WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 7, 9-17. Research Article ISSN 2277– 7105

PHYTOCHEMICAL AND ANTIBACTERIAL ANALYSIS OF AQUEOUS AND ALCOHOLIC EXTRACTS OF VERNONIA AMYGDALINA (DEL.) LEAF

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Article Received on
04 Feb. 2018,
Revised on 24 Feb. 2018,
Accepted on 15 Mar. 2018,
DOI: 10.20959/wjpr20187-11567

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ABSTRACT

Medicinal plants offer an attractive array of phytochemicals with novel microbial disease – controlling potentials, due to the spectrum of secondary metabolites present in their extracts. The aim of this study was to investigate the phytochemicals present in leaf extracts of *V. amygdalina* and to examine its antibacterial properties against some clinical bacterial isolates. Ethanol, methanol and water were used as solvents in the extraction. The phytochemical screening showed that the ethanolic and aqueous extracts had tannins, flavonoids, alkaloids, saponins, phytate and oxalate in varying proportions while alkaloid was the only exclusion in the methanolic extract. The antibacterial activity of the ethanolic extract showed an average diameter inhibition zone between 9.0 mm to 24.0 mm against some clinical bacterial isolates such as, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. The methanolic extracts showed significant level of activity against the bacterial isolates tested, with average diameter inhibition zones between 10.0 mm – 17.0 mm. The aqueous extracts showed antibacterial activity against the clinical bacterial isolates tested with average diameter inhibition of 9.0 mm – 19.0 mm. The plant leaf is recommended as a source of broad spectrum antibiotic for treatment of Gram positive and negative bacterial infections.

KEYWORDS: Vernonia amygdalina, phytochemical, plant extract, antibacterial activity.

INTRODUCTION

In recent times, a great deal of interest has been shown on the role of complementary and alternative medicines (CAM) for the treatment of various acute and chronic infections and diseases. The medicinal use of plants, vegetables and spices has become widely acceptable as major sources of prophylactic and chemopreventive drug discovery and development.[1,2] It has been reported that over 60% of the world human population depends directly on plants for medicinal purposes.[3] It has also been estimated that about 25 % of the drugs prescribed worldwide are derived from *plants*, and 121 such active compounds are in *use*.[4] An estimated 90 % of the African population is reckoned to depend on plants sources for their medicinal needs.[5]

Phytochemicals, as defined by Murugan et al., are non – nutritive plant chemicals that have protective or disease preventive properties.[6] Phytochemicals are natural bioactive compounds of secondary metabolite found in plants that work with nutrients and fibres to act as a defence system against disease.[7] Although, recent researches have demonstrated that many phytochemicals can protect humans against diseases, they are originally produced for self – protection by the plants.[8]

These phytochemicals are divided into two groups based on their functions in plant metabolism which are primary and secondary constituents. Primary constituents comprises of common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds and many more such as flavonoids and tannins.[7]

Many plant extracts have been shown to inhibit the growth of microorganisms.[2] These extracts consist of chemicals and are usually considered originally to play a significant role in defense reactions of plants against infections by pathogenic microorganisms.[2,6,9]

Vernonia amygdalina is commonly called bitter leaf in English due to its bitter taste. It is a tropical shrub that grows in some parts of Africa, particularly, Nigeria, Cameroon and Zimbabwe, averaging about 3 metres in height.[10] Its prepared leaves are used as staple vegetable in soups and stews of various cultures throughout equatorial Africa. African common names of *V. amygdalina* include *chusar-doki* (Hausa), *grawa* (Amharic), *ewuro* (Yoruba), *etidot* (Ibibio), *onugbu* (Igbo), *ityuna* (Tiv), *oriwo* (Edo), *mululuza* (Luganda),

labwori (Acholi), *olusia* (Luo) and *ndoleh* (Cameroon).[11,12] It is reputed to have several health benefits.[10]

Some antimicrobial activities of extracts of *V. amygdalina* have been reported.[10,13,14] Also, the plant has been shown to possess cytotoxic effects towards human carcinoma cells of the nasopharynx,[15] and the human glioblastoma brain cancer cells.[16] Medicinal benefits of *V. amygdalina* in treatment of ailments include amoebic dysentery (16) and gastrointestinal disorders (17) have also been documented. It is also submitted that it has some hepatoprotective effects.[17,18,19]

Therefore, this study investigates the phytochemical properties and antibacterial activity of *V. amygdalina* leaf extracts against some clinical bacterial isolates.

MATERIAL AND METHODS

Collection of Samples and Preparation

Fresh and healthy leaves of *V. amygdalina* were obtained from the herbarium of the Plant Science and Biotechnology Department of Nasarawa State University, Keffi, Nigeria.

Extraction of V. amygdalina leaves

Distilled water was used to wash the leaves and rinsed in deionised water, and then allowed to air – dry for a period of 7 days. The leaves were then pulverized into coarse powder using clean mortar and pestle, and then extracted using the Soxhlet method. Water, ethanol and methanol were used as solvents.[19,20]

Phytochemical Analysis

Test for Alkaloid

The extracts (20 μL) was applied on TLC plate (Silica Gel 60G, 5 × 10 cm) and eluted using toluene – ethyl acetatediethylamine (70: 20: 10) as solvent system. Alkaloid was detected after spraying Dragendorff's reagent as orange – brown spots on the TLC plate.[2,21]

Test for Tannin

Extracts were treated with 1mL of 5% ferric chloride. The presence of tannin was indicated by the formation of bluish black or greenish black precipitate.[2,22]

Test for flavonoid

Few fragments of magnesium metal ribbon (3-4 pieces) was added to 1 mL of the extracts, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink or red colour indicated the presence of flavonoids.[2,23]

Test for Saponin

The 2 mL of distilled water was added to extracts suspended in ethanol and was shaken vigorously. The formation of profuse foam layer indicated the presence of saponins.[2,23]

Test for Phytic Acid

The method of Ebana et al.[24] was adopted for the extraction of phytic acids in the extract. Ferric chloride was used to precipitate the phytic acid to ferric phytate.

Test for Oxalate

The oxalic acid in the extracts was precipitated with calcium chloride as calcium salts.[24]

Antibacterial Screening

Isolation and Identification of Bacterial Species

Bacterial species were isolated from different clinical samples from patients at a tertiary healthcare centre, Nasarawa state, Nigeria. The isolation was carried out as described by Ekeleme et al.[2]

Determination of Antibacterial Activity of *V. amygdalina* Leaf Extracts Using Cup – Plate Agar Diffusion Bioassay

The antibacterial activity of extracts of *V. amygdalina* leaf was carried out using cup – plate agar diffusion bioassay as follows; 100 μL of fresh culture (Standardized to 0.5 McFarland) was spread uniformly on a sterile Mueller-Hinton agar (MHA) plates and allowed to air – dry. Wells of 6 mm in diameter were made in the MHA plates and the base was seeded with molten MHA and approximately 100 μL for each concentration (50 mg/L, 25 mg/L, 12.5 mg/L, 6.25 mg/L, 3.125 mg/L, 1.56 mg/L and 0.78 mg/L) of the extracts was dispensed into the wells and the plates were allowed to stand for 1 hr at room temperature for pre-diffusion and then incubated at 37 °C for 24 hrs. The diameter zone of inhibition was then measured and recorded. Ciprofloxacin (5µg) was used as control.[2,25]

RESULTS AND DISCUSSION

The phytochemical screening of *V. amygdalina* showed that the ethanolic and aqueous extracts had tannins, flavonoids, alkaloids, saponins, phytate and oxalate in varying proportions. All these phytochemicals with the exception of alkaloid were also detected in the methanolic extracts (Table 1).

Table 1: Phytochemical Analysis on solvent fractions of *V. amygdalina* leaf extracts.

Fractions	Tannins	Flavoniods	Alkaloids	Saponins	Phytate	Oxalate
Ethanol	++	++	+	+	++	++
Methanol	++	++	-	+	+	++
Aqueous	+	+	+	+	++	+

= Absent; + = Present in moderate quantity; ++ = Present in large quantity

The presence of the different phytochemicals such as flavonoids, tannins, saponins and alkaloids in the leaf extracts of *V. amygdalina* is confirmatory to previous reports.[26] These phytochemicals present in the plant leaves may have link with the numerous pharmacological properties such as: anti-carcinogenic, hypoglycemic, antioxidative, anti-inflammatory, antibacterial, antimalarial, anti-helminthic amongst several others attributed to *V. amygdalina* leaves.[10,13,14,15,17,18,19,27,28,29]

Asfour (2017) asserted that, "Medicinal plants offer an attractive repertoire of phytochemicals with novel microbial disease-controlling potential, due to the spectrum of secondary metabolites present in extracts, which include phenolics, quinones, flavonoids, alkaloids, terpenoids, and polyacetylenes. They have recently received considerable attention as a new source of safe and effective quorum sensing inhibitory substances."[30]

The antibacterial activity of the three extracts of *V. amygdalina* leaf showed that the ethanolic extract was more effective, having an average diameter inhibition zone ranging between 9.0 mm to 24.0 mm against *P. aeruginosa* at different concentrations between 6.25 mg – 50mg. The ethanolic extract also showed mean inhibition zone ranging between 9.5 mm – 20.0 mm against *E. coli*, 8.5 mm – 19.0 mm against *S. aureus*, 14.0 mm – 15.0 mm against *S. pneumoniae* and 8.5 mm – 18.5 mm against *K. pneumoniae*.

The methanolic extracts showed significant level of activity against the bacterial isolates tested, with average diameter inhibition zone ranging between 10.0 mm – 17.0 mm against *P. aeruginosa*, 8.5 mm – 14.5 mm against *E. coli*, 10.5 mm to 17.0 mm against *S. aureus*, 9.0 –

13.0 mm against *S. pneumoniae* and 12.5 mm – 15.5 mm against *K. pneumoniae* at concentrations ranging between 6.25 mg to 50 mg.

The aqueous extracts of *V. amygdalina* leaf showed antibacterial activity against the clinical bacterial isolates tested with average diameter inhibition zone between 9.0 mm – 13.5 mm, 11.2 mm – 15.5 mm, 9.5 mm – 19.0 mm, 13.5 mm – 17.5 mm and 8.5 mm – 12.5 mm against *P. aeruginosa, E. coli, S. aureus, S. pneumoniae* and *K. pneumoniae* respectively (Table 2).

Table 2: Antibacterial activity of ethanolic, methanolic and aqueous leaf extracts of *V. amygdalina* against clinical bacterial isolates.

Bacterial isolates	Diameter zone of inhibition (mm) (mean ± SD)			
	50 mg	25 mg	12.5 mg	6.25 mg
Ethanolic extracts				
P. aeruginosa	24.0 ± 1.58	18.0 ± 0.71	14.0 ± 2.0	9.0 ± 21.1
E. coli	20.0 ± 0.14	13.5 ± 2.14	9.5 ± 2.11	0.00
S. aureus	19.0 ± 1.40	14.0 ± 1.45	10.5 ±.72	8.5 ± 0.77
S. pneumoniae	15.0 ± 1.41	14.0 ± 1.45	0.00	0.00
K. pneumoniae	18.5 ± 2.11	14.0 ± 1.44	8.5 ± 2.10	0.00
Methanolic extracts				
P. aeruginosa	17.0 ± 1.40	10.0 ± 1.41	0.00	0.00
E. coli	14.5 ± 0.74	11.0 ± 1.40	8.5 ± 0.70	0.00
S. aureus	17.0 ± 1.44	13.5 ± 0.71	10.5 ±0.70	0.00
S. pneumoniae	13.0 ± 1.49	9.0 ± 1.47	0.00	0.00
K. pneumoniae	15.5 ± 2.12	12.5 ± 0.77	0.00	0.00
Aqueous extracts				
P. aeruginosa	13.5 ± 2.11	9.0 ± 1.9	0.00	0.00
E. coli	15.5 ± 2.10	11.2 ±1.20	0.00	0.00
S. aureus	19.0 ± 1.48	14.5 ± 0.98	9.5 ± 0.99	0.00
S. pneumoniae	17.5 ± 1.81	13.5 ± 3.10	0.00	0.00
K. pneumoniae	12.5 ± 2.15	8.5 ± 0.74	0.00	0.00

Our findings revealed that the ethanolic extract has significantly higher antibacterial activity when compared with the methanolic and the aqueous extracts. This report suggests the affirmative when compared with the study by Oshim et al.[26] who studied the antibacterial activity by ethanolic extracts of *V. amygdalina* against *E. coli, S. aureus, K. pneumoniae* and *P. aeruginosa*. This study further corroborates the numerous assertions about the usefulness of *V. amygdalina* as a source of food and medicine for chemopreventive and chemotherapeutic purposes.[1,10,14,19,27]

CONCLUSION

This study confirmed the presence of various phytochemicals such as tannins, flavoniods, alkaloids, saponins, phytate and oxalate in *V. amygdalina* leaf extracts. Furthermore, these extracts exhibited some significant antibacterial activity against some clinical bacterial isolates. We recommend that this plant leaf be explored as a source of broad – spectrum antibiotic for the treatment of infections caused by gram negative and gram positive bacteria.

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