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Antibacterial Effect of *Vernonia Amygdalina* and *Ocimum Gratissimum* on Some Bacteria that Cause Opportunistic Infections in HIV/AIDS Patients

Kolawole Sunday[1*], Obueh Henrietta[2] and Ukachi Augusta[3]

[1, 3] National Open University of Nigeria, Benin City Study Centre, Benin City Nigeria

[2] Biology Department, College of Education, P.M.B 1144, Ekiadolor, Benin City

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***Corresponding author:** Kolawole Sunday, National Open University of Nigeria, Benin City Study Centre, Benin City Nigeria, E-mail: kolasunde@gmail.com*

Abstract

Antibacterial effect of *Vernonia amygdalina* (Bitter leaf) and *Ocimum gratissimum* (Scent leaf) on *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* that cause opportunistic infections in HIV/AIDS patients was studied. The extract of *Ocimum gratissimum* had higher antibacterial effect, though not significant (*P>0.05*), on the bacterial isolates than the extract of *Vernonia amygdalina.* The *Ocimum gratissimum* extract had higher quantity of phytonutrients (alkaloid, steroid, tannin and flavonoid) than the extract of *Vernonia amygdalina.* Susceptibility of the bacterial isolates was in the order *Escherichia coli > Pseudomonas aeruginosa > Staphylococcus aureus* to the *Ocimum gratissimum* and *Vernonia amygdalina* extracts at concentrations 30 mg/ml to 150 mg/ml. At concentration of 30 mg/ml, the *Vernonia amygdalina* extract was not inhibitory to *S. aureus* and *P. aeruginosa*. However, the extract of *Ocimum gratissimum* inhibited all the test organisms with higher diameter of the zones of inhibition. The use of *Ocimum gratissimum* extract could help reduce the effect and complications of opportunistic infections caused by *E. coli, S. aureus* and *P. aeruginosa* in HIV/AIDS patients.

Keywords: Antibacterial, V*ernonia amygdalina, Ocimum gratissimum*, opportunistic infections, HIV/AIDS patients.

Introduction

The Human Immunodeficiency Virus (HIV) results in life – threatening opportunistic infections and malignancies [14]. It destroys the body's ability to fight against infections, and reduces the functional impairment and damages the immune system. Opportunistic infections are caused by pathogen such as bacteria, viruses, fungi and protozoans. The CD4 lymphocyte count plays a major role in HIV/AIDS patients [22]. Most opportunistic infections occur when the CD4 lymphocyte count is below 200cells/mm3 and this CD4 cell decrease has been traced to the activity of oxidative cells, thereby affecting the immune system and patients' recovery rate on antiretroviral therapy [23]. Some of the opportunistic infections include tuberculosis, pneumonia, diarrrhoea, malaria, candidiasis, cancers, cardiovascular disease, osteoporosis, lung or kidney disease (WHO, 2013). Hence, the exogenous supply of antioxidants, as natural compounds that scavenge free radicals might represent an important additional strategy for the treatment of HIV infection [30].

Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic infections in people living with HIV/AIDS [4]. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [15]. Plants contain a wide variety of free radical scavenging molecules such as flavonoids, anthocyanins, cartenoids, dietary glutathionine, alkaloids, tannins, saponins, steroids, terpenoids and rotenoids which are rich in antioxidants activities [31]. Many of these plant- derived substances show anti-HIV activity e.g. alkaloids, flavonoids, coumarins, tannins, catechin, steroids and terpenoids [23]. *Vernonia amygdalina* (bitter leaf) is a small shrub that grows predominantly in tropical Africa. It is called bitter leaf because of its bitter taste. The bitterness is as a result of the presence of the sesquiterpenes lactones and steroid glycosides [13]. The leaf also contains some phytochemical compounds such as alkaloid, flavonoids, glycosides, saponin, tannin, steroids, proteins and carbohydrates in high concentration [38]. The aqueous extraction of bitter leaf could be used as adjuvant in the management of people living with HIV/AIDS as well as boosting immunity and a panacea for skin blemishes [7]. *Ocimum gratissimum* (scent leaf) is commonly used in cooking due to its minty and aromatic flavor. It is rich in alkaloids, tannins, flavonoids, phytates, resins, glucosides oligosaccharides and essential oils such as eligenol and thymol, which are responsible for its antibacterial, anti- diabetic and anti-malaria properties [27]. Study by Ayisi and Nyadedzor (2003) showed that extracts of scent leaf had inhibited HIV-I strain HTLVIII (B) cytopathicity and had high antiviral indices. Scent leaf has been described to be active against several species of bacteria and fungi and has proved useful in the medication for people living with HIV/AIDS [26, 11]. Therefore, this study is to investigate the antibacterial effect of the extracts of *Vernonia*

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amygdalina and *Ocimum gratissimum* on clinical isolates of *E. coli, S. aureus and P. aeruginosa.*

Materials and Methods

Sample Collection and Preparation of Extracts

Fresh leaves of *Vernonia amygdalina* (bitter leaf) and *Ocimum gratissimum* (scent leaf) were collected from Koko village (Latitude 6°0 4 N and Longitude 5°28 3 E) in Warri North Local Government Area of Delta State, Nigeria. The plants were identified and authenticated. The fresh leaves were washed with distilled water, sun dried for fifteen days and then milled into powder using an electrical grinder. Thirty grams (30 g) of the ground bitter leaf were placed in 100 ml of deionised water in a 500 ml beaker and stirred vigorously. This was then filtered using Whatmann No1 filter paper. The filtrate was evaporated to dryness using a rotatory evaporator [2]. The same procedure was carried out for the scent leaf. The extracts were scored at 4°C, until analyses were done.

Preparation of Test Organisms

The pure clinical isolates of the experimental bacterial strains of *E. coli, S. aureus and P. aeruginosa* were obtained from the department of medical microbiology and parasitology, Central Hospital, Benin City. They were isolated and purified on Nutrient Agar slants and identified using their cultural, morphological and biochemical characteristics. The agar slants were preserved in the incubator at 37°C.

Phytochemical Analyses

Phytochemical analyses were conducted on the plant extracts to determine the presence of alkaloid, glycosides, flavonoids, tannins, terpenoid, steroids, and reducing sugar. Glycoside content was determined by the method of tannin content was determined by the spectrophotometric method of alkaloid content was determined by the method of reducing sugar content was determined by the method of flavonoid content was determined by the method of terpenoid and steroid were determined by the method of [16,3,29,19,33,18].

Determination of Antibacterial Activity

Serial Dilution of Extracts

Preparations of fivefold serial dilution from the extracts were carried out. One millimeter (1 ml) of each prepared extract was added to 9 ml of sterile distilled water in a test tube to make a 10^{-1} dilution (150 mg/ml concentration). This was then serially diluted to get 10^{-2} (120 mg/ml), 10^{-3} (90 mg/ml), 10^{-4} (60 mg/ml) and 10^{-5} (30 mg/ml) concentrations.

Preparation of Disks

Filter papers were perforated into 6mm sizes. They were sterilized in an autoclaved at 121°C for 15 min. A known volume of 0.1 ml of each leaf extract was placed on different disks from the 30 mg/ml to 150 mg/ml concentrations of the serial dilution. They were allowed to dry in an incubator for 37°C.

Microbiological Assay

Pure isolates of *E. coli, S. aureus and P. aeruginosa* were streaked into different prepared nutrient agar plates in triplicates and labeled. Some of the plates were incubated at 37°C for 24 h to determine microbial count. The disks containing the extracts of *Vernonia amygdalina* and *Ocimum gratissimum* were placed aseptically into the Nutrient Agar plates containing the pure isolates. They were incubated at 37°C for 24 h until marked decline in the potency of the extracts to inhibit the growth of the test organisms were noticed [26]. Zones of inhibition and the diameter of such zones were measured in millimeter. The Minimum Inhibitory Concentration (MIC) was taken as the lowest concentration of the extracts that prevented growth of the isolates after comparing the different concentration of the extracts from the zones of inhibition [1].

Statistical Analysis

Data obtained were analyzed using a two way analysis of variance (ANOVA) and values for $P < 0.05$ were considered statistically significant.

Results

The result of the phytochemical analysis is presented in table 1. The presence of all the phytochemical content tested was observed in the extracts of *Vernonia amygdalina* and *Ocimum gratissimum* which is the basis of the therapeutic potentials of the extracts. The extract of *Ocimum gratissimum* had higher presence of alkaloid, steroid, flavoid and tannin than the extract of *Vernonia amygdalina* by colour indication. The bacterial count (cfu/ml) of the isolates in the Nutrient Agar containing the extracts is presented in table 2. The bacterial counts of the isolates ranged from 0.8 ± 0.01 to 1.5 ± 0.08 cfu/ml, 1.3 ± 0.05 to 1.9 ± 0.01 cfu/ml and 1.2 ± 0.05 to 1.6 ± 0.04 cfu/ml in the extract of *Vernonia amygdalina* and the bacterial counts of the isolates ranged from 0.6 ± 0.03 to 1.2 ± 0.01 cfu/ml, 1.0 ± 0.04 to 1.7 ± 1.30 cfu/ml and 0.8 ± 0.01 to 1.5 ± 1.00 cfu/ml in the extract of *Ocimum gratissimum.* The aqueous extracts had higher antibacterial activity against the gram negative *E.coli and P. aeruginosa* than the gram positive *S. aureus.*

S. aureus was the least susceptible organism to the leaf extracts while *E. coli* was the most susceptible organism. The bacterial isolates were more susceptible to the extract of *Ocimum gratissimum* than the extract of *Vernonia amygdalina.* The result of the antibacterial activity of the extracts against the test organisms is show in table 3.

The mean zone of inhibition of the leaf extracts determines the antibacterial activity of the extracts. The highest antibacterial activity was shown by the leaf extracts at 150 mg/ml concentration against all the test organisms. The scent leaf extract exhibited higher antibacterial activity on the test organisms. The zone of inhibition in mm decreased as the dilution factor increased. The MIC was 30 mg/ml for the scent leaf (*Ocimum gratissimum*) extract and 60 mg/ml the bitter leaf (*Vernonia amygdalina*)

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extract. The bitter leaf extract did not show antibacterial activity against *S. aureus and P. aeruginosa* at 30 mg/ml concentration in table 4.

Table 1: Quantitative phytochemical analysis of the extracts of *Vernonia amygdalina* and *Ocimum gratissimum*

Extract	Alkaloid	Glycosides	Terpenoid	Steroid	Flavonoid	Tannin	Reducing sugar
Scent leaf	++	+	+	++	++	++	+
Bitter leaf	+	++	+	+	+	+	++
Colour indication	yellow	red	reddish violet	bluish green	red	deep blue	brick red

Key: ++ = Highly Present
+ = Moderately present

Table 2: Bacterial counts (cfu/ml) of isolates in extracts of *Vernonia amygdalina* and *Ocimum gratissimum*

	Bacterial Count (cfu/ml) x 102					
	E. coli		*S. aureus*		*P. aeruginosa*	
Concentration of extract (mg/ml)	**Bitter leaf extract**	**Scent leaf extract**	**Bitter leaf extract**	**Scent leaf extract**	**Bitter leaf extract**	**Scent leaf extract**
150	0.8 ± 0.01	0.6± 0.03	1.3± 0.05	1.0± 0.04	1.2± 0.05	0.8± 0.01
120	1.0± 0.32	0.8± 0.01	1.8± 0.09	1.3± 0.08	1.5± 0.50	0.9± 0.02
90	1.0± 0.50	1.0± 0.30	1.7± 0.35	1.3± 0.04	1.4± 0.07	1.0± 0.05
60	1.1± 0.10	1.1± 0.06	1.7± 1.07	1.5± 0.08	1.6± 0.04	1.3± 0.08
30	1.5± 0.08	1.2± 0.01	1.9± 0.01	1.7± 1.30	1.6± 0.08	1.5±1.00

Table 3: Antibacterial activity of the extracts of *Vernonia amygdalina* and *Ocimum gratissimum* against the test organisms

	Mean zones of inhibition (mm)					
	E. coli		*S. aureus*		*P. aeruginosa*	
Concentration of extract (mg/ml)	**Bitter leaf extract**	**Scent leaf extract**	**Bitter leaf extract**	**Scent leaf extract**	**Bitter leaf extract**	**Scent leaf extract**
150	7.0 ± 0.03	14.0± 0.10	3.0± 0.05	9.0± 0.05	4.0± 0.08	10.0± 0.50
120	6.0± 0.10	12.0± 0.05	3.0± 0.01	7.0± 0.10	7.0± 0.17	9.0± 0.10
90	4.0± 0.05	10.0± 0.08	2.0± 0.05	5.0± 0.07	3.0± 0.03	8.0± 0.05
60	2.0± 0.01	7.0± 0.10	2.0± 0.0	5.0± 0.01	3.0± 0.01	6.0± 0.08
30	1.0± 0.01	5.0± 0.01	-	2.0± 0.05	-	4.0±0.01

Note: - = No zone of inhibition

Table 4: Minimum inhibitory concentration (MIC) of the extracts of *Vernonia amygdalina* and *Ocimum gratissimum*

	MIC (mg/ml)	
Test organisms	Bitter leaf extract	Scent leaf extract
E. coli	30	30
S. aureus	60	30
P. aeruginosa	60	30

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Discussion

The higher presence of steroids, flavonoids, alkaloids and tannins in *Ocimum gratissimum* (scent leaf) than in *Vernonia amygdalina* (bitter leaf) could have attributed to its higher antibacterial activity against the test organisms. This agreed with report of [25]. The presence of these antibacterial components may have invariably led to the degree of antibacterial activity on the test organisms. An important characteristic of the plant extracts and their components is their hydrophobicity, which enabled them to partition the lipids of the bacterial cell membranes and mitochondria, disturbing the cell structures and rendering them more permeable [36]. The inhibition produced by the plant extracts against particular organism depends upon various extrinsic and intrinsic parameters [17]. S. aureus was the least susceptible organism while E. coli was the most susceptible organism with the action of the leaf extracts. This is in disagreement with the report of where ethanol extracts of *O. gratissimum* showed more antibacterial activity against *S. aureus* than *E. coli* [26]. The result of this study is in agreement with the study of that showed that *E. coli* was more sensitive to the deleterious effect of the extract of *O. gratissimum* than *S. aureus* due to presence of different phytochemicals contained in the plant [2]. Flavonoids which are also known as vitamin p or plan modifier elicits a wide range of therapeutic activities as antihypertensive, antirheumatic as well as antimicrobial [39]. Plants containing flavonoids have diuretic and antioxidant properties [12]. Glycosides have been used for over two centuries as stimulant in cases of cardiac failure and disease [37]. Alkaloids have many pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial activity and anticancer actions [34]. Some alkaloids are useful against HIV infections as well as intestinal infection associated with AIDS [35]. Tannins play an important role in promoting wound healing [28]. They are a major group of compounds that act as primary antioxidants or free radical scavengers [32]. Tannin containing plant extracts are used as astringents against diarrhoea, as diuretics, against stomach and duodenal tumors and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals [8, 9]. Terpenoids have medicinal properties such as anticarcinogenic, antimalarial, antiulcer, hepaticidal, antimicrobial or diuretic activity and the sesquiterpenoid antimalarial drug artemisinin and the diterpenoid anticancer drug taxol [10]. Steroids are important compounds due to their relationship with such compounds as sex hormones [6].

The bacterial counts in cfu/ml reduced with increased concentration of the leaf extracts for all the test organisms with *E. coli* having the lowest bacterial counts. This could be attributed to the effect of the leaf extracts on the carbon metabolism, respiratory activities and inhibition of dehydrogenase activities of the bacterial strains [24, 2]. The diameter zones of inhibition reported in the study is higher compared to that reported in the study of [26]. The aqueous extract of *O. gratissimum* exhibited higher inhibitory effects at all concentrations than the aqueous extract of *V. amygdalina* due to the fact that *O. gratissimum* oils have properties that can inhibit the growth of psychrophiles and heat resistant organisms [20]. It is suggested that there is need for the use of this plant and its derivative for the primary purpose of flavouring foods and exhibiting antimicrobial activities. Also, the result established a good support to the use of this plant in herbal medicine and as base for the development of new drugs in phyto-medicine especially in the prevention and treatment of opportunistic infections in HIV/AIDS patients [20, 4].

Conclusion

The plant extracts, especially *Ocimum gratissimum*, are sources of important chemical compounds that could inhibit the growth of the test organisms that cause some opportunistic infections in people living with HIV/AIDS. It is therefore necessary to isolate these active compounds and possibly develop then into drugs to manage HIV/AIDS.

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