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ASSESSING THE PHYTOCHEMICAL CONTENTS AND ANTIMICROBIAL ACTIVITY OF BITTER LEAF (VERNONIA AMYGDALINA) ON MICRO-ORGANISMS

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Abstract
Medicinal plants have assumed the basis of traditional medicine and have proven effective in human health care across the world. There is a growing interest in the role of complementary and alternative medicines to treat various illnesses. The continued consumption of antibiotic with little or no impact on bacteria have negatively affected health care delivery. Bitter leaf (Vernonia amygdalina) is a popular shrub belonging to the family Asteraceae and a perennial shrub commonly cultivated as a homestead vegetable and fodder tree in various parts of Nigeria. The current study was conducted to assess the phytochemical constituents and antimicrobial activity of bitter leaf on Candida albicans, Pseudomonas aeruginosa and Staphylococcus aureus. The result of the phytochemical analysis conducted on the root and stem bark of V. amygdalina revealed the presence of alkaloids, tannin, steroids, flavonoids, phenol, and saponins. However, it was found that tannins and phenol were mainly deposited in the plant's stem bark. Also, the antimicrobial analysis conducted revealed that S. aureus was more vulnerable to the ethanol, acetone, and hot aqueous extracts of V. amygdalina. while, P. aeruginosa, and C. Albicans showed sensitivity to ethanol and acetone exposure but failed to react to the hot aqueous extracts. The study concludes that the observed biological reactions shown by the aqueous extracts of V. amygdalina corroborate the traditional application of this plant as an alternative antibiotic.

Keywords: Bitter leaf, Phytochemical, Ethanol, Acetone, Antimicrobial

Introduction
Over the years, medicinal plants have played a substantial role in Nigeria's health sector.
Medicinal plants have assumed the basis of traditional medicine and have proven effective in human health care across the world (Asif, 2013; Hamayun et al., 2006; Johnson et al., 2015; Kumar et al., 2013; Tripathi & Pandey, 2017). There is a growing interest in the role of complementary and alternative medicines to treat various illnesses (Albejo et al., 2015; S. Ali et al., 2020; Baars et al., 2019; James et al., 2018; Mordeniz, 2019). Plant's secondary metabolites and phytochemicals are the essential elements in the curative success of plants. However, the mechanisms underlying most medicinal plant-related cure is still unclear. The success of complementary and alternative medicines in treating infectious diseases indicates the effectiveness of plants in treating different types of fungal, bacterial, and other infections (el Hajj & Holst, 2020; Kamatenesi-Mugisha et al., 2008; Kayanja, 2008; Martin & Ernst, 2003; Wang et al., 2014). Complementary and alternative medicine is increasingly employed to curb infectious disease in Nigeria. The trend has continued to attract wide patronage following most conventional antibiotics' ineffectiveness due to the antimicrobial resistance by bacteria.

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The growing occurrence of antimicrobial drug resistance by most bacteria has triggered a public health concern (Bennani et al., 2020; Buchy et al., 2020; Christaki et al., 2020; Dadgostar, 2019; Didier et al., 2019; Lowy, 2003; Taneja & Sharma, 2019; Vidovic & Vidovic, 2020; WHO, 2014). The continued consumption of antibiotics with little or no impact on bacteria has negatively affected health care delivery worldwide. Perhaps, extensive research has been dedicated to searching for an alternative remedy to bacterial infections. (Alsheikh et al., 2020; Chandra et al., 2017; Esmael et al., 2020; Nawab et al., 2020; Santos et al., 2019; Septama et al., 2020; Shin et al., 2018; Singh et al., 2020; Srivastava et al., 2014; Yang et al., 2018). Mostly, the research in alternative to the chemically powered antibiotics has focused on plant bioactive components. Phytochemical contents of many plants such as alkaloids, essential oils, resins, lactose, and saponins have proved relevant in curbing bacterial activities (Igbinaduwa et al., 2012; Mujeeb et al., 2014; Muthu et al., 2006; Steenkamp et al., 2004; Umaru et al., 2019). Thus, this study is aimed to ascertain the aqueous constituents and the antimicrobial properties of a bitter leaf on micro-organisms.

Bitter leaf (*Vernonia amygdalina*) is a popular shrub belonging to the family Asteraceae (Okwuzu et al., 2017). *V. amygdalina* is a perennial shrub commonly cultivated as a homestead vegetable and fodder tree (Ndaeyo, 2007). It is widely found in various parts of Nigeria. Bitter leaf is one of the leafy vegetables crucial in curbing micronutrient malnutrition (Tonukari et al., 2015). The plant is an essential protective vegetable and a popular ingredient used in making soup (Onabanjo & Oguntona, 2003; Shokunbi et al., 2011; Uchechukwu Anastasia, 2011). The leaves are widely considered appetizers and used to aid digestion (Oyeyemi et al., 2018). It is beneficial for human health care and the treatment of various diseases. Bitter leaf is widely recognized due to the vast bioactive compounds obtained in various plant parts (Oyeyemi et al., 2018).

Research on the pharmacological potentials of *V. amygdalina* indicates that the plant possesses immunomodulatory activities (Setiawan et al., 2018), antimicrobial properties (Ijeh & Ejike, 2011; Salawu et al., 2011), antibacterial activity (Habtamu & Melaku, 2018), insecticidal properties (Green et al., 2017; Ileke Kayode & Olabimi Isaac, 2019), Antidiabetic (Owen et al., 2011), Anthelmintic (Nalule et al., 2011), Anticancer (Izevbigie et al., 2008; Yedjou et al., 2015), Antihelmitic and Antimalarial properties (Oboh, 2006), anxiolytic, sedative and hypothermic effects (Oloruntobi et al., 2014). Although *V. amygdalina* has been widely studied and its bioactive components and antimicrobial effects are well documented. This study aimed to further evaluate existing evidence from the literature on the antimicrobial effects of V. amygdalina on bacteria. Thus, the current study's primary purpose is to determine the phytochemical contents and the antimicrobial activity of *V. amygdalina* on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.

Materials and Methods

Collection and Preparation of Plant Materials

Fresh samples of *V. amygdalina* plant was collected from the open market in Umunze, Anambra State, Nigeria, and were taken to the lab for proper identification and authentication. The samples were washed and prepared according to the method described in (Alara et al., 2019).

Sample Extraction

Aqueous Extract

Ten grams of the ground sample of the plant stem bark and leaf were extracted was added to 100ml of sterile distilled. The extraction of the plant's aqueous components was done following the method adopted in (Abdulmalik et al., 2016; Ali et al., 2017).

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Phytochemical Screening of *V. amygdalina*

Phytochemical screening of the *V. amygdalina* stem bark and root extracts was conducted to ascertain bioactive components such as alkaloids, tannins, saponins, steroids, phenols, and flavonoids using the standard qualitative method as previously described by Trease and Evans (1989).

Sterility Test of the Plant Extracts

The plant's extracted constituents were tested for sterility following sterilization by inoculating 1 mL of each extract on sterile nutrient agar incubated at 37°C for 24 hours. Perhaps, the plates were carefully observed for growth.

Standardization of the Bacterial Cell Suspension

McFarland standard was adopted to test for the standardization of the bacterial suspension. Colonies of the tested organism were picked into a sterile test-tube containing sterile nutrient broth and incubated for one day.

Determination of Antimicrobial Activities

The crude extracts' antimicrobial activity was conducted using the agar well diffusion method described by (Gashe & Zeleke, 2017) with few modifications.

Table 1:

Table showing the phytochemical contents of the root and stem bark of *V. amygdalina*

Phytochemical compounds	Root	Stem bark
Alkaloids	+ +	+ +
Saponins	+ +	+ + +
Phenolic	-	+ + +
Steroid	+ + +	+
Tannin	-	+ + +
Flavonoids	+ +	+

Key: + = Positive, + + = Moderate + + + = High, - = Negative

The above table shows the outcome of the aqueous screening conducted on the root and stem bark of V. *amygdalina* indicates that the plant contains a significant number of alkaloids, tannin, steroids, flavonoids, phenol, and saponins. However, the screening revealed tannins and phenols in the stem bark and not the plant's root.

Table 2:

Table showing the sensitivity parameter of the organisms to ethanol extract.

Organisms	Stem bark	%	Root	%
C. albicans	8mm	36.89	2mm	12.45
P.aeruginosa	5mm	18.21	1mm	6.78
S. aureus	8mm	37.10	7mm	36.26

Table showing the observed zone of inhibition of ethanol extract on *V. amygdalina* stem bark and root against some pathogenic organisms. Exposing the organisms to the aqueous extract of stem bark for sensitivity revealed the same 8mm diameter of inhibition zone (36.89% and 37.10%), respectively, for *C.*

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Albicans and *S. aureus.* However, *P. aeruginosa* possessed a minimal 5mm (6.78%) diameter inhibition zone compared to *C. Albicans* and *S. aureus*. The test on an ethanol extract of the root shows that *S. aureus* produced a high diameter of inhibition zone of 7mm (36.26%) while *C. albican*s produced lower with 3mm (12.45%) diameter of inhibition zone.

Table 3:
Table showing the sensitivity parameter of organism to acetone extract of the plant.

Organisms	Stem bark	%	Root	%
C. albicans	8mm	21.38	7mm	41.19
P.aeruginosa	15mm	31.36	3mm	8.82
S. aureus	15mm	36.10	5mm	26.16

The table above shows the test organisms' sensitivity parameter when exposed to an acetone extract of *V. amygdalina* stem bark and root. The result indicated a high diameter of inhibition zones of 15mm for *P. aeruginosa* and *S. aureus* (31.36% and 36.10%), respectively, when tested with acetone stem bark extract of *V. amygdalina*. However, *C. Albican*s produced a reduced diameter of inhibition zones of 8mm (21.38%). Furthermore, it was revealed that *C. Albican*s produced an increased diameter of inhibition zones of 7mm (41.19%) when subjected to acetone root extract of *V. amygdalina*.

Table 4:
Table showing the sensitivity position of the test organisms to hot aqueous extract

Organisms	Stem bark	%	Root	%
C. albicans	r	-	r	-
P.aeruginosa	r	-	r	-
S. aureus	8mm	96.10	r	-

The organisms were exposed to hot aqueous extract of the plant. Only one pathogenic organism (*S. aureus*) exhibited a reaction with the 8mm diameter of inhibition zone. Thus, other organisms maintained a resistant position towards the extract.

Discussion

The current study was conducted to assess the phytochemical constituents and antimicrobial activity of bitter leaf on *Candida albicans, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. The result of the phytochemical analysis conducted on the root and stem bark of *V. amygdalina* revealed the presence of alkaloids, tannin, steroids, flavonoids, phenol, and saponins. However, it was found that tannins and phenol were mainly deposited in the plant's stem bark. Thus, the study is consistent with previous studies (Alara et al., 2019; Asfere et al., 2018; Oyeyemi et al., 2018). The plant's bioactive content has been implicated in the antimicrobial potentials of *V. amygdalina* (Jin et al., 2017; Nenaah, 2013).

Furthermore, the antimicrobial analysis conducted revealed that *S. aureus* was more vulnerable to the ethanol, acetone, and hot aqueous extracts of *V. amygdalina*. This is indicated in the increased size of the inhibition zones' diameter, as shown in the tables. However, *P. aeruginosa,* and *C. Albicans* showed sensitivity to ethanol and acetone exposure. However, they failed to react to the hot aqueous extracts. Consistent with (Adetutu et al., 2011; Moreno et al., 2006), the findings affirmed ethanol and acetone extracts' antimicrobial potentials compared to aqueous extracts.

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Conclusion

The present study assessed the antimicrobial potentials of *V. amygdalina* extracts on *Candida albicans, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. The result confirmed that the stem bark and root extracts of the plant possess antimicrobial tendencies. Thus, the observed biological reactions shown by the aqueous extracts of *V. amygdalina* corroborate the traditional application of this plant as an alternative antibiotic.

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