Synergistic Effects of Plants Extracts on Bacteria

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ABSTRACT: The antibacterial activities of ethanol and aqueous extracts of *Ocimum gratissimum* and *Vernonia amygdalina* were tested *invitro* against seven bacterial species, Pro*teus vulgaris, Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Klebsiella pneumonia* by agar diffusion method. The pattern of inhibition varied with the plant extract, the solvent used for extraction and the organisms tested. The antibacterial activities of the ethanol extracts were significantly higher ($p<0.05$) than the antibacterial activities of the aqueous extracts of the two plants. Among the individual samples, *V. amygdalina* had the most impressive activities and compares favorably with that of the combination of *V. amygdalina* and *O. gratissimum.* The combinations of the leaf extracts exhibited a higher effect on the test bacterial species (16mm to 24mm) than any of the individual plant extracts (12mm to 16mm). Results of this kind herald the interesting promise of designing a potentially active antibacterial synergized agent of plant origin.
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1. INTRODUCTION

Natural compounds of plants origin are biodegradable, often of low mammalian toxicity and pose low danger to the environment (Kiesta *et al.*, 2000). They have therapeutic properties of being antileukemic, antidiabetic, antitumor, aperitive, cytotoxic, laxative, purgative and immuno modulatory among many others. This characteristics exhibited by medicinal plants is of great importance and has led to various researches carried out on them.

According to Olukoya *et al*., (1993), a number of plants have been used in traditional medicine for many years. Examples of such are *Vernonia amygdalina, Paulinia pinnata, Carica papaya, Ocimum gratissimum, Azadirachta indca, cymbopogon citratus* among many others. Akerele (1993) wrote that 80% of the world's total population depends on traditional medicine for the treatment of their infections. It can thus be assumed that the major part of traditional therapy involves the use of plant extracts or their active components. These active components have antimicrobial properties which make them effective.

As a result of the presence of these active components, most of these plants have been known to be toxic to various forms of microorganisms, thus reducing diseases caused by microorganisms to a minimal level. The antimicrobial activity of medicinal plants is known to be obtained from the chemical compound, which are referred to as the secondary metabolite (Oyagade *et al*., 1999). These substances include phenolic compounds, steroids, glycosides and a host of others.

This research work is aimed at determining the antibacterial effect of the aqueous and ethanol extract of *Ocimum gratissimum* and *Vernonia amygdalina*, evaluating their single and combined effects. The pyhtochemical screening for active component in the extracts, sensitivity pattern of both gram positive and negative bacteria to these extracts will also be determined.

2. MATERIAL AND METHODS

Fresh leaves of *Ocimum gratissimum* and *Vernonia amygdalina* were collected at different locations in Ado-Ekiti. Ekiti State, Nigeria. The leaves were harvested in the early hours of the day to guard against wilting and possible loss of active principles due to evaporation. Identification of the leaves was done in the herbarium unit of the department of Plant Sciences, University of Ado-Ekiti, Nigeria.

2.1 Ethanol Extraction

Fifty grams of dried powdered leaves of *Ocimum gratissinum* and *Vernonia amygdalina* were extracted in 200ml of 70% ethanol in 250ml conical flasks for 5 days. The extracts were obtained by filtration using filter paper into sterile crucibles. The extracts were allowed to evaporate to dryness in crucibles and were kept in sterile bottles at 4^0C until used.

2.1 Aqueous Extraction

Fifty grams of dried powdered leaves of *Ocimum gratissinum* and *Vernonia amygdalina* were soaked in 200ml of distilled water in 250ml conical flasks for 5 days. The resulting liquor was filtered through Whatman no 1 filter paper, evaporated to dryness under vacuum at 45^0C, poured into clean sterile bottles, labeled and kept at 4^0C until used.

2.3 Sources of test Organisms.

All the tested organisms i.e *Proteus vulgaris, Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Klebsiella pneumonia* were obtained from the Microbiology Laboratory of the Department of Microbiology, University of Ado-Ekiti, Nigeria. They were all maintained on agar slants.

2.4 Antimicrobial assay

The antimicrobial assay was done using the agar diffusion method. The various test bacteria were standardized using the 0.5 McFarland turbidity standards. These standardized strains were inoculated onto the surface of sterile plates of Diagnostic sensitivity test agar (DST). The assay were carried out using extracts concentrations that ranged from 15.62mg/ml to 250mg/ml. Cork borer (6mm) was used to make wells on the inoculated DST agar . One milliliter of each concentration of extracts was introduced into designated wells. These were allowed to be absorbed into the agar, and then incubated at 35^0C for 24h. The antimicrobial activities were determined by the width of the zone of growth inhibition (Bauer, 1996).

3. Results

The antibacterial activities of the individual extract and combination of extract of plant parts (*O. gratissiumum* and *V. amygdalina*) were very encouraging. Broad antimicrobial activities against both Gram positive and negative test bacteria were demonstrated.

3.1 Effects of extracts of *V. amygdalina* on test bacterial species.

Results obtained with the ethanol extracts as shown in table 1 implicated *Bacillus subtilis* as the least susceptible, showing no zone of growth inhibition at 31.25mg/ml and 15.62mg/ml extracts concentrations. *Salmonella typhimurium* also shows no zone of growth inhibition at 15.62mg/ml concentration. *Klebsiella pneumonia* shows the highest zone of growth inhibition ranging from 12.0mm to 21.0mm. This is closely followed by *Proteus vulgaris* and *Pseudomonas aeruginosa* with zones of growth inhibition ranging from 10mm - 20mm. *Staphylococcus aureus* had zones of growth inhibition ranging from 10.0mm to 16.0mm while *E. coli* had zone of growth inhibition that ranges from 10.0mm to 17.0mm. The antibacterial effect decreased with decrease in concentration.

There were no significant differences when the antibacterial effect of the aqueous extracts was compared with that of the ethanol extract as shown in table 2. However, *S. aureus* had the least susceptibility to the aqueous extract. The highest zone of growth inhibition ranging from 20mm to 15mm was recorded for *K. pneumoniae*. The aqueous extract inhibited the growth of *Proteus species* to a zone of 18.0mm to 14.0mm. All the bacteria were more sensitive to ethanol extract than the aqueous extract. Activities were observed against both Gram positive and negative bacteria. This showed that the extracts had broad spectrum activities.

3.2 Effects of extracts of *Ocimum gratissimum* on test bacterial species.

Ethanol extraction of ***Ocimum gratissimum*** showed that all the test bacteria were sensitive to the extract except *Staphyloccocus aureus* which showed no zone of growth inhibition at all the varying concentration used .(Table 1). The test bacteria were observed to show very low susceptibility to the extract with most of them *Proteus vulgaris, Klebsiella pneumoniae, Bacillus subtilis,* and *Pseudomoas aeruginosa*, showing activities within the range 10.0mm to 14.0mm. *Escherichia coli* showed the least zone of growth inhibition between the ranges of 12.0mm- 10.0mm.

The highest zone of growth inhibition was recorded with *Salmonella typhimurium* and *Pseudomonas aeruginosa* (10.0mm to 16.0mm). *Klebsiella pneumoniae* and *Bacillus subtilis* responded by presenting with similar growth inhibition pattern (11.0mm to 14.0mm).

3.3 Effect of the combination of *Ocimum gratissimum* and *Vernonia amygdalina* on test bacterial species.

As shown in tables 3, the combination of the ethanol extracts of *Ocimum gratissimum* and *Vernonia amygydalina* at equal ratio exert a much higher activities on *Proteus vulgaris* and *Pseudomonas aeruginosa* (13.0mm to 22.0mm). *Bacillus subtilis* was least susceptible (10.0mm to 14.0mm) at concentrations of 31.25mg/ml and 15.62mg/ml. However the combination of the aqueous extracts revealed *Pseudomonas aeruginosa* as the most susceptible (14mm to 22mm) while *Staphylococcus aureus* was the least susceptible. No activity was recorded at extracts concentration of 62.5mg/ml, 32.25mg/ml and 15.62mg/ml.

Table 1: Antibacterial activities of ethanol extracts of *Vernonia amygdalina* and *Ocimum gratissimum*

	Vernonia amygdalina						*Ocimum gratissimum*					
	Zones of Growth Inhibition						Zones of Growth Inhibition					
Test bacteria	**250 mg/ml**	**125 mg/ml**	**62.5 mg/ml**	**31.25 mg/ml**	**15.62 mg/ml**	**Control**	**250 mg/ml**	**125 mg/ml**	**62.5 mg/ml**	**31.25 mg/ml**	**15.62 mg/ml**	**Control**
P. vulgaris	20.00	18.00	14.00	12.00	10.00	0.00	14.00	11.00	10.00	10.00	0.00	0.00
S. typhi	17.00	13.00	12.00	10.00	0.00	0.00	22.00	20.00	18.00	17.00	15.00	0.00
S. aureus	16.00	14.00	11.00	10.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E. coli	17.00	15.00	11.00	10.00	10.00	0.00	12.00	11.00	11.00	10.00	10.00	0.00
K. pneumonia	21.00	18.00	17.00	14.00	12.00	0.00	14.00	12.00	10.00	10.00	0.00	0.00
B. subtilis	15.00	12.00	11.00	0.00	0.00	0.00	14.00	13.00	12.00	11.00	10.00	0.00
P. aeruginosa	20.00	18.00	17.00	16.00	10.00	0.00	14.00	12.00	11.00	0.00	0.00	0.00

Table 2: Antibacterial activities of aqueous extracts of *Vernonia amygdalina* and *Ocimum gratissimum*

	Vernonia amygdalina						*Ocimum gratissimum*					
	Zones of Growth Inhibition						Zones of Growth Inhibition					
Test bacteria	**250 mg/ml**	**125 mg/ml**	**62.5 mg/ml**	**31.25 mg/ml**	**15.62 mg/ml**	**Control**	**250 mg/ml**	**125 mg/ml**	**62.5 mg/ml**	**31.25 mg/ml**	**15.62 mg/ml**	**Control**
P. vulgaris	18.00	17.00	16.00	15.00	14.00	0.00	15.00	14.00	12.00	16.00	10.00	0.00
S. typhi	15.00	14.00	13.00	12.00	10.00	0.00	16.00	16.00	15.00	14.00	10.00	0.00
S. aureus	12.00	10.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E. coli	16.00	15.00	14.00	12.00	10.00	0.00	12.00	10.00	10.00	0.00	0.00	0.00
K. pneumonia	20.00	19.00	18.00	16.00	15.00	0.00	14.00	12.00	11.00	0.00	0.00	0.00
B. subtilis	14.00	13.00	12.00	11.00	10.00	0.00	14.00	12.00	11.00	0.00	0.00	0.00
P. aeruginosa	17.00	16.00	15.00	14.00	12.00	0.00	16.00	14.00	13.00	12.00	10.00	0.00

Table 3: Synergistic effects of ethanol extracts of *V. amygdalina* and *O. gratissimum* on test bacterial species

Test bacteria	*V. amygdalina*: *O. gratissimum* (1:1) Zone of growth inhibition (mm)					*V. amygdalina*: *O. gratissimum* (1:2) Zone of growth inhibition (mm)					*V. amygdalina*: *O. gratissimum* (2:1) Zone of growth inhibition (mm)				
	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.62 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.62 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.62 mg/ml
P. vulgaris	22.00	18.00	16.00	15.00	14.00	18.00	17.00	16.00	15.00	0.00	20.00	20.00	20.00	18.00	14.00
S. typhi	20.00	18.00	16.00	15.00	10.00	15.00	14.00	13.00	12.00	0.00	20.00	18.00	18.00	15.00	10.00
S. aureus	15.00	13.00	12.00	12.00	0.00	12.00	10.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E. coli	20.00	16.00	14.00	13.00	0.00	16.00	15.00	14.00	12.00	0.00	18.00	14.00	12.00	12.00	0.00
K. pneumoniae	18.00	16.00	15.00	12.00	11.00	20.00	19.00	18.00	16.00	0.00	24.00	18.00	15.00	15.00	11.00
B. subtilis	14.00	12.00	10.00	0.00	12.00	14.00	13.00	12.00	11.00	0.00	17.00	15.00	14.00	14.00	12.00
P. aeruginosa	22.00	16.00	15.00	14.00	15.00	17.00	16.00	15.00	14.00	0.00	22.00	20.00	20.00	20.00	15.00

4. Discussion

The result showed that the ethanol and aqueous extracts of the leaves of *V. amygdalina* and *O. gratissium* possessed appreciable antimicrobial activity against *Proteus vulgaris*, *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The antimicrobial activities of the plant extracts appeared to be broad spectrum since both Gram positive and negative bacteria were sensitive to the extracts. The results showed that the ethanol extract of the leaves appeared to be more effective in inhibiting the growth of the test organisms. This confirmed that the work of Cowan (1999) who stated that the reason for the effectiveness of ethanol extraction could be that most of the identified active components from plants, aromatic or saturated organic compounds, are most often obtained through ethanol or methanol extraction.

The single effect of the extracts on the test bacterial species revealed that *V. amygdalina* was more active on the test organisms than *O. gratissimum.*. The sensitivity of the test organisms to *V. amygdalina* may be due to the presence of active saponins and essential oils (Desta, 1993). The susceptibility of the test organisms to the leaf extract of *V. amygdalina* agreed with the findings of Scalbert (1991) that demonstrated the antimicrobial activity of some medicinal plants against bacteria by using the extract of *V. amygdalina* as one of the samples. In similar studies, Aina and Uko, (1990), reported that *V. amygdalina* possess bacteriostatic activity but has little or no effect on yeast.

However, the results suggested that when the leaf extracts were used in combination, they had wider antibacterial activities than when used singly.

The very low antibacterial activities observed with *Staphylococcus aureus* is not surprising since multiple antibiotic resistant strains of *S. aureus* exist in clinical settings worldwide as well as Nigeria (Karen and Edzard, 2003; Kesah *et al*., 2003). The absence of activity against the test organisms as observed with *O. gratissimum* when used singly and in combination may suggest that the concentration of active constituents in the extracts are too low for any appreciable antimicrobial activity. Furthermore, this may be due to factors such as time of collection of plant material and climate which might in turn affect the concentration of constituents in the plant material. In addition, low concentration of diffusible water soluble active constituents which often affect biologically active substances such as flavonoid, essential oils and other heterogeneous phyto-constituents present in the extracts might also influence their respective activity. Finally, the result obtained indicated that the activity against test bacteria is enhanced when *Vernonia amygdalina* and *Ocimum gratissimum* are combined in a ratio of 2: 1 and 1: 1 respectively.

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