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Asian Journal of Plant Science and Research, 2013, 3(5):65-69

ISSN : 2249-7412
CODEN (USA): AJPSKY

Comparative analysis of *Ageratum conyzoides* L. and *Ocimum gratissimum* extracts on some clinical bacterial isolates

[#1]Kelly Osezele Elimian, [2]Ebakota Omonigho Daniel, [1]Emmanuel Ademola Adesanmi and *[#3]Joseph Osamudiamen Osazee

[1]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
[2]Microbiology Option, Faculty of Basic and Applied Sciences, Benson Idahosa University, Benin City, Nigeria
[3]Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

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ABSTRACT

The antibacterial efficacy of crude aqueous, ethanolic and methanolic extracts of Ocimum gratissimum and Ageratum conyzoides L. against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli were determined using disc diffusion method. All the extracts (aqueous, ethanol, methanol extracts) showed marked antibacterial activity. Results obtained revealed that methanolic extracts were more potent inhibiting all isolates with zones of inhibition ranging from 7 mm – 17 mm for A. conyzoides L. and 9 mm – 19 mm for O. gratissimum. Ethanolic extracts showed 7 mm – 16 mm for both plants. Aqueous extracts showed the least with diameter zones of inhibition ranging from 7mm – 12 mm for A. conyzoides L. and 6 mm – 10 mm for O. gratissimum. When the antibacterial activity of each of the plant extracts were compared for aqueous, ethanol and methanol extracts, there was no observable difference noticed in their spectra of activity. The efficacy of both plants towards inhibition of the micro organisms increased with increased concentration. The antibacterial activity of the extracts could be enhanced if the components were purified. These plants therefore, are potential sources of new drugs for treating infections caused by these clinical pathogens.

Keywords: Disk diffusion method, antimicrobial activity, *A. conyzoides*, *O. gratissimum*, methanolic extract.

INTRODUCTION

The use of plant materials as spices, condiments and for medicinal purposes dates back to the history of mankind [4]. The antimicrobial properties of many plants have been investigated by a number of researchers worldwide. Medical uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoction from the plants. Medicinal plants are excellent antimicrobial agents because they possess a variety of chemical constituents that are antimicrobial in nature. Recently, much attention has been directed towards extracts and biologically active compounds isolated from popular plant species [10] because of the need for alternative sources of the antibiotics as the pathogenic microbes are gaining resistance against standard antibiotics [2]. Medicinal plants belonging to the genus *Ocimum and Ageratum* have been ascertained to provide various culinary and medicinal properties. These medicinal properties exert bacteriostatic and bacteriocidal effects on some bacteria [19].

O. gratissimum has been reported to yield oil of diverse nature, with some chemical components and active ingredients known to possess antimicrobial properties [5]. A wide range of allelochemicals including alkaloids, flavonoids, chromenes, benzofurans and terpenoids known to have antimicrobial effects has also been isolated from *A. conyzoides* L. [1].

The aim therefore this work was to compare the antibacterial efficacy of *A. conyzoides* L. and *O. gratissimum* extracts on clinical bacterial isolates. Previous studies have proven that extracts using different solvent of *A.*

conyzoides L. and *O. gratissimum* have different Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on test isolates thus this study was also to investigate the MIC and MBC of three solvent extractions of the two plants on three clinical isolates.

MATERIALS AND METHODS

Collection and identification of plant sample: The plants, *O. gratissimum* were purchased from a local market (Uselu) in Benin City, Edo State while the fresh leaves of *A. conyzoides* L. were collected from the surroundings of University of Benin, Edo state, Nigeria. Both plants were properly identified and authenticated with reference to the Herbarium sheets available at the Herbarium of the Department of Plant Biology, University of Benin, Benin, and Nigeria.

Source and maintenance of test organisms: The clinical isolates (*S. aureus*, *E. coli* and *P. aeruginosa*) used in the work were obtained from the Microbiology laboratory of the University Benin Teaching Hospital (U.B.T.H), Benin, Edo State, Nigeria. Viability test of each isolate was carried out by resuscitating the organisms in buffered peptone broth and thereafter subcultured into nutrient agar medium and incubated at 37 ° C for 24 hours. Identification of microbial isolates was done using the Analytic Profile Index (API).

Extracts Preparation: The leaves of *O. gratissimum and A. conyzoides* L. were air dried for 4 days and finely ground using a sterile grinder mill. Twenty grams of the fine powder from *O. gratissimum and A. conyzoides* L. leaves were placed in 250 ml of solvents (95 % ethanol, 95 % methanol, distilled water), placed in three different conical flasks and refluxed at 50℃ for 60 minutes. The extracts were filtered through Whatman filter paper No. 1 and were evaporated to dryness using a hot air oven at a much reduced temperature (40 ℃). The residues obtained were dissolved in 1 % dimethyl sulphoxide (DMSO). The weights of the extracts were determined and stored below ambient temperature.

Antibacterial Assay: Disk diffusion method using Müeller-Hinton agar plates was used to demonstrate the antimicrobial properties of the crude extracts. A suspension of the bacteria compared to 0.5 Macfarland standard was seeded on the Mueller - Hinton agar plates. 10 mm discs were cut from Whatman No.1 filter paper and sterilized in the oven at 160 ^{o} C for 2 hours. The disks were then impregnated with the extract by soaking in the extract for 24 hours of different concentrations of the extracts (that is 25, 50, 100 and 200 mg/ml respectively). Each of the disc contained approximately 200 mg/ml hot distilled water, ethanolic and methanolic extract. Using sterilized forceps, each disc was recovered from the extract and applied aseptically unto the agar plates already inoculated with a pure culture of the test organisms and the plates were incubated overnight at 37 ° C. The solvents were used as the negative controls whiles 10 µg ampicillin disk (Oxoid) was used as the positive control. The control zones of the solvents were deducted from the zones of inhibition created by the crude extracts. Growth was determined by measuring the diameter of the zone of inhibition.

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 ^{o} C for 24 hours. The MICs 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 sub cultured onto extract free agar plates, incubated for further 24 hours at 37 ^{o} C. The concentration, at which no growth was observed, was noted as the MBC.

RESULTS

Table 1 and 2 showed the antibacterial activity of the crude aqueous, ethanolic and methanolic extracts of *A. conyzoides and O. gratissimum* on *S. aureus*, *P. aeruginosa* and *E. coli*. The methanolic extract of *A. conyzoides* showed the highest antibacterial activity with the diameter of zone of inhibition of 17 mm against *S. aureus*, *P. aeruginosa* showed the least with 15 mm as the zone of inhibition at 200 mg/ml. For *O. gratissimum* the highest zone was 19 mm for *S. aureus*. *P. aeruginosa* also showed the least zone of inhibition (17 mm) at 200 mg/ml concentration.

The MIC and MBC of the ethanolic, methanolic and aqueous extracts of the plants are shown in Table 3 and 4. Generally the methanolic extract showed greater antibacterial activity compared to its corresponding extracts. The methanolic extracts showed the highest activity against *S. aureus* and *E. coli* followed by *P. aeruginosa.* All the methanolic plant extracts have antibacterial activity at 200 mg/ml and in the descending order at double dilutions; 100 mg/ml, 50 mg/ml and 25 mg/ml.

Table 1: Antibacterial Activity of *A. conyzoides* L. Extracts

CONCENTRATIONS (mg/ml)	*P. aeruginosa* Zones of Inhibition (mm)			*E. coli* Zones of Inhibition (mm)			*S. aureus* Zones of Inhibition (mm)		
	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol
200	9	15	14	11	16	16	12	17	15
100	8	12	13	9	15	11	7	14	13
50	NS	11	11	8	13	8	8	12	10
25	NS	7	NS	NS	8	NS	NS	9	7
Positive control (Ampicilin)		NS			15			13.5	
Negative control		NS			NS			NS	

NS – Not Sensitive

Table 2: Antibacterial Activity of *Ocimum gratissimum* Extracts

CONCENTRATION (mg/ml)	***P. aeruginosa* Zones of Inhibition (mm)**			***E. coli* Zones of Inhibition (mm)**			***S. aureus* Zones of Inhibition (mm)**		
	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol
200	10	17	14	8	18	16	9	19	16
100	8	16	13	7	14	11	8	17	12
50	6	14	11	NS	12	10	6	13	11
25	NS	9	NS	NS	10	8	NS	11	7
Positive control (Ampicilin)		NS			15			13.5	
Negative control		NS			NS			NS	

NS – Not Sensitive

Table 3: M.I.C. and M.B.C. of the various leaf extracts of *A. conyzoides* L. (mg/ml)

ISOLATES	**Aqueous**		**Methanol**		**Ethanol**	
	MIC	MBC	MIC	MBC	MIC	MBC
P. aeruginosa	100	200	25	50	50	100
E. coli	50	50	25	25	25	50
S. aureus	50	100	25	25	25	50

Table 4: M.I.C. and M.B.C. of the various leaf extracts of *O. gratissimum* (mg/ml)

ISOLATES	Aqueous		Methanol		Ethanol	
	MIC	MBC	MIC	MBC	MIC	MBC
P. aeruginosa	50	100	25	25	50	100
E. coli	50	50	25	25	25	50
S. aureus	25	25	25	25	25	50

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. 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 [21].

The increasing resistance to antibiotic represents the main factor justifying the need to find and develop new antimicrobial agents. Thus, some studies have focused on the antimicrobial agents and on the antimicrobial properties of plant-derived active principles, which have been used for a long time in traditional medicine to overcome infections [12]. Thus, plant foods would be useful as medicine, in addition to their nutritional potentials.

The results obtained in this study revealed antimicrobial efficacy of extracts of *A. conyzoides* L. and *O. gratissimum* leaves. The active components of these plants may be due to their high non polar compounds. This is similar to the findings of Ijeh et al. (2006) [6], but in contrast to the findings of Obi and Onuoha, (2000) [20], who documented ethanol as the best solvent for the extraction of plant active substances of medical importance. Methanol extracts were the most potent of all the extracts suggesting that the active component must be a highly non polar compound. At 200 mg/ml, the methanolic extracts of both plants did better than the positive control which was the antibiotic Ampicillin.

The efficacy of *O. gratissimum and A. conyzoides* L. on bacterial isolates has been reported by Adebolu and Salau, (2005) [18], [7]. The antimicrobial activities of these extracts (ethanol, methanol and aqueous) appeared to be broad spectrum since both the Gram-positive and Gram negative bacteria were sensitive to their inhibitory effects.

The tested plants extracts were most active against Gram positive (*S. aureus*) than most of the Gram negative microorganisms (*P. aeruginosa* and *E. coli*). This is in an agreement with the previous reports of several workers [11]. The difference in antimicrobial properties of the plants extracts might be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), contamination by field microbes, substitution of plants, incorrect preparation and dosage [16], [17]. In the same vein, Okigbo and Ajalie, (2005) [15] reported that inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials. Subsequently, Chukwuka *et al*. (2011) [9] also noted that medicinal plants with no antibacterial activity may be due to astringent properties possessed by extract of the plants.

The choice of these microorganisms used in the work was made due to the fact that some of them are causative agents of intestinal infection in human. The high zones of inhibition exhibited by *O. gratissimum* and *A. conyzoides* L. extracts against the isolates could mean that these extracts contain active ingredients which qualify their use in the treatment of wound infections, plaster to cover wound surfaces and baby cord. The results of the zones of inhibition obtained in the two plants extracts indicate a higher inhibition at a concentration of 200 mg/ml. *S. aureus* showed more sensitivity to the aqueous extract of *A. conyzoides* L. compare to that of *O. gratissimum.* At 25 mg/ml *the* aqueous extracts of the plants were not effective on all the isolates except for *S.* aureus with the zone of inhibition of 6mm. Concentration of aqueous extract of *A. conyzoides* L. at 50 mg/ml did not inhibit the growth of *P. aeruginosa* while at that same concentration *O. gratissimum* inhibited it's growth with a diameter of 6 mm. At a concentration of 25 mg/ml, there were no zones of inhibition for both *A. conyzoides* L. and *O. gratissimum* aqueous extract on *Pseudomonas.* At 200 mg/ml concentration of *Ageratum* ethanol extract, the largest zones of inhibition occurred with *E. coli* while for *O. gratissimum, S. aureus* showed the largest zones of inhibition. For *A. conyzoides* L., *S. aureus* showed the highest zones of inhibition at high concentration of 100 mg/ml while *Pseudomonas* showed the highest zones for *O. gratissimum* with the same ethanolic extract. At the concentration of 25 mg/ml, ethanol extract of *O. gratissimum* inhibited the growth of *S. aureus, P. aeruginosa* and *E. coli* with inhibition zone diameter of 8 mm, 7 mm and 9 mm respectively while for *A. conyzoides* L., it is 9 mm, 7 mm and 8 mm. This is similar to the findings of Nwinyi *et al*. (2009) [14] who reported that ethanol extracts of *O. gratissimum* showed more antibacterial activity than aqueous extract against *E. coli*. At 25 mg/ml concentration, the methanolic extract showed greater antimicrobial activity than the aqueous and ethanol extracts as indicated by zones of inhibition. The minimum inhibitory concentrations observed for the aqueous, ethanolic and methanolic extracts of *A. conyzoides* L. are quite high between ranges of 25 – 100 mg/ml, while that of *O. gratissimum* extracts are between ranges of 25 – 50 mg/ml. The results obtained for the minimum bactericidal concentration (MBC) gave a range of 50 – 200 mg/ml for aqueous, 25 – 100 mg/ml for ethanol and 25 – 50 mg/ml for methanol extracts of *A. conyzoides* L. while for the aqueous, ethanol and methanol extracts of *O. gratissimum,* a range of 25 – 100 mg/ml, 25 – 50 mg/ml and 25 mg/ml was established respectively. Extracts varied considerably from the results obtained for the minimum inhibitory concentrations (MICs). 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. The findings of this study work agrees with that of several workers that demonstrated that *O. gratissimum* and *A. conyzoides* L. plants have activity against several species of bacteria including *S. aureus, P. aeruginosa, E. coli, Salmonella* sp, *Shigella* sp [8][13]. Iwalokun *et al*., (2003) [3] stated that the future use of *O. gratissimum and A. conyzoides* L. antibiotic combinations as a therapeutic measure against shigellosis can be envisaged in the nearest future.

CONCLUSION

It was clearly evident from the study that both *A. conyzoides* L. and *O. gratissimum* possess antibacterial properties. When the antibacterial activity of each of the plant extracts were compared for aqueous, ethanol and methanol extracts, no significant difference was noticed in their activity. The antibacterial activity of the extracts could be enhanced if the components are purified. These plants therefore, are potential sources of new drugs for treating infections caused by these clinical pathogens.

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