

The Antibacterial Activity of Leaf Extracts of *Ocimum Gratissimum* and *Sida Acuta*

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Abstract: The antibacterial activities of ethanolic and aqueous extracts of *Sida acuta* and *Ocimum gratissimum* on *Escherichia coli*, *Staphylococcus faecalis* and *Pseudomonas aeruginosa* were determined using disc diffusion assays. All the extracts exhibited moderate to high level of inhibition against the four microorganisms. The antibacterial activities were measured by the diameter of inhibition zone, in which ethanolic extracts of *Sida acuta* exhibit the highest yield which is 18mm and the lowest yield in aqueous (cold) extract 7mm. The minimum inhibitory concentration (MIC) is 0.125mg/ml on both plant extracts using the ethanolic extracts of *Sida acuta* and *Ocimum gratissimum* were positive on both the four microorganism and the minimum inhibitory concentration (MIC) were also positive and gram negative organisms. This research is discussed in relation to the acceptable herbal medicines as a means of disease control.

Keywords: *Ocimum gratissimum*, *Sida acuta*, Minimum Inhibitory Concentration, *Escherichia coli*, *Staphylococcus faecalis* and *Pseudomonas aeruginosa*.

I. Introduction

Medicinal plants are distributed worldwide, but they are most abundant in the tropical countries (Calixto, 2000). It is estimated that plant materials are presently in or have provided the models for 50% western drugs. A relative small percentage of medicinal plants are used as food for both humans and other animals species, it is possible that even more are used for medicinal purposes.

Plants based on antimicrobials have therapeutic potentials. They are used for effective treatment of infectious disease yet gently. An example is *Hydratic canadensis*, not only does it have antimicrobial activity but also increase blood supply to the spleen releasing mediating compounds (Murray et al., 1995). Also, *Xytopia aethiopia* has an attractive aroma and has been applied in ethno medicine in the treatment of cough, bronchitis, dysentery and female fertilization.

Iwu (1993) reported that infectious diseases account for one half of all deaths in the tropical countries irrespective of efforts made in controlling the incidence of epidemic. Most food-borne diseases due to poor hygiene can be life threatening and need antibiotic therapy but most of the causative agents have already developed resistance to common antibiotics in many countries (Iwu, 1993). This resistance has been reported in Ethiopia in Africa. Due to absence of modern healthcare system in most rural areas, people decided to visit traditional healers who use medicinal plants to treat their patients with the recent advances made in using plant extracts in inhibiting microbial growth, it was observed that phytochemicals have antimicrobial effect against some human pathogens such as *Staphylococcus aureus*, *Escherichia coli*, etc.

Many plant extracts have shown to acquire antibacterial properties active against many microorganisms inside the body or in vitro for example *Garcinia biflavonone* have been found to be active against variety of organisms like *Staphylococcus aureus* and *Escherichia coli* (Iwu, 1993). It is used in the treatment of liver disorder, bronchitis as a chewing stick and throat infections (Lamidi et al., 1995). Some extracts of green pepper, garlic and onion have been noticed to inhibit the growths of *Shigella dysenteriae*, *Salmonella typhosia* (Sofowora, 1983). *Ocimum gratissimum* and *Sida acuta* are also medicinal herbs in Nigeria used in treatment of some infectious diseases.

Ocimum gratissimum is used as a vegetable for soup preparations which exhibit hot and spicy taste and are consumed during the cold season. It is claimed that species and herbs assist in the concentration of the uterus in postpartum women (Okwu, 2003).

However, it is generally assumed that the active dietary constituents contributing to those medicinal properties exhibited by herbs and species are the phytochemical vitamins and minerals. The *Ocimum* oil is active against several species of bacteria and fungi, for example *Shigella*, *Salmonella*, *Proteus*, *Trichophyton rubrum* etc (El-said

et al., 2000). *Ocimum gratissimum* is rich in alkaloids, tannins, phylates flavonoids and Oligosaccharides and it has tolerable cyanogenic glycoside content (Ijeh *et al.*, 2004) which is the chemical compound active against microorganisms.

Sida acuta is a marvelous weed that frequently dominates improved pastures, waste and disturbed places roadsides (Mann *et al.*, 2003). The described pharmacological properties of the plants involve the antiplasmodial, antimicrobial, antioxidant, and many other properties. Some studies resulted in the isolation of single compounds while the others just demonstrated the activity of the crude extracts.

Medicinal plants as *Sida acuta* and *ocimum gratissimum* have been asserted to provide various culinary and medicinal properties. These medicinal properties exert bacteriostatic and bacteriocidal effects on some bacteria. These effects have been attributed to the peptides, alkaloids essential oil, flavonoids etc which are the major compounds in these plants (Okigbo and Igwe, 2007).

This study was done to determine the inhibitory properties of *ocimum gratissimum* and *Sida acuta* on four strains of bacteria of which are *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli* using ethanol and aqueous as solvent for extraction. It is also used to measure the zone of inhibition of these plants on the organism.

II. Materials And Methods

MATERIALS

Materials used in this study were collected in the laboratories of Nation Root Crop Research Institute (NRCRI) Umudike and the department of Microbiology in Michael Okpara University of Agriculture, Umudike. Except the two plants which were collected from the laboratory of the Federal Medical Centre, Umuahia.

METHODS

COLLECTION OF PLANT MATERIALS

The medicinal plants used in this study were the leaf of *Ocimum gratissimum* and *Sida acuta*. Fresh sample of *Ocimum gratissimum* leaves were collected from an uncultivated farmland at Nsukka in Enugu State, while the fresh leaves of *Sida acuta* were collected from road side in Nsukka, Enugu State. The plants were identified and authenticated at the Botany Department, Michael Okpara University of Agriculture, Umudike by a taxonomist Dr. I. C. Okwulehe. The leaves of each plant was washed thoroughly under running water and dried under room temperature. They were grinded into powder and stored in air tight bottles.

STERILIZATION OF MATERIALS

All material were washed the detergent and rinsed thoroughly. They were placed in a rack to dry and then autoclaved at 121°C for 15 minutes to kill microorganisms.

TEST MICROORGANISM

The strain used in this work was *Staphylococcus aureus*, *streptococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli*. They were all collected at laboratory in Federal Medical Centre Umuahia and their viability were tested by resuscitating them in buffered peptone broth and then sub-cultured in Nutrient agar medium at 37°C for 3 hours prior to antibacterial testing.

PREPARATION AND EXTRACTION OF THE PLANTS

ETHANOL EXTRACTS

The ethanol extracts was prepared by weighing out twenty grams (20g) of the powdered leaves of *Ocimum gratissimum* and *Sida acuta* in different conical flask and adding 200ml of ethanol in each and stirring vigorously with a glass rod and left for 24 hours. They were filtered off with the sterile filter paper (Whatman No 1 filter paper) into a clean conical flask and filtrate was transferred into the sample holder of the rotary vacuum evaporator where the solvent was evaluated at room temperature of 28°C. The standard extracts obtained were then stored in refrigerator at 4°C until when required for use.

COLD WATER EXTRACTS

The cold water extracts was prepared by weighing out twenty grams (20g) of the powdered leaves of *Ocimum gratissimum* and *Sida acuta* in different conical flask and adding 200ml of cold water in each stirring vigorously with a glass rod and left for 24 hours. They were filtered off with sterile filter paper (Whatman No 1 filter paper) into a clean conical flask and the filtrate was transferred into a sample holder of the rotary vacuum evaporation where the cold water solvent was evaporated at its room temperature at 28°C. The standard extracts obtained were then stored in a refrigerator at 4°C until when required for use.

DISC DIFFUSION ASSAY

The disc diffusion method was adopted for the determination of the antibacterial activity of extract. Whatman No 1 filter paper was used with slight modification. The filter papers were cut into disc of 6mm in diameter using a perforator. The discs were treated by boiling for 30 minutes, the reason was to destroy chemicals used in preserving the filter paper and also to avoid the inhibitor of the antimicrobial action of the extracts on test organisms. After boiling, the disc was sterilized by autoclaving for 15 minutes and were stored in a sterile bottle for use.

PREPARATION FOR CULTURE MEDIA

Muella Hinton agar was prepared by weighing 38 grams of the powdered agar into 100ml of distilled water in a clean conical flask. It was soaked for 20 minutes and then covered with a foil and was autoclaved at 121⁰C, 115 atmospheric pressure for 15 minutes. The medium was cooled to 50⁰C and 20ml of the medium was poured into a sterile glass petri dish and allowed to solidify. The sterility of the medium was tested by allowing it to stay overnight and checking for contamination.

A flaming wire loop is being used to pick an organism and stick it well on a prepared media. After that the paper disc is picked with a sterilized wire loop and use it to collect the extract then place it on the media in which the organism is stick on. Then covered well and incubated for 24 hours at 37⁰C.

After an overnight incubation at 37⁰C, the zone inhibition was measured and recorded. The test was carried out in duplicates of different organisms with different extracts.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

A stock solution of 100mg/ml of ethanol and cold water extracts were prepared. 0.4ml of the solution of *Sida acuta* and *Ocimum gratissimum* is diluted to 20ml with the nutrient broth. Serial dilutions were prepared to obtain the following concentrations: 400, 200, 100, 50, 25, 12.25mg/ml. 0.0ml of the suspensions of overnight cultures of the test organisms which was adjusted to McFarland standard. The organism is streaked on the agar medium, using a cut borer to make wholes on the medium. Different concentrations of the extracts were added into the wholes and were covered and incubated for 24 hours at 37⁰C (Ekundayo and Ezeogu, 2006). The zones of inhibition were measured, and the least concentration of each extracts that inhibited microbial growth after the incubation period was taken on the MIC.

CONTROL EXPERIMENT USING GENTAMYCIN AND TETRACYCLINE

Gentamycin and Tetracycline were used as a control in order to compare the diameter of zone of inhibition from the extracts and already standardized antibiotics and it was carried out aseptically. This is to ensure the prescription of either antibiotics or plants herbs for antibacterial activities.

Gentamycin and tetracycline (250mg) capsule was dissolved to get a stock solution of 50,000mg/ml. 0.4ml of these solutions were taken to make up to 10ml (0.4ml+9.6ml) of distilled water. 20ml (0.2ml) of the dilution was dropped on each disc. These discs were placed on the inoculated culture medium along side the aqueous and ethanol extracts.

III. Results

PERCENTAGE YIELD OF PLANT EXTRACT

The yields of the plant extracts (ethanol and aqueous) were calculated as percentages of the initial powdered sample of plant materials shown in table 3 below. The ethanol extracts of *Ocimum gratissimum* gave the highest yield which is 8.5g representing 40%, while the ethanol extract of *Sida acuta* yields 7.5g representing 35%. The percentage yields of aqueous extracts of *Ocimum gratissimum* and *Sida acuta* were 30% and 25% respectively.

ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS

The antibacterial activities of the extraction of two plant extracts were measured by the diameter zone of inhibition using the disc agar assay and by using serial dilution to determine the minimum inhibitory concentration (MIC) of the extracts. The summary of the diameter zone of inhibition produced by the plants extract against the organisms is shown below in table 4. The ethanol extract of *Sida acuta* had the zone of inhibition of 18mm on *Staphylococcus aureus* and *Streptococcus faecalis* it yielded 14mm. The diameter zone of inhibition of the ethanol extracts on *Escherichia coli* is 16mm, while *Pseudomonas aeruginosa* yielded 14mm. The aqueous extract of *Sida acuta* had diameter zone of inhibition of 10mm, on both *Staphylococcus aureus* and *Pseudomonas aeruginosa* and 12mm on both *Escherichia coli* and *Streptococcus faecalis*. On *Ocimum gratissimum* in the ethanol extract yields 13mm, 15mm and 12mm on *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* respectively while its aqueous extract

yield 7mm,6mm,8mm,and 8mm on *Staphylococcus aureus*, *Streptococcus faecalis*. *Escherichia coli* and *Pseudomonas aeruginosa* respectively

Table 3 shows the minimum inhibition concentration in *Staphylococcus aureus* using the plant extracts in which its ethanolic extracts had minimum inhibition concentration of 0.125 on both extracts. Tables 4,5,and 6 also shows the minimum inhibition concentration on *Streptococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. *Escherichia coli* had the lowest minimum inhibition concentration which is 0.0625 on ethanolic extracts of *Sida acuta*.

Gentamycin(control) show a wide zone of inhibition on both *Escherichia coli* and *Staphylococcus aureus* as 32mm and 24mm or *Ocimum gratissimum* and 25mm and 20mm on both organisms but in *Sida acuta*. While tetracycline(control) yield 22mm and 10mm on both organisms in *Ocimum gratissimum* and 30mm and 26mm on both organisms by *Sida acuta*

TABLE 1:THE PERCENTAGE OF THE CRUDE EXTRACTS OF *Ocimum gratissimum* and *Sida acuta*

Plant species	Extracts	weight of the Powdered sample	weight of the extracts recovered	percentage yield of extracts
<i>Ocimum</i>	Aqueous	20.0g	6.0g	30%
<i>Gratissimum</i>	ethanol	20.0g	8.5g	40%
<i>Sida acuta</i>	Aqueous	20.0g	5.0g	25%
	Ethanol	20.0g	7.5g	35%

TABLE 2:ANTIBACTERIAL ACTIVITY OF THE EXTRACTS WITH DIAMETER ZONE OF INHIBITION

Plant Species	Extract type	<i>E.coli</i>	<i>S.faecalis</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>
	Aqueous	8mm	6mm	8mm	7mm
<i>Ocimum gratissimum</i>	Ethanol	15mm	10mm	12mm	13mm
	Gentamycin	32mm	-	-	24mm
	Tetracycline	22mm	-	-	10mm
<i>Sida acuta</i>	Aqueous	12mm	12mm	10mm	10mm
	Ethanol	16mm	14mm	14mm	18mm
	Gentamycin	25mm	-	-	20mm
	Tetracycline	30mm	-	-	26mm

TABLE 3:THE MINIMUM INHIBITION CONCENTRATIONS ON *Staphylococcus aureus* by the two PLANT EXTRACTS USED.

Plant extracts	Different concentration of the extracts								
	1.0	0.50	0.25	0.125	0.0625	0.0315	MIC		
<i>Sida acuta</i>	Aqueous		10.0	7.6	5.6	0	0	0	0.25
	(Cold)								
Ethanol		20.6	14.6	11.0	7.3	0	0	0.125	
<i>Ocimum Gratissimum</i>	Aqueous		7.0	5.6	3.3	0	0	0	0.25
	(Cold)								
Ethanol		13.0	9.5	7.6	5.3	0	0	0.125	

KEYS : MIC=MINIMUM INHIBITION CONCENTRATION

TABLE 4:THE MINIMUM INHIBITION CONCENTRATIONS OF THE EXTRACTS ON *Streptococcus faecalis* by two PLANT EXTRACTS

Plant extracts	Different concentration of the extracts								
	1.0	0.50	0.25	0.125	0.0625	0.0315	MIC		
<i>Sida acuta</i>	Aqueous		12.0	9.6	5.6	0	0	0	0.25
	(Cold)								
Ethanol		14.0	10.3	6.3	0	0	0	0.25	
<i>Ocimum Gratissimum</i>	Aqueous		6.0	5.6	0	0	0	0	0.5
	(Cold)								
Ethanol		10.0	7.6	6.3	5.6	0	0	0.125	

TABLE 5:THE MINIMUM INHIBITION CONCENTRATIONS OF THE EXTRACTS ON *Escherichia coli* by two PLANT EXTRACTS

Plant extracts	Different concentration of the extracts								
	1.0	0.50	0.25	0.125	0.0625	0.0315	MIC		
<i>Sida acuta</i>	Aqueous		12.0	9.6	5.6	0	0	0	0.125
	(Cold)								
	Ethanol	18.0	14.6	10.3	9.5	6.6	0	0	0.0625
<i>Ocimum</i>	Aqueous	12.0	9.6	8.0	5.6	0	0	0	0.125
<i>Gratissimum</i>	(Cold)								
	Ethanol	15.0	12.0	9.3	6.6	0	0	0	0.125

TABLE 6:THE MINIMUM INHIBITION CONCENTRATIONS OF THE EXTRACTS ON *Pseudomonas aeruginosa* by two PLANT EXTRACTS

Plant extracts	Different concentration of the extracts								
	1.0	0.50	0.25	0.125	0.0625	0.0315	MIC		
<i>Sida acuta</i>	Aqueous		10.0	9.6	6.3	0	0	0	0.25
	(Cold)								
	Ethanol	14.0	11.3	8.6	6.3	0	0	0	0.125
<i>Ocimum</i>	Aqueous	5.0	0	0	0	0	0	0	1.0
<i>Gratissimum</i>	(Cold)								
	Ethanol	8.0	5.6	5.3	0	0	0	0	0.25

IV. Discussion

The inhibitory activities of ethanolic extracts of *Ocimum gratissimum* and *Sida acuta* were found to be little greater than aqueous(cold) crude extracts according to Sofowora(1993) the active principles of the plant herb may be more soluble in ethanol as employed in traditional medicine.

In this study reported here,the aqueous(cold) extracts of *Ocimum gratissimum* and *Sida acuta* showed the zone of inhibition to all the organisms used which are *Pseudomonas aeruginosa*,*Escherichia coli*,*Streptococcus faecalis* and *Staphylococcus aureus*.This result almost correspond to the findings of Lewis and Elvin(1995) who in thier work found the weak antibacterial activity on the extracts on both gram positive and gram negative organisms.

The ethanol extracts of *Ocimum gratissimum* and *Sida acuta* which showed a greater zone of inhibition to all organisms used.This also correspond to the findings of(Onajobi,1986) who in their work found that this plants can be useful in treating some infectious disease such as diarrhoea,haedache,skin disease,pneumonia,fever,gum disorder etc.

The effectiveness of antimicrobial agent varies with the nature of organisms being treated.Since microorganisms differ markedly in their susceptibility.The presence of the active principles in plants is influenced by several factors such as age of the plants,method of extraction and extracting solvent.It is possible that the leaves of the *Sida acuta* and *Ocimum gratissimum* contains high concentration of the antimicrobial compounds or different antimicrobial agents based on the values of minimum inhibition concentration and the zone of inhibitions.

However,there was a based a broad spectrum activity observed on the two plants extracts which are *Ocimum gratissimum* and *Sida acuta* as it showed high activity against both gram negative and gram positive tested.

V. Conclusion

Ocimum gratissimum and *Sida acuta* are plant of wide usage in traditional medicine.The results of this work,now demonstrated that these plants were active on several bacterial strains such as *Pseudomonas aeruginosa*,*Escherichia coli*,*Streptococcus faecalis* and *Staphylococcus aureus*.

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