

Evaluation of preliminary phytochemical and antibacterial activity of *Ageratum conyzoides* (L) on some clinical bacterial isolates

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Abstract

The antimicrobial effects of ethanolic extract of *Ageratum conyzoides* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Shigella dysenteriae* was determined using the Agar-well diffusion technique. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as well as the phytochemical properties of the extract on the test isolates were also examined using the standard methods. The phytochemical components of the ethanolic extract of the *A. conyzoides* include tannin, alkaloids, steroid, saponin, phenol, flavonoids, triterpenes glycosides and carbohydrate. All the test organisms were susceptible to ≥ 50 mg/ml of the extract. The MIC and MBC of the ethanolic extract of the *A. conyzoides* against *S. aureus* and *E. coli* was 120mg/ml, while that of *P. aeruginosa* and *Shigella dysenteriae* were 160mg/ml and 200mg/ml of the extract, respectively. The result of this study suggests that the ethanolic extracts of *A. conyzoides* could be suitable for the treatment of diseases/infections caused by *S. aureus*, *P. aeruginosa* and *E. coli* and *Shigella dysenteriae*.

Keywords: Antimicrobial, Phytochemical, Ethanolic, Susceptible. Minimum inhibitory Concentration, Minimum bactericidal concentration

Date of Submission: 14 April 2014



Date of Publication: 10 June 2014

I. INTRODUCTION

Plant based antimicrobials represent a vast untapped source for medicines. Plants as gift of nature have many therapeutic properties combined with much nutritive value which have made their use in chemotherapy as valuable as the synthetic drugs. 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). Medicinal plants constitute an effective source of both traditional and modern medicines and about 80% of rural populations depend on it as their primary health care (Ilori *et al.*, 1996). The use of plant extracts and phyto-products is gaining attention due to their availability, cost effectiveness, proven nature of specificity, biodegradability, low toxicity and minimum residual toxicity in the ecosystem (Maji *et al.*, 2005). In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens which have led to the emergence of new bacterial strains that are multi-resistant (WHO, 2001; Aibinu *et al.*, 2003; Aibinu *et al.*, 2004). The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality rates (Williams, 2000) which has led to the search for more effective antimicrobial agents among materials of plant origins with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (Pretorius *et al.*, 2003; Moreillion *et al.*, 2005).

Infectious diseases are caused by some microorganisms and these diseases account for approximately 66.67% of all deaths in tropical countries (Akinnibosun *et al.*, 2009). The increase in the trend of infectious diseases in the tropics call for a renewed interest in infectious disease in the medical and public health communities and renewed strategies on treatment and prevention with the development of new antimicrobials (Iwu *et al.*, 1999). Historically, plants have provided a good source of anti-infective agents with compounds which are highly effective instrument in the fight against microbial infections. Phytomedicine derived from plants have shown great promise in the treatment of intractable infectious disease including opportunistic AIDS infections (Iwu *et al.*, 1999)

In this study, the plant *Ageratum conyzoides* is studied for its medicinal properties by assay for its antibacterial properties on selected human pathogenic bacteria of the family enterobacteriaceae namely

Escherichia coli, *Shigella dysenteriae*, *Staphylococcus aureus* and as well as *Pseudomonas aeruginosa*. The plant is widely utilized in traditional medicine by various cultures and tribe worldwide, although applications vary by regions. In Nigeria, it is used traditionally in the treatment of different kinds of ailments such as gastrointestinal pains, diarrhoea, sore throat and skin infection. In central Africa, it is used to treat pneumonia, but the most common use is to cure wounds and burns (Durodola, 1977). Traditional communities in India used it as a bacteriocide, antidiysenteric and antilithic. In Cameroon and Congo, it is traditionally used to treat fever, rheumatism, headache and colic (Menut *et al.*, 1993). Therefore, this research was carried out to determine the phytochemical constituents and antibacterial potential/activity of the ethanolic extracts of *Ageratum conyzoides* on some selected Gram-negative bacteria.

II. MATERIALS AND METHODS

Collections and identification of research plant

Fresh leaves, stem and roots of *Ageratum conyzoides* were collected from Iworoko-Ekiti, Ekiti State, Nigeria and was authenticated by comparison with herbarium samples at the Department of Plant science and Forestry, Faculty of Science, University of Ado-Ekiti, Nigeria. The fresh plants were allowed to air dry at room temperature for five weeks. The dried plants were blended into powder and kept in clean air-tight containers for further use (Shahidi, 2004).

Extraction procedure of plant materials

Twenty grams (20g) of the powdered plant was suspended in 200ml of Ethanol in 500ml sized conical flasks. The extracts in the conical flasks were allowed to infuse for two days at room temperature on a rotary shaker. The ethanolic extracts was obtained by soxhlet extraction (Voskresenky, 1972) and concentrated by evaporation using a rotary vacuum extractor.

Test organisms

The test bacteria used include *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Pure culture of these bacteria were obtained and confirmed at the research laboratory of the Department of Obstetrics and Gynaecology, College of Medicine, University of Lagos. The bacteria were maintained on nutrient agar slants and stored at 4°C in a refrigerator until required.

Phytochemical screening of the extracts

The phytochemical screening of the plant extract was carried out according to the method described by Harborne (1998) and Onwuka (2005) for the purpose of detecting active components of the plant.

Antibacterial bioassay of crude extracts

Agar-well diffusion technique was used for this purpose. A stock solution of 200mg/ml of the ethanolic extract was prepared using the extracting solvent and was serially diluted to obtain to 100 mg/ml, 50 mg/ml, 25 mg/ml and 5mg/ml (NCCLS, 2000). Each labelled medium plate was uniformly inoculated with a standardized inoculum (10^5 cfu/ml) of test organism and a sterile cork borer of 5mm diameter was used to bore wells on the medium into which 0.1ml of the various extract concentration were added (Shahidi, 2004). The inoculated plates were kept on the bench for 30mins to allow the extracts to diffuse into the agar medium (Atata, 2003). The agar plates were incubated at 37°C for 24hours. Antibacterial activities were determined by measuring the diameter of the zones of inhibition (mm) of the extract against the test organisms after incubation (Junaid *et al.*, 2006).

Minimum Inhibitory Concentration (MIC)

The MIC of the potent extracts was determined according to the macro broth dilution technique. Standardized suspensions of the test organism was inoculated into a series of sterile tubes of nutrient broth containing two-fold dilutions of leaf extracts and incubated at 37 ° C for 24 hours. The MICs of the ethanolic extract were read as the least concentration that inhibited the growth of the test organisms.

Minimum Bactericidal Concentration (MBC)

The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube was subcultured onto extract-free agar plates, incubated for further 24 hours at 37°C. The concentration at which no growth was observed was taken as the MBCs of the extract against the test organisms.

III. RESULTS

Table 1 shows the phytochemical screening of the ethanolic extracts of *Ageratum conyzoides*. The result indicates the presence of tannin, steroids, saponins, alkaloids, phenol, flavonoids and carbohydrate.

Table 1. Phytochemical Constituents of ethanolic extract of *Ageratum conyzoides*

Phytochemicals	Bioassay
Alkaloids	+
Carbohydrates	+
Glycosides	+
Phenol	+
Flavonoids	+
Saponins	+
Tannins	+
Steroid	+
Triterpenes	+

Key: + = positive - = negative

Table 2 shows the zones of inhibitions (mm) of ethanolic extracts of *A. conyzoides* on *P. aeruginosa*, *E.coli*, *S. aureus* and *S. dysenteriae* at concentrations 5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml.

Table 2: Antimicrobial activity of Ethanolic extracts of *A. conyzoides*.

Test Organism	Concentration (mg/ml)				
	200	100	50	25	5
<i>Staphylococcus aureus</i>	18.0	13.0	10.0	6.0	0.0
<i>Pseudomonas aeruginosa</i>	16.0	11.0	9.0	0.0	0.0
<i>Escherichia coli</i>	10.0	8.0	4.0	0.0	0.0
<i>Shigella dysenteriae</i>	16.0	14.0	8.0	6.0	0.0

Table 3 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the test organisms on the ethanolic extract of *A. conyzoides*. The MIC and MBC were 160mg/ml for *P. aeruginosa*, 200mg/ml for *Shigella dysenteriae* and 120mg/ml for *E. coli* and *Staphylococcus aureus* respectively.

Table 3: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanolic extracts of *A. conyzoides*.

Test Organism	MIC (mg/ml)	MBC (mg/ml)
<i>Staphylococcus aureus</i>	120	120
<i>Pseudomonas aeruginosa</i>	160	160
<i>Escherichia coli</i>	120	120
<i>Shigella dysenteriae</i>	200	200

IV. DISCUSSION

Phytochemicals act in numerous ways to assist the body in combating diseases and health problems. The consumption of phytochemicals enhances reduction in the emergence of degenerating diseases (Alan, 1996). In the present study, the phytochemical analysis of the ethanolic extract of *A. conyzoides* revealed the presence of alkaloids, saponins, flavonoids, phenol, tannins, steroids and glycosides (Table 1). The result of this study is in agreement with other previous work (Amadi *et al.*, 2012; Borkataky *et al.*, 2013). The antibacterial activity of the extract (Table 2) showed that the extract had a broad spectrum of antibacterial activities, inhibiting *P. aeruginosa*, *E. coli*, *S. dysenteriae* and *S. aureus* at concentration of $\geq 50\text{mg/ml}$. The extract at 25mg/ml was observed to have some activities on *S. dysenteriae* and *S. aureus* with zone of inhibition 6.0mm for both. The antimicrobial activity of *A. conyzoides* could be due to the abundant presence of phytochemicals (Phadungkit, *et al.*, 2012) which include alkaloids, flavonoids, tannin, saponins and phenol. Flavonoids according to Alan, (1996) have shown antibacterial, anti-inflammatory, antiallergic, anti-mutagenic, antiviral, anti-thrombotic and vasodilatory activity. The presence of the flavonoids may have aided the antibacterial activity of the plant. Tannins on the other hand have astringent properties and hasten the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004) while studies have shown that saponins exhibit cytotoxic effect and the growth inhibition against a variety of cell, making them have anti-inflammatory and anticancer properties. They also show tumour inhibiting activity in animals (Iwu, 1989). The presence of tannins and saponins in the present study could be attributed to the use of *A. conyzoides* in treating wounds (Kamboj and Saluja, 2008). The presence of phenol in the plant extract further explains the antibacterial properties of the plant as phenols and phenolic compounds have been extensively used in disinfection and remain the standard with which other bactericides are compared (Okwu, 2001; Okwute and Yakubu, 1998).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. aureus* and *E. coli* were 120mg/ml , while for *P. aeruginosa* and *S. dysenteriae* is 160mg/ml and 200mg/ml respectively. The result of this study showed that *P. aeruginosa*, *E. coli*, *S. aureus* and *S. dysenteriae* were susceptible to the ethanolic extract of *A. conyzoides* which is in support of the study of Akinyemi *et al.*, 2005; Onuoha *et al.*, 2013 and Elimian, *et al.*, (2013).

V. CONCLUSION

The world has entered the era when health is increasingly managed with an eye to cost containment. The emergence of bacteria strains that are resistance to many conventional antibacterial agents (antibiotics) means that treatment failures may become more common. This study as shown that *Ageratum conyzoides* have potential antibacterial component and activity for the treatment of diarrhoea, wounds, sores and gastrointestinal tract infections.

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