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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Ageratum conyzoides* L.

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ABSTRACT

The need for cheap drug and the necessity to combat drug resistance have led to *Ageratum conyzoides L* being investigated against *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans, C. stellatoidea* and *C. (torulopsis) glabrata* using well diffusion methods and minimum inhibitory concentrations. The sensitivity of the bacteria and yeasts to the essential oil from the stem, leaves and root were determined. *Pseudomonas aeruginosa* was not sensitive to the essential oil. The minimum inhibitory concentrations ranged between 2.0 mg/ml and 4.0mg/ml and these were recorded for *Bacillus subtilis, Klebsiella pneumoniae,* and *Staphylococcus aureus* in the stem, leaves and the root extracts. All the microbes tested were sensitive to the essential oils of *Ageratum conyzoides* except *Pseudomonas aeruginosa* The observable inhibition of selected bacteria by oils of *Ageratum conyzoides* makes it a promising alternative antimicrobial agent.

Key words: *Ageratum conyzoides,* photoblastic, antidysentric, bacteriocide and spasms

INTRODUCTION

Ageratum conyzoides L. (Astreracae) is an annual herb, which is erect, 30 – 80 cm tall; with fine white hairs on the stem and pink flowers (Kaul and Neelangini 1989). It is a weed generally found in cultivated fields and other ecosystems such as pastures, grasslands, wastelands and even forest areas (Batish *et al*., 2006). The plant is known to have originated from tropical America and now spread to various tropical and subtropical parts of the world (Juliana *et al*., 2010). The control of the growth of the fruit is difficult because it is dispersed by wind and the seeds are photoblastic (Marks and Nwachuku 1986; Ladeira *et al*., 1987; Kalia and Singh 1993; Lam *et al*., 1993, Paradkar *et al*. 1993; Waterhouse, 1993).

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Kong *et al*. (2004) has attributed the successful invasion of *A. conyzoides* to its wide range of environmental adaptability, higher reproductive potential and allelopathy. It is commonly called Billygoat-weed, Goatweed *etc*. In southwest Nigeria, it is s called "Imiesu" and has been noted as a medicinal plant (Ming, 1999). Pharmacological investigations by Durodola (1977) confirmed that ether and chloroform extracts of *Ageratum conyzoides* has inhibitory activities against *in vitro* development of *Staphylococcus aureus.* Extracts of the whole plant *in vitro* studies have an antibacterial activity against *Bacillus subtilis, Escherischia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Almagboul *et al*., 1985). Marques Neto *et al*. (1988) reported that 66% of patients observed analgesic effect when administered on aqueous extract of the whole plant in trials of patients with

arthrosis. The antimicrobial activity of the essential oils of A. conyzoides has been a subject of investigation for its aflatoxin suppression of *Aspergillus flavus* (Juliana *et al*., 2010) and inhibition of other fungal growth such as *Penicillium chrysogenum, P. javanicum* and *Didymella bryoniae* (Okunade, 2002). This study was set out to determine the effectiveness of the antibacterial and the antifungal properties of the essential oils obtained from the root and the aerial part of *Argeratum conyzoides* against some selected *Candida* species (*C. stellatoidea, C. albicans and C. glabrata*) and bacterial species (B. *subtilis, P. aeruginosa, K. pneumoniae and S. aureus*)

MATERIALS AND METHODS

Aerial and roots parts *Ageratum conyzoides* were collected from Sagamu (46km to Lagos, Nigeria) in May 2008 and was authenticated by Elkalf Herbarium at Plant and Applied science department of Olabisi Onabanjo University, Ago-Iwoye. Nigeria. The plant materials were washed with clean water and air-dried. This plant was selected based on folklore medicinal uses.

Microbial Strain

The microorganisms were supplied from the Department of medical Microbiology of the University and maintained on Nutrient agar (Merck, Darmasadt, Germany). The bacteria and fungi used were selected because they have been implicated with skin, oral and intestinal tract of man. Four species of bacteria *Klebsiella pneumonia* (ATCC 35657), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 24213), and *Pseudomonas aeruginosa* (ATCC 9027) and three species of fungi *Candida albicans* (ATCC 10231), *C. stellatoidea* (ATCC 20408), and *C.glabrata* (ATCC 15126) were used in this study.

Preparation of Inoculum

The modified method of Benkeblia (2004) was used in which Young actively growing cells were generated by growing cells in Brain-Heart Infusion broth (Merck) for 24 h at 37^0C. The cell suspensions were diluted with peptone water to provide initial cell counts of about $3x10^8$ CFU/ml. While An aliquot of 1 ml (is) was used for antimicrobial test

Essential oil isolation

The air-dried plant materials (100 g) was introduced into conical flask with 100 ml of water and plugged with cotton wool. (It was hydrodistilled for 3hrs using a Clevenger type apparatus with n-Hexane (3ml) as the extractant to get a yield of 4.2(v/w). The oil was dried over anhydrous sodium sulfate and kept in a sealed vial at 4^0C until analysis and tests.

Antimicrobial assays

The methods of Hufford *et al.* (1975) were used with some modification. Agar-well diffusion assay was used to evaluate the antimicrobial activities of the essential oil. Mueller–Hinton agar (Scharlau Chemie) was used for the culture of bacteria while Sabouraud Dextrose agar (Difco) was used for that of (the) fungi. Twenty milliliters of the specified molten agar (45 ◦C) was aseptically mixed with 1 ml of (a) each bacterial suspension and same was done with 1ml of fungal suspension which was mixed with twenty litres of molten Sabouraud Dextrose (3×10^8 CFU/ml) and poured into 100 mm ×15 mm sterile Petri dishes (Rosario rojas *et al*., 2003). Once the agar has hardened, 6 mm wells were bored using a sterile cork borer. A 0.1 ml of the oils [in 75% methanol (v/v)] was placed into the wells and the plates were incubated for 24 h at 37^0C for the bacteria and 24–72 h at room temperature for the fungi. Ampicillin (10 µg) serve as positive control for the bacteria species while Ketoconazole (10µg) (Pfizer) serves as positive control for the Candida species. The antimicrobial activity was measured as the diameter (mm) of clear zone of growth inhibition. Solvent that served as controls (75% methanol) was included in every experiment as negative controls.

Minimum inhibitory concentration (MIC) was determined by incorporating various amounts (1–256 mg/ml) of reconstituted essential oils into the medium (Jennifer, 2001). The plates were incubated at 37°C for 24hrs for bacteria and 48hrs

for the fungi isolates. The antimicrobial activity was determined by measurement of the inhibition zone. The experiment had three replicates, while the mean values were presented. The MIC was interpreted as the lowest concentration of the extracts that did not permit any visible growth when compared with that of the control. The Negative control was 75% methanol. The result of inhibition of the extract was compared with standard antibiotics ampicillin (10 µg) and ketoconazole (10µg)

RESULTS

The antimicrobial activity of *Ageratum conyzoides* at different concentrations was determined by agar well diffusion method as stated in Table 1. A total of 7 microorganisms that consisted of four bacterial and 3 fungi were assayed. Standard antibiotics (Ampicillin and ketoconazole) were used as positive control while 75% methanol as negative control.

Table 1. Antifungal activity of the essential oil of the aerial and root parts of Ageratum *conyzoides* L

	Inhibition Zones (mm)								
	C.albicans			*C.stellatoidea*			*C.glabrata*		
Part of Plant	Leaves	Stem	Root	Leaves	Stem	Root	Leaves	Stem	Root
Essential oil (10ul/ml)	15±0.3	6.0±0.3	7.0 ± 0.3	19±0.4	7.0±0.3	11 ± 0.3	18±0.4	6.0±0.4	9.0 ± 0.4
MIC (mg/ml)	2.0	Na	Na	2.0	Na	4.0	2.0	Na	4.0
Control (Ampicillin 10 ug/ml)	13±0.3	13.3±0.3	12 ± 0.3	11±0.3	11.0±0.3	13 ± 0.4	10.0±0.4	10±0.4	10.0 ± 0.4

MIC, Minimum Inhibitory Concentration, Na, Not active

Table 2. Antibacterial activity of the essential oil of the aerial and root parts of *Ageratum conyzoides* L

	Inhibition Zones (mm)											
	B.subtilis			*P.aeruginosa*			*K. pneumoniae*			*S. aureus*		
Part of Plant	Leaves	Stem	Root	Leaves	Stem	Root	Leaves	Stem	Root	Leaves	Stem	Root
Essential oil (10ul/ml)	24.0±0.1	18.0±0.2	19.0±0.2	Na	Na	na	16.0±0.2	Na	12.0±0.3	18.0±0.4	19.0±0.2	7.0±0.2
MIC (mg/ml)	2.0	2.0	2.0	Na	Na	na	2.0	Na	4.0	2.0	2.0	Na
Control (Ampicillin 10 ug/ml)	25.0±0.2	20.0±0.2	20.0±0.2	18.0±0.2	20.0±0.2	18±0.2	22.0±0.3	22.0±0.2	22.3±0.3	23.0±0.1	23.0±0.2	23.0±0.1

MIC, Minimum Inhibitory Concentration, Na, Not active

The essential oil obtained from hydrodistillation of the leaves, stem and root parts of *Ageratum conyzoides* was 1.5 %[(w/w) based on the dry weight of the plant]. Table 1 shows that the essential oils obtained from the leaves of *Ageratum conyzoides* L were the most effective against the tested yeasts while essential oils from the stem and root were not as active showing no activity in some cases against the tested *Candida* spp. The largest inhibition zone of 19 ± 0.4 mm was obtained against *C. stellatoidea* while the essential oil from stem shows little or no activity against *C. glabrata.*

Also the essential oils obtained from the leave proved to be the best as shown in table 2. All the tested bacteria were sensitive to the essential oil from the leave except for *P. aeruginosa* that was completely resistant to the essential oil from *Ageratum conizoides* L. Also, the essential oil from the stem of *A. conyzoides* showed no activity against *K. pneumonae.* The most susceptible of all the bacteria was B. subtilis Analysis of the results (Table 2) indicates that the inhibition zone sizes were nearly similar except for 7.0 ± 0.2 mm that was recorded for the root of the plant against *S. aureus.* This occurrence might be as a result of similar composition in the constituent of the plant. However, there is need to establish this assertion with further research on the constituent of the essential oil of this plant..

DISCUSSION

The two screening methods employed in this study show perfect correlation, from tables 1 and 2; it could be observed that higher zones of inhibition resulted in lower MIC values. The antimicrobial property of the essential oil of *Ageratum conyzoides* has earlier been confirmed by the study conducted by Viuda-Martos *et al.* (2007) and

Dikbas *et al*. (2008). Although in their studies the essential oil was only screened against *Aspergillus flavus*, but nonetheless the studies confirm the antimicrobial activity of the plant which is in agreement with the observation made in this study Also, the demonstration of sensitivity by *Bacillus subtillis, Klebsiella pneumoniae and Staphlococcus aureus* to essential oils of *Ageratum conyzoides* confirmed its usefulness in treament of wounds and burns (Durodola, 1977 and Manandhar, 2002). The observed inhibition of these bacteria by the essential oil of *Ageratum conyzoides* shows that it could be of importance in the pharmaceutical industry, especially for the treatment of diseases caused by bacteria and fungi studied in this work.

The performance of essential oil justifies its ethno pharmacological claims as it competes favorably with the commercially available drugs such as ampicillin and ketoconazole that were used as positive controls in this study. In conclusion, the antibacterial activity is more concentrated in the leave than in any other part of the plant, but the difference between the leave and any other part of the plant may be due to the higher concentration of antimicrobial constituents in the leave since the same volume of oil was used. *Ageratum conyzoides* is a medicinal plant with high antibacterial activity and this justifies its use for treatment of sores, body rash, sore throat, spasms, diarrhoea rheumatism etc. Pharmacological and toxicological studies of the plant extract can be done in future to identify its pure compound and elucidate the components responsible for these antimicrobial activities.

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