of *Euphorbia heterophylla*. **Materials and methods:** Clinical isolates of *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were incubated in agar plates after serial dilutions (100, 50, 25, 12.5, 6.25, and 3.16 mg/ml) of the extract were added in wells made in the agar and incubated at 37°C. After 24 hours, zones of inhibition (in mm) around the wells were measured. The same organisms were tested for sensitivity against standard antibiotics (ceftriaxone, ciprofloxacin, gentamicin, lincocin, and ofloxacin) using disk diffusion method. Microsoft Excel 2010 and Graph Pad Prism 6.0 were used for statistical analysis. **Results:** Phytochemical test showed that the extract contained saponins, tannins, alkaloids, resins, sterols and terpenoids. The extract demonstrated dose dependent antibacterial activity which was, however, apparently lower than that of the standard antibiotics. **Conclusion:** It was concluded that the extract had significant antibacterial activity against test organisms with minimum inhibitory concentration (MIC) of 6.25 mg/ml. Its use for treatment of infections by traditional healers is justified. **Key words:** Antibiotics, aqueous extract, bacteria, *Euphorbia heterophylla*, inhibition.

I. Introduction

Euphorbia heterophylla (*E. heterophylla*) is an annual weed. Alternative names of this weed include *Euphorbia geniculata*, *Euphorbia pronifolia*, *Poinsettia geniculata* and *Poinsettia heterophylla*^[1]. It grows mainly in tropical and sub-tropical climates. In Nigeria, it is mainly found in cassava farms during the planting season.

In Igbo traditional medicine, the leaf extract is used as a purgative as well as for the treatment of gonorrhoea, respiratory tract infection, malaria, and skin infections^{[1], [2]}. It is commonly called nono-kunchiya in Hausa, egeleor aka- itoin Igbo and adimeru in Yoruba, Nigeria^[2].

Screenings of medicinal plants for antimicrobial activities are important for finding potential new compounds for therapeutic use. Medicinal plants readily provide sources of such novel agents. Herbs known to have antibacterial properties include *Acacia arabica*, *Nymphaea lotus*, *Sphararanthus hirtus*, *Emblica officinalis*, *Cinchoriumintybus*, *Cardus marianum*, and *Ficus exasperata*^{[3], [4]}. However, most of the antimicrobial studies on this plant were done using the ethanol extract. Even in traditional medical practice most of these plants are usually soaked in local gin to extract the active constituents. Knowing the toxic effect of alcohol on the liver, the aqueous extract will be safer. However, it is known that the extraction of the active constituents and therefore the efficacy of herbal remedy depend on the extracting solvent, geographical location, and soil characteristics among other factors^[5].

The aim of the study is to confirm the antibacterial activity of aqueous extract of *E. heterophylla*. Specific objectives of the study include:

- To determine the inhibitory effect of *E. heterophylla* on the growth of *Klebsiella pneumoniae* (*K. pneumoniae*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*Ps. aeruginosa*).
- To compare the inhibitory effect of different concentrations of the extract on the growth of *K. pneumoniae*, *S. aureus*, *E. coli*, and *Ps. aeruginosa*.
- To compare the inhibitory effect of this extract and some standard antibiotics on the growth of *K. pneumoniae*, *S. aureus*, *E. coli*, and *Ps. aeruginosa*.

II. Materials And Methods

This study was conducted at the Pharmacology and Medical Microbiology laboratories of NnamdiAzikiwe University, Nnewi Campus between February and July, 2012.

2.1. Plant source

Fresh leaves of *E. heterophylla* were collected at Umuoji, Idemili North Local Government, Anambra State, Nigeria in the month of March 2012. The leaves were identified by Prof. C. U. Okeke of Botany Department, NnamdiAzikiwe University, Awka.

2.2. Preparation of aqueous extract

Fresh leaves of *E. heterophylla* were air-dried at room temperature. Thereafter, 200 gm of the dry leaves were mashed in 250 mls of water, filtered, and the filtrate evaporated to dryness using Soxhlet extracting apparatus.

2.3. Phytochemical analysis

Preliminary phytochemical test as described by Harbone (1973) showed that the extract contained saponins, tannins, alkaloids, resins, sterols and terpenoids^[6].

2.4. Preparation of extract solutions

Six (6) g of extract was dissolved in 60 ml of water giving a concentration of 100 mg/ml. This was taken as stock solution. Serial dilutions of the stock solution were prepared by mixing 1ml of stock solution with 1, 2, 3, 4 and 5 ml of distilled water giving 50, 25, 12.5, 6.25 and 3.16 mg/ml respectively.

2.5. Laboratory procedures

Isolates of *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* were obtained from NnamdiAzikiwe University Teaching Hospital Clinics and stored in Oxford nutrient agar slants for one week before use. Twenty microliter nutrient agar was prepared according to the manufacturer's (Oxford) instructions. The agar was poured into six Petri dishes and allowed to set. The agar in Petri dishes were sterilized and allowed to cool. The plates were inoculated with test organisms. Seven equal holes (6 mm) were made in each agar plate with sterile cork borer (one of the holes being central in position); two plates each for *E. coli*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*. The Petri dishes were labeled with their respective organisms and the wells marked 100, 50, 25, 12.5, 6.25 and 3.1 mg/ml respectively for easy identification. The wells were filled with 5 drops of corresponding dilution of the extract. The central well was filled with distilled water to act as the control. They were allowed to diffuse well into the agar medium and subsequently incubated at 37°C for 24 hours. Thereafter, the zones of inhibition produced by the different concentrations of the extract against the growth of the bacteria were measured and recorded. For comparison, the bacteria from the same clinical specimens were tested for sensitivity using standard antibiotic (ceftriaxone, ciprofloxacin, gentamicin, lincocin, and ofloxacin) diffusion discs.

2.6. Statistical analysis

Data generated from the measurement of zones of inhibition were subjected to analysis for analysis using Microsoft excel 2010. The data were further subjected to analysis for evidence of statistically significant differences in the zones of inhibition produced by the extract and those produced by some standard antibiotics. Results of the analyses were taken as statistically significant at p -value ≤ 0.05 .

III. Results

3.1. Aqueous extraction

Aqueous extraction of 200 gm of the dry leaves of *E. heterophylla* yielded 11.5 gm extract (giving extractive value of 5.75%).

3.2. Phytochemical test

Preliminary phytochemical test showed that the extract contained saponins, tannins, alkaloids, resins, sterols and terpenoids.

3.3. Antibacterial activity

The extract exhibited inhibitory effect on the growth of *K. pneumoniae*, *S. aureus*, *E. coli*, and *P. aeruginosa* as shown in Table 1 and Fig. 1. The extract also exhibited wide spectrum antibacterial activity against the said bacteria when compared to most of the orthodox antibiotics with which it was compared (ceftriaxone, ciprofloxacin, gentamicin, lincocin, and ofloxacin) as shown in Table 2 and Fig. 2. However, these

antibiotics produced higher zones of inhibition when compared with the extract (Table 2, Fig. 2, and Fig. 3). There was a statistically significant difference in the zones of inhibition produced by the extract against these bacteria, with S.aureus being the most susceptible (Fig. 4). There were also statistically significant differences between the zones of inhibition produced by the standard antibiotics and those produced by the extract (Fig. 5).

Table 1: Inhibitory effect of increasing concentrations of ethanol leaf extract of E. heterophylla on the growth of different bacteria

Test organism	Concentration of extract (mg/ml)	Size of well in mm (A) of inhibition (B)	Size of well +area (B-A)	Zone of inhibition
K. pneumonia	100	6	10	4
	50	6	9	3
	25	6	9	3
	12.5	6	8	2
	6.25	6	6	0
	3.16	6	6	0
	* C	6	6	0
	Mean zone of inhibition = 2.00			
S.aureus	100	6	11	5
	50	6	12	6
	25	6	11	5
	12.5	6	11	5
	6.25	6	10	4
	3.16	6	6	0
	* C	6	6	0
	Mean zone of inhibition = 4.16			
E. coli	100	6	10	4
	50	6	10	4
	25	6	9	3
	12.5	6	8.5	2.5
	6.25	6	6	0
	3.16	6	6	0
	* C	6	6	0
	Mean zone of inhibition = 2.25			
Ps. aeruginosa	100	6	9	3
	50	6	9	3
	25	6	10	4
	12.5	6	9	3
	6.25	6	8	2
	3.16	6	6	0
	* C	6	6	0
	Mean zone of inhibition = 2.50			

* C = absolute alcohol. This was used to fill the central well to act as a control.

Table 2: Comparison of the effects of some orthodox antibiotics and ethanol leaf extract of E. heterophylla on the growth of different bacteria

Concentration of antibiotics and extract	Bacteria and corresponding zones of inhibition (in mm)			
	K.pneumoniae	S.aureus	E.coli	Ps.aeruginosa
Ceftriaxone (10 µg)	8mm	11mm	27mm	-
Ciprofloxacin (5 µg)	5mm	2mm	25mm	-
Gentamicin (10 µg)	4 mm	9 mm	8 mm	7 mm
Lincocin (10 µg)	-	-	9 mm	-
Ofloxacin (5 µg)	-	-	20 mm	10 mm
Extract (100 mg/ml)	* 4 mm	5 mm	4 mm	3 mm

*The growth inhibitions against the bacteria produced by the highest concentration of the extract (100mg/ml).

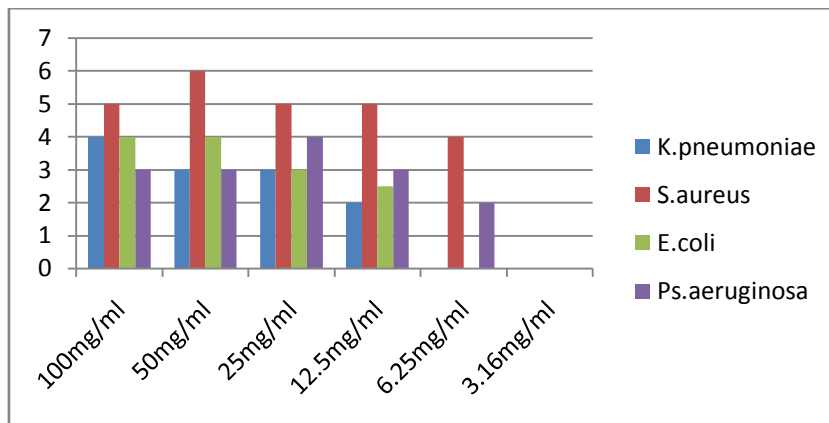


Figure 1: Bar chart showing dose dependent inhibition (in mm) produced by aqueous leaf extract of *E.heterophylla* against some bacteria.

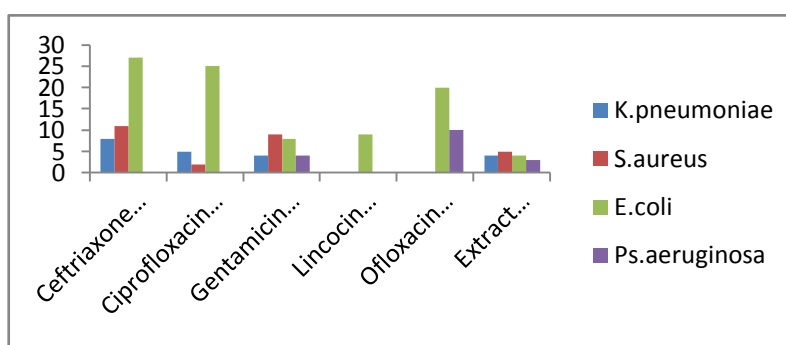


Figure 2: Bar chart comparing the zones of inhibition produced by some orthodox antibiotics and aqueous leaf extract of *E.heterophylla* against the growth of some bacteria

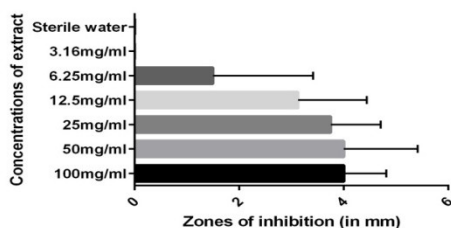


Table Analyzed	Data 1				
Repeated measures ANOVA summary					
Assume sphericity?	No				
F	19.57				
P value	0.0054				
P value summary	**				
Statistically significant (P < 0.05)?	Yes				
Geisser-Greenhouse's epsilon	0.2690				
R square	0.8671				
Was the matching effective?					
F	7.330				
P value	0.0021				
P value summary	**				
Is there significant matching (P < 0.05)?	Yes				
R square	0.1397				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	79.09	6	13.18	F (1.614, 4.842) = 19.57	P = 0.0054
Individual (between rows)	14.81	3	4.938	F (3, 18) = 7.330	P = 0.0021
Residual (random)	12.13	18	0.6736		
Total	106.0	27			
Data summary					
Number of treatments (columns)	7				
Number of subjects (rows)	4				

Figure 3: Analysis showing statistically significant differences in the zones of inhibition produced by different concentrations of aqueous leaf extract of *E.heterophylla* against some bacteria.

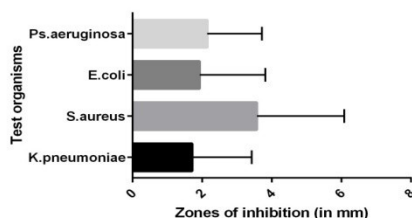


Table Analyzed		Data 1			
Repeated measures ANOVA summary					
Assume sphericity?	No				
F	7.330				
P value	0.0144				
P value summary	*				
Statistically significant (P < 0.05)?	Yes				
Geisser-Greenhouse's epsilon	0.5380				
R square	0.5499				
Was the matching effective?					
F	19.57				
P value	< 0.0001				
P value summary	****				
Is there significant matching (P < 0.05)?	Yes				
R square	0.7459				
ANOVA table					
Treatment (between columns)	SS	DF	MS	F (DFn, DFd)	P value
Individual (between rows)	14.81	3	4.938	F (1.614, 9.684) = 7.330	P = 0.0144
Residual (random)	79.09	6	13.18	F (6, 18) = 19.57	P < 0.0001
Total	12.13	18	0.6736		
	106.0	27			
Data summary					
Number of treatments (columns)	4				
Number of subjects (rows)	7				

Figure 4: Analysis showing statistically significant differences in the zones of inhibition produced by aqueous leaf extract of *E.heterophylla* against some bacteria.

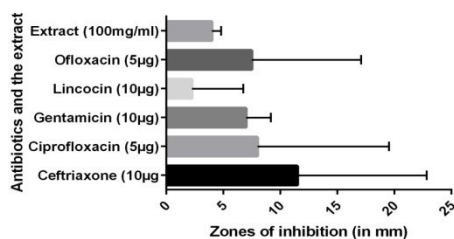


Table Analyzed		Data 1			
Repeated measures ANOVA summary					
Assume sphericity?	No				
F	1.233				
P value	0.3555				
P value summary	ns				
Statistically significant (P < 0.05)?	No				
Geisser-Greenhouse's epsilon	0.3398				
R square	0.2912				
Was the matching effective?					
F	6.090				
P value	0.0064				
P value summary	**				
Is there significant matching (P < 0.05)?	Yes				
R square	0.4633				
ANOVA table					
Treatment (between columns)	SS	DF	MS	F (DFn, DFd)	P value
Individual (between rows)	210.2	5	42.04	F (1.699, 5.097) = 1.233	P = 0.3555
Residual (random)	623.1	3	207.7	F (3, 15) = 6.090	P = 0.0064
Total	511.6	15	34.11		
	1345	23			
Data summary					
Number of treatments (columns)	6				
Number of subjects (rows)	4				

Figure 5: Analysis showing statistically significant differences in the zones of inhibition produced by some standard antibiotics and aqueous leaf extract of *E.heterophylla* against some bacteria.

IV. Discussion

As shown in the results section, this extract exhibited a wide spectrum of antibacterial action against *K.pneumoniae*, *S.aureus*, *E.coli*, and *Ps.aeruginosa*. The inhibition of growth of the test organisms by the extract was dose dependent with the lowest effective concentration being 6.25mg/ml. This can be considered the minimum inhibitory concentration (MIC) of this extract against for *S.aureus* and *Ps.aeruginosa*. For *K.pneumoniae* and *E.coli*, the MIC was 12.5mg/ml. Odunbaku et al. had earlier found the MIC of this extract against *E.coli* to be 300mg/ml^[7]. This high MIC (low activity) could be due to differences in geographical location, season of plant, age of the plant, and method of extraction, all of which affect the yield and the active constituents of medicinal plants^{[8], [9]}. It could also be due to differences in laboratory procedures and reagents used^{[10], [11]}. The MICs observed in this study were also lower than those of another medicinal plant, *Ficusexasperata* whose MIC was 100mg/ml against *K.pneumoniae* and 25mg/ml against *E.coli*, *S.aureus*, and *Ps.aeruginosa*^[4]. Minimum inhibitory concentrations (the lowest concentration of drug which inhibits growth) are used by diagnostic laboratories to determine in vitro activity or resistance to both old and new antimicrobial agents^[12].

The extract exhibited a wide spectrum of antibacterial activity when compared with most of the orthodox antibiotics tested as shown in Table 2 and Fig. 2. However, these antibiotics produced higher zones of inhibition when compared with the extract (Table 2, Fig. 2, and Fig. 3).

Looking at the zones of inhibition produced by the extract and those produced by the standard antibiotics, one may be tempted to conclude that the latter are more efficacious in the treatment of infections caused by the above mention bacteria. However, it must be noted that the extract was used in its crude form, unrefined form. Therefore, the concentration of the active constituents in each given dose of the extract may actually be lower than those found in the orthodox antibiotics. Also, being crude, this extract could contain active constituents that interacted with one another thereby reducing its total inhibitory effect on the organisms. In fact, Kafaru et al. had earlier shown that purification of crude extract leads to loss of medicinal value^[13]. On the other hand, Okoli and Iregbudemonstrated that the crude ethanolic extract of *Synclisia scabrida* had no antibacterial activity against clinical isolates of *Proteus sp.* which was susceptible to the purification product of the same plant^[14]. The observed antibacterial actions of this extract could be attributed to the presence of saponins, tannins, and alkaloids which have proven antimicrobial properties^{[15], [16]}.

It is interesting to note that *S.aureus* is Gram-positive while the rest are Gram-negative. Whereas *K.pneumoniae* is an important cause of respiratory infection, *E.coli* causes gastroenteritis especially in children. *E. coli*, *S. aureus*, and *Ps.aeruginosa* are implicated in the aetiology of urinary tract infections, the latter two bacteria usually after instrumentation or in the immunocompromised^[17]. It is important to note that the extract exhibited antibacterial activity against all bacterial species tested especially *Ps.aeruginosa* which is known to be resistant to many conventional antibiotics. Therefore, the use of the extracts of the leaves of this plant in the treatment of diarrhea, as well as urinary and respiratory infections in traditional medical practice appears justified.

V. Conclusion

The aqueous leaf extract of *E.heterophylla* produced dose dependent inhibitory effect on the growth of *K.pneumoniae*, *S.aureus*, *E.coli*, and *Ps. aeruginosa*, with *S.aureus* being the most susceptible. However, the standard antibiotics produced higher zones of inhibition when compared with the extract. Therefore the use of extracts of this plant in the treatment of respiratory, urinary tract, and gastrointestinal infections in traditional medical practice is justified. Clinical trials using refined products of this extract are strongly recommended especially as this extract exhibited broad spectrum antibacterial action against the organisms tested. This plant being readily available in commercial quantity in most tropical regions will provide alternative and cheap sources of antimicrobial agents. Also, because the extract has not been abused, drug resistance to it will not be a problem.

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