PHYTOCHEMICAL ANALYSIS OF *Vernonia amygdalina* AND *Ocimum gratissimum* EXTRACTS AND THEIR ANTIBACTERIAL ACTIVITY ON SOME DRUG RESISTANT BACTERIA

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ABSTRACT

The Phytochemical analysis and antibacterial activity of *Ocimum gratissimum* (scent leaf) and *Vernonia amygdalina* (bitter leaf) extracts was carried and it showed that the extracts had antibacterial effects on some drug resistant bacteria. The antibacterial properties of *O. gratissimum* and *V. amygdalina* was determined and tested against pure cultures of clinical isolates of *Stphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Water and ethanol were used for the plants' active constituents' extraction. The method used was Agar diffusion method to determine the antibacterial effects of both plant extract on the test organisms. Phytochemical analysis of *O. gratissimum* and *V. amygdalina* (bitter leaf) revealed that it contains bioactive compounds which include: oxalate, phylate, tannins, saponins, flavonoid, cyanogenic glycosides, alkaloids, anthraquinone, steroid and phenol. The minimum inhibitory concentrations (MIC) of water and ethanol extracts on the test organisms ranged between 25µl/ml – 50µl/ml. Similarly, the zone of inhibition of the plant extract diameters at concentration of 100µl/ml ranged between 7.5 – 5.0mm and 11.5 – 7.0mm for water and ethanol extracts respectively on the test organisms. Water extract of *V. amygdalina* was more effective on *Pseudomonas aeruginosa* than that of *O. gratissimum*, also ethanolic extract of *O. gratissimum* was more effective than ethanolic extract of *V. amygdalina* on the same bacteria. These extracts showed zones of inhibition higher than some

selected antibiotics (Amoxicillin, Tetracycline, Doxycycline, Ampiclox and Septrin) at 400µl/ml. This suggests the possibility of using the ethanol extracts of *O. gratissimum* in treating the diseases caused by the test organisms.

Keywords: Phytochemical analysis, Antibacterial activity, Vernonia amygdalina, Ocimum gratissimum, Drug resistant bacteria

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1. INTRODUCTION

Medicinal plants are known to contain substances which could be used for treatment purposes or used to produce drugs[1]. Many of such plants known to be used primitively to alleviate symptoms of illnesses have been screened to have medicinal importance, some of which include: *Azadirachta indica* (Dogonyaro), *V. amygdalina* (Bitter leaf), *Allium sativum* (Garlic), *O. gratissimum* (Scent leaf), and *Zingiber officinale* (Ginger). These plants have been reportedly used in the treatment of ailments such as stomach disorder, fever symptoms and cough traditionaliy[2]. Medicinal plants play vital roles in the health of individuals and the communities. The medicinal value of some plants lies in some chemical substances that produce definite physiological actions in the human body. Examples of these most important bioactive constituents are alkaloids, tannis, flavonoids and phenolic compound. The leaves of *Vernonia amygdalina* are green with a characteristic odour and bitter taste. *Vernonia amygdalina* is a valuable medicinal plant that is widespread in West Africa, it is known as bitter leaf due to its characteristic bitter taste and flavour, and can be used as an active anticancer, antibacterial, antimalarial and antiparastic agent. This plant contains complex active components that are useful pharmacologically. In ethno medicine, the roots and the leaves are used to treat fever, hiccups, kidney problems and stomach discomfort. Many West African countries like Cameroon, Ghana and Nigeria use the stem and root as chewing sticks[3]. It is also documented that *V. amygdalina* has been used traditionally in blood clothing

and has elicited a substantial reduction in the level of glucose in the blood at post-prandial time point. [4] Reported that *V. amygdalina* has hypoglycaemic activity. They observed a close-dependent reduction in fasting blood sugar level in alloxan-induced diabetic rats after treatment with different concentrations of the aqueous leaf extracts. [5]Also demonstrated *V. amygdalina* leaf extracts as a DNA-damaging of anticancer agent in the management of breast cancer. The wide use of antibiotics (drugs) in the treatment of bacterial infections has emergence and spread of resistant strains. The emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious disease [6]. Recently, [7]assessed the antibacterial activity of *V. amygdalina and O. gratissimum* leaves extract on selected food borne pathogens. The high zone of inhibition at low concentration proved the plants to be medically useful. [8]Tested the antibacterial activity of the extract of leaves of *O. gratissimum* on *Listeria monocytogens*. Their findings yielded great significance in health delivery system, since it could be used as an alternative treatment to orthodox antibiotics in the treatment of diseases caused by the bacterial isolates especially as they frequently develop resistance to known antibiotics and reduce the cost of obtaining health care as observed by [9]. [10] documented antibacterial activity of these plants on selected Gram positive and negative bacterial isolates. [11] showed that extracts of *Zingiber officinale, Myristica fragrans, Ocimum gratissimum*, thyme, sage, rosemary, yarrow and guava showed antibacterial activity against antibiotic resistant bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus* sp. and *Shigella* sp. [12] found ethanolic extracts and essential oil of *Zingiber officinale* and *Myristica fragrans* to be effective against the Enterobactericae. Also [13] tested the methanolic extract of *Z. officinale* to be effective against *Proteus* sp., *Bacillus* sp. *and Staphylococcus* sp. [14] found the ethanolic extract of *Z. officinale* and *M. fragrans* to be effective on *Bacillus* sp., *Pseudomonas* sp. and *Staphylococcus* sp. For these reasons, researchers are increasingly turning their attention to herbal products for new leads to develop much better drugs against MDR microbial strains [15].

2. MATERIALS AND METHODS

The bitter leaf and scent leaf were air-dried for two weeks and mashed into powder and sieved with mesh of size 0.50mm. The leaves were collected from a market at Uselu, Ugbowo, Benin City. The leaf samples were then stored in clean brown bottles at room temperature. The bacteria isolates used were *Staphylococcus aureus*, *Pseudomonas*

aeruginosa and *Escherichia coli*. Each of the leaves was dispensed 50g in 500ml of distilled water in a litre conical flask. The mixture was vigorously stirred intermittently with a magnetic stirrer and then allowed to stand for 48 hours. It was stirred the second time and filtered through a Whatman filter paper-lined funnel into a conical flask. The filtrate was evaporated at 40^{o}C with a water bath to obtain the solid crude extract. The same procedure was carried out for ethanol extraction except that the crude solid extract was obtained by concentrating the filtrate with a rotary evaporator. All extracts obtained were stored in a refrigerator until required for use. The extracts of both leaves were analysed for alkaloids, tannins, glycosides, steroids, flavonoids, saponins, volatile oil and resins using standard procedures. The method used for the test of the phytochemical components was according to [1], [16] and [17]. Antibacterial activity determination was done using the agar well diffusion technique. The tube dilution technique was used to determine the minimum inhibitory concentrations (MIC) of the extracts. Proximate analysis was carried out to determine the moisture content, ash content, crude fibre, fat content and protein content. The oven method was used to determine percentage moisture content. Kjeldahl method was used to determine the percentage protein content, Furnace method was used to determine the percentage of ash content, Cleg Anthrone method was used to determine the percentage carbohydrate content, Soxhlet Extraction method was used to determine the percentage lipid content and the percentage fibre content was determined by subtracting the summation of the percentage composition of moisture, protein, lipid, carbohydrate, and ash contents from 100.

The antibiotic sensitivity test was carried out using already prepared antibiotic disc standard, the diameter was taken (5mm) with this, filter paper disc of about 5mm in diameter were prepared from bund filter paper using a model 330 paper punch and the disc placed inside a glass container were sterilized using the autoclave at 121^{0}c for 30 minutes. 400µg of antibiotic each were diluted with 1ml of sterile water into each McCartney bottle; 1ml of the antibiotic was pipetted and mixed overnight. Nutrient agar was poured into clean Petri-dishes and allowed to set. Duplicate agar plates were then inoculated with 0.1ml broth culture of each test organism (less than 24hour) using aseptic spreading method. The agar was then inoculated with the filter paper after absorbing the antibiotics; this was done using a flamed but cooled forceps and pressed down firmly. The Petri dishes were incubated at 37^{0}C for 24 hours after which the zones of inhibition were observed, measured and recorded Antibiotics used were Doxycycline, Amoxicillin, Ampiclox, Tetracycline and Septrin.

3. RESULTS

This study showed that the phytochemical components of *V. amygdalina* and *O. gratissimum* includes; oxalate, phytate, tannins, saponins, flavonoids, cyanogenic glycosides, alkaloids, anthraquinone, steroid and phenol. In terms of concentration in (mg/100g), *V. amygdalina* contained higher levels of bioactive compounds than *O. gratissimum* save for phytate and cyanogenic glycosides (Table 1). Antibacterial sensitivity tests revealed that the ethanolic extracts of *V. amygdalina* and *O. gratissimum* had higher inhibitory zones than that of the water extracts (Table 2). This observed difference between these plant extracts could be as a result of insolubility of the active compounds in water [18]. Both extracts (ethanol and water) inhibited the growth of test organisms (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus).* Ethanolic *V. amygdalina* extract had a greater zone of inhibition of (11.5mm) on *Pseudomonas aeruginosa;* this may be due to ability of ethanol to extract bioactive compounds like tannis, saponnins, flavonoid, alkaloid, anthraquinone, phenol and steroid which have higher concentrations in *V. amygdalina.* Water extracts of *O. gratissimum* had the same zone of inhibition as ethanol extract on *Escherichia coli* (7.5mm) and *Staphylococcus aureus* (10mm); this may also be due to compounds like phytate and cyanogenic glycosides which are completely soluble in water and slightly soluble or insoluble in ethanol. *O. gratissimum* contains higher concentration of phytate and cyanogenic glycosides (5.56mg/100g and 2.38 mg/100g) respectively as compared to (3.95mg/100mg and 1.11mg/100g) in *V.* amygdalina. The minimum inhibitory concentrations of *V. amygdalina* and *O. gratissimum* were ranged between 25µl/ml – 50µl/ml. MIC for *V. amygdalina* varied between 25-50µl/ml (ethanol) and 50µl/ml (water), while *O. gratissimum* had its MIC as 50µl/ml (ethanol) and 25µl/ml (water). When compared to standard antibiotics such as Amoxicillin, Tetracycline, Doxycycline, Ampiclox and Septrin at (400µl/ml), only Doxycycline had an appreciable zone of inhibition of the test organisms (*E. coli* – 11mm, *S aureus* – 9mm and *P. aeruginosa* – 10mm). Other antibiotics used had zones of inhibition ranging between 1mm – 4mm. Proximate analysis of *V. amygdalina* and *O. gratissimum* had moisture content of (86.2% and 82.6%), protein (12.5% and 8.5%), fat (11.3% and 9.5%), carbohydrate(28.5% and 64.9%), ash (15.2% and 13.6%) and fibre (11.8% and9.5%) respectively.

Figure 1: Bitter Leaf (*Vernonia amygdalina*).

Figure 2: Scent Leaf (*Ocimum gratissimum*).

Table 1: Phytochemical Components of Ethanolic Extracts of *V. amygdalina* and *O. gratissimum* (mg/100g)

Phytochemical	*V. amygdalina*	*O. gratissimum*
Oxalate	3.48	0.75
Phytate	3.95	5.56
Tannins	9.62	2.48
Saponins	5.97	3.52
Flavonoid	4.89	1.74
Cyanogenic glycoside	1.11	2.38
Alkaloids	2.16	1.07
Anthraquinone	0.14	0.31
Steroid	0.38	0.30
Phenol	3.24	0.73

Table 2: The Antibacterial Activities of Ethanol and Water Extracts of *V. amygdalina* and *O. gratissimum* at 100µl/ml

	Zone of Inhibition (mm)			
Organisms	*V. amygdalina*		*O. gratissimum*	
	Ethanol	Water	Ethanol	Water
Escherichia coli	7.0	7.0	7.5	7.5
Pseudomonas aeruginosa	11.5	5.0	9.0	7.5
Staphylococcus aureus	7.5	6.0	10	10

Table 3: The Minimum Inhibitory Concentration of *V. amygdalina* and *O. gratissimum*

Concentration (µl/ml)				
Organisms	*V. amygdalina*		*O. gratissimum*	
	Ethanol	Water	Ethanol	Water
Escherichia coli	50	25	50	25
Pseudomonas aeruginosa	25	50	50	25
Staphylococcus aureus	50	25	50	25

Table 4: Proximate Analysis of *V. amygdalina* and *O. gratissimum* Leave Extract (%/100g)

Components	*V. amygdalina*	*O. gratissimum*
Moisture	86.2	82.6
Protein	12.5	8.5
Fat	11.3	9.5
Carbohydrate	28.5	64.9
Fibre	11.8	9.5
Ash	15.2	13.6

Table 5: Antibiotic Sensitivity on Test organisms (mm)

Organisms	DOX	AMP	AMO	TET	SEP
E.coli	11	2	2	4	2
S.aureus	9	1	1	3	1
P.aeruginosa	10	2	1	3	1

Key: Dox = doxycycline, AMP = Ampiclox, AMO = Amoxicillin, TET = Tetracycline, SEP = Septrin.

4. DISCUSION

The findings of this study agrees with that of several researchers that demonstrated that *V. amygdalina* and *O. gratissimum* extracts have an antibacterial activity against several species of bacteria and even fungi. Therefore, the efficacy of these extracts as reported by [18] may be due to the age of the plant, solvent extraction, extraction method and the period of harvest of plant materials. The statistical analysis (t-test) shows that there is significant difference in the ethanolic extracts of the two leaves. From this study, it was observed that bioactive components are abundant in these leaves and that the ethanol extracts exhibited higher inhibitory activity on the test organisms. This can be deduced to the ability of ethanol to extract more of the essential oil and secondary plant metabolites which are believed to exert antibacterial activity on test organisms. Traditionally, water extracts of *O. gratissimum* can

be used orally as it is also as effective as the ethanol extract especially for people who do not take alcohol.

5. CONCLUSION

This study however can justify the use of the leaf in traditional medicine practice as a therapeutic agent and can explain the traditional use of these plants. In cases where possible, the ethanol extracts of these plants should be used at a concentration up to 100µl /ml so as to give a better treatment margin.

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