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Full Length Research Paper

Antimicrobial Properties, Phytochemical Composition, and Phenotypic Resistance Pattern of Selected Enteropathogenic Microorganism on *Ageratum conyzoides* (Goat Weed) Leaf Extract

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ABSTRACT

The aim of this study is to determine the phytochemical composition, antimicrobial properties and phenotypic resistance pattern of selected enteropathogenic microorganism on Ageratum conyzoides leaf extract. Enteropathogenic isolates were collected from Adekunle Ajasin University Health Centre, Akungba Akoko, Ondo State and were identified using Microbact[™] 24E Identification kit. Antibiotic sensitivity testing was carried out against the enteropathogenic organism using Kirby-Bauer disc diffusion method, imipenem was active against Proteus mirabilis, Enterobacter agglomerans, Enterobacter gerogoviae, with diameter zone of inhibition of 41 mm, 26 mm and 30 mm. ofloxacin were active against Proteus mirabilis, Enterobacter agglomerans, Enterobacter gergoviae, with diameter zone of inhibition of 21, 31 and 21 mm. All enteropathogenic organisms shows high resistant rate against oxacilin, vancomycin, amoxicillin, ceftazidime, piperacillin, cefoxitin, tazobactam. Antimicrobial screening of the leaf extract of Ageratum conyzoides revealed high bioactivity against Serratia marcescens, Proteus vulgaris, Enterobacter agglomerans and Proteus mirabilis isolates with diameter zone of inhibition ranging from 15 mm to 22 mm at 100 mg/ml. Using broth dilution method, the minimum inhibitory concentrations (MIC) of the Ageratum convzoides (Goatweed) extract range from 25 mg/ml and 100 mg/ml. The phytochemical screening results shows that Ageratum conyzoides extract contained alkaloid, pholobotannins, Cardiac glycoside, phenol, tannins, saponin, and flavonoids. It can be deduced from this research work that Ageratum conyzoides has better antimicrobial properties and it contains very important phytochemical constituents which can be a magic bullet to deal with the menace of antimicrobial resistance enteropathogenic microorganism.

Keywords: Antimicrobial properties, Ageratum conyzoides, Phenotypic resistance pattern, Enteropathogenic bacteria

INTRODUCTION

Ageratum conyzoides (Billy goat-weed, Chick weed, Goat weed, White weed; Ageratum conyzoides L., Ageratum obtusifolium Lam., Cacaliamentrasto Vell) is native to tropical America, especially Brazil. The herb 0.5–1 m. high, with ovate leaves 2–6 cm long, and flowers are white to mauve. In Vietnamese, the plant is called curtlon (Pig faeces) due to its growth in dirty areas. The plant belongs to the order asterales, family asteraceae, tribe eupatorieae, genus Ageratum, and species conyzoides. It is a common weed, having spread from its native range to all areas of the Tropics within 20° of the Equator to an altitude of 2,500 metres.

The leaves and the flowers yield 0.2% with a powerful nauseating odour. The oil contains 5% eugenol, which has a pleasant odour. The oil from plants growing in Africa has an agreeable odour, consisting almost entirely of eugenol. A decoction of the fresh plant is used as a hair wash, leaving the soft, fragrant and dandruff-free (Adak et al., 2002). The plant survived in full sun and under sheltered position in any reasonably

fertile moisture retentive soil that does not dry out in the summer. Plant vigour and flowering period are much reduced on dry soils. This species is not frost hardy, though it can be grown as a summer annual in Britain (Akinyemi et al., 2005). *Ageratum conyzoides* can complete its life cycle in less than two months. Although it can flower when less than two months, true leaves have expanded, it is more commonly to seen in favourable conditions as a well-branched plant up to 9cm tall with hundreds of flower heads (Chanda et al. 2006). Antimicrobial resistance is one of the world's most serious public health problems, many of the microbes (bacteria, viruses, protozoa) that cause infectious disease no longer respond to common antimicrobial drugs.



Figure 1. Ageratum conyzoides leaf.

The problem is so serious that unless concerted action is taken worldwide, we run the risk of returning to the pre-antibiotic era when many more children than now died of infectious diseases. The major infectious diseases kill over 11 million people per year. The prevalence rate of antimicrobial resistance all overall the world of diarrheal shigellosis is 10-90% for ampicillin 5-95% and for trimethoprim/ sulfamethoxazole (Dandekar et al., 2010). For this reason the microbial antibiotics resistance is receiving increasing attention in light of the increasing incidence of human bacterial infections resistant to antibiotic treatment (Dahiru et al., 2006). The resistance of enteropathogenic bacteria to commonly prescribed antibiotics is increasing both in developing as well as in developed countries; resistance has emerged even to newer, more potent antimicrobial agents and is commonly seen in organism like Salmonella, Shigella, Vibrio cholerae (Darudoal 2006).

Multiple-drug resistant organisms (MDRO) are defined as bacteria that have become resistant to more than one class of antimicrobial agents and usually are resistant to all but one or two commercially treatment of illness they cause. The emergence of MDRO is increasingly recognized as a major public health threat based on data from the centres for Disease Control and Prevention (DCP) costing the United States Healthcare system approximately 3.2billion dollars annually with increasing mortality rates (Edeoga et al., 2005). Currently, two-thirds of all health careassociated infections (HAIs) are caused by just six MDRO referred to by the acronym ESKAPE: Enterococcus species (vancomycin-VISA/VERSA), Klebsiella pneumonia, Acinetobacter baumanni, Pseudomonas aeruginosa and Enterobacter species (extended-spectrum beta-lactamase-producing gramnegative bacilli-ESBLs and fluoroquinolone-resistant P. aeruginosa) (Hazra et al., 2007).

MATERIAL AND METHODS

Description of the Study Area

The study area was Adekunle Ajasin University Health Akungba Akoko, Ondo State, Nigeria. The study was conducted between October 2017 and June 2018. Patients from Adekunle Ajasin University Health Centre Akungba Akoko, one of the major Towns in Ondo State. Ondo State lies between latitude 5° 45' and 8° 15' North and longitude 4° 45' and 6° East were used for this research work (Kamboj et al., 2008).

Collection and Processing of Samples

A total of 20 stool samples were collected from patients with acute diarrhoea (Gastroenteritis). About five to ten grams (or millilitres) fresh stool samples without any preservatives were collected in sterile plastic universal specimen bottles. All stool samples were transported to the laboratory in the sterile container, and all the stool specimens are stored at -20°C freezer for further analysis. Samples were completely labelled by the necessary data (date, time of collection, sample type, patient name).

Test Organisms

The test organisms used were standard strains of pathogenic enteric bacteria isolates. They including Proteus vulgaris, Stenotrophomonas maltophilia, Hafnia alvei, Proteus mirabilis, Serratia marcescens, Enterobacter agglomerans, Citrobacter freundii, Proteus luminescence, Samonella substilis, Serratia rubidaea and Enterobacter gergoviae as they are identified via microbat[™] 24E identification kit. The bacterial isolates were cultured in slanted Mueller Hinton agar and transported at a low temperature to Microbiology Laboratory, Adekunle Ajasin University, Akungba-Akoko, Ondo State and incubated in an incubator for reactivation of the bacteria. They were then sub-cultured and stored -4°C prior to bioassay of the extracts (Kumar et al., 2007).

Identification of the Enteropathogenic Bacterial Isolates

The enteropathogenic bacterial isolates were identified using their colony morphological characteristics. The appearance of each colony on the agar media and characteristics such as shape, edge, colour, elevation and texture were observed as described by (Mcarvalho et al., 2010). The isolates were there after subjected to relevant biochemical tests and identified using the taxonomic scheme of Bergey's Manual of and **Microbact™** Determinative Bacteriology 24E identification kit test



Figure 2. Microbact[™] 24E identification kit result for isolated bacteria.

Standardization of Test Organisms

Slants of the various organisms were reconstituted at aseptic conditions; using a sterile wire loop approximately one isolated colony of each pure culture was transferred into 5 ml of sterile nutrient broth and incubated for 24 hours. After incubation, 0.1 ml of the isolated colony was transferred into 9.9 ml of sterile distilled water contained in each test tube using a sterile needle and syringe, and the mixed properly. The liquid now serve as source of inoculums containing approximately 106 cfu/ml of bacterial suspension (Metchock et al., 2011, Milstone et al., 2010).

Antibiotic Susceptibility Test for the Bacterial Isolates

Single disc diffusion method were employed as described by Bauer, was used to examine bacterial susceptibility to antimicrobial agents. The antibiotic sensitivity discs used were oxacillin (1 μ g), imipenem (10 μ g), vancomycin (30 μ g), amoxicillin (30 μ g), ceftazidime (30 μ g), ofloxacin (5 μ g), piperacillin (100 μ g), cefoxitin (30 μ g) and tazobactam (110 μ g) (Becton Dickson, USA). Single bacterial colonies from overnight culture were suspended in 5 ml normal saline. The surface of Mueller Hinton agar plates was evenly

inoculated; the antibiotics discs were applied on the surface of the inoculated agar plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface; plates were incubated at 370 °C for 24 hours. After incubation, the plates were examined and the diameter of the zones of inhibition was measured. Susceptibility data were interpreted according to the Clinical and Laboratory Standard Institute (Onwukaeme et al., 2007, Osuntokun 2015).

Plant Sample- Source and Collection of Plant Sample

The leaves of *Ageratum conyzoides* used in this study were collected from the campus environment, Adekunle Ajasin University Akungba Akoko, Ondo State, Nigeria.

Authentication of plant sample: the plants were authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Preparation of plant sample: The leaves of *Ageratum conyzoides* after collection were first washed thoroughly with sterile distilled water to lose contamination and appropriately air dried at room temperature for two weeks to ensure the sample loss of their moisture content. The leaves of *Ageratum conyzoides* after being air dried, was milled to powder at the Department of Microbiology Laboratory, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Preparation of plant extracts: Five hundred gram (500 g) of the fine powder plant (*Ageratum conyzoides*) was weighed into corked container containing 1500 ml of dichloromethane, the mixture were initially shaken vigorously and left for 7 Days. Mixture was filtered using whatman filter (No 1) papers, and the filtrates were collected directly into sterile crucibles. The filtrate was extracted using rotary evaporator, and the residues obtained were kept at room temperature (Metchock et al., 2011).

Standardization of plant extracts: At aseptic condition, the extracts are reconstituted by adding 1 g of extract, 2.5 ml of Dimethyl sulfoxide (DMSO) and 7.5 ml of sterile distilled water, making it 100 mg/ml. 5 ml of distilled water is measured into four bijou sterile bottles. In bijou bottle, a 5 ml from the 100 mg/ml was drawn and added, making it 50 mg/ml. The serial concentration was prepared to get concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml respectively (Mcarvalho et al., 2010, Olajubu et al., 2015).

Antimicrobial assay of the plant extracts: Susceptibility screening test using agar well diffusion method, all the test bacteria, were sub-culture onto sterile Molarity

agar plates, and incubated at 37°C for 24 hours, five distinct colonies for each organisms were inoculated for 3-4 hours. All inocula were standardized accordingly to match the 0.5 McFarland standards and this standard was used for all susceptibility tests. The leaf extracts were reconstituted accordingly into the following concentrations; 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, using Dimethyl sulfoxide (DMSO). 0.1 ml of 1:10,000 dilutions (equivalent to106 cfu/ml) of fresh overnight culture of the enteric bacteria isolates grown in Nutrient broth was seeded into 40 ml of Molarity agar, and properly mixed in universal bottles. The mixture was aseptically poured into petri dishes and allowed to set. Using a sterile cork borer of 6 mm diameter, equidistant wells were made in the agar. Drop of the re-suspended, (2 ml per well) extracts with concentration between 100 mg/ml to 12.5 mg/ml were introduced into the wells till it was filled. Levofloxacin 50 mg/ml was used as the control experiment. The plates were allowed to stand on a bench for an hour, to allow per diffusion of the extracts before incubation at 37°C for 24 hours. The zone of inhibition was measured to the nearest millimetre (mm) using a standard transparent meter rule. All the experiments were performed in duplicates (Milstone et al., 2010).

Phytochemical Screening of Plant Extract

The extract was subjected to phytochemical tests of qualitative and quantitative screening for plant secondary metabolites in accordance with (Onwukaeme et al., 2007).

RESULT

Table 1 shows the data of patients, collected isolates and laboratory codes, specimen Microbact[™] 24E code, percentage of the organism and identified organisms. Isolates collected were identified with Microbact[™] 24E identification kit which is incorporated with different biochemical tests like indole, catalase, citrate, urease, lysine, hydrogen sulphide, gelatin, malonate, inositol, sorbitol, rhamnose, arabinose, raftinose, orninine, salicine, arginine, glucose, mannitol, xylose, sucrose, lactose, ONPG, VP, and TDA.

Table 2 shows the diameter (in mm) of the zone of inhibition of the conventional antibiotics against the tested isolates. All the isolates were resistant to ceftazidime. oxacillin. vaconmycin. cefoxitin. piperacillin, amoxicillin, and tazobactam, while 50% of the isolates were susceptible to imipenem, and The Proteus ofloxacin. mirabilis isolates are susceptible to imipenem with the following diameter 26 mm, 24 mm, 41 mm and 20 mm; Samonella subtilis was susceptible to imipenem with diameter of 26 mm; Enterobacter agglomerans was susceptible to imipenem with diameter of 26 mm; Enterobacter gergoviae was susceptible to imipenem with the diameter of 30 mm; Serratia marcescens was susceptible to imipenem with the diameter of 17 mm; *Proteus luminescence* are susceptible to imipenem with diameter 19 mm. Proteus mirabilis isolates were susceptible to ofloxacin with the diameter of 16 mm, 25 mm, 21 mm and 33 mm; Samonella subtilis were susceptible to ofloxacin with the diameter of 23 mm; *Enterobacter agglomerans* were susceptible to ofloxacin with the diameter of 33 mm and 31 mm; *Enterobacter gergoviae* was susceptible to ofloxacin with the diameter of 21 mm; Serratia rubidaea is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 23 mm; *Proteus luminescens* is susceptible to ofloxacin with the diameter of 25 mm.

Table 1. Identification of microorganisms using microbact24E and their clinical details.

S/N	Lab code	Specim en	Result code	Percenta ge probabili ty	Probable organism
1	D1	Stool	1673610 4	94.56%	Proteus vulgaris
2	D2	Stool	5016600 0	88.70%	Stenotrophomonas maltophilia
3	D3	Stool	7076622 1	98.97%	Hafnia alvei
4	D4	Stool	7057700 0	94.86%	Serratia marcescens
5	D5	Stool	7476406 0	80.58%	Proteus mirabilis
6	D6	Stool	5356616 6	76.98%	Serratia rubidaea
7	D7	Stool	1257620 1	90.76%	Enterobacter agglomerans
8	D8	Stool	3676611 1	84.01%	Proteus mirabilis
9	D9	Stool	3457612 3	99.99%	Proteus mirabilis
10	D10	Stool	1677630 2	92.68%	Enterobacter agglomerans
11	D11	Stool	1037600 1	75.56%	Proteus mirabilis
12	D12	Stool	1036466 0	81.01%	Citrobacter freundii
13	D13	Stool	5006400 1	92.10%	Stenotrophomonas maltophilia
14	D14	Stool	1437610 2	99.86%	Proteus vulgaris
15	D15	Stool	7256602 5	72.54%	Enterobacter gergoviae
16	D16	Stool	6256612 1	89.76%	Serratia rubidaea

17	D17	Stool	7477634 1	96.76%	Ptoteus mirabilis	-	19	D19	Stool	1036600 0	94.78%	Proteus luminescens
18	D18	Stool	7777672 1	97.86%	Salmonella subtilis		20	D20	Stool	3757617 1	91.42%	Proteus mirabilis

Table 2. Susceptibility test result of the test organisms.

Enteropathogenic bacteria		Antibiotics							
N = 20	IPM	OFX	CAZ	ох	VA	TZP	AMC	FOX	PRL
Proteus vulgaris	0	0	0	0	0	0	-	-	-
Stenotrophomonas maltophilia	0	0	0	0	0	0	-	-	-
Hafnia alvei	0	0	0	0	0	0	-	-	-
Serratia marcescens	17	0	0	0	0	0	-	-	-
Proteus mirabilis	26	16	0	0	0	0	-	-	-
Serratia rubidaea	0	0	0	0	0	0	-	-	-
Enterobacter agglomerans	22	33	0	0	0	0	-	-	-
Proteus mirabilis	24	25	0	0	0	0	-	-	-
Proteus mirabilis	41	21	0	0	0	0	-	-	-
Enterobacter agglomerians	26	31	0	0	0	0	-	-	-
Proteus mirabilis	0	0	0	0	0	0	-	-	-
Citrobacter freundii	0	0	0	0	0	0	-	-	-
Serratia maltophilia	0	0	0	0	0	0	-	-	-
Proteus vulgaris	0	0	0	0	0	0	-	-	-
Enterobacter gergoviae	30	21	0	0	0	0	-	-	-
Serratia rubidaea	0	23	0	0	0	0	-	-	-
Proteus mirabilis	0	0	-	0	-	-	0.0	0	0
Salmonella subtilis	26	23	-	0	-	-	0	0	0
Proteus luminescence	19	25	-	0	-	-	0	0	0
Proteus mirabilis	20	33	-	0	-	-	0	0	0

OFX=Ofloxacin (5 μg) TZP=Tazobactam (110 μg) VA=Vancomycin (30 μg) 0.0=No Inhibition FOX=Cefoxitin (30 μg) AMC=Amoxillin (30 μg) CAZ=Ceftazidime (30 μg) IPM=Imipenem (10 μg) PRL=Piperacillin (100 μg) OX=Oxacillin (1 μg)



Figure 3. Antibiotic susceptibility test.

Table 3 shows the antimicrobial activity of ethanol extract of Ageratum conyzoides leaf against test enteropathogenic organisms at different concentrations (100, 50, 25, 12.5 mg/ml, and Control 5 mm/ml levofloxacin) measured in diameter (mm). Proteus vulgaris shows the susceptibility diameter zone of inhibition of 22.0 mm at 100 mg/ml, 20.0 mm at 50 mg/ml, 18.0 mm at 25 mg/ml, 12.0 mm at 12.5 mg/ml; Enterobacter agglomerans showed the susceptibility diameter zone of inhibition of 22.0 mm at 100 mg/ml, 17.0 mm at 50 mg/ml, 15.0 mm at 25 mg/ml, 10.0 mm at 12.5 mg/ml; Proteus mirabilis isolates shows the susceptibility diameter zone of inhibition of 15.0 mm, 19.0 mm, 18.0 mm at 100 mg/ml, 15.0 mm, 15.0 mm, 14.0 mm at 50 mg/ml, 10.0 mm, 15.0 mm, 8.0 mm at 25 mg/ml and 0.0 mm, 12.0 mm, 0.0 mm at 12.5 mg/ml. Enterobacter agglomerans and Proteus vulgaris were observed to have the highest susceptibility to the leaf extract with 22.0 mm at 100 mg/ml, 17.0 mm at 50 mg/ml, 15.0 mm at 25 mg/ml, 10.0 mm at 12.5 mg/ml and 22.0 mm at 100 mg/ml, 20.0 mm at 50 mg/ml, 18.0 mm at 25 mg/ml, 12.0 mm at 12.5 mg/ml diameter zone inhibition. P. mirabilis was observed to have the lowest susceptibility to the leaf extract of the plant with diameter 15 mm at 100 mg/ml, 15 mm at 50 mg/ml, 10 mm at 25 mg/ml and 0.0 mm at 12.5 mg/ml.

Table 3. Antimicrobia	l activity of ethanol	extract of Ageratum	conyzoides.
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Test Organisms			Ethan	ol extract of A	Ageratum conyzoides concentration (mg/ml)
	100	50	25	12.5	Levofloxacin 5 mg/ml
Proteus vulgaris	22	20	18	12	31
S. maltophilia	0	0	0	0	0
Hafnia alvei	0	0	0	0	0
S. marcescens	0	0	0	0	0
proteus mirabilis	15	15	10	0	32
Serratia rubidaea	0	0	0	0	0
E. agglomerans	22	17	15	12	31
Proteus mirabilis	19	15	15	12	33
Proteus mirabilis	18	14	8	0	36
E. agglomerans	0	0	0	0	0
Proteus mirabilis	0	0	0	0	0
Citrobacter freundii	0	0	0	0	25
S. maltophilia	0	0	0	0	30
Proteus vulgaris	0	0	0	0	28
E. gergoviae	0	0	0	0	23
Serratia rubidaea	0	0	0	0	0

Proteus mirabilis	0	0	0	0	0
Salmonella subtilis	0	0	0	0	0
P. luminescence	0	0	0	0	0
Proteus mirabilis	0	0	0	0	32

Data are presented as mean of measurement of zone of inhibition of two replicates measured in mm. Zone of inhibition does not include the diameter of the cork borer (6 mm). Levofloxacin is the control, Key: 0.0=No Inhibition.



Figure 4. Antimicrobial susceptibility test.

Table 4 shows the minimum inhibitory concentration (MIC) value of the ethanol extract of *Ageratum conyzoides* leaf against the test organisms used in the study. From this table, *E. agglomerans* strain was the test organism with the least MIC values at 50 mg/ml while *P. mirabilis* was the test organism that was inhibited throughout the values measured. *P. mirabilis* isolates were the bacteria with the highest MIC value among the bacteria isolates with its MIC value at approximately 100 mg/ml.

Table 5 shows the qualitative phytochemical analysis of *Ageratum conyzoides* using methanol, ethyl acetate, dichloromethane and acetone solvents. From this

table, it was observed that alkaloid cardiac glycoside, phenol, tannins, and saponin were found in all the solvents used. Steroids were absent in acetone while, it was present in dichloromethane, methanol and ethylalkaloid. cardiac acetate. The glycoside. anthraguinone, tannins, saponin constituents were present in all the solvents used. Flavonoids was absent in methanol while present in ethyl-acetate, dichloromethane and acetate and reducing sugar was absent in ethyl-acetate while present in methanol, dichloromethane and acetate.

Table 4. Minimum Inhibitory Concentration of EthanolExtracts of Ageratum conyzoides.

Lab code	25 mg/ml	50 mg/ml	100 mg/ml
Proteus vulgaris	+ve	-ve	-ve
Proteus mirabilis	+ve	-ve	-ve
Enterobacter agglomerians	+ve	-ve	-ve
Proteus mirabilis	+ve	-ve	-ve
Proteus mirabilis	-ve	-ve	-ve

Key: ve+=Growth observed ve-=No growth observed

Table 5. Qualitative phytochemical analysis of Ageratum conyzoides.

Phytochemical	Methanol Solvent	Ethyl-acetate solvent	Dichloromethane solvent	Acetone solvent			
Alkaloid	+ve	+ve	+ve	+ve			
Cardiac glycolside	+ve	+ve	+ve	+ve			
Steroid	+ve	+ve	±ve	-ve			
Anthraquinone	+ve	ND	+ve	+ve			
Phenol	+ve	+ve	+ve	+ve			
Tannins	+ve	+ve	+ve	+ve			
Saponin	+ve	+ve	+ve	+ve			
Flavonoids	-ve	+ve	+ve	+ve			
Reducing sugar	+ve	-ve	+ve	+ve			
Keys: +ve=Positive -ve=Negative							

Table 6 shows the quantitative analysis of the phytochemical constituents of *Ageratum conyzoides* using four different solvents which are methanol, ethyl-

acetate, dichloromethane and acetone, showed the presence of different phytoconstituents in different quantities. For leaf using methanol, alkaloid was

shown to be present in the largest quantity with 9.10, and saponin was found to be the least abundantly present with 0.3. The quantitative phytochemical screening of A. conyzoides leaf using ethyl acetate, alkaloid was shown to be present in the largest quantity with 11.3, and cardiac glycoside was found to be the least abundantly present with 0.6. The quantitative phytochemical screening of A. conyzoides leaf using dichloromethane (DCM), alkaloid was shown to be present in the largest quantity with 13.8, and flavonoids was found to be the least abundantly present with 3.21. The quantitative phytochemical screening of A. conyzoides leaf using acetone, cardiac glycoside was shown to be present in the largest quantity with 9.71, and flavonoids was found to be the least abundantly present with 2.90.

Table 6. Quantitative phytochemical analysis of Ageratumconyzoides.

Phytochemical	Methanol Solvent	Ethyl- acetate solvent	Dichloromethane solvent	Acetone solvent
Alkaloid	9.1	11.3	13.8	3.89
Cardiac glycoside	9.35	0.6	9.71	9.71
Phlobatannins	3.11	7.5	14	4.01
Phenol	4.7	9.52	9.7	9.7
Tannins	7.37	0.3	7.51	7.51
Saponin	0.32	7.34	7.6	7.6
Flavonoids	2.71	7.23	3.21	2.9

DISCUSSION

The aim of this study is to determine the phytochemical composition, antimicrobial properties, and Phenotypic Resistance Pattern of Selected Enteropathogenic microorganism on *Ageratum conyzoides* leaf extract. For centuries, medicinal plants have been the main source for drugs in many centuries, and it is estimated that at least 25% of modern medicine are derived either directly or indirectly from medicinal plants (Olajubu et al., 2015, Prasannabalaji et al., 2006).Herbal medicine has been shown to have genuine utility and about 80% of rural dwellers depend on its efficacy for their primary health care. Medicinal plants contribute an effective source of both traditional and modern medicines (Ram et al., 2008).

Ageratum conyzoidesis commonly found in the rain forest region of Nigeria, along with other plants with good medicinal properties has plays a significant role in the eradication of Enteropathogenic Microorganism causing Gastroenteritis (Onwukaeme et al., 2007, Ram et al., 2008). In this study, the leaf of *Ageratum conyzoidesis* plant were extracted using ethanol, and were tested for their antibacterial properties against Proteus vulgaris, Stenotrophomonas maltophilia, Hafnia alvei, Proteus mirabilis, serratia marcescens, Enterobacter agglomerans, Serratia rubidaea, Enterobacter gergoviae, Proteus luminescence, Salmonella subtilis, and Citrobacter freundii.

Gastroenteritis caused by Enteropathogenic microorganism is one of the most popular sicknesses among children and can be caused by many infectious agents, varying from bacteria, parasites and viruses, whose etiology and prevalence varies among developing and developed countries. However, patients may show more severe symptoms ranging from relatively mild upper gastroenteritis symptoms, such as nausea and vomiting, to severe symptoms, such as profuse diarrhoea that leads to dehydration or death (Osuntokun et al., 2015). Each year, an estimated 2.5 billion cases of diarrhoea occurs among children under five years of age, and estimates suggest that overall incidence has remained relatively stable over the past two decades. Infectious diarrhoea affects mainly children who are at risk of complications, especially when they suffer from malnutrition, which is common in Palestinian children (Olajubu et al., 2015, Oyedele et al., 2006). The crude plants extract tested in this study showed antimicrobial activities against Proteus vulgaris, Enterobacter agglomerans and Proteus mirabilis isolates with diameter zone of inhibition of 22.0 mm at 100 mg/ml, 20.0 mm at 50 mg/ml, 18.0 mm at 25 mg/ml, 12.0 mm at 12.5 mg/ml; 22.0 mm at 100 mg/ml, 17.0 mm at 50 mg/ml, 15.0 mm at 25 mg/ml, 10.0 mm at 12.5 mg/ml; and 15.0 mm, 19.0 mm, 18.0 mmat 100 mg/ml, 15.0 mm, 15.0 mm, 14.0 mm at 50 mg/ml, 10.0 mm, 15.0 mm, 8.0 mm at 25 mg/ml, 10.0 mm, 12.0 mm, 0.0 mm at 12.5 mg/ml. However, differences were observed between their antimicrobial activities.

Ethanol extract of *Ageratum conyzoides* is leaf, as observed from this study, can be a source of a novel antimicrobial agent, especially with good activities against organisms like Proteus vulgaris (Raquel 2007). The minimum inhibitory concentration of the extract range from 25 mg/ml to 100 mg/ml. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (antibiotic or bacteriostatic, antifungal) drug that will inhibit the visible growth of a microorganism after overnight incubation (Tripathi et al., 2013).

The quantitative phytochemical screening of *Ageratum conyzoides* leaf using different solvents, showed the presence of different phytoconstituents in different quantities. For leaf using methanol, alkaloid was shown to be present in the largest quantity with 9.10, and saponin was found to be the least abundantly present with 0.3. The quantitative phytochemical screening of *A. conyzoides* leaf using ethyl acetate, alkaloid was shown to be present in the largest

quantity with 11.3, and cardiac glycoside was found to be the least abundantly present with 0.6. The quantitative phytochemical screening of *A. conyzoides* leaf using dichloromethane (DCM), alkaloid was shown to be present in the largest quantity with 13.8, and flavonoids was found to be the least abundantly present with 3.21. The quantitative phytochemical screening of *A. conyzoides* leaf using acetone, cardiac glycoside was shown to be present in the largest quantity with 9.71, and flavonoids was found to be the least abundantly present with 2.90 (Table 6) (Trease et al., 2006, Wang et al., 2002).

The qualitative phytochemical screening of A. conyzoides contains alkaloid, flavonoid, tannins, and saponin, reducing sugars, steroids, phenol, cardiac glycoside and anthraguinone (Table 5). These biologically active constituent is known to act by different mechanism and exert antimicrobial action (WHO 2005). Alkaloids are medicinally useful, possessing analgesic, antispasmodic and bactericidal effects. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Wang et al., 2002).

The antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell (Trease et al., 2006). Steroids have been reported to have antibacterial properties the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes(Xuan et al., 2004). These differences could be attributed to the differences in their chemical composition and amount of the bioactive compounds extracted by the solvent. These compounds usually accumulate in different parts of the plants (Tripathi et al., 2013). Proteus vulgaris was observed to be the most susceptible organism to the ethanol extract of Ageratum conyzoidesis leaf with diameter zone of inhibition of 22 mm at 100 mg/ml while Proteus mirabilis was seen to be the least susceptible to this extract with diameter zone of inhibition of 15 mm at 100 mg/ml respectively as shown in Table 3. The greater abundance of the dichloromethane (DCM) extract of A. conyzoides leaf could indicate that, the crude dichloromethane (DCM) extract possess antimicrobial properties than any others extracts of this same plant.

The importance of *Ageratum conyzoides* must be mentioned in order to encourage its uses. *Ageratum*

conyzoides is used as an infusion with the leave or the entire plant; this is employed in the treatment of colic, fever, diarrhoea, rheumatism and spasms. It can also be used as blood tonic (Xuan et al., 2004).it is highly employed in the treatment of burns and infected wound. In some country like Nigeria, the plant can be used as antibiotics because of its antimicrobial properties and various clinical microorganism especially the enteropathogenic microbes and infection (Akubugwo et al., 2006).

CONCLUSION

Plants are found in nearly all the regions of the world. The prevailing climatic, soil and environmental conditions often play a vital role in determining the type of plant species that could be found in such region. Due to the challenges associated with drug resistance, which have made scientists to search for effective and sustainable means of managing the problem. Plants have emerged as an alternative to synthetic antibiotics which is prone to reoccurring drug resistance. The result of antimicrobial susceptibility assay showed promising evidence for the antimicrobial effects of A. conyzoides against bacterial Proteus vulgaris, Enterobacter agglomerans, and Proteus mirabilis isolates used in this study. The MIC value of different organisms are verified, and thus, MIC are assays capable of verifying that the compound has antimicrobial activities, and that it gives reliable indication of the concentration of medicine required to inhibit the growth of microorganisms. Phytochemical analysis is responsible for the identification of components which are responsible for antimicrobial activity of plant, thus these traditional species can be used as a potential source of medicine against various diseases.

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