



Preliminary Phytochemical screening and *in vitro* antibacterial activities of aqueous and ethanol extracts of *Ageratum conyzoides* L. Leaf, Stem, Flower and Root on some Bacterial isolates associated with Diarrhoea

*ENERIJI OFI, K. E.¹ AND ISOLA, O. B.²

¹Department of Biological Sciences, Samuel Adegboyega University, P. M. B.001, Ogwa, Edo State.

²Department of Chemical Sciences, Samuel Adegboyega University, P. M. B.001, Ogwa, Edo State.

ABSTRACT

There is renewed interest in the use of medicinal plants to search for novel drugs especially as over 80% of the developing world population depend on them for their first line of treatment. This study aimed at evaluating the phytochemical and antibacterial activities of ethanol and aqueous extracts of parts of *Ageratum conyzoides* L. Thirty grams of the fine powdered plant parts were soaked in 300ml of ethanol and water for 48 hours respectively before extraction using Soxhlet extractor. Mueller Hinton agar was used to determine the antibacterial activity of the extracts with concentration ranging from (25 - 300mg/ml) by agar well diffusion method. Phytochemical screening revealed Tannins, Alkaloids, Flavonoids, Phenol, Steroids, Phlobatannins, Saponins, and Anthraquinones. Ethanol extracts revealed that Leaf of 300mg/ml gave the highest inhibition of 25mm, 27mm, 24mm and 26mm against *Salmonella* sp., *Enterobacter* sp., *E. coli* and *P. aeruginosa*. Aqueous extracts concentration of 300mg/ml gave the highest inhibition of 13mm, against *Salmonella* sp., 11mm against *Enterobacter* sp. and *P. aeruginosa* while 12mm was recorded against *E. coli*. However, the control did not show any antimicrobial activity. The minimum inhibitory concentration of the ethanol extract of the various plants' parts ranged from 50 - 75mg/ml while the aqueous extract recorded 100mg/ml against all bacterial isolates tested. The Minimum bactericidal concentration of ethanol extract for leaf and stem was 50mg/ml while flower and root was 75mg/ml. The MBC for aqueous extract of leaf, flower, stem and root was 100mg/ml for the four bacterial isolates. The results confirm the potential of the extracts from *Ageratum conyzoides* L. against pathogenic bacterial isolates from diarrhoea patients.

Keywords: *Ageratum conyzoides* L., bioactive constituents, diarrhoea, inhibition, phytochemical screening.

Introduction

Africa and indeed Nigeria is endowed with plants resources, unfortunately, these natural gifts by God have been neglected due to over reliance on Western products (Odunayo 2008; Onuoha *et al.*,

2013). *Ageratum conyzoides*, mostly known as goat weed is given different names in various communities in Nigeria. The Yorubas call it *Imi-esu*; Igbo, *Ula ujula*; Hausa, *Ahenhen* and Igedes,

Corresponding Author: Enerijiofi, K. E

Department of Biological Sciences, Samuel Adegboyega University, P. M. B.001, Ogwa, Edo State. Phone: +2348034553580; Email: kingsmore1luv@gmail.com



Ufuopioko (Amadi *et al.*, 2012; Onuoha *et al.*, 2013). According to the world health organization (2008), about 80% of the rural population living in developing countries relies essentially on plant products for their first line of treatment (Mckay and Blumberg, 2007; Nasrin, 2013). Plant extract have been used for a long time in developed countries because they are natural and relatively safe (Gurib-Fakim, 2006). The medicinal properties of plants are mainly attributed to the phytochemicals which may be influenced by organic and inorganic compounds like phenolic acid and antioxidant micro nutrients like Copper and Zinc (WHO, 2008).

Medicinal plants are rich as biological homes for traditional and modern medicine, food supplements and pharmaceutical intermediates (Dash and Murthy, 2011; Onuoha *et al.*, 2013). *Ageratum conyzoides* is a medicinal herb which is domiciled in the tropics has its components, pharmacologically active (Mantle *et al.*, 2000; Pribitkin, 2005). It has been utilized in treatment from different parts of the world for curing various diseases. In Cameroon, India and Brazil, it is a local remedy for external ailments like craw-craw, boils, wounds, leprosy and internally for uterine troubles, purulent ophthalmia and gynecological diseases (Dash and Murthy, 2011). Furthermore, its popularly used in treating of skin diseases and wound healing in Nigeria, a decoction of the extract is taken internally to treat diarrhoea and to get rid of the pains associated with navel in children (Okunade, 2002; Souza *et al.*, 2006). There are lots of issues arising from antimicrobial resistance in which antimicrobial drugs can no longer treat infections arising from infectious organisms (Osuntokun *et al.*, 2018). Over the years, the WHO (2008) has encouraged countries to interact with traditional medicinal practitioners on better ways to identify and exploit areas that relatively provide safe and current solutions for diseases of both microbial and non-microbial origins.

Esan land occupying Edo central senatorial district of Edo State, Nigeria endowed with forests is blessed with the abundant and rich varieties of plants and shrubs including *Ageratum conyzoides*. It is worthwhile to study this plant that is common and traditionally utilized in curbing lots of ailments. This study was set out to investigate the phytochemical composition and *invitro* antimicrobial activity of the different parts of *A. conyzoides* crude extracts against some bacterial isolates obtained from stool samples of patients attending the University of Benin Teaching Hospital, Benin City.

Materials and Methods

Sample Collection and Authentication

Fresh matured plants of *Ageratum conyzoides* were collected in large quantities from the premises of Samuel Adegboyega University, Ogwa, Edo State. The plant was authenticated by Prof. (Mrs.) F. M. Ogbe, a botanist and Head, Department of Biological Sciences, Samuel Adegboyega University.

Sample preparation

The plant was separated into the flowers, leaves, stems and roots. They were washed with distilled water; air - dried and crushed mechanically using a mortar and pestle to fine powder. The weight of samples were taken using analytical balance Setra model BL2005. The fine powdered samples were stored in airtight bottles for phytochemical and antimicrobial analyses. Thirty grams of fine powdered flowers, leaves, stems and roots were each successively extracted after soaking with 300mls of ethanol and water respectively for 48 hours using a sohxlet extractor. The liquid extracts were concentrated under vacuum to yield dry extracts (Azwanida, 2015).

Source and Confirmation of Bacterial Isolates

The pathogenic bacterial isolates; *Salmonella* sp., *Shigella dysenteriae*, *Escherichia coli* and *Pseudomonas aeruginosa* associated with

diarrhoea patients were collected from Microbiology laboratory of the University of Benin Teaching Hospital, Benin City and preserved on Nutrient Agar (NA) slants in the refrigerator at 4°C prior to use. The identities of the bacterial isolates were further re- confirmed by morphological and biochemical tests.

Phytochemical analysis of the Plant parts

This was done using the methods of (Dash and Murthy, 2011; Amadi *et al.*, 2012). The dried fine powdered form of *A. conyzoides* flowers, leaves, stems and roots were analysed for the following phytochemicals.

Tannins: One millilitre of 10% KOH was added to 1ml of the extract, the production of dirty white precipitate was indicative of tannins.

Alkaloids: Five millilitres of 1% HCl was added to 3mls of the extract, followed by heating for 20 minutes and allowed to cool and filtered. Thereafter, 1ml of the filtrate was measured and added to picric acid solution. Turbidity or precipitation confirmed alkaloids.

Flavonoids: One millilitre of 10% NaOH was added to 3mls of the extract, a yellow colouration depicted Flavonoids.

Phenol: Two drops of 5% FeCl₃ was added to 1ml of the extract in a test tube, a green precipitate means that phenol was available.

Glycosidase: Ten millilitres of 50% H₂SO₄ was added to 1ml of the extract, followed by boiling in water for 15 minutes. Thereafter, 10cm³ of Fehling's solution was added, and boiled to give a brick - red precipitate which showed the availability of glycosides.

Steroids: Five drops of concentrated H₂SO₄ was mixed with 1ml of the extract, a red colouration indicated that steroids was present.

Phlobatannins: One millilitre of the extract was added to 1% HCl, the formation of a red precipitate showed that the extract possessed phlobatannins.

Saponins: Zero-point five gram of crude powder was shaken with water in a test tube followed by warming in a water bath. The persistence of froth meant that saponins was available.

Anthraquinones: Zero-point five gram of crude powder was mixed with 10ml of benzene, thereafter filtered, followed by the addition of 0.5ml, 10 % ammonia solution to the filtrate. This mixture was rocked vigorously, the production of violet colour in the layer phase revealed the availability of anthraquinones.

Determination of antibacterial activity

Antibacterial activity of the different concentrations of ethanol and aqueous extracts were done using agar well diffusion method (CLSI, 2014). The test bacterial cultures were adjusted to 0.5 McFarland standard with normal saline. Two hundred microlitres (200ul) of the different bacterial suspensions were spread evenly on the surface of the already solidified Mueller Hinton Agar (MHA) plate. Cock borers of 5mm in diameter were used to bore holes inside the already solidified MHA plate. A total of 200ul of ethanol and water extracts of the different concentrations of the plant's parts were dispensed into the bored holes. Distilled water was used as the control. The plates were left for 4hours to allow diffusion of the extracts into the solidified MHA in the petridish. The plates were incubated at 37°C for 24hrs, thereafter checked for zones of inhibition around the wells which was measured, recorded and interpreted appropriately.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The concentrations of ethanol and aqueous extracts that showed significant activities in the primary screen were used to carry out the MIC and MBC.

The MIC was determined using serially diluted plant extracts as described by Lar *et al.* (2011). Thirteen test tubes labelled 1 -13 containing 5ml of Mueller Hinton Broth were used. One millilitre of the extract that showed substantial activity was introduced into the thirteen test tubes respectively and mixed. The broth cultures (0.1ml) equivalent to 0.5Macfarland of the test bacterial isolates were added to 12 test tubes with the 13th serving as the control. They test tubes were incubated at 37°C for 24hours thereafter, checked for bacterial growth. The test tube containing the least concentration in which there was no bacterial growth was taken as the MIC. The MBC was determined by sub culturing 0.1ml of the assay tubes that showed no growth on a fresh solid MHA agar. The inoculum were spread out using a smooth sterile glass rod for each isolate followed by incubation at 37°C for 24hours. Bacteria colonies were observed for growth in the solid medium plate. The highest dilution that did not give any bacterial growth on the medium was recorded as the MBC.

Results

The phytochemicals were available in the different parts of *Ageratum conyzoides* though there were some inconsistencies. However, ethanol extracts had Tannins, Alkaloids, Flavonoids, Phenols, Glycosidase, Steroids, Phlobatannins, Saponins and Anthraquinones in flower, leaf and root (Table 1).

Table 2a reveals the antibacterial activity of the different concentrations (25 – 300mg/ml) of ethanol extracts on the bacterial isolates. Leaf extract of 300mg/ml gave the highest zone of inhibition of 25mm against *Salmonella* sp., 27mm for

Enterobacter sp., 24mm for *E. coli* and 26mm against *P. aeruginosa*. Root extract of 25mg/ml gave the least zone of inhibition of 3mm against *Enterobacter* sp. while 4mm was recorded against *Salmonella* sp., *E. coli* and *P. aeruginosa*.

Table 2b reveals the antibacterial activity of the different concentrations (25 – 300mg/ml) of aqueous extracts on the bacterial isolates. Leaf extract of 300mg/ml gave the highest zone of inhibition of 13mm against *Salmonella* sp., 11mm against *Enterobacter* sp. and *P. aeruginosa* while 12mm was recorded against *E. coli*. Root extract of 25mg/ml gave the least zone of inhibition for 1mm against the four tested bacterial isolates. The control did not give any antimicrobial activity.

The result of the MIC of ethanol and aqueous extracts of the leaf, stem, root and flower are presented in tables 4a and 4b respectively. The MIC of ethanol extract for leaf was 50mg/ml for all bacterial isolates tested except *Salmonella* sp. which was 75mg/ml. The MIC for flower was 75mg/ml in all isolates. The MIC for stem was 75mg/ml for all bacterial isolates except *Enterobacter* sp. which was 50mg/ml. The MIC for root extracts was 75mg/ml against *Salmonella* sp. and *Pseudomonas aeruginosa* while *Enterobacter* sp. and *Escherichia coli* displayed MIC at 100mg/ml (Table 3a). In table 3b, the MIC for aqueous extract recorded was 100mg/ml against all bacterial isolates tested. The Minimum bactericidal concentration (MBC) of ethanol extract for leaf and stem was 50mg/ml while flower and root was 75mg/ml. (Table 4a). The MBC for aqueous extract of leaf, flower, stem and root was 100mg/ml for the four bacterial isolates tested (Table 4b).

Table 1: Phytochemical screening of leaf, stem, root and flower of *Ageratum conyzoides*.

Phytochemicals	Leaf		Flower		Stem		Roots	
	E	W	E	W	E	W	E	W
Tannins	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve
Alkaloids	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve
Flavonoids	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve
Phenol	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve
Glycosidase	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve
Steroids	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve
Phlobatannins	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve
Saponins	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve
Anthraquinones	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve

Legend: +ve = Present -ve = Absent E= ethanol W= water

Table 2a: Antibacterial Activity of Ethanol extracts of *A. conyzoides* L. on test bacterial isolates (mm).

Bacterial Isolates Conc. (mg/ml)	<i>Salmonella</i> sp.				<i>Enterobacter</i> sp.				<i>Escherichia coli</i>				<i>P. aeruginosa</i>			
	L	F	S	R	L	F	S	R	L	F	S	R	L	F	S	R
25	7	4	3	4	8	4	6	3	8	4	6	4	7	4	6	4
50	10	8	5	7	10	6	6	3	11	5	7	4	9	5	7	6
75	12	8	5	7	11	6	6	3	10	6	7	7	13	6	8	6
100	12	9	7	7	11	8	6	4	11	9	7	8	11	6	8	6
125	13	10	7	8	12	10	6	4	12	10	8	8	12	6	9	7
150	13	10	7	8	14	10	7	6	13	11	10	8	13	7	10	7
175	14	12	8	9	16	13	7	6	15	12	10	9	15	7	12	7
200	15	13	8	9	17	13	8	6	15	17	11	10	16	8	12	8
225	18	13	8	10	20	15	8	7	16	14	12	14	16	7	13	9
250	20	14	10	10	23	18	10	7	19	16	14	10	19	12	15	9
275	23	16	12	14	25	18	15	9	21	18	16	18	22	14	17	14
300	25	20	13	14	27	20	17	12	24	22	17	18	26	15	22	18
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend: L = Leaf; F = Flower; S = Stem; R = Root**Table 2b: Antibacterial Activity of water extracts of *A. conyzoides* L. on test bacterial isolates (mm)**

Bacterial Isolates Conc. (mg/ml)	<i>Salmonella</i> sp.				<i>Enterobacter</i> sp.				<i>Escherichia coli</i>				<i>P. aeruginosa</i>			
	L	F	S	R	L	F	S	R	L	F	S	R	L	F	S	R
25	3	2	3	1	3	1	3	1	4	2	3	1	2	1	1	1
50	5	2	4	1	4	1	3	1	4	2	3	2	2	1	1	1
75	6	2	4	2	4	1	3	1	5	2	3	2	4	1	1	1
100	5	3	4	2	5	2	4	1	5	3	3	2	3	1	1	1
125	6	3	4	2	5	2	4	2	4	3	4	3	4	2	2	1
150	7	3	5	3	6	2	4	3	5	3	4	2	5	2	4	2
175	8	4	5	3	8	3	5	4	6	3	4	2	6	3	4	2
200	7	5	5	4	8	4	5	4	8	4	5	3	6	3	4	2
225	8	5	6	4	9	5	6	4	8	4	5	4	7	3	5	2
250	11	7	6	6	9	5	6	5	8	5	5	4	9	4	5	3
275	11	7	8	7	10	7	8	6	12	6	7	5	9	4	6	4
300	13	9	10	8	11	9	9	7	12	6	7	5	11	4	8	5
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend: L = Leaf; F = Flower; S = Stem; R = Root

Table 3a: Minimum Inhibitory Concentration (MIC) of the plants part of *Ageratum conyzoides* L. ethanol extracts on test bacterial isolates.

Bacterial Isolates	Leaf				Flower				Stem				Root			
	Conc. (mg/ml)				Conc. (mg/ml)				Conc. (mg/ml)				Conc. (mg/ml)			
	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25
<i>Salmonella sp.</i>	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve
<i>Enterobacter sp.</i>	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve
<i>E. coli</i>	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve
<i>P. aeruginosa</i>	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve

Legend: +ve: Growth observed, -ve: No Growth observed

Table 3b: Minimum Inhibitory Concentration (MIC) of the plants part of *Ageratum conyzoides* L. aqueous extracts on test bacterial isolates.

Bacterial Isolates	Leaf				Flower				Stem				Root			
	Conc. (mg/ml)				Conc. (mg/ml)				Conc. (mg/ml)				Conc. (mg/ml)			
	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25
<i>Salmonella sp.</i>	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
<i>Enterobacter sp.</i>	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
<i>E. coli</i>	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
<i>P. aeruginosa</i>	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve

Legend: +ve: Growth observed, -ve: No Growth observed

Table 4a: Minimum Bactericidal Concentration (MBC) of the plants part of *Ageratum conyzoides* L. ethanol extracts on test bacterial isolates.

Bacterial Isolates	Leaf			Flower			Stem			Root		
	Conc. (mg/ml)			Conc. (mg/ml)			Conc. (mg/ml)			Conc. (mg/ml)		
	100	75	50	100	75	50	100	75	50	100	75	50
<i>Salmonella sp.</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
<i>Enterobacter sp.</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
<i>E. coli</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
<i>P. aeruginosa</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Legend: +ve: Growth observed, -ve: No Growth observed

Table 4b: Minimum Bactericidal Concentration (MBC) of the plants part of *Ageratum conyzoides* L. aqueous extracts on test bacterial isolates.

Bacterial Isolates	Leaf Conc. (mg/ml)	Flower Conc. (mg/ml)	Stem Conc. (mg/ml)	Root Conc. (mg/ml)
<i>Salmonella sp.</i>	-ve	-ve	-ve	-ve
<i>Enterobacter sp.</i>	-ve	-ve	-ve	-ve
<i>E. coli</i>	-ve	-ve	-ve	-ve
<i>P. aeruginosa</i>	-ve	-ve	-ve	-ve

Legend: +ve: Growth observed, -ve: No Growth observed

Discussion

The phytochemicals revealed in *A. conyzoides* L. plant extract had been reported by earlier studies (Anjoo and Ajay, 2008; Ezeokeke *et al.*, 2015; Wuyep *et al.*, 2017). The variations in this study and those of earlier authors could have arose from differences in extraction methods, nature of solvent used and its concentration, polarity, part of plant used and age (Folashade *et al.*, 2012). The ethanol extraction was more effective and reliable as it enabled easy filtration and contained more phytochemicals than aqueous extract which was difficult to filter due to inability of the plant materials to completely dissolve in it. In like manner, Vongsak *et al.* (2013) demonstrated that diverse extraction methods affect the total content of phytochemical compounds and that maceration and extraction with 70% ethanol was the most effective.

This study shows that aqueous extract had the tendency to easily ferment which was suspected to have led to the loss of some of the phytochemicals available in the ethanol extract. The phytochemicals reported are known to exhibit medicinal and physiological activities which indicates the continuous relevance of the herb. Many of these phytochemicals detected have pharmacological applications. It is known that saponins, tannins and resins have antimicrobial activities while glycoside is rich in vitamins (Onuoha *et al.*, 2013). Flavonoids, alkaloids and phenols have antibacterial, anti-inflammatory, antiallergic, anti-mutagenic, antiviral, anti-thrombotic and vasodilatory activities while Flavonoid have the potential to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Amadi *et al.*, 2012). Tannins have astringent abilities and accelerate healing of wounds and swellings of mucous membrane (Iwu, *et al.*, 1999). Saponins can damage cells, giving them anti-inflammatory and anticancer capabilities. They also prevent tumour formation in animals (Odeleye *et al.*, 2014). The spectra of

antimicrobial activities of both extracts of *Ageratum conyzoides* Linn against diarrhoea causing bacterial isolates increased with increasing concentration. However, ethanol extracts displayed wider spectrum than aqueous extract. In the ethanol extracts, all bacterial isolates displayed resistance at concentrations below 175mg/ml while at concentrations above 175mg/ml, they displayed susceptibility. In the aqueous extract, all isolates displayed resistance even at the highest concentration of 300mg/ml with antibacterial activities ranging from 2 – 13mg/ml. This means that with increasing concentration, more of the ethanol extracts will be able to prevent the growth of bacteria isolates associated with diarrhea as against the aqueous extract. However, the control did not give any antimicrobial activity. The high concentrations of phytochemical constituents observed in the ethanol extract may have accounted for the better antimicrobial properties in ethanol extract of the studied herb.

Some authors; Lar *et al.* (2011); Odeleye *et al.* (2014) and Osuntokun *et al.* (2018) reported that antimicrobial activity is stronger when extractions are done using organic solvents. In like manner Raquel *et al.* (2007) reported that ethanol extract of *Ageratum conyzoides* leaf can be a source of a novel antimicrobial agent, especially with good activities against *Proteus vulgaris*. Dash and Murthy (2011) reported that *A. conyzoides* has proved that the control of microbial infection is necessary for better wound healing and its management. The use of medicinal plants for prophylactic and cure of ailments and diseases will continue to enhance the health of individuals as they possess a great potential in the search for new antimicrobial agents. (Ere *et al.*, 2014). This could be the reason why Pandey *et al.* (2011) documented that 80% of the population in the world rely on plant derived medicine for first line of treatment.

The minimum bactericidal concentration (MIC) of the ethanol extract ranged from 25mg/ml to 75mg/ml while aqueous extract was 100mg/ml. In agreement, Osuntokun *et al.* (2018) reported a MIC of dichloromethane extract which ranging from 25mg/ml to 100mg/ml in diarrhoea associated bacteria. The MIC is the lowest concentration of an antimicrobial drug that inhibits the growth of microorganisms after 24hours of incubation (Tripathi, 2013). The MIC for the ethanol extract of the various plant parts ranged from 50 - 75mg/ml while minimum bactericidal concentration (MBC) for aqueous extract of the various plant parts was 100mg/ml for the four bacterial isolates tested. The value recorded for MIC is the same with the MBC values. This suggest that the extracts were both bacteriostatic and bactericidal at the same concentration. The implication of this result is that ethanol and aqueous extracts from leaves, flowers, stem and roots of *Ageratum conyzoides* could be used for treatment of bacterial isolates associated with diarrhoea although ethanol extracts were more active.

Conclusion

In rural areas of developing countries, the reliance on herbal remedies is very pronounced and the communities in Esan land are not exempted from these practices. The results revealed the presence in varying quantities of phytochemicals in the leaf, flower, stem and roots of *Ageratum conyzoides* and their potency in controlling bacterial isolates associated with diarrhoea.

References

- Amadi, B. A., Duru, M.K.C., and Agomuo, E.N. (2012) Chemical profiles of leaf, stem, root and flower of *Ageratum conyzoides*. *Asian J. Plant Sci. and Res.*, **2**(4):428-432
- Amadi, B. A., Duru, M.K.C., and Agomuo, E.N. (2012) Chemical profiles of leaf, stem, root and flower of *Ageratum conyzoides*. *Asian J. Plant Sci. and Res.*, **2**(4):428-432
- Anjoo, K. and Ajay, K.S. 2008. *Ageratum conyzoides* L.: A Review on Its Phytochemical and Pharmacological Profile. *Int. J. Green Pharm.* **2**(2): 59-68.
- Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants*, **4**: 196.
- CLSI, (2014). Clinical Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing: Approved Standard. 11th Edition.
- Dash, G. K. and Murthy, P.N. (2011). Wound healing effects of *Ageratum conyzoides* linn *Inter. J. Pharm. and Biosci.*, **2**(2): 369-383.
- Ere, D., Pondei, K., Inaibo, Q. and Orutugu, L. (2014). Phytochemicals and Antimicrobial activity of plant parts of *Ageratum conyzoides* extracted using different solvents. *J. of Chem., Biol. and Phy. Sci.*, **4**(4): 3429 – 3434.
- Ezeokeke, E.E., Ene, A.C. and Igwe, C.U. (2015). *In vivo* antiplasmodial effect of ethanol and aqueous extracts of *Alchornea cordifolia*. *Biochem. Analyt. Biochem.* **4**: (4):1 - 4.
- Folashade KO, Omoregie EH, Ochogu AP. (2012). Standardization of herbal medicines – A review. *International J. Biodiver. Conser.*, **4** (3):101-112.
- Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med*, **27**: 1-93. In: Broodstock Management and Egg and Larval Quality, (eds:
- Bromage, N. and Roberts, J.), Blackwell Sc. Ltd, Osney Mead, Oxford, Pp25-52.

- Iwu, M.W., Duncan, A.R. and Okunji, C.O. (1999). *New antimicrobials of plant origin*. In: Perspectives on new crops and new uses. J. Janick (ed.), ASHS Press, Alexandria, VA. pp. 457–462.
- Lar, P. M., Ojile, E. E., Dashe, E. and Oluoma, J. N. (2011). Antibacterial activity of *Moringa oleifera* seed extracts on some gram negative bacterial isolates. *Afr. J. Nat. Sci.*, **14**: 57 – 62.
- McKay D, L. and Blumberg, J. B. (2007). A review of the bioactivity of South African herbal teas: Rooibos (*Aspalathus linearis*) and Honey comb (*Cyclopia intermedia*). *Phytother. Res.*, **21**: 1-16.
- Nasrin, F. (2013). Antioxidant and cytotoxic activities of *Ageratum conyzoides* stems. *Intl. Curr. Pharm. J.*, **2**(2): 33-37.
- Odeleye, O. P., Oluyeye, J. O., Aregbesola, O. A. and Odeleye, P. O. (2014). Evaluation of preliminary phytochemical and antibacterial activity of *Ageratum conyzoides* (L) on some clinical bacterial isolates. *Intl. J. Engr. and Sci.*, **3**: (6)1 - 5.
- Odunayo, A. (2008). *Plants in Nigeria*. University of Lagos press, Yaba Pp 53-60
- Ojiako, O. A. and Akubugwo, E. I. (1997). An Introductory approach to practical Biochemistry. 1st edition, CRC Publication Owerri, Nigeria. 103-105.
- Okunade, A. L. (2002). *Ageratum conyzoides* L.- A Review. *Fitoterapia*. **73**: 1-16
- Onuoha, O.G., Ayo, J.A, Osuagwu V, Iruolaje, F.O. (2013). Investigation of the antibacterial activity of *Ageratum Conyzoides* extract on microorganisms isolated from Septic wound. *Topclass. J. Herbl Med.*, **2** (8):182-188.
- Osuntokun, O. T., Jemilaiye, T. A., Yusuf-Babatunde, A. M. and Akele, E. O. (2018). Antimicrobial Properties, Phytochemical Composition, and Phenotypic Resistance Pattern of Selected Enteropathogenic Microorganism on *Ageratum conyzoides* (Goat Weed) Leaf Extract *Intl. Res. J. Microbiol.*, **7**(2): 18-28.
- Pandey, M., Debnath, M., Gupta, S and Chikara, S. K. (2011). Phytomedicine: An ancient approach turning into future potential source of therapeutics. *J. Pharmaco. and Phytother.*, **3**(3): 27-37.
- Pribitkin E. A. (2005). Herbal Medicine and Surgery. *Seminars in Integrative Med.*, **3**: 17-23.
- Raquel, F. E., Savage, P. B. and Epand, R. M. (2007). Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). *Biochimica et Biophysica Acta*. **1768**:2500 - 2509.
- Sofowra, A. 1993. Medicinal Plants And traditional Medicine. In: Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.
- Souza, S.M.C., Aquino, L.C., Milach Jr, A.C., Bandeira, M.A., Nobre, M.G. and Viana, G.S. (2006). Anti-inflammatory and antiulcer properties of tannins from myracrodruonurundeuva *Allemae* (Anacardiaceae) in Rodents. *Phytother. Res.*, **21** (3): 220-225.
- Shekhar, T. C. and Anj, G. (2012). A comprehensive review on *Ageratum conyzoides* Linn (Goat weed). *International. J. Pharm. Phytopharmacol. Research*, **1** (6): 391 - 395.
- Tripathi, K. D. (2013). *Essentials of Medical Pharmacology* 7th ed. Jaypee Brother Medical Publishers, New Delhi, India. pp. 696 - 697.

Wang, Z. G and Ren, J. (2002). Current status and future direction of Chinese herbal Medicine. *Trends in Pharma. Sci.*, **23**: 347 - 348.

World Health Organization (2008) Fact sheet: traditional medicine. Geneva: World Health Organization.

Wuyep, P. A., Musa, H. D., Ezemokwe, G. C., Nyam, D. D. and SilaGyang, M. D. (2017).

Phytochemicals from *Ageratum conyzoides* L. extracts and their antifungal activity against virulent *Aspergillus* sp. *J. of Acad. and Indust. Res.*, **6**(3): 32 -39.

Zani, C. L., Chaves, P. P. G., Queiroz, R., Deoliveira, A. B., Cardoso, J. E., Anjos, A. M. G. and Grandi, T. S. M. (1995). Brine shrimp lethality assay as a prescreening system for anti- *Trypanosoma cruzi* activity. *Phytomedicine*, **21**: 47 - 50.