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Antimicrobial Activity of Ethanol Extract of Four Indigenous Plants From South Eastern Nigeria

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ABSTRACT

The Antimicrobial properties of the ethanolic leaf extract of four indigenous plants, *Laportia ovalifolia, Spondias mombin, Ficus exasperata* and *Ageratum conyzoides*, on four bacterial isolates *(Stapshylococcus aureus, P aeruginosa, Escherichia coli, and Salmonella taphi),*were investigated, The minimum inhibitory concentration (MIC) of these plants for each of the test organisms was evaluated. The antimicrobial activity of the plant extracts was determined by the Well diffusion method. The ethanolic leaf extract of all the plants inhibited the test organisms. The widest spectrum of antimicrobial activity was recorded for the ethanolic leaf extracts of *S. monbin* against *P aeruginosa* (17.5±0.7 mm) *S. aureus* (12.0±1.4 mm), *E. coli* (11.5±0.7 mm), and *S. typhi* (9.0±0.7mm). This was followed by the ethanolic leaf extract of *F. exasperata* which showed zone of inhibition of (10.0±1.4 mm) on *E. coli* (8.0±0.7 mm) on *S. aureus,* (6.5.5±0.7 mm) on , *P. aeruginosa* and (6.0±0.7mm) on *S. typhi.* The antimicrobial activity of *Laportia ovalifolia* against the organisms was minimal compared to that of S. *mombin and F. exasperata.* The ethanolic leaf extract of this plant had a narrow antibacterial spectrum against the tested organisms ranging from 8.0 ±1.4mm against *S. aureus* to 3.0± 1.4mm against *S. typhi.* The MIC showed that the extracts exhibited antimicrobial properties at concentrations ranging from 12.5ml-200ml and compared favourably with Ciprofloxacine (the positive control) at a concentration of 200 ml. The results obtained in this investigation were discussed in line with the use of the plants under investigation as medicinal plants.

Keywords: *Laportia ovalifolia, Spondias mombin, Ficus exasperate, Ageratum conyzoides,* Antimicrobial properties.

1. INTRODUCTION

Diseases caused by micro-organisms remain one of the major threats to human health. Although a number of natural-synthetic antimicrobial agents have been isolated and developed to kill pathogenic microorganisms effectively, global antimicrobial resistance is an increasing public health problem. Various specific plants have continued to be an important therapeutic aid for alleviating the ailments of humankind. Therefore, novel antimicrobial agents from different biological sources are continuously sought (Yamac and Bilgili 2006). There is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects, (Nair and Chanda, 2007). This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants. A number of medicinal plants, such a *Boerhavia diffusa, Commelina nodiflorum* (obogu), *Erythrina senegalensis* (echichi) or (echichili), *Mucuna pruriens* (akugba), *Sida cordifolia, Ricinus communis* (ugba) or (ogiri-ugba), *Ageratum conyzoides* (akwukwo nwaosinaka), *Paullinia pinnata, Spathodea campanulata* (utu ogbolo mmiri), *Tetrapleura tetraptera* (oshosho), *Vitex doniana* (elili) or (ukoro), *Monodora myristica* (eruru), *Strophanthus hispidus* (anu mmii) *Euphorbia kamerunica* (abananya), and many others have been listed against the various ailments they cure. The antimicrobial activity of some of these plants has been investigated by many researchers in Nigeria and all over the world. Plant species have been shown to possess antagonistic effects against bacteria, fungi, and viruses. Researchers have shown that among 122 known plant species tested against bacteria, fungi and viral activities, the extracts of these plants were inhibitory against the growth of the microorganisms (Anesiniet and Perez, 1993). Bryanygaba (2004) investigated the antimicrobial activity of the compounds from *Partheum argentatum* against *Kleibsiella pneumonia* and *Pseudomonas aeruginosa* and discovered that the plant extracts were potent *macrocarp* on *S. aureus, E. coli* and *K. pneumonia* and the inhibitory effects of the extract from *Eucalyptus species* against soil fungi, (Bruna *et al*; 1989). A more detailed study on the antimicrobial activities of compounds from extracts of over 120 plant species has revealed that the extracts obtained from 58 of the plants were shown to possess inhibitory effects against *S. auerus, E. coli* and *P. aeruginosa* (Santos *et al* ., 1990). The essential oils obtained from *Croton triangularis* and *Lippia gracilis* leaves have been reported to have antimicrobial properties against wide range of bacteria. Nascimento and Chiappeta, (1990), investigated the effects of the ethanol extracts of *Combretum duarteanum* against five bacterial isolates and fungal species. The results showed that the extracts of the plant were potent against the organisms. Similariy, Cruz *et al*; (1996), reported the antimicrobial activity of *Mikania triangularis* against five bacteria and three genera of yeast and showed that the plant extract exhibited inhibitory effect against *S. aureus, Bacillus cereus, E. coli, P. aeruginosa* and *S. epidermidis.*

The present study is aimed at evaluating the antimicrobial activity of the ethanolic extract of the leaf of *L. ovalifolia, S. mombin, F. exasperata* and *A. conyzoides* at the concentrations of 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml and to find out the minimum inhibitory concentration (MIC) of these extracts on the tested organisms.

2. MATERIALS AND METHODS

2.1 Collection And Identification Of Plant Material

Four plant species *(Laportea ovalifolia, Spondias mombin, Ficus exasperata and Ageratum conyyzoides*) used for this investigation were collected from Ogwe Asa in Ukwa-West Local Government Area of Abia State, Nigeria. They were authenthicated by Mr Oriaku Williams (A laboratory technician in the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike, Abia State,Nigeria.

2.2 Extraction Procedure

The Fresh leaves of the various plants were dried separately at room temperature for five days. The dried leaves were powdered using the laboratory mortar. The powdered material was stored in air tight sterile containers protected from sunlight until required for analysis. A slightly modified method of extraction by Mehmet *et al*; (2010) was followed. Fifty grams (50g) of the processed plant materials each were mixed with 20 ml ethanol solvent in a beaker and placed on a rotary shaker for 24 hours so that the phytochemicals such as tannins, flavonoids, alkaloids, terpenoids and other constituents present would get dissolved. The aqueous solutions were filtered using Whatman filter paper (No 1) and then concentrated in vacuum for 15 min at 37°C using a Rotary evaporator. The concentration was then dissolved in 15 w.w. of 1% dimethylsulfoxide and stored at 4°C for further study.

2.3 Test Organisms

A total 4 species of human pathogenic bacteria (*Staphylococcus aureus*, *Salmonella typhi (*Gram-positive), *Escherichia coli* and *Pseudomonas aruginosa (*Gram-negative)) were used in this study. These microorganisms were obtained from the Microbiology Laboratory of Federal Medical Center (FMC), Umuahia, Abia State, Nigeria. The bacteria strains were maintained on nutrient agar medium at 37^0C for further study.

2.4 Preparation of Media

To prepare the medium, 12g of Muller Hinton Media was mixed with 250ml of distilled water and sterilized in an autoclave immediately at 103 KNM-2 (121^0C and 15lb pressure for 15 minutes). The freshly prepared medium was poured into disposable Petri–dishes. The agar medium was allowed to cool and gel at room temperature.

2.5 Preparation Of Antibiotic Stock Solution

The antibiotic used for this investigation is Ciprofloxacin (500mg). A tablet of the antibiotic was dissolved in 5ml of distilled water (500mg/5ml) in a sterile test tube and was used for the antimicrobial susceptibility test on each of the bacterial isolates *(Staphyloccocus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi)).* Various concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml were prepared and were used to determine the minimum inhibitory concentration (MIC) of the antibiotic on the test organisms. This was done using double dilution method.

2.6 Antimicrobial Activity Of The Plant Extracts

The ethanolic extract of *L. ovalifolia, F. exasperate, S. mombin and A. Conyzoides were tested against four bacterial pathogens (S. aureus, E.c* oli, *P. aeruginosa and S. typhi*). The antimicrobial tests were carried out by the disc diffusion method (Collins, and Lyne, 1987). The prepared culture plates were inoculated with different selected strains of bacteria using swab stick. This procedure was repeated by streaking two lines to ensure an even distribution of the organism. Wells were created on the agar surface with 6mm cork borer. The plates were turned upside down and the wells labeled with a marker. The extracts were poured into the wells using sterile Pasteur pipette. The plates were incubated at 37^0C for 24 hours. The zone of inhibition was calculated by measuring the diameter of the inhibition (mm). The readings were taken in three different directions on all 3 replicates and the average value was tabulated. Ciprofloxacin was used as the positive control whereas sterile distilled water served as the negative control.

2.7 Minimum Inhibitory Concentration

The minimum inhibitory concentration of the extracts was investigated by diluting a given volume of the extract to various concentrations according to Macro-broth dilution technique (Baron and Finegold, 1990). One millilitre of distilled water was measured into five different appropriately labeled test tubes. This was followed by double dilution of the plant extracts to obtain dilutions such as 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml. Six wells were made on the agar surface with a sterile cork borer (6mm) and the wells were appropriately labeled. The concentrations were used to fill up the wells using Pasteur pipette. This was followed by the incubation of the plates at 37^0C for 24 hours. The resulting zones of inhibition were measured using a ruler calibrated in millimetre. The average of the three reading were taken to be the three zones of inhibition of the bacterial isolates in question at that particular concentration (Abayomi, 1982; Junaid *et al.*, 2006).

3. STATISTICAL ANALYSIS

All the results obtained were analyzed using simple statistics of ANOVA. The mean was separated using Least Significant Difference (LSD). (> 0.5)

4. RESULTS AND DISCUSSION

The results of the antimicrobial activity of the ethanolic plant extract of *L. ovalifolia, F. exasperate, S. mombin* and *A. conyzoides* against some bacterial isolates (*S.aureus, E. coli, P. aeruginosa* and *S. typhi*) are presented in Table 1. The results showed that the four possess antimicrobial activity. The of *S. mombin* extract showed more antimicrobial efficacy against *P. aeruginosa*, and *S aureus* recording 17.0mm, 12.5mm inhibitory zones respectively, followed by its effects on, *E. coli* and *S. typhi* with zones of 11.5mm and 9.0mm, respectively. *F. exasperata* was second in efficacy and showed a mean inhibition zone of 10.0mm, 8.0mm, 6.0mm and 6.0mm on *E.coli, S. aureus, P. aeruginosa* and *S. typhi* respectively. The following zones of inhibition were observed in *L. ovalifolia* against the tested organisms (8.0mm, 8.0mm, 6.0mm and 3.0mm) this extract recorded the lowest zone of inhibition. *A. conyzoides* recoreded 7.0mm, 6.5mm, 6.0mm and 5.0mm respectively on the organisms. The highest zone of inhibition was observed in *P. aeruginosa* while the least zone was observed in *S. typhi* (3.0mm) by the leaf extract of *L. ovallifolia*. The antibiotic Ciprofloxacine used as positive control at the concentration of 500mg/5ml showed the widest range of inhibition (37.0mm) on *E. coli*, 36.0mm on *S. aureus*, 27.0mm on *S. typhi* and 23.0mm *on P. aeruginosa*. There was no zone of inhibition observed in the distilled water used as negative control (0.0mm).

The minimum inhibitory concentration (MIC) of the leaf extracts of the four plants on each of the tested organisms at different concentration (6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) are presented in Tables 2-5. *L. ovalifolia* recorded no inhibition at the concentrations of 12.5 mg/ml, 25 mg/ml, and 50 mg/ml and highest inhibition at 12.5ml. Likewise *A. conyzoides* did not inhibit the growth of the organism at 12.5mgml/ and 25 mg/ml, while *F. exasperate* did not inhibit it at 12.5 mg/ml. *S. mombin* recorded the lowest zone of inhibition of 1.5mm on *S. typhi* at a concentration of 12.5 mg/ml and highest at a concentration of 200 mg/ml and (Table 2).

P. aeruginosa was not sensitive to *S. mombin* at 6.25 mg/ml, *F. exasperate* at 6.25 mg/ml and 12.5 mg/ml, *A. Conyzoides* and *L.ovalifolia* at 6.26 mg/ml-25 mg/ml respectively (Table 3). While the highest sensitivity was recorded in *S. mombin* (17.0±0.7), followed by that in *F. exasperate (*10.5±0.7) and in *L. ovalifolia and A. Conyzoides* (6.5±0.7 respectively.

The minimum inhibitory concentration (MIC) of the leaf extracts of the four plants on *E.Coli* (Table 4), showed total inhibition at the concentrations of 6.25 mg/ml- 12.5 mg/ml, and highest at 200 mg/ml. However *F. Exasperrata* and *S. mombin* had highest and equal inhibition of the organism at 200 mg/ml (12.0±1.4).

At 6.25 mg/ml and 12.5 mg/ml, all four plant extracts recorded 0.0mm inhibition zone for the bacterium *S. aureus*.(Table 5). The plant extract of *S. mombin* gave inhibition of 4.0mm at 25 mg/ml at which concentration all the other three plant extracts gave mean zones of 0.0mm inhibition.

5. DISCUSSION

Researchers have shown that among 122 known plant species tested against bacterial, fungal and viral activities, the extracts of these plants were inhibitory to the growth of the microorganisms (Anesiniet and Perez, 1993). Bryanygaba (2004) investigated the antimicrobial activity of the compounds from *Partheum argentatum* against *Kleibsiella pneumonia* and *Pseudomonas aeruginosa* and discovered that the plant extract was potent against the organisms. Studies also have shown the inhibitory activity of *Vatairea macrocarp* on *S. aureus, E. coli* and *K. pneumonia* and the inhibitory effect of the extract from *Eucalyptus species* against soil fungi (Bruna, *et al*., 1989).

Hence, many medicinal plants may be used as a response to specific health problems. As can be seen in this study all the plant extracts examined showed different levels of potency against all the tested organisms. The ethanolic leaf extract of *S. mombin* showed a greater antimicrobial potency against *P .aeruginosa*, It was also potent against *S. aureus*, *E. coli* and *S. typhi* and may help in the cure of diseases caused by these organisms. *S. aureus* for instance causes gonorrhea and other infectious disorders of the skin. Thus, the leaf extract of *S. mombin* may be used as agent for the treatment of these diseases. *E. coli* can also be controlled by the leaf extract of *F. exasperrata*. This observation agrees with the previous studies of Akindele and Adeyemi (2007). The antibiotic Ciprofloxacine (500mg/5ml) used as positive control showed the widest zone of inhibition on all the tested organisms. This confirms the effectiveness of the drug in combating or treating infections associated with the tested organisms (Adebayo *et al*., 2009).

Medicinal plants can be poisonous if wrong plant parts or wrong concentrations are used (Frohne, 1999). The results of the minimum inhibitory concentration (MIC) of the ethanolic plant leaf extracts on the tested organisms have revealed that the extracts possess antimicrobial activity at the various concentrations but highest at 200 mg/ml. The inhibitory effects of the extracts on the tested organisms increase with increase in the concentrations. The non-inhibitory effect of all four extracts at 6.25 mg/ml on the four bacterial pathogens implies that they cannot serve as good antimicrobial at that concentration in the treatment of diseases associated with the tested organisms.

From these results, the observed antimicrobial properties could be attributed to the presence of bioactive compounds present in them. These include tannins, flavonoids, saponin, alkaloids and phenolic compounds

which have been shown to possess antimicrobial properties (Edeoga *et al*., 2005). However, the inability of the extracts to inhibit the growth of the tested organisms at lower concentrations may be due to the low levels ctive ingredient (the bioactive compounds) in the concentration of the extracts. The inhibitory effect of the plant extracts at the various concentrations against the tested organisms has established antimicrobial potentials of the plants.

Table 1: Mean zones of inhibition of the ethanolic leaf extracts of *L. ovalifolia A. conyzoides F. exasperata and S.mombin*

Test organism	Plant extracts and their zones of inhibition (mm)				Ciprofloxacin+ve control 500mg/5ml	Sterile distilled water –ve control
	L. ovalifolia	***A. conyzoides***	***F. exasperata***	***S. mombin***		
S. aureus	8.0 ±1.4	7.0 ±1.4	8.0 ±0.7	12.0 ±1.4	36.0 ±0.7	0.0 ±0.0
E. coli	6.0 ±1.4	6.0 ±0.7	10.0 ±1.4	11.5 ±0.7	37.0±0.7	0.0 ±0.0
P. aeruginosa	8.0 ±1.4	4.5 ±0.7	6.5 ± 1.4	17.5 ±0.7	23.0±0.7	0.0 ±0.0
S. typhi	3.0 ±1.4	5.0 ±0.7	6.0 ±1.4	9.0 ±0.7	27.0±0.7	0.0 ±0.0

Values are means inhibition zone (mm) ± standard deviation of three replicates

Table 2: Minimum inhibitory concentration (MIC) of plant extracts on *Salmonella typhi*

Plant extracts	Concentration of plant extracts (ml)						Ciprofloxacin +ve control (ml)						Sterile distilled water –ve control
	6.25ml	12.50ml	25.0ml	50.0ml	100.0ml	200.0ml	0.0±0.0	1.5±0.7	3.5±0.7	5.5±0.7	8.5±0.7	10.0±0.7	0.0±0.0
A. conyzoides	0.0±0.0	0.0±0.0	0.0±0.0	3.5±0.7	6.0±1.4	8.5±1.4	0.0±0.0	1.5±0.7	3.5±0.7	5.5±0.7	8.5±0.7	10.0±0.7	0.0±0.0
F. exasperata	0.0±0.0	0.0±0.0	1.5±0.7	4.0±1.4	6.0±1.4	8.0±1.4	0.0±0.0	1.5±0.7	3.5±0.7	5.5±0.7	8.5±0.7	10.0±0.7	0.0±0.0
S. mombin	0.0±0.0	1.5±0.7	4.5±0.7	7.5±0.7	9.5±0.7	12.5±1.4	0.0±0.0	1.5±0.7	3.5±0.7	5.5±0.7	8.5±0.7	10.0±0.7	0.0±0.0
L. ovalifolia	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.5±0.7	3.0±1.4	0.0±0.0	1.5±0.7	3.5±0.7	5.5±0.7	8.5±0.7	10.0±0.7	0.0±0.0

Values are means inhibition zone (mm) ± standard deviation of three replicates

Table 3: Minimum inhibition concentration of plants extracts on *P. aeruginosa*

Plant extracts	Concentration of plant extracts (ml)						Ciprofloxacin +ve control (ml)						Sterile distilled water – ve control
	6.25ml	12.5ml	25.0ml	50.0ml	100ml	200ml	6.25ml	12.5ml	25.0ml	50.0ml	100ml	200ml	
L. ovalifolia	0.0±0.0	0.0±0.0	0.0±0.0	1.5±0.7	3.5±0.7	6.5±0.7	2.5±0.7	4.5±0.7	6.5±0.7	8.5±0.7	10.5±0.7	11.5±0.7	0.0±0.0
A. conyzoides	0.0±0.0	0.0±0.0	0.0±0.0	1.5±0.7	3.5±0.7	6.0±0.7	2.5±0.7	4.5±0.7	6.5±0.7	8.5±0.7	10.5±0.7	11.5±0.7	0.0±0.0
F. exasperata	0.0±0.0	0.0±0.0	4.0±1.4	5.5±0.7	8.0±0.7	10.0±0.7	2.5±0.7	4.5±0.7	6.5±0.7	8.5±0.7	10.5±0.7	11.5±0.7	0.0±0.0
S. mombin	0.0±0.0	1.5±0.7	4.0±1.4	7.5±0.7	14.0±0.7	17.0±0.7	2.5±0.7	4.5±0.7	6.5±0.7	8.5±0.7	10.5±0.7	11.5±0.7	0.0±0.0

Values are mean zones of inhibition (mm) ± standard deviation of three replicates

Table 4: Minimum inhibitory concentration (MIC) of plant extracts *on E. coli*

Plant extracts	Concentration of plant extracts (ml)						Ciprofloxacine +ve control (ml)					Sterile distilled water –ve control	
	6.25 ml	12.5ml	25.0 ml	50.0 ml	100ml	200ml	6.25ml	12.5ml	25.0ml	50.0ml	100ml	200ml	
L. ovalifolia	0.0±0.0	0.0±0.0	1.5±0.7	3.5±0.7	4.5±0.7	6.5±0.7	0.0±0.0	0.0±0.0	3.5±0.7	5.0±0.7	7.0±0.7	9.5±0.7	0.0±0.0
A. conyzoides	0.0±0.0	0.0±0.0	0.0±0.0	3.5±0.7	5.5±0.7	8.0±1.4	0.0±0.0	0.0±0.0	3.5±0.7	5.0±0.7	7.0±0.7	9.5±0.7	0.0±0.0
F. exasperata	0.0±0.0	0.0±0.0	4.0±1.4	6.5±0.7	9.5±0.7	12.0±1.4	0.0±0.0	0.0±0.0	3.5±0.7	5.0±0.7	7.0±0.7	9.5±0.7	0.0±0.0
S. mombin	0.0±0.0	0.0±0.0	4.0±1.4	7.0±1.4	10.0±1.4	12.0±1.4	0.0±0.0	0.0±0.0	3.5±0.7	5.0±0.7	7.0±0.7	11.5±0.7	0.0±0.0

Values are mean zones of inhibition (mm) ± standard deviation of three replicates

Table 5: Minimum inhibitory concentration (MIC) of plant extracts *on S .aureus*

Plant extracts	Concentration of plant extracts (ml)						Ciprofloxacin +ve control (ml)						Sterile distilled water –ve control
	6.25mg /ml	12.5mg /ml	25mg/ ml	50mg/ ml	100mg/ ml	200mg/ ml	6.25mg/ ml	12.5mg/ ml	25mg/ ml	50mg/ ml	100mg/ ml	200mg/ml	
L. ovalifolia	0.0±0.0	0.0±0.0	0.0±0.0	3.0±1.4	4.5±0.7	6.5±0.7	0.0±0.0	0.0±0.0	2.5±0.7	5.5±0.7	8.5±0.7	10.5±0.7	0.0±0.0
A. conyzoides	0.0±0.0	0.0±0.0	0.0±0.0	2.5±0.7	5.0±1.4	7.0±1.4	0.0±0.0	0.0±0.0	2.5±0.7	5.5±0.7	8.5±0.7	10.5±0.7	0.0±0.0
F. exasperata	0.0±0.0	0.0±0.0	0.0±0.0	4.0±1.4	6.0±1.4	9.0±1.4	0.0±0.0	0.0±0.0	2.5±0.7	5.5±0.7	8.5±0.7	10.5±0.7	0.0±0.0
S. mombin	0.0±0.0	0.0±0.0	4.0±1.4	8.0±1.4	14.5±0.7	18.±0.7	0.0±0.0	0.0±0.0	2.5±0.7	5.5±0.7	8.5±0.7	10.5±0.7	0.0±0.0

Values are mean zones of inhibition zone (mm) ± standard deviation of three replicates

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