PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *Vernonia amygdalina* AGAINST SOME CLINICAL ISOLATES

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ABSTRACT

This study evaluates the phytochemical profile and antimicrobial activity of *V. amygdalina* against some clinical isolates, with a view of determining its potential therapeutic application and justifying its use in folklore medicine. Phytochemical screening, biochemical and agar diffusion standard procedures were employed. Four phytochemicals namely: Alkaloids, flavonoids, saponins and tannins were found in *V. amygdalina* leaf extract with absence of cardiac glycosides. *V. amygdalina* ethanol leaf extract was found promising against *Pseudomonas aeruginosa* and *Staphylococcus aureus* at concentrations of 25, 50 and 100 mg/ml. No activity was exhibited against *Candida albicans* at all the tested concentrations. Furthermore, the extract was also found promising against both *S. aureus* and *P. aeruginosa* with a similar MIC of 50mg/ml and an MBC of 25mg/ml. These findings suggest the potential of *V. amygdalina* leaf extract to be used in the management of infection due to *P. aeruginosa* and *S. aureus*.

Key words: Antimicrobial activity, clinical isolates, leaf extract, phytochemical profile, *V. amygdalina*.

INTRODUCTION

Vernonia amygdalina, commonly known as bitter leaf, is an important medicinal plant that belongs to the family *Compositae* (Olusola-Makinde *et al*., 2021; Abayomi, 1993). It is the largest family in flowering plants comprising 950 genera, and about 23000 species. The family is of cosmopolitan distribution covering almost all habitats (Adesanoye & Farombi, 2010). *V. amygdalina* occurs widely in most countries of tropical Africa, from Guinea east to Somalia and South to North eastern south Africa and Yemen (Adesanoye & Farombi, 2010). It commonly grows as a vegetable in Benin, Nigeria, Cameroon, Gabon and DR Congo and to a lesser extent in their neighboring countries (Huffman, 2003). Economically, *Compositae* family is of considerable importance. It is found useful as food, insecticide, medicinal preparation as well as ornamentals (Fred *et al.,* 2010). Thus, may have vast array potentials in improvement of animal health and welfare as functional food.

Plants are sources of a large number of drugs, comprising groups such as antifungal, antispasmodics, emetics, anticancer and antibacterial (Dhanavade *et al.,* 2011). A large number of plants are claimed to possess antibiotic properties in the traditional system and are used extensively in folklore medicine worldwide (Dhanavade *et al.,* 2011; Kawai *et al.,* 2000). Researchers today are emphasizing on evaluation and characterization of various plants and plants constituents against a vast array of microbial pathogens (Tamotake *et al.,* 2006). Biologically active compounds present in medicinal plants have always been of great interest to scientists as microorganisms are becoming resistant to commonly used antibiotics (Yedjou *et al.,* 2008). Medicinal plants are the richest bio-resources of drugs in traditional medicine, modern medicine, pharmaceutical intermediates and chemical entities for synthetic drugs (Probhakar *et al*., 2006).

Some previous studies have hinted towards the antimicrobial activity of extracts of *V. amygdalina* against both Gram positive bacteria (*Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis, Bacillus megaterium, and Serratia marcescens*), Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), and fungi (*Aspergillus niger, Candida albicans and Penicillium chrysogenum*) (Anibijuwon *et al*. 2012; Evbuomwan *et al*. 2018; Bamigboye and Ahmed, 2019), hence it is necessary for further studies on the antimicrobial activity of various extracts from this plant to be carried out, to further consolidate its therapeutic potential.

MATERIALS AND METHODS

Sample collection and processing

Leaves of *V. amygdalina* were collected from Katsina layout, Katsina State. The taxonomic identity of *V. anygdalina* was confirmed by a botanist in the Department of Biological Sciences Umaru Musa Yaradua University, Katsina. A voucher specimen with reference number VI603 was deposited in the Herbarium of Biological Sciences Department, Umaru Musa 'Yar'adua University, Katsina. The leaves were air dried at room

temperature (28±2°C) for two weeks. The air dried leaves of *V. amygdalina* were pulverized into powder using clean mortar and pestle. The powdered leaf was stored in clean plastic container prior to extraction.

Test isolates

S. aureus, P. aeruginosa and *C. albicans* isolates were obtained from Microbiology laboratory, Umaru Musa 'Yar'adua University, Katsina. The taxonomic identity of the isolates was confirmed using Gram's staining and biochemical tests. Inocula of the test isolates were prepared and standardized using McFarland standard (Olusola-Makinde *et al*., 2021; Ibrahim *et al.*, 2009)

EXTRACTION OF *V. amygdalina* LEAF

Fifty grams of pulverized *V. amygdalina* leaf were weighed using electric weighing balance, percolated in 500ml ethanol in a 1000ml conical flask, covered with cotton wool and aluminium foil and the preparation was allowed to stand for 72 hours, before being subsequently filtered using Whatman's No. 1 filter paper. The filtrate was concentrated on a water bath set at 78.5°C. The extract was transferred into a sterile, airtight bijou bottle and stored in the refrigerator at 4°C until use (Ibrahim *et al;* 2009).

PHYTOCHEMICAL SCREENING OF *V. amygdalina* ETHANOL LEAF EXTRACT

The *V.amygdalina* leaf extract was screened for the presence of alkaloids, cardiac glycosides, flavonoids, saponins and tannins (Mustapha *et al*; 2007).

ANTIMICROBIAL ACTIVITY OF *V. amygdalina* ETHANOL LEAF EXTRACT

This was done using agar well diffusion method as described by Ibrahim *et al;* 2009. Three concentrations, namely: 100mg/ml, 50mg/ml, and 25mg/ml of the plant extract were prepared and introduced into different holes bored onto sterile medium containing the test isolates. The plates were incubated uninvertedly at 37ºC for 24 hours. Areas that showed clear zones around the bored holes indicate the susceptibility of the test isolates to the extract and these were measured using centimeter rule and recorded in mm (Mukhtar & Ghori, 2012; Mustapha *et al*; 2007).

MINIMUM INHIBITORY CONCENTRATIONS

Three concentrations (100mg/ml, 50mg/ml, 25mg/ml) of the *V. amygdalina* ethanol leaf extract were introduced into different test tubes in the sterile medium containing the standardized inocula of the test isolates. The test tubes were incubated at 37ºC for 24 hours. The dilutions that first showed no sign of visible turbidity were taken as minimum inhibitory concentration (Mustapha *et al*; 2007).

MINIMUM FUNGICIDAL CONCENTRATIONS

The test tubes with fungal standardized inocula that showed no visible turbidity after overnight incubation were subcultured on sterile potatoes dextrose agar plates and incubated at 35°C for 24 hours. The concentration that showed no visible growth after incubation was considered as minimum fungicidal concentration (Mustapha *et al*; 2007).

MINIMUM BACTERICIDAL CONCENTRATIONS

The test tubes of the MIC that showed no visible turbidity were sub cultured on sterile nutrient agar plates and incubated at 37ºC for 24 hours. The plates that showed no visible growth were the minimum bactericidal concentrations (Mustapha *et al*; 2007).

RESULTS

Phytochemical profile of *V. amygdalina* ethanol leaf extract

The result of phytochemical screening of *V. amygdalina* ethanol leaf extract demonstrated the presence of four phytochemicals, namely: Alkaloid, Flavonoid, Saponins, and Tannins with absence of Cardiac glycosides (Table 1).

Table 1: Phytochemical profile of *V. amygdalina* ethanol leaf extract.

Extract	Phytochemical profiles				
	Alkaloid	Cardiac glycosides	Flavonoid	Saponins	Tannins
V.amygdalina ethanol leaves	+	–	+	+	+

Key: + indicates presence of phytochemical, – indicates absence of phytochemical.

Antimicrobial Activity of the Extract

The *V. amygdalina* ethanol leaf extract was found promising as it inhibited the growth of *S. aureus* at 50mg/ml (zone of inhibition = 16 mm) and *P. aeruginosa* at 100mg/ml and 50mg/ml, with 20.5 and 18 zones of inhibition (mm) respectively. *C. albicans* was found to be resistant at 100 mg/ml, 50mg/ml and 25mg/ml.

Table 2: Antimicrobial activity of *V.amygdalina* ethanol leaf extract against *P.aeruginosa, S.aureus* and *C.albicans*.

Clinical isolates	Zones of inhibition (mm) at different concentrations (mg/ml) of *V.amygdalina* ethanol leaf extract.		
	100	50	25
S.aureus	13.5	16	12.3
P.aeruginosa	20.5	18	9.2
C.albicans	0	0	0

Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) of *V. amygdalina* ethanol leaf extract against *P. aeruginosa, S. aureus* and *C. albicans.*
The MIC, MBC and MFC results indicated that *V. amygdalina* was found promising against both *S. aureus* and *P. aeruginosa*, with a similar MIC of 50mg/ml and an MBC of 25mg/ml.
Table 3: MIC, MBC and MFC of *V.amygdalina* ethanol leaf extract against *S.aureus, P.aeruginosa,* and *C.albicans*

Test isolates	Varied concentrations (mg/ml) of *V .amygdalina* ethanol leaf extract		
	MFC	MIC	MBC
S.aureus	NA	50	25
P.aeruginosa	NA	50	25
C.albicans	0	0	NA

KEY: NA-means not applicable.

DISCUSSION

This study investigates the phytochemical profile and antimicrobial activity of *V. amygdalina* ethanol leaf extract against *P. aeruginosa, S. aureus* and *C. albicans* with a view of determining the potential of the plant part for possible application as candidate for drug development to be used in the management of infections due to the test isolates. Generally, green plants are known to represent a reservoir of effective chemotherapeutic agents with more systemic and easily biodegradable potentials (Olusola-Makinde *et al*., 2021).
Previous studies had confirmed that the antimicrobial activity of *V. amygdalina* seemed to be more dependent on the solvent used for extraction (Teia *et al*., 2021; Evbuomwan *et al*. 2018), therefore, the choice of solvent to be used in extraction is highly paramount. Ethanol is preferred in extracting phytochemicals because the higher volatility of ethanol tends to extract more active compounds from the samples than water (Olusola-Makinde *et al*., 2021), however, extracts obtained using other solvents have also been reported to possess antibacterial activity. For instance, Teia *et al.* (2021) reported the antimicrobial activity of *Vernonia amygdalina* chloroform (CHCl3) and methanol leaves extracts against *Bacillus subtilis, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans*. The occurrence of four phytochemicals in *V. amygdalina* ethanol leaf extract suggests the potential of this part of the plant to be used for drug discovery. This is also in agreement with previous findings, since a vast array of phytochemicals (more than 30), including alkaloids, saponins, flavonoids, terpenes, steroids, anthraquinone, lignans, phenolic acids, coumarins, xanthones, edotides and sesquiterpenes, (Izevbigie, 2003; Cimanga *et al*. 2004; Muraina *et al*. 2010), sesquiterpene lactones, steroidal saponins (vernoniosides) and steroid glycosides have been reported to be detected in the plant (Erasto *et al*. 2006; Owoeye *et al*. 2010; Luo *et al*. 2011, Teia *et al.*, 2021).
Typically, sapponins have inflammatory properties, and alkaloids have been reported to be responsible for some antibacterial activities (Yar'adua *et al.,* 2015). Furthermore, many of the biological functions of flavonoids, including their antibacterial properties, have been attributed to their free radical scavenging, metal ion chelating and antioxidant activities (Olusola-Makinde *et al*., 2021). Generally, plants in the genus *Vernonia* are known to possess potent bioactive compounds. Ogundare *et al*. (2006) had shown that exthanol extracts from another species within this genus, namely*: V. tenoreana,* contained saponins, flavonoids, tannins and anthraquinones, and was found to have very potent antibacterial as well as antifungal activities, which can be attributed to the ability of the extracts to elicit protein denaturation of the microbial cell, and the destruction of the cell wall/membrane of the bacterial isolates.

Variation in the zone of inhibition exhibited by the extracts against the test bacteria can possibly be due to their relative composition of cell wall components, i.e. the major difference between gram positive and gram negative bacteria (Evbuomwan *et al*., 2018).

The *V. amygdalina* ethanol leaves extract promising antibacterial effects against the bacterial test isolates, further buttressing the use of this plant in folklore medicine. This finding is similar to that of Kawai *et al.* (2000) who reported that *V. amygdalina* ethanol leaf extract was found promising on *P. aeruginosa* at the concentrations tested. Moreover, this is also in agreement with the findings of Udochukwu *et al*. (2015) who reported that the aqueous and ethanol extracts of *V. amygdalina* possess antibacterial action against pure cultures of isolates of clinical bacteria. Likewise, Olusola-Makinde *et al*. (2021) had also reported the antibacterial activity of aqueous and ethanolic leaf extracts of *V. amygdalina* against *S. aureus, P. aeruginosa* and other bacteria (*Bacillus subtilis, Streptococcus pyrogens and Corynebacterium diphitheriae*). Additionally, a prior study by Ghamba *et al*. (2014) reported that the extracts of *V. amygdalina* leaves showed strong antimicrobial activities against the tested isolates.

The resistance phenomenon exhibited by all the tested concentrations may be attributed to the absence of other phytochemicals that may inhibit fungus due to the presence of certain cell constituent presence in the *C. albicans* cells that may protect it from being inhibited by the *V. amygdalina* ethanol leaf extract as earlier observed by WHO (2007). The current MIC and MBC result agreed with the report of Owoeye (2010), furthermore, Oboh and Enobhayisobo (2009) and Udochukwu *et al.* (2015) had previously observed inhibitory effects of aqueous and ethanolic extracts of this plant on the growth of Gram-positive bacterium, *Staphylococcus aureus,*will go a long way in providing indices that will be used to provide standard dosage that will be very useful in effective management of infections due to the test isolates.

CONCLUSION

V. amygdalina leaf extract demonstrates promising antibacterial activity against *S. aureus* and *P. aeruginosa* due to the presence of a vast array of phytochemicals.

RECOMMENDATION

Based on the findings of the current study the following recommendations are offered.

1. There is need to purify, isolate and characterize the phytochemical constituents responsible for the observed bioactivity, with a view to supplementing conventional drug development and/or probably serving as principal compounds for various pharmaceuticals especially in developing countries like Nigeria.
2. Further research should be carried out to determine most promising phytochemicals between alkaloids, flavanoids, saponins and tannins.
3. Since four phytochemicals was found present in *V. amygdalina* ethanol leaf extract, it is recommended that further research should be carried out to determine which among of these four compounds or their combination is responsible for the inhibition of the bacteria.
4. Toxicity studies of *V. amygdalina* should also be done to determine the safety indices of its extracts. Clinical trials should be carried out to explore the potential of the extract in the treatment of these infectious diseases as well as its potentials in improvement of animal health and welfare as functional food.

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