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In vitro antimicrobial evaluation of whole-plant extracts of *Eleucine indica*

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Abstract
The Ibibio people of Southern Nigeria have a great wealth of knowledge of medicinal plants used in the treatment of wounds, skin infections and various ailments. *Eleucine indica* is widely used in various disease states, especially in feverish conditions. This study was aimed at evaluating the *in vitro* antimicrobial potentials of whole-plant extracts of *Eleucine indica.* Four typed cultures of bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and one clinical strain of fungi, *Candida albicans,* were assessed using agar well diffusion method. The extracts that exhibited antimicrobial activity were then tested to determine the Minimum Inhibitory Concentration for each bacterial or fungal sample. The ethyl acetate extract showed the widest zone of inhibition (25.0 mm), followed by chloroform when tested against *Staphilococcus aureus.* The ethyl acetate extract also exhibited broadspectrum antibacterial activity against *Pseudomonas aeruginosa*, *E. coli* and *B. subtilis.* None of the extracts showed any inhibitory effect against the fungal strain of *Candida albicans.* Both chloroform and ethyl acetate extracts exhibited similar potencies having MIC of 50 mg/ml against *E. coli and P. aeruginosa* respectively. The antibacterial activities of this plant may be ascribed, at least in part, to the presence of phytochemical constituents such as flavonoids, alkaloids and tannins in the extracts.

Keywords: Antimicrobial, Eleucine indica, Whole-plant, *In vitro*.

Introduction
Eleucine indica, L. Gaertn (Poaceae) is called nkimenang (Ibibios) and crowsfoot or goose grass (English). It is an annual growing to 0.45m and is considered as an adventitious species that is native in the tropics and subtropical regions [1]. It is generally considered as an adventitious species that is native in the tropics and subtropical regions [2]. It is an annual growing to 0.45m. It is in flower from July to August and the seeds ripen from August to October. The flowers are monaceous (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by wind. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well drained soil. The plant prefers acid, neutral and basic (alkaline) soil [1]. This plant is used for the treatment of malaria among the Ibibios of Southern Nigeria. The whole plant, especially the root, is depurative, diuretic, febrifuge and laxative, and hence is used for the treatment of influenza, hypertension, oliguria and urinary retention [3]. It is also used for kidney problems in Trinidad and Tobago [4]. Two main flavonoids have been isolated: schaftoside (6-C-β-glucopyranosyl-8-C-α-arabinopyranosylapigenin) and vitexin (8- C-β-glucopyranosylapigenin) based on ^{1}H and ^{13}C NMR spectra and found to have strong anti-inflammatory activities [5]. *Eleucine indica* has been reported to have phytochemical content of sterol glucoside forms [6] and C-glycosyl-flavone possessing anti-inflammatory activities [7]. *Eleucine indica* leaves are reported to have good bactericidal activity towards methicillin-resistant *staph aureus* (MRSA), *B. subtilis*, *P. aeruginosa* and *S. choleraesuis*, antioxidant and non-cytotoxic properties [8].
Phytochemical screening has indicated the presence of alkaloids, tannins, flavonoids, cardiac glycosides, terpenes and simple sugar.The extract has antiplasmodial [9], analgesic, anti-inflammatory [10], antipyretic and antioxidant and anticonvulsant activities [11, 12]. While the leaves of this plant have been reported to have antimicrobial properties, there is little or no scientific report on the whole plant and its effect on fungal clinical isolates. This study was aimed at determining the effect of the whole plant extract on both bacterial and fungal organisms.

Materials and Methods

Plant materials

Collection and Identification of Plant Material

The plant material (*Eleucine indica*) was collected in April 2009 from Uyo, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. (Mrs.) Margaret Bassey (a plant Taxonomist) in the Department of Botany and Ecological Studies, University of Uyo, where a voucher specimen (UUH 1409) was deposited.

Test organisms

Five organisms used in this study as test organisms were three typed cultures of bacteria: *Eschericia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (NCTC 8853) and one clinical isolate of fungi (*Candida albicans*) obtained from the Pharmaceutical Microbiology and Parasitology Unit of the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo. The typed cultures of bacteria and clinical isolates of fungi were sub-cultured on Nutrient agar (Oxoid) and Saboraud dextrose agar (Oxoid) slants respectively and stored at 4°C until used.

Antimicrobial agents

Drugs used as in the test as positive controls: streptomycin 0.4mg/ml and Nystatin 50,000 I.U/ml.

Preparation of Extracts

The plant material was air-dried and then oven-dried at reduced temperature. It was thereafter ground into powder using a mixer grinder and successively extracted in chloroform, ethylacetate and methanol at room temperature for 72 h, and filtered. The filtrate was dried *in vacuo* using the rotary evaporator to obtain dried extract. The extracts were stored in a refrigerator at – 4 °C until required for use. These extracts were thereafter kept in sterile bottles at – 4 °C and were then used to determine the concentration in mg/ml.

Evaluation of antimicrobial potentials of extracts

Mueller Hinton Agar (MHA) medium was used to evaluate the antibacterial activity. The method used was agar well diffusion method as described by Omenka and Osuba and Nwafor *et al*., [13, 14]. Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. The culture plates were each seeded with test organisms and allowed to solidify. They were then punched with a sterile cork -borer (5.0mm diameter) thereby cuting uniform wells. Open wells were then filled with 0.05ml of the extract. All plates were thereafter incubated at 37 °C for 24 hours. Antimicrobial test for fungi was done using Saboraud dextrose agar (SDA) plates incubated at 30°C for 72 hours. Zones of inhibition were then measured and recorded and compared with positive standard controls streptomycin (0.4mg/ml) for bacteria and nystatin (50,000 I.U/ml) for fungi.

Minimum inhibitory concentration (MIC)

In order to determine the MIC, various concentrations of the extracts were prepared (6.25mg/ml, 12.5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml.). The culture plates were then seeded with test bacterial organisms and allowed to solidify and then punched with a sterile cork- borer. They were then filled with 0.05ml of the extract. The plates were then incubated at 37°C for 24h. The lowest concentration of the extract that showed inhibition of growth of the test organisms was read and taken as the minimum inhibitory concentration (MIC) [13, 14].

Results and discussion

The results of the antimicrobial potentials of three extracts of *Eleucine indica* are as shown in Table 1. The ethyl acetate extract showed the widest zone of inhibition (25.0 mm), followed by chloroform when tested against *Staphilococcus aureus.* The ethyl acetate extract also exhibited broadspectrum antibacterial activity against *Pseudomonas aeruginosa*, *E. coli* and *B. subtilis.* The chloroform extract similarly showed broadspectrum activity except that it had no effect on *B. subtilis*. None of the extracts had activity against *Candida albicans.* Methanol extract showed no activity at all against gram-positive and gram-negative bacteria as well as fungal organism tested. The ethyl acetate extract has more effective against gram-positive organisms than gram-negative organism.

Table 1: Antimicrobial Activity of Three Extracts of Eleucine indica

Bacterium / Isolate	Extract Concentration mg/ml	Diameter of Growth Inhibition Zone (mm)				
		Chloroform	Ethyl acetate	Methanol	Streptomycin 0.4mg/ml	Nystatin 50,000 (i.u./ml)
S. aureus	100	12.0	25.0	-	50.0	-
	50	9.0	10	-	-	-
	25	-	-	-	-	-
	12.5	-	-	-	-	-
P. aeruginosa	100	10.0	12.0	-	34.0	-
	50	-	-	-	-	-
	25	-	-	-	-	-
	12.5	-	-	-	-	-
E. coli	100	15.5	15.0	-	40.0	-
	50	7.0	10.5	-	-	-
	25	-	-	-	-	-
	12.5	-	-	-	-	-
B. subtilis	100	-	12.0	-	45.0	-
	50	-	7.0	-	-	-
	25	-	-	-	-	-
	12.5	-	-	-	-	-
B. albicans	100	-	-	-	-	-
	50	-	-	-	-	-
	25	-	-	-	-	20
	12.5	-	-	-	-	-

The Minimum Inhibitory Concentrations (MIC) values of the extracts are shown in Table 2. Against *Staphylococcus aureus*, the minimum inhibitory concentration for both ethyl acetate and chloroformv was 100 mg/ml. When tested against *E. coli*, the MIC for ethyl acetate was 100 mg/ml while chloroform was 50 mg/ml. When tested against *B. subtilis* the MIC for ethyl acetate extract was 100 mg/ml but 50 mg/ml when tested against *Pseudomonas aeruginosa*. Chloroform extract gave an MIC of 100 mg/ml when tested against *P. aeruginosa*. Both chloroform and ethyl acetate extracts exhibited similar potencies having MIC of 50 mg/ml against *E. coli and P. aeruginosa* respectively.as shown in Table 2.

Table 2: Minimum Inhibitory Concentration (MIC) of Three Extracts of Eleucine indica

Bacterium/Isolate	Extract Concentration mg/ml	Diameter of Growth Inhibition Zone (mm)		
		Chloroform	Ethyl acetate	Methanol
S. aureus	100	10.5	15.0	-
	50	-	-	-
	25	-	-	-
	12.5	-	-	-
P. aerueginosa	100	9.0	-	-
	50	-	9.5	-
	25	-	-	-
	12.5	-	-	-
E. coli	100	-	10	-
	50	9.5	-	-
	25	-	-	-
	12.5	-	-	-
B. subtilis	100	-	10.0	-
	50	-	-	-
	25	-	-	-
	12.5	-	-	-

Higher plants have great potentials for the development of antimicrobials of the future. This becomes moreso, as there is increasing resistance to previously effective antimicrobials. ATP-dependent efflux pumps are one of the major mechanisms of preventing accumulation of effective concentrations of antibiotics at molecular target sites within the bacterial cell. The main efflux pump systems observed in Gram-negative strains like *P. aeruginosa* are MexXY-OprM or MexCD-OprJ Which have been associated with acquired multidrug resistance [15, 16, 17]. Organisms found to be susceptible in this study are *S. aureus, P. aureginosa, B. subtilis and E.coli* all of which are implicated in systemic infections. *P. aeruginosa* is involved in pneumonia, bacteremia, wound infection, endocarditis,,meningitis, urinary tract infections, brain abscess, osteomyelitis and otitis media. [18, 19]. The phytochemical screening of this plant showed the presence of flavonoids and tannins [9] both of which have been shown to possess antibacterial properties [20, 21, 22]. Flavonoids are known for their anti-inflammatory, anti-arthritic and antibacterial properties [23]. *Eleucine indica* extract has alkaloids as one of the phytochemical constituents [9]. Alkaloids are a large and structurally diverse group of compounds that have served as scaffolds for important antibacterial drugs such as metronidazole and the quinolones [24]. Therefore, the antibacterial activities of this plant may be ascribed, at least in part, to the presence of tannins and flavonoids in the extracts [25].

Conclusion

In conclusion, the results of this study show that the extracts of *Eleucine indica* possess antimicrobial activity. The ethyl acetate extract showed the widest zone of inhibition (25.0 mm), followed by chloroform when tested against *Staphilococcus aureus.* The ethyl acetate extract also exhibited broadspectrum antibacterial activity against *Pseudomonas aeruginosa*, *E. coli* and *B. subtilis.* None of the extracts showed any inhibitory effect against the fungal strain of *Candida albicans.* Both chloroform and ethyl acetate extracts exhibited similar potencies having MIC of 50 mg/ml against *E. coli and P. aeruginosa* respectively. The antibacterial activities of this plant may be ascribed, at least in part, to the presence of phytochemical constituents such as flavonoids, alkaloids and tannins in the extracts.

References

1. Morris R. Plants for A Future – Species Database. 2000. *http//www.ibiblio.org/pfaf/cgi-bin*/arr_html. Date of Access: January 30, 2009): 1-3.
2. Haber RM, Seaman MT. Two new records from Lebanon: Chamacyse nutans (Lag.) Small (Euphorbiaceae) and Eleucine indica (L) Gaertus (Poaceae). Turkish Journal of Botany 2007; 31: 341-343.
3. Leach GE, Devine MD, Kirkwood RC, Marshall G. Target Enzyme-based Resistance to Acetyl-coenzyme A Carboxylase Inhibitors in *Eleucine indica.* Pesticide Biochemistry and Physiology. 1995; 51:129-136.
4. Lans CA. Ethnomedicines Used in Trinidad and Tobago for Urinary Problems and Diabetes Mellitus. Journal of. Ethnobiology and Ethnomedicine. 2006; 13:45.
5. Agrawal PK. *Carbon -13 NMR of Flavonoids*. Amsterdam, The Netherlands: Elsevier Science Publishers B. V. 1989; pp. 332-333.
6. Phuong NM, Sung TV, Ripperger H, Adam G. Sterol Glucosides from *Eleucine indica. Planta Medica*. 1994; 60:498.
7. De-Melo GO, Muzitano MF, Legora-Machado A, Almeida TA, De Oliveira DB, Kaiser CR. C-glycosyl Flavones From the Aerial Parts of *Eleucine indica* Inhibit LPS – induced Mouse Lung Inflammation. Planta Medica. 2005; 7:362-363.
8. Adel SA, Ahmad BA, Siddig IA, Chew YP, Syam M, Manal ME. *Eleucine indica* possesses Antioxidant, Antibacterial and Cytotoxic properties. Hindawi Publishing Corporation. Evidence-Based Complementary and Alternative Medicine. 2011. Article ID 965370, doi:10.1093/ecam/ nep091: 1-6.
9. Ettebong EO, Nwafor PA, Okokon JE. *In vivo* antiplasmodial activities of ethanolic extract and fractions of *Eleucine indica*. Asian Pac J Trop Med. 2012; 5(9):673-6.

10. Ettebong EO, Nwafor PA. Anti-inflammatory and analgesic potentials of *Eleucine indica*. J Phytopharmacol 2014; 3(2):130-8.
11. Ettebong EO, Nwafor PA. Antipyretic and antioxidant activities of *Eleucine indica*. J of Phytopharmacology 2015; 4(4):235-42.
12. Ettebong EO, Edidiong E, Augustine B. Anticonvulsant potentials of ethanolic extract of *Eleusine indica.* Journal of Coastal Life Medicine. 2016; 4(12):965-968.
13. Omenka CA, Osuoha JO. Antimicrobial potency of grape fruit seed extract of five selected pathogens. Nig. J. Microbiol. 2000; 14(2):39-42.
14. Nwafor IB, Amadi ES, Nwaziri AA, Nwuzo AC. Prelimary antimicrobial activities of crude extract of some medicinal plants on otitis media pathogen. Proceedings of Annual Conference. Biotechnology Society of Nigeria (BSN), 2007, 17-79.
15. Schweizer HP. Efflux as a mechanism of resistance to antimicrobials in Pseudomonas aeruginosa and related bacteria: unanswered questions. Genetics and Molecular Research. 2003; 2(1):48-62.
16. Nikaido H. Multidrug resistance in bacteria. Annual Review of Biochemistry. 2009: 78:119-146.
17. Mujeeb F, Bajpai P, Pathak N. Phytochemical Evaluation, Antimicrobial Activity, and Determination of Bioactive Components from Leaves of *Aegle marmelos.* Biomed -ical Research International. 2014; 2014:497-606.
18. Turner KH, Everett J, Trivedi U, Rumbaugh KP, Whiteley M. Requirements for *Pseudomonas aeruginosa* Acute Burn and Chronic Surgical Wound Infection. PLoS Genet. 2014; 10(7):e1004518. https://doi.org/10.1371/journal.pgen.1004518
19. Harith JFand Saba NA. Urinary tract infections caused by *staphylococcus aureus* DNA in comparison to the *candida albicans* DNA. North American Journal of Medical sciences. 2011; 3(12):565-569.
20. Hostettman K, Martson AJ, Wolfender I, Maillard M. Screening for flavonoids and related compounds in medicinal plants by LC-UV-MS and subsequent isolation of bioactive compounds. Akademiai, Kiaho, Budapest. 1995, 35-52.
21. Oboh G, Akindahunsi AA, Famutimi R, Adetuyi FC. Antimicrobial activity of saponin extracts from two wild yams (*Dioscorea spp.*). Nigerian Journal of Biochemstry and Molecular Biology. 1998; 13:47-50.
22. Akter K, Barnes EC, Brophy JJ, Harrington D, Community Elder Y, Vemulpad SR, *et al*. Phytochemical profile and antibacterial and antioxidant activities of medicinal plants used by aboriginal people of New South Wales, Australia. Evidence-Based Complementary and Alternative Medicine. 2016, Article ID 4683059, 1-14.
23. Cushnie TP, Cushnie B, Lamb AJ. Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. International journal of Antimicrobial agents. 2014; 44(5):377-86. doi: 10.1016/j.ijantimicag.2014.06.001.
24. Trease GE, Evans WC. Pharmacognosy. English Language Book Society, 13th ed. Bailliere Tindall, London, 1989; pp.683-684.
25. Mabhiza D, Chitemerere T, Mukananyama S. Antibacterial Properties of Alkaloid Extracts from Callistemon citrinus and Vernonia adoensis against Staphylococcus aureus and Pseudomonas aeruginosa. International Journal of Medicinal Chemistry. 2016; Article ID 6304163, 1-7.