academicJournals

Vol. 5(6), pp. 60-64, June 2013
DOI:10.5897/JMA11.033
ISSN 2141-2308 ©2013 AcademicJournals
http://www.academicjournals.org/JMA

Journal of Microbiology and Antimicrobials

Full Length Research Paper

Bacterial activity of crude extracts of *Vernonia amygdalina* on clinical isolates

Adetunji C. O.[1]*, Olaniyi O.O.[2] and Ogunkunle A.T.J.

[1]Nigerian Stored Product Research Institute, Km 3 Asa Dam Road, P.M.B. 1489, Ilorin, Kwara State, Nigeria.
[2]Microbiology Department, Federal University of Technology, Akure, Ondo State, Nigeria.
[3]Pure and Applied Biology, LAUTECH,PMB 4000, Ogbomoso, Nigeria.

Accepted 15 June, 2013

The antibacterial activities and preliminary phytochemical screening of ethanolic and aqeous extract of *Vernonia amygdalina* was performed against clinical isolates obtained from University of Ilorin Teaching Hospital (UITH) which included *Escherichia coil*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Generally, the ethanol extracts showed more activities than aqueous extracts in both samples. The extracts exhibited antibacterial activity against the test organisms (0.5 to 15.50 mm). The phytochemical screening of *Vernonia amydalina* showed the presence of tannins, cardiac glycosides, saponins and alkaloids. The minimum inhibitory concentration (MIC) ranged between 25 and 200 mg/ml. *V. amydalina* exhibited a minimum bacteriocidal concentration (MBC) of 50 mg/ml for *P. aeruginosa* and 125 mg/ml for *S. aureus* while *E. coil* was bacteriostastic. The crude extracts exhibited strong potency against the clinical bacteria with *P. aeruginosa* being the most susceptible. The antibacterial efficacy of the crude extracts is therefore discussed.

Key words: Antimicrobial activity, minimum inhibitory concentration, phytochemical, *Vernonia amygdalina*.

INTRODUCTION

Botany and medicine have been closely linked throughout history. Prior to this century, medical practitioners whether allopath (medical doctors), homeopaths, naturopaths, herbalist or shamans had to know the plants in the area and how to use them since many of their drugs were derived from plants (Akujobi et al., 2004). Around 1900, 80% of the drugs were derived from plants. However, in the decades that followed, the development of synthetic drugs from petroleum products caused a sharp decline in the pre-eminence of drugs from live plant sources (Akujobi et al., 2004).

But with the recent trend of high percentage resistance of microorganisms to the present day antibiotics (Ibekwe et al., 2000), efforts have been intensified by researchers towards a search for more sources of antimicrobial agents.

Vernonia amygdalina is a tropical plant belonging to the family Compositae and is used widely as vegetable and medicinal plant. It has the common name bitter leaf (Ibrahim et al., 2000). It is a shrub of about 2 to 5m with a petiolate leaf of about 6 mm in diameter and elliptic shape. The leaves are green with a characteristic odour and bitter taste. It does not produce seeds and has to be distributed or propagated through cutting. It grows under a range of ecological zones in Africa and produces a lager mass of forage and it is drought tolerant, with about 200 species of Vernonia. It is majorly used for human consumption and has to be washed to remove the bitter taste. Its bitter taste is due to antinutritional factors such as alkaloids, saponins, tannins and glycosides. It stimulates the digestive system as well as reduces fever. It is differentiated from its counterpart *V. colorata*, which grows

*Corresponding author. E-mail:charliguitar@yahoo.com. Tel: 08039120079.

wildly by hairy leaves of the latter (Iwu, 1993).

This plant contains complex active components that are pharmacologically useful. The roots and the leaves are used in ethno-medicine to treat fever, hiccups, kidney problems and stomach discomfort. The stem and root divested of the bark are used as chewsticks in many West African countries like Cameroon, Ghana and Nigeria (Burkill, 1985; Hamowia, 1994).

However, extract of bitter leaf had been reported to exert antibiotic action against drug resistant microorganisms and possess antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities (Akinpelu, 1999; Dahanukar et al., 2000). Furthermore, the root provides one of the commonly used chew sticks in Nigeria due to alleged beneficial effect on dental caries (Aregheore et al., 1998). The leaves and bark in Ethiopian local medicine are used as purgative, against menstrual pain and wound dressing (Akah and Okafor, 1992; Uhegbu and Ogbuchi, 2004). In Nigeria, the research for new drugs is on course hence the aim of this study was to verify the antibacterial activities of the plant so as to find an alternative for the common antibiotics present in use.

MATERIALS AND METHODS

Collection of plants materials

Fresh leaf samples of *V. amygdalina* were purchased from Oja Oba market at Ilorin, Kwara State, Nigeria. The plant was identified at the Herbarium Unit of the Department of Plant Biology, University of Ilorin. The plants were dried in the sun until the moisture content was reduced. The plants were then mashed in a mortal with pestle and further ground to powder using an electric blender and stored in polythene bags until use. A certain percentage of the yield was obtained.

Preparation of plant material and plant extracts

Four different extracts namely cold ethanol, hot ethanol (80°C), cold water and hot water (80°C) were used for plant.

Hot ethanolic and hot aqueous extracts

Fifty grams of the finely ground powder was introduced into two conical flask and 200 ml of absolute ethanol and distilled water was added to the ground *V. amygdalina* respectively. The mixture was put in a water bath and heated to 80°C for 1 h. The mixture was allowed to cool and passed through a muslin cloth and later filtered with a Whatman No .1 filter paper (110 mm). The filtrate obtained was evaporated to dryness at 45°C, and the residue obtained was kept inside aluminum foil. The residue was later reconstituted in 95% ethanol as stock concentration of 250 mg\ml at 4°C.

Cold ethanolic and cold aqueuos extracts

Fifty grams of the finely ground powder was introduce into a conical flask and 200 ml of absolute ethanol and distilled water was added to the ground *V. amygdalina*, respectively. After 48 h, the extract was decanted and passed through a muslin cloth and later filtered with a Whatman No .1 filter paper (110 mm). The filtrate obtained was evaporated to dryness at 45°C, and the obtained residue was reconstituted in 95% ethanol as stock concentration of 250 mg/ml.

Collection and maintenance of the test organisms

Three bacteria clinical isolates were used for the tests. They were collected from the Department of Medical Microbiology and Parasitology of University Ilorin Teaching Hospital. The organisms comprise of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. All the bacterial species used were maintained on nutrient agar slopes and stored in the refrigerator. All inoculums were standardized using the method described by Bauer et al. (1966).

Antibacterial assay

Agar dilution method was used to determine the antibacterial activity of the plant extracts. Isolates were inoculated into Mueller Hinton Agar (MHA) andincubated at 37°C for 18 to 24 h. The MIC for the plant extracts was determined using the method of Oyagade et al. (1999).

Phytochemical screening of the leaves extracts

Phytochemical screening was done in order to detect the presence of plant constituents such as alkaloids, tannins, saponins, phenols, glycosides, phlobatannins, flavonoids and glycosides using the methods described by Odebiyi and Sofowora (1978).

Test for saponins

Two milliliter of the aqueous and ethanolic extracts in a test tube was shaken for two minutes. Fronthing which persisted on shaking was taken as evidence for the presence of saponins.

Test for alkaloids

Three milliliter of the ethanolic and aqueous extracts was stirred with 5 ml of 1% HCL on a steam bath for twenty minutes. The solution obtained was cooled and filtered and few drops of Mayer's reagent/picric acid was added to the filtrate. A cream precipitate indicated the presence of alkaloid.

Test for phenolics

Two drops of 5% ferric chloride were added to 5 ml of the ethanolic and aqueous extracts in a test tube. A greenish precipitate was taken as indication of phenolics.

Test for tannins

A volume of 1 ml of freshly prepared 10% potassium hydroxide was added to a volume of 1 ml of the ethanolic extracts and aqueous extracts. The presence of a dirty white precipitate was taken as indication of tannins.

Test for steroids

To a volume of 1 ml of the extracts, five drops of concentrate tetra-oxoiosulphate VI acid was added. Red coloration indicated the presence of steroids.

Table 1. Antimicrobial activity of *V. amygdalina* against *P. aeruginosa*, *S. aureus* and *E. coli.*

Test organism	Diameter of inhibition(mm)																			
	Cold Aqueous extract					Hot aqueous extract					Hot ethanolic extract					Cold ethanolic extract				
	C	25	50	100	200	C	25	50	100	2000	C	25	50	100	200	C	25	50	100	200
P. aeruginosa	-	0.5	2.00	3.00	3.50	-	-	-	-	-	-	1.50	4.00	11.00	13.00	-	2.00	3.00	6.00	10.50
S. aureus	-	3.00	5.00	6.00	7.50	-	-	-	-	-	-	-	2.00	5.00	6.00	-	-	1.00	2.00	4.50
E. coli	-	0.5	2.50	3.50	5.00	-	-	-	-	-	-	-	2.00	7.00	9.50	-	-	1.00	3.50	8.00

- = No zone of inhibition; C = control of each extract.

Test for phlobatanins

To a volume of 1 ml of the ethanolic and aqueous extracts, 1% hydrochloric acid was added. A red precipitate was taken as the presence of phlobatannins.

Test for flavonoids

To a volume of three milliliter of the ethanolic and aqueous extract, a volume of 1 ml of 10% sodium hydroxide was added. A yellow coloration indicated the presence of flavonoids.

Test for glycosides

To a volume of 3 ml of the ethanolic and aqueous extract, 2 ml of chloroform was added. Tetraoxiosulphate VI acid was carefully added to form a lower layer. A reddish brown color at interface indicated the presence of a steroidal ring.

RESULTS

Table 1 shows the data obtained from the antimicrobial activities of the hot ethanolic and cold ethanolic extract with hot and cold aqueous extract of *V. amygdalina.* The determination of the antimicrobial activity of hot and cold ethanolic extract of *V. amygdalina* showed that the extracts possess antibacterial activity against *S. aureus, P. aeruginosa and E. coli.*

P. aeruginosa was the most sensitive organisms to the hot ethanolic extract of *V. amygdalina* with the zone of inhibition of 13.00 mm and the least sensitive organisms to the same extract was *S. aureus* with the zone of inhibition of 6.00 mm at the same concentration (200 mg/ml). The cold ethanolic etract of *V. amygdalina* has the highest zone of inhibition on *P. aeruginosa* with a diameter of 10.50 mm while other test organisms are less sensitive. *E. coli and P. aeruginosa* are less sensitive to cold aqueous extract of *V. amygdalina* but *S. aureus* was sensitive to the extract as shown in Table 1.

Table 2 shows the minimum inhibitory concentration (MIC) of the plant extracts on *P. aeruginosa, S .aureus and E. coli*. The MIC values obtained on the test organisms varied from one plant to another .The hot ethanolic extract of *V. amydalina* has the lowest minimum inhibitory concentration on *P. aeruginosa* at 25 mg/ml while cold aqueous extract of *V. amydalina* had the highest value of MIC on *P. aeruginosa* at 55 mg/ml. The cold ethanolic extract and cold aqueous extract of *V. amgydalina* recorded the highest MIC at 60 mg/ml on *S. aureus*. However, cold ethanolic extract of *V. amydalina* had the lowest values on *S. aureus* at 40 mg/ml. In all, *V. amydalina* extract had bacteriostatic effect on all test organisms. The *V. amydalina* extract had bacteriostatic effects on *E. coli.*

But *V. amydalina* of hot ethanolic extract was bacteriocidal on *P. aruginosa* and *S.aureus* at 50 and 125 mg/mg .

The result of the phytochemical analysis of the ethanolic extract of *V. amygdalina* leaf is shown in Table 3. Plant constituents such as saponins, cardiac glycosides, tannins and steroids were detected while components such as flavonoids and phlobatannins were not detected. The results show that there was variation in the degree of antibacterial activities of the extracts. The results indicated that the cold and hot ethanolic extract rather than the aqueous extract produced effecttive antimicrobial activities in *V. amygdalina*. The antimicrobial activities of the extracts possess antibacterial activity against *S. aureus*, *P. aeruginosa* and *E. coli.*

Ethanol extracts showed more activity against the bacteria isolates than the water extracts. This may be due to the higher volatility of the ethanol which tends to extract more active compounds from the samples than water (Ibekwe et al., 2001; Dutta, 1993). The plant extracts from the two plants have a profound activity against both Gram-negative and positive bacteria. There was however, more activity against the Gram negative organism than the Gram positive. The minimum bactericidal concentration (MBC) from the *V. amygdalina* on *P. aueriginosa* was found to be 50 mg/ml while *E. coli* exhibited a bacteriostastic activity.

Table 2. Minimum inhibitory and bactericidal concentration of plant extracts on *P. aeruginosa*, *S. aureus and E. coli.*

Test Organism	Plant	Plant extract	MIC (mg/ml)	MBC (mg/ml)
		Hot ethanol	25	50
P. aeruginosa		Cold ethanol	30	-
		Cold aqueous	55	-
		Hot ethanol	45	125
S. aureus	*V. amygdalina*	Cold ethanol	60	-
		Cold aqueous	60	-
		Hot ethanol	35	-
E. coil		Cold ethanol	60	-
		Cold aqueous	120	-

MIC = Minimum inhibitory concentration; MBC = minimum bactericidal concentration; - = not sensitive.

Table 3. Phytochemical analysis of the *V. amygdalina*.

Sample/Table	Hot ethanol	Cold ethanol	Hot water	Cold water
Saponins	+	+	+	+
Cardiac glycosides	+	+	-	+
Tannins	+	+	+	+
Steroids	+	+	-	+
Alkaloids	-	-	-	-
Phlobatinins	-	-	-	-
Phenolics	-	-	-	-

+ = Positive, - = not detected.

Results of the preliminary phytochemical screening revealed the presence of these compounds in the extracts of *V. amygdalina* (Table 3). Kaufman et al. (1988) and Dutta (1993) also reported some compounds as an indication of the potential medicinal value of the plants in which they appear. The ethanolic extract have the largest zones of inhibition in all the results obtained from this study probably because of the higher concentration except in Table 1 where cold aqueous extract had a higher activity of *V. amygdalina* against *S. aureus*. Goodman et al. (1980) reported that one of the factors that affect microbial susceptibility is the concentration of the active component; the more the concentration, the higher the activity of the chemical substance.

The MBC of the *V. amygdalina* on *P. aueriginosa* was found to be 50 mg/ml while *E. coli* exhibited a bacteriostastic activity. Results of the preliminary phytochemical screening revealed the presence of these compounds in the extracts of *V. amygdalina* (Table 3).

Since all the organisms were affected in one way or the other by exposure to different extracts, it is very possible that at much higher concentrations and observable time limit, there could be bacteriocidal effect on the organisms. The susceptibility of all the test organisms especially *P. aeruginosa* that has resistance against many antibiotics goes further to prove that the plant have potentials as alternative source of antimicrobial agent in the advert of problems of resistance to chemotherapeutic drugs. It should be noted however, that just as there is the potentially advantageous medicinal use of this plant to some extents on it antimicrobial property, studies have shown that the leaf crude extract has induced abortion in goats and mice. The extract also reduced the rate of isolated frog heart and in cat caused marked fall in blood pressure (Iwu, 1993). However, this should not be discouraging since many antibiotic that are in use today, have their own side effects.

DISCUSSION

Since all the organisms were affected in one way or the other by exposure to different extracts, it is very possible that at much high concentrations and observable time limit, there could be bactericidal effect on the organisms. The susceptibility of all the test organisms especially *P. aeruginosa* that has resistance against many antibiotics goes further to prove that the plant have potentials as alternative source of antimicrobial agent in the growing bacterial resistance world.

Ogundare et al. (2006) showed that a similar plant species: *V. tenoreana* containing saponins, flavonoids, tannins and anthraquinones was found to have very potent

antibacterial as well as antifungal activities. These phytochemical constituents were further reported to be responsible for many antimicrobial activities of different plant species (Ghoshal et al., 1996; Iwu et al., 1999). Flavonoids have been reported to be synthesized by plants in response to microbial infections and are good antibacterial agents; tannins have been demonstrated to have antibacterial activities (Akiyama et al., 2001).

Conclusively, most of the sensitive extracts were only able to inhibit the growth of the organism which is known as bacteriostatic in action while some exert a killing effect on the test organism and suggests that the crude extracts could also be bactericidal.

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