

Antibacterial Activities Of *Ageratum conyzoides* Extracts On Selected Bacterial Pathogens

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Abstract

In-vitro antibacterial activities of *Ageratum conyzoides* extracts on some selected bacterial pathogens were investigated. The susceptibility of *Staphylococcus aureus*, *Yersinia enterocolitica*, *Salmonella gallinarum* and *Escherichia coli* to the various extracts as well as the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were studied using standard methods. Results obtained showed that the hexane extracts of the leaf, stem and root had 100% susceptibility to all the bacterial isolates used. The aqueous leaf extracts gave 75% susceptibility, while methanolic leaf extract gave 50%. The extracts inhibited the growth of the bacterial isolates in a concentration dependent manner. Phytochemical analysis of the dried leaves revealed the presence of Resins, Alkaloids, Tannins, Glycosides and Flavonoids. The dried stems revealed the presence of Resins Saponins, Tannins, Glycosides and Flavonoids while the dried roots contained Resins, Alkaloids Saponins and Flavonoids. The MIC(s) ranged between (6.25-100) mg/ml while the MBC(s) gave a range of (3.13-50) mg/ml. This study shows that *Ageratum conyzoides* extracts carries the potentials of introducing new template into modern medicine and would require further investigation in the empirical development of new antibacterials.

INTRODUCTION

The African environment is probably the least explored in terms of available untapped resources. Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potentials of introducing new templates into modern medicine.

The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into African continent (Akinyemi et al, 2005). Herbal medicine has been wisely used and formed an integral part of primary health care in China (Liu, 1987) Ethiopia (Desta, 1993) Argentina (Anesini and Perez, 1993) and Papua New Guinea (Nick et al, 1995). A significant proportion of pharmaceutical products in current use are designed from plants (Cowan, 1999 and Raskin et al, 2002). A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effect on all types of micro-organisms in vitro (Cowan, 1999) and some plant extracts have shown activity on both gram negative and gram positive organism (Nascimento et al, 2000)

The use of various plant parts in the treatment of the sick developed into tradition which was handed down from one

generation to another over the years verbally or written (Sofowora, 1982; Akinyanju, 1986). For thousand of years, medicine depended exclusively on leaves, flowers and barks of plants, only recently have synthetic drugs come into use and in many instances, these are carbon copies of chemicals identified in plants (Conway, 1973). In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients is extracted refined and made ready for consumption, while in traditional medicine, a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food (Conway, 1973).

Even before the discovery of modern antibiotics and other chemotherapeutic agents, traditional medicine has served as man's resort when attacked by infective agents such as bacteria and fungi (Crafton, 1983).

Ageratum conyzoides is derived from the Greek word *agera* meaning non-aging referring to longevity of the flowers or the whole plant, commonly called goat or billy goat weed. It belongs to the family *Asteraceae*, which is an annual herbaceous plant with a long history of traditional use in several countries of the world (Ming, 1999). The specific epithet *conyzoides* is derived from *konz* the Greek name of

Inula helenium which it resembles (Kissman and Groth, 1993).

Traditional communities in India use this species as an antidiarrhetic and antilithic (Borthakur and Basu, 1987) and in Asia, South America and Africa aqueous extract of this plant is used as an antibacterial agent (Almagboul, 1985; Ekundayo et al, 1987). In Cameroon and Congo traditional use is to treat fever, rheumatism, headache, and Colic (Menut et al, 1993; Bioka et al, 1992). *Ageratum conyzoides* has long been known in herbal or folk medicine as a remedy for various ailments in Africa (Almagboul et al, 1985) Asia and south America (Ekundayo et al, 1987; Borthakur and Baruah, 1987). The plant is used by the Fipa in South Africa as application to fresh wounds and in central Africa, the leaf is used to aid the healing of wound especially those caused by burn (Watt et al, 1962). The reputed efficacies of these plants have been experienced and passed on from one generation to the other.

This knowledge could enable more rational exploitation of the plant both in traditional medicine and in the empirical development of new antibacterials.

This work was based on scientific investigation of the widely acclaimed medicinal value of *Ageratum conyzoides* to establishing its antimicrobial activity on some selected bacterial pathogens.

MATERIALS AND METHODS

SOURCE OF AGERATUM CONYZOIDES

Fresh plant materials were collected from a nearby farmland of the Root-crop Research Institute, near Vom. Their botanical identities were determined and authenticated at the Department of Botany, University of Jos and the Federal College of Forestry, Jos, Nigeria.

PROCESSING OF THE PLANT

The fresh leaves, stems and roots were harvested, washed properly with tap water and rinsed with sterile distilled water. The various parts of the plants were dried separately using the hot air oven at 40 °C for three days.

The dried plants were pulverized using sterile laboratory mortar to obtain the powdered form. These were stored in air tight sterile containers protected from sunlight until required for analyses.

EXTRACTION

Cold/hot water and the methanolic/hexane soxhlet extraction

as described by AOAC (1980) Akinyemi et al, (2005) and Junaid et al, (2006) were adopted for this study.

PHYTOCHEMICAL SCREENING METHODS

The presence of alkaloids, resins, saponins, glycosides, tannins, flavonoids, cardiac glycoside, steroidal ring, steroidal terpenes, anthraquinone and carbohydrates were determined as described by Wall et al (1952), Kapoor et al (1969), Harborne (1973), Sofowora (1982), Trease and Evans (1989), Akinyemi et al (2005) and Junaid et al (2006).

SOURCES OF BACTERIAL ISOLATES

The stock clinical isolates of *Yersinia enterocolitica*, *Escherichia coli*, *Salmonella gallinarum*, *Staphylococcus aureus* used were obtained from the Department of Medical Microbiology, Federal College Veterinary Medical Laboratory Technology (FCVMLT), Vom and the Bacterial Research Division, National Veterinary Research Institute (NVRI), Vom.

PREPARATION OF THE TEST ORGANISMS

The isolates were subcultured onto selective and differential solid media and re-identified using API 20E strips (Biomérieux, Marcy l'Etoile, France).

Colonies of fresh cultures of the different bacterial isolates were selected and suspended in 10ml of nutrient broth (Biotec, Suffolk, UK). Using tenfold serial dilution, in sterile normal saline the population density for all the test organisms were determined (Miles and Misra, 1938). All cultures were incubated for 24hrs at 37 °C except for *Yersinia enterocolitica* at 30 °C.

BACTERIAL SUSCEPTIBILITY TESTING

AGAR DIFFUSION TEST

A standardized inoculum of $1 - 2 \times 10^7$ cfu/ml was used for the inoculation of plates. The plates were allowed to dry in the incubator for 30 minutes at 37 °C and with the aid of a sterile standard cork borer, 6 wells were bored at equidistant. The bottoms of the wells were sealed with sterile molten nutrient agar (Biotec, Suffolk, UK) to prevent seepage of the extract under the agar. The 5th and 6th wells served as positive and negative controls. The sterile distilled water served as negative control. Ciprofloxacin (10ug/ml) was used as the positive control. 0.2ml of each prepared concentration of the extracts was aseptically introduced into wells 1 – 4. The plates were allowed on the bench for 40 minutes for pre-diffusion followed by an overnight incubation at 37 °C. The resulting zones of inhibition were

measured using a ruler calibrated in millimeters. The average of the three readings was taken to be zone of inhibition of the bacterial isolates in question at that particular concentration (Abayomi, 1982; Junaid et al, 2006).

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC)

MIC of the extracts was determined by dilution to various concentration according to the macro broth dilution technique (Baron and Finegold, 1990; Akinyemi et al, 2005). Standardized inoculum was added to series of sterile tubes of nutrient broth containing two fold dilution of the extracts and incubated at 37 ° C for 24hours. The MIC was read as the least concentration that inhibited the growth of the test organisms. However, the MBC was determined by subculturing the test dilutions onto fresh drug-free solid medium and incubated further for 18 – 24hours. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

RESULTS

The antibacterial activities of aqueous methanolic and hexane extract of *Ageratum conyzoides* leaves gave different mean zone diameter of inhibition on the bacterial isolates tested (Table 1). The aqueous leaf extract gave the mean zone diameter of inhibitions ranging from 7 – 15mm for *Staphylococcus aureus*, 8 – 12mm for *Yersinia enterocolitica* and 6 – 12mm for *Escherichia coli*, *S. gallinarum* was not inhibited. The methanolic leaf extract did not inhibit the growth of *S. gallinarum* and *Y. enterocolitica* but inhibited *Staphylococcus aureus* and *Escherichia coli* at a range of 10 – 14mm and 10 – 16mm mean zone diameter respectively. While the hexane leaf extracts inhibited the growth of all the isolates giving a mean zone diameter of inhibition ranging from 9 – 16mm for *S. aureus*, 10 – 14mm for *Y. enterocolitica*, 9 – 17mm for *S. gallinarum* and 7 – 16mm for *Escherichia coli*.

Aqueous methanol and hexane extracts of *Ageratum conyzoides* stem gave the mean zone diameters of inhibition ranging from 7 – 14mm for *S. aureus*, 6 – 10mm for *Y. enterocolitica*, 6 – 13mm for *S. gallinarum* and 8 – 11mm for *Escherichia coli*. The methanolic stem extracts did not inhibit any of the bacterial isolates. The hexane stem extract gave mean zone diameters ranging from 8 – 15mm for *S. aureus*, 7 – 11mm for *Y. enterocolitica*, 10 – 14mm for *S. gallinarum* and 6 - 11mm for *E. coli* (Table 2).

The aqueous root extracts showed no inhibition on the

bacterial isolates except for *E. coli* which was inhibited with a mean zone diameter of 7-12mm. The hexane root extracts inhibited the growth of the bacterial isolates giving a range of 7 – 12mm for *S. aureus*, 7 – 14mm for *Y. enterocolitica*, 7 – 10mm for *S. gallinarum* and 9 – 13mm for *Escherichia coli* (Table 3).

The aqueous leaf extracts gave MIC results of (100, 25 and 50mg/ml for *Staphylococcus aureus*, *Y. enterocolitica* and *Escherichia coli* respectively. The methanolic leaf extracts also gave MIC of (50 and 100) mg/ml for *Staphylococcus aureus* and *Escherichia coli* respectively, while hexane leaf gave MIC of (6.3, 25, 25 and 12.5) mg/ml for *Staphylococcus aureus*, *Y. enterocolitica*, *S. gallinarum* and *Escherichia coli* respectively.

The aqueous stem extracts gave MIC of (25,25,50 and 50) mg/ml for *Staphylococcus aureus*, *Y. enterocolitica*, *S. gallinarum*, and *Escherichia coli* respectively. While the hexane stem extracts gave MIC of 12.5, 12.5, 25 and 25 mg/ml for *Staphylococcus aureus*, *Y. enterocolitica*, *S. gallinarum*, and *Escherichia coli* respectively.

The aqueous root extracts gave MIC of 100mg/ml for *Escherichia coli* only. While hexane root extract gave MIC of 12.5, 12.5. 50 and 6.25 mg/ml for *Staphylococcus aureus*, *Y. enterocolitica*, *S. gallinarum*, and *Escherichia coli* respectively.

The aqueous leaf extracts gave MBC of 50, 25 and 25mg/ml for *Staphylococcus aureus*, *Y. enterocolitica*, *S. gallinarum*, and *Escherichia coli* respectively. The methanolic leaf extract gave MBC of 25 and 50mg/ml for *Staphylococcus aureus* and *Escherichia coli* respectively. While hexane leaf extracts gave MBC of 3.13, 12.5, 25 and 6.25mg/ml for *Staphylococcus aureus*, *Y. enterocolitica*, *S. gallinarum*, and *Escherichia coli* respectively (Table 4).

The aqueous stem extracts gave MBC(s) of 50, 25, 25 and 50 mg/ml for *Staphylococcus aureus*, *Y. enterocolitica*, *S. gallinarum*, and *Escherichia coli* respectively. Hexane stem extract gave MBC(s) of (6.25, 6.25, 12.5 and 12.5)mg/ml for *Staphylococcus aureus*, *Y. enterocolitica*, *S. gallinarum*, and *Escherichia coli* respectively (Table 5).

The aqueous root extract gave MBC(s) of (12.5, 6.25, 25 and 6.25)mg/ml for *Staphylococcus aureus*, *Y. enterocolitica*, *S. gallinarum*, and *Escherichia coli* respectively (Table 6)

The percentage effectiveness of the leaf extract on the bacterial isolates used in this work was 75%, 50% and 100%

respectively for the aqueous, methanolic and hexane leaf extracts. Also from this finding the root extract of Ageratum conyzoides gave 25% effectiveness for the aqueous extract and 100% for the hexane extract. The methanolic root extract was not effective against any of the bacteria isolates tested in this work. The negative control showed no inhibition on any of the bacteria isolates while the positive control inhibited all the bacterial isolates.

The phytochemical screening showed that Ageratum conyzoides contains alkaloids, Resins, Saponins, Tannins, Glycosides and Flavonoids. The leaves of Ageratum conyzoides contains Resins, Alkaloids, Tannins, Glycosides and Flavonoids. The dry stem powder contains Resins Saponins, Tannins, Glycosides and Flavonoids, while the dry root powder of Ageratum conyzoides contains Resins, Alkaloids, Saponins and Flavonods.

Figure 1

Table 1: Antibacterial Activity Of Leaf Extracts Of Ageratum Conyzoides

Isolate	Mean Zone Diameter Of Inhibition (Mm)						Extract
<i>S. aureus</i>	15	11	9	7	0	21	Aqueous
<i>S. aureus</i>	14	12	12	10	0	19	Methanolic
<i>S. aureus</i>	16	13	11	9	0	22	Hexane
<i>Y. enterocolitica</i>	12	12	11	8	0	17	Aqueous
<i>Y. enterocolitica</i>	0	0	0	0	0	17	Methanolic
<i>Y. enterocolitica</i>	14	12	10	10	0	15	Hexane
<i>S. gallinarum</i>	0	0	0	0	0	24	Aqueous
<i>S. gallinarum</i>	10	0	0	0	0	22	Methanolic
<i>S. gallinarum</i>	17	15	11	9	0	24	Hexane
<i>E. coli</i>	12	10	9	6	0	18	Aqueous
<i>E. coli</i>	16	14	12	10	0	16	Methanolic
<i>E. coli</i>	16	13	10	7	0	19	Hexane
Concentration of Extracts (mg/ml)	100	50	25	12.5	-ve contr	-ve contr	

Controls:

Negative- Sterile distilled water for aqueous and methanolic extracts

Negative- 50%DMSO (dimethyl sulphoxide) for hexane extract

Postive- 10µg/ml ciprofloxacin for all extracts

Figure 2

Table 2: Antibacterial Activity Of Stem Extracts Of

Isolate	Mean Zone Diameter Of Inhibition (Mm)						Extract
<i>S. aureus</i>	14	12	12	7	0	18	Aqueous
<i>S. aureus</i>	0	0	0	0	0	19	Methanolic
<i>S. aureus</i>	15	13	10	8	0	21	Hexane
<i>Y. enterocolitica</i>	10	9	6	6	0	16	Aqueous
<i>Y. enterocolitica</i>	0	0	0	0	0	17	Methanolic
<i>Y. enterocolitica</i>	11	9	9	7	0	14	Hexane
<i>S. gallinarum</i>	13	11	10	8	0	21	Aqueous
<i>S. gallinarum</i>	0	0	0	0	0	23	Methanolic
<i>S. gallinarum</i>	14	12	12	10	0	25	Hexane
<i>E. coli</i>	11	9	8	8	0	17	Aqueous
<i>E. coli</i>	0	0	0	0	0	19	Methanolic
<i>E. coli</i>	11	9	9	6	0	15	Hexane
Concentration of Extracts (mg/ml)	100	50	25	12.5	-ve contr	-ve contr	

Figure 3

Table 3: Antibacterial Activity Of Root Extracts Of

Isolate	Mean Zone Diameter of Inhibition (mm)						Extract
<i>S. aureus</i>	0	0	0	0	0	17	Aqueous
<i>S. aureus</i>	0	0	0	0	0	20	Methanolic
<i>S. aureus</i>	12	10	8	7	0	19	Hexane
<i>Y. enterocolitica</i>	0	0	0	0	0	17	Aqueous
<i>Y. enterocolitica</i>	0	0	0	0	0	17	Methanolic
<i>Y. enterocolitica</i>	14	12	10	7	0	17	Hexane
<i>S. gallinarum</i>	0	0	0	0	0	23	Aqueous
<i>S. gallinarum</i>	0	0	0	0	0	25	Methanolic
<i>S. gallinarum</i>	10	10	9	7	0	22	Hexane
<i>E. coli</i>	12	10	9	7	0	19	Aqueous
<i>E. coli</i>	0	0	0	0	0	17	Methanolic
<i>E. coli</i>	13	12	11	9	0	19	Hexane
Concentration of Extracts (mg/ml)	100	50	25	12.5	-ve contr	-ve contr	

Figure 4

Table 4: Minimum Bactericidal Concentration (Mbc) Of Leaf Extracts Of

Isolate	Concentration of extracts (mg/ml)							Extracts	MBC (mg/ml)
	100	50	25	12.5	6.25	3.13	1.56		
<i>S. aureus</i>	-	-	+	+	+	+	+	Aqueous	50
<i>S. aureus</i>	-	-	-	+	+	+	+	Methanolic	25
<i>S. aureus</i>	-	-	-	-	-	-	+	Hexane	3.13
<i>Y. enterocolitica</i>	-	-	-	+	+	+	+	Aqueous	25
<i>Y. enterocolitica</i>	-	-	-	-	+	+	+	Hexane	12.5
<i>S. gallinarum</i>	-	-	-	+	+	+	+	Hexane	25
<i>E. coli</i>	-	-	-	+	+	+	+	Aqueous	25
<i>E. coli</i>	-	-	+	+	+	+	+	Methanolic	50
<i>E. coli</i>	-	-	-	-	-	+	+	Hexane	6.25

- No growth
+ Growth

Figure 5

Table 5: Minimum Bactericidal Concentration (Mbc) Of Stem Extracts Of

Isolate	Concentration of extracts (mg/ml)							Extracts	MBC (mg/ml)
	100	50	25	12.5	6.25	3.13	1.56		
<i>S. aureus</i>	-	-	+	+	+	+	+	Aqueous	50
<i>S. aureus</i>	-	-	-	-	-	+	+	Hexane	6.25
<i>Y. enterocolitica</i>	-	-	-	+	+	+	+	Aqueous	25
<i>Y. enterocolitica</i>	-	-	-	-	-	+	+	Hexane	6.25
<i>S. gallinarum</i>	-	-	-	+	+	+	+	Aqueous	25
<i>S. gallinarum</i>	-	-	-	-	+	+	+	Hexane	12.5
<i>E. coli</i>	-	-	+	+	+	+	+	Aqueous	50
<i>E. coli</i>	-	-	-	-	+	+	+	Hexane	12.5

- No bacterial growth
+ Bacterial growth

Figure 6

Table 6: Minimum Bactericidal Concentration (Mbc) Of Root Extracts Of

Isolates	Concentration of extracts (mg/ml)							Extracts	MBC (mg/ml)
	100	50	25	12.5	6.25	3.13	1.56		
<i>S. aureus</i>	-	-	-	-	+	+	+	Hexane	12.5
<i>Y. enterocolitica</i>	-	-	-	-	-	+	+	Hexane	6.25
<i>S. gallinarum</i>	-	-	-	+	+	+	+	Hexane	25.0
<i>E. coli</i>	-	-	+	+	+	+	+	Aqueous	50
<i>E. coli</i>	-	-	-	-	-	+	+	Hexane	6.25

- No bacterial growth
+ Bacterial growth

DISCUSSION

Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been shown to have genuine utility and about 80% rural

population depends on its efficacy for their primary health care. Scientist from divergent fields in a similar efforts are investigating plants a new with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Over the years, the WHO advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins (WHO 1978). The results obtained in the study revealed antimicrobial efficacy of extract of *A. conyzoides* leaves, roots and stems. The active component of this plant may be due to its high non polar compounds. This is similar to the findings of Ijeh et al. (2005), and Junaid et al (2006) but in contrast to the findings of Obi and Onuoha (2000), who documented alcohol as the best solvent for the extraction of plant active substances of medical importance. Hexane extract was the most potent of all the extract suggesting that the active component must be a highly non polar compound.

Although the inhibitory effects of aqueous and methanolic extracts of medicinal plants are usually reported (Tignokpa et al, 1986; Omer et al,1998; and Olayinka et al, 1992)).

In this study, the methanolic, stem or root extracts were non inhibitory to all the bacteria isolates used (*S. aureus*, *Yersinia enterocolitica*, *Salmonella gallinarum* and *E. Coli*) however it is similar to the findings of Durudola (1997) and Almagboul (1985) and more recently, Umeh et al (2005) who documented non inhibitory effects of methanolic extract in Benue state Nigeria. The antibacterial activity of the hexane extracts against all the bacterial isolates used suggest that the principal active ingredients of the plant must be more of lipid soluble or non polar, since hexane is a non-polar solvent which must have easily extracted the lipid soluble phytochemicals such as essential oils and coumarins. The diffusion rates of these phytochemicals within the agar matrix may explain the wider zone of inhibition observed (Cowan, 1999).

The minimum inhibitory concentrations observed for the aqueous and methanolic (leaf, stem and root) extracts are quite high between a range of (25-100) mg/ml, while that of hexane (leaf, stem and root) extract are between a range of (6.25-50) mg/ml. This is further supported by claims that the active antimicrobial phytochemicals of the plant *Ageratum conyzoides* must be more lipid soluble or non-polar.

The result obtained for the minimum bactericidal concentration (MBC) which gave a range of (25-50) mg/ml

for aqueous and methanolic (leaf, stem and root) extract and a range of (3.13-25) mg/ml for hexane (leaf, stem and root), extracts varied considerably from the results obtained for the minimum inhibitory concentrations (MIC(s)). These variations in results implies that the MBCs result obtained from plate cultures after plating on various dilutions of extracts is more reliable and accurate compared to MICs results obtained visually using turbidity as an index.

Interestingly, the observed antibacterial effects of *Ageratum conyzoides* plant parts on the selected bacterial isolates though in vitro implies that the extracts may indeed be effective in vivo as claimed by traditional healers.

In central Africa, particularly Liberia, *A. conyzoides* is used to treat pneumonia, while in Congo, its leaf sap is used to treat trachycardia (Burkhill, 1985) though the most common use is to cure wounds and burns, as claimed by Oladejo et al, 2003. Hence the inclusion of gram negative organisms in this study. In Nigeria, it is reportedly used in the treatment of typhoid fever and diarrhoea (Adodo, 2002).

The world has entered an era when health is increasingly managed with an eye to cost containment. Critical to developing a cost effective approach to the evaluation and management of clinical illness is the selective use of available diagnostic methods, therapies and preventive measure.

The emergence of bacterial strains that are resistant to many commonly used antibacterial agents means that treatment failures may become more common. Appropriate antimicrobial therapy can shorten illness and reduce morbidity in some bacterial and parastic infections and can be life saving in invasive infection.

This work shows that *Ageratum conyzoides* is a potential antibacterial agent for the treatment of gastrointestinal tract infection and diarrhea and wound infections caused by *Staphylococcus aureus*. Herbal therapies are generally available and cheaper and offer important alternatives to more expensive allopathic medicine. The use of *A. conyzoides* extracts or leaves to feed infected birds with fowl typhoid is highly supported by our findings (Aon, 2007).

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