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Antibacterial and Phytochemical Activities of the Crude Extracts of *Vernoniaamygdalina*Del. and *Ocimumgratissimum*L.

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Abstract
The antibacterial and phytochemical activities of ethanolic, methanolic and water extracts of *Vernoniaamygdalina*Del. and *Ocimumgratissimum*L.was investigated invitro on *Staphylococcus aureus,Proteus vulgaris Pseudomonas aeruginosa, Escherichia coli,* and *Citrobacter spp*. Cold maceration method was used to extract the active components from plants. Susceptibility testing was carried out using agar diffusion and broth dilution technique. Phytochemical activity of crude extracts were carried out using standard procedures. All the bacterial isolates were susceptible to at least one plant extract except *Proteus vulgaris* which was resistant to all plant extracts. *Staphylococcus aureus* was susceptible to all the plant extracts. The minimum inhibitory concentration and minimum bactericidal concentration of extracts of these plants ranged from 12.5 to 50mg/ml and 25 to 100 mg/ml respectively.Anthraquinone was present in leaves of both plants however, absent in *O.gratissimum* seeds. The study offer a scientific basis for the traditional use of water and ethanol extracts of *V. amygdalina* and *O. gratissimum*for treating diseases.*Staphylococcus aureus* was the most susceptible organism to the extracts which suggests that *Vernoniaamygdalina* and *Ocimumgrattisimum* could be used to treat diseases caused by this microorganism.

Keyword: *Vernonia amagdalina, Ocimum gratissimum*, antibacterial, crude extracts

INTRODUCTION

Vernoniaamygdalina belongs to the family, Compositae (Asteraceae) and was named after William Vernona 17th century botanist (Anibijuwon,*et al.*, 2012; Keay, 1989). *Vernoniaamygdalina* is plant that originated from tropicalAfrica and common grownall over sub-Saharan Africa (Bosch *et al*., 2005). The shrub is 5m high, leaves are simple and entire (5x15cm), finely grandular underneath, with few lateral nerves. The flowers occur in a panicle white and fragnant. It is differentiated from its counterpart *Vernoniacolorata* which grows wildly hairly leaves of the latter (Iwu and Kokwaro, 1996). It is commonly called bitter leaf in English "Oriwo" in Edo

"Oringbo"inUrhobo, "ewuro" in Yoruba, "shekawu" in Hausa and "onugbu" Igbo (Oboh and Masodje, 2009). The plant has a bitter taste, however, the leaves are used as condiments and vegetables after removing the bitterness by washing in water. The bitterness of this plant is due to antimicrobial factors such as alkaloids, saponins, tannins and glycosides (Akahand Okafor, 1992; Abosi and Raserola, 2003; Izevbigie*et al*., 2004). The roots and leaves are used traditionally to treat fever, kidney disease hiccups, and stomach upset (Gill, 1992). The peeled stem has anticaries activity and often used as chewing stick for cleaning the teeth (Sadhan and Almas, 1999). The bitter juice from leaves are exploited by nursing mothers to assist in weaning their babies by rubbing it on their breast (Iwu and Kokwaro, 1996). The plant is often added to other plants for treatment of various illness. It is often used as sugar level control in diabetes patients. A cup full daily is prescribed to patients in serious cases (Anibijuwon*et al*., 2012).

Ocimumgratissimum is an aromatic medicinal plant in the family Lamiaceae. It is an important herbal medicine found in the tropical and warm region such as Kenya and Nigeria (Lexa*et al*., 2008; Aguiyi*et al*., 2000; Okigbo and Ogbonnanya 2006). *Ocimumgratissimum* is useful in the treatment of upper respiratory tract. It is also useful in the treatment of conjunctivitis, skin diseases, pneumonia and diarrhea (Ilori*et al*.,1996; Nwiyi*et al*.,2009) .

This study was conducted to test the antibacterial activities of crude extracts of *Vernoniaamygdalina* and *Ocimumgratissimum* against some microorganisms previously, isolated from clinical samples with a view to ascertain their medicinal values.

MATERIALS AND METHODS

Study Area

Plants used in the study were collected from their natural habitat in Abraka. Abraka is a university town located in Ethiope East local government area of Delta State, Nigeria. It is situated approximately on latitude 5'°48'N and longitude 6° 06"E (Osakwe, 2012).

Sample Collection

Fresh seeds and leaves of *O. gratissimum* and leaves of *Vernoniaamygdalina* were collected from the natural habitat and identified at the Botany Department, Delta State University Abraka. *Escherichia coli*, *Citrobacterspp, Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus aureus* were collected from stock of previously isolated and identified bacteria from clinical specimens. All the bacterial species used were maintained on nutrient agar slopes and stored at 4^0C until required.

Extract Preparation

Air dried and powdered samples (40g) of *Ocimumgratissimum* seeds and *Vernoniaamygdalina* leaves were soaked in 150mls of water, 70% ethanol and methanol and on separate conical flask then filtered using Whatman No 1 filter paper after 24 hours for water extract and 72 hours for methanol and ethanol extracts respectively. The filtrates were evaporated in waterbath maintained between 60-70^0C. The extracts obtained were placed in sterile labelled containers. Their weights was noted.

Antibacterial Test

A known weight of each extract was mixed in a little volume of sterile distilled water or ethanol and methanol to give the desired concentration of extract in milligram. The bacterial suspensions were cultured in Mueller Hinton broth for 24 hours. Serial dilution was carried out to 10^8cfu/ml as McFarland standard. A sterile cotton swab containing the organismswere streaked on the plate surfaces of Mueller Hinton agar medium then well(5mm) were made on agar at different points. Different concentrations of extracts were introduced into wells and inoculated plates were left on the laboratory bench for one hour to allow prediffusion of extracts before incubation at 37^oC of 18-24 hours.

Plates were incubated in duplicates for each concentration for each organism and the diameter of zones of inhibition was measured in millimetres after incubation period. The zone of inhibition shows the effect of the extract on the bacteria isolates. Gentamicin (10µg) and the solventsused for extraction were used as control.

Determination of Minimum Inhibiting Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined by transferring 5ml of the sterile plant extract into 5ml of sterile Muller Hinton broth to obtain 50mg/ml concentration. Other dilution such as 25mg/ml, 12.5mg/ml and 6.25mg/ml was carried out(Kolawale*et al.*, 2018) and0.1ml of the standard bacterial cell suspension was inoculated into each of the test tube, and incubated for 24 hours at 37^{o}C. Duplicate tubes were maintained for each test. The antibacterial control andorganism control were also inoculated. The test tube containing the

lowest concentration of the extract with no turbidity when compared with the control test tube indicated theMIC. The tube showing the MIC was subcultured onto a fresh culture medium and incubated for 24 hours. The highest dilution that did not produce any bacterial colony on the solid medium was taken as MBC.

Phytochemical Screening

Phytochemical analysis of the plant extract were carried out in order to determine the presence of Alkaloids, cardiac glycosides, Anthraquinones. Saponins, Tannins and Phlobatanins were also analysed using standard phytochemical methods as described by Sofowara, (1993), Culer (1982) and (Trease and Evans, 2002)

RESULTS

The antibacterial activities of water, methanol and ethanol extracts of *O. gratissimum*, *V. amygdalina* and standard antibiotics is presented in Table 1. All the bacterial isolate, were susceptible to at least one plant extract except *Proteus vulgaris* that was resistant to all the extracts tested, *Staphylococcus aureus* was susceptible to all the extracts except water extract of *O. gratissimum*at 100mg/ml. *Citrobacter*spp produced the highest zone of inhibition for gentamicin the standard antibiotic used for control. The zone of inhibition of *S. aureus* for ethanol extract of *O. gratissimum* was comparable to gentamicin the standard antibiotic. Table 2 shows the diameter of

zones of inhibition of extracts at 50mg/ml on the test organisms. The results of the MIC and MBC is presented in table 3 and 4. The MIC values varied from one organism to another while methanol leaf extract of *O. gratissimum* has the lowest minimum inhibitory concentration of 12.5mg/ml for *E. coli* and *O. gratissimum* seed, ethanol extract had the lowest MIC of 12.5mg/ml for *S. aureus*. The minimum inhibitory concentration water and methanol extracts for *O. gratissimum* is 50mg/ml and 25mg/ml respectively for *Pseudomonas aeruginosa*. The minimum bactericidal concentration of the extracts ranged from 25mg/ml to 50mg/ml. The phytochemical analyses *O.gratissimum* seeds, and leaves and *V. amygdalina* leavesare presented in Table 5. The seeds and leaves of these plants contain alkaloids, flavonoids, tannins, terpenoids, reducing sugar, phenol and saponin. The phytochemical compounds in O. gratissimum seeds and leaves varied. The seeds lack cardiac glycosides and phlobatanins, and anthraquinone, but the leaves possessed anthraquinone, *Vernoniaamygdalina* contains all, i.eanthraquinones, cardiac glycosides and phlobatanins in addition to aforementioned components.

Table 1: Diameter of Zone of Inhibition of Extracts (100mg/ml) on Test Organisms

	Ocimumgratissimum						*Vernoniaamygdalina*			
	Seeds			Leaves						
Organisms	W	M	E	W	M	E	W	M	E	Gentamycin
Escherichia coli	NI	NI	NI	16	22	20	NI	NI	NI	15
Citrobacterspp	12	NI	16	18	20	18	NI	NI	NI	25
Staphylococcus aureus	10	12	20	NI	18	22	12	18.5	20	22
Proteus spp	NI	NI	NI	NI	NI	NI	NI	NI	NI	22
Pseudomonas aeruginosa	NI	NI	NI	NI	17	17	12	NI	NI	20

Diameter of inhibition is (mm)

Key :W=Water, M =Methanol, E = Ethanol, NI = No inhibition

Table 2: Diameters of zones of inhibition of extracts (50mg/ml) on the test organisms

	Ocimumgratissimum						*Vernoniaamygdalina*		
	Seeds			Leaves					
Organisms	W	M	E	W	M	E	W	M	E
Escherichia coli	NI	NI	NI	8	12	10	NI	NI	NI
Citrobacterspp	7	NI	10	8	14	10	NI	NI	NI
Staphylococcus aureus	7	10	12	NI	8	14	6	8	9
Pseudomonas aeruginosa	NI	NI	NI	8	10	NI	4	NI	NI

Key :W=Water, M =Methanol, E = Ethanol, NI = No inhibition

Table 3: Minimum Inhibitory Concentration (MIC) and Extracts (mg/ml)

	Ocimumgratissimum						*Vernoniaamygdalina*		
	Seeds			Leaves					
Organism	W	M	E	W	M	E	W	M	E
Escherichia coli	-	-	-	25	12.5	25	-	-	-
Citrobacterspp	25	-	25	12.5	25	25	-	-	-
Staphylococcus aureus	25	25	12.5	-	25	12.5	25	25	25
Pseudomonas aeruginosa	-	-	-	50	25	-	25	-	-

Key :W=Water, M =Methanol, E = Ethanol

Table 4: Minimal bacteriacidal concentration of extracts (mg/ml)

	Ocimumgratissimum						*Vernoniaamygdalina*		
	Seeds			Leaves					
Organism	W	M	E	W	M	E	W	M	E
Escherichia coli	-	-	-	-	50	-	-	-	-
Citrobacterspp	50	-	-	25	50	50	-	-	-
Staphylococcus aureus	50	50	25	-	25	25	50	50	25
Pseudomonas aeruginosa	-	-	-	100	-	-	-	-	-

Key :W=Water, M =Methanol, E = Ethanol

Table 5: Phytochemical results of plants

	Ocimumgratissimum		*Vernoniaamygdalina*
Phytochemical Constituents	Seeds	Leaves	
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Terpenoid	+	+	+
Saponin	+	+	+
Cardiac glycoside	-	-	+
Anthraquinone	-	+	+
Phlobatanins	-	-	+
Reducing sugar	+	+	+
Phenol	+	+	+

DISCUSSION

Extracts obtained by cold extraction of *Vernoniaamygdalina* and *Ocimumgratissimum* using methanol, water and ethanol were investigated. *Staphylococcus aureus* showed appreciable susceptibility to the various extracts and the diameter of zone obtained from ethanolic leaf extracts for both plants were comparable to the zone of inhibition obtained from gentamicin, the standard antibiotic used. This is in agreement with Adebayo and Adegoke, (2008). Their study revealed that ethanol extracts of *V. amygdalina* and *Ocimumgratissimum* were very potent on *Staphylococcus aureus* where zones of inhibition obtained for *O. gratissimum*at 80mg/ml and 40mg/ml were 20mm and 10mm respectivelyand inhibition zone obtained for*V. amygdalina* was 10mm at 80mg/ml. Also, in this study *S. aureus* was susceptible to all crude extracts obtained from*O. gratissimum* seeds contrasting lesser activity obtained for water extract of *V. amygdalina.*

Escherichia coli was only susceptible to leaves of *O. gratissimum*, the zones of inhibition obtained forethanolic and methanolic extracts were comparable to that obtained for gentamycin the standard antibiotics. This organism was not susceptible to *V. amygdalina* compared to other findings (Akinjogunla, *et al*., 2011, Udochukwu, *et al*., 2015) however was sensitive to *Ocimumgratissimum* as reported by Kolawale (2018). A number of factors may be responsible for the resistance. Plants are weak antibacterial agents, and thus have bacteriostatic effect. This could be readily visible in the sense that the first eight to twelve hours of incubation of plates containing test organisms and plant extracts, the inhibition zone can be readily visible. After 24 hours however, the plates would virtually be flooded with inoculum used. This is probably why in traditional medicine, concoction (more than one plant) rather than decoction (only one plant) is usually used to combat diseases. Synergism has also been reported as the mode of action

of medicinal plants as different parts of the plant is normally used. However in this study one (leaf or seed) was used. From this study, the phytochemical components of *O. gratissimum* seeds and leaf was not the same. In traditional medicine the traditional practitioner usually use different parts of different plants to prepare herbal remedies used for treating diseases.

From this study, the gram positive isolate was susceptible to the crude extracts than the gram negative bacteria. The cell wall of gram positive is simple compared to gram negative cell wall (Bhatia and Ichhpujani, 2008). Seasons and ectotypecould be other factors bacteria are resistant to plant extracts as osbserved by Makinde*et al* using *Morindalucida* leaves against *Plasmodium bergheiberghei*in mice (Makinde and Salako, (1994).

The ectotype of plant play a major role in the activity of plant extract. This is manifested in the phytochemistry test results. Adebayo and Adegoke, (2008) reported similar results for *V.* a*mygdalina* and *Ocimumgratissimum* in their report both plants possessed saponins, tannins but lack alkaloids, anthraquinone, phlobatannin and cardiac glycosides. *Proteus vulgaris* was not sensitive to any of the extracts which concord to other reports (Menn,2012).

The MIC values obtained for the test organisms varied from one organismsto another. The ethanolic extracts of *O. gratissimum* had the lowest MIC for *Staphylococcus aureus* which was 12.5mg/ml. Similarly, MIC for*O. gratissimum* leaf was 12.5mg/mlfor *E. coli* and 50mg/ml for *Pseudomonas aeruginosa*. The MBC of organism varied from 25mg/ml to100mg/ml for the plants studied.

Vernoniaamygdalina possess alkaloids, flavonoids, tannins, terpenoid, saponins, cardiac glycosides, anthraquinones, Phlobatannins, reducing sugar and phenols in this study contrasting the previous reports (Adebayo and Adegoke, 2008 ; Akinjogunla*et al.*, 2011). While Akinjogunla*et al* (2011)*)* reported the presence of saponins, tannins, phlobatannin, cardiac glycoside, anthraquinone, flavonoids, terpenes, alkaloids for the two plants. However, in their study, phlobatanninswas absent in*V. amygdalina*.

The phytochemical test results obtained in this study showed the absence of cardiac glycoside and phlobatannin and thus consistent with other reports (Adebayo andAdegoke, 2008).

CONCLUSION

The antibacterial and phytochemical analysis of *O. gratissimum* seeds and leaves of *V. amygdalina* and *O. gratissimum*were investigated.*Proteus vulgaris* was resistant to all plant extracts. *Staphylococcusaureus* was the most susceptible organism to the crude extracts. The three crude extracts contained phytochemical compounds such as alkaloids, flavonoids, tannins, terpenoids, reducing sugar, phenol and saponin and others however,*Ocimumgratissmum*leaves possess anthraquinone while the seeds lacked it , thus showng variation of phytochemical compounds in same plant.

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