International Journal of Interdisciplinary Innovative Research &Development (IJIIRD)

ISSN: 2456-236X

Vol.04 Issue 01|2019

Antibacterial and Cytotoxic Activities of Amaranthus Spinosus Extract

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ABSTRACT

This study was carried out to assess the antibacterial activity of Amaranthus spinosus extract against clinical isolates of Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, and Salmonella typhi as well as its cytotoxic effect onArtemisia salina nauplii. The result showed that none of the test bacteria were susceptible to the extracts at the lowest concentration used (10 mg/ml) whereas at the highest concentration used (50mg/ml), there were appreciable activity of the extracts against the test organisms with E. coli recording the highest zone of inhibition (14.33mm) against the extract while S. typhi (7.33mm) recorded the least zone of inhibition. The MIC obtained in this study ranged between 15 mg/ml and 30 mg/ml. Percentage lethality of brine shrimp at four different concentrations (1, 10, 100, 1000 ppm/ml) of A. spinosus extracts showed that higher concentration of extract leads to higher mortality of the shrimps as 0, 15, 45 and 65 % mortality was recorded at the respective concentration. These results have lent credence to the folkloric therapeutic claim of Amaranthus spinosus.

Keywords: ***Amaranthus spinosus, antimicrobial, cytotoxicity, LC_{50}, MIC***

1. INTRODUCTION

Traditional medicine has been used for thousands of years with great contributions made by practitioners to human health, particularly as primary health care providers at the community level. Countries in Africa, Asia, and Latin America use traditional medicine to help meet some of their primary healthcare needs. In Nigeria, for example, herbal medicine is the first line of treatment for 60% of children with high fever from malaria, while 85% of Nigerians use and consult traditional medicine for health care, social and psychological benefits [1]. The world Health organization (WHO) has since urged developing countries to utilize the resources of traditional medicine for achieving the goals of Primary Healthcare. This has been due to the various advantages of traditional medicine namely: low cost, afford ability, accessibility, acceptability and perhaps low toxicity [2].

The plant kingdom has been the best source of remedies for curing a variety of diseases. This is why medicinal plants have played a key role in the worldwide maintenance of health. Similarly, many plants have known efficacy for control of diseases and disease vectors. Infectious diseases are caused by pathogenic microorganisms and are the second leading cause of death worldwide [3]. As a result of this, a lot of interests have been focused in herbal medicine as antimicrobials on pathogenic microorganisms. Moreover, there has been an increasing interest in the radical scavenging activities of some natural antioxidants, especially those found in medicinal plants, which may play a role in preventing these various chronic diseases. There is also increasing need to search for new compounds with cytotoxic activity as the treatment of cancer with the available anticancer drugs is often unsatisfactory due to the problem of toxicity to the normal cells [4].

Amaranthus spinosus Linn are erect, monoecious perennial, up to 1 m. Stem are terete or obtusely angular, glabrous or slightly pubescent, green, reddish-brown, glabrous, and branched. The leaves alternate and are simple without stipules; petiole is approximately as long as the leaf blade. The blade shape is ovate-lanceolate to rhomboid, acute and often slightly decurrent at base, obtuse, rounded or slightly ret use and often short mucronate at apex, entire, glabrous or slightly pubescent on veins when young [5].

The plant is used in the treatment of abdominal pain, chicken pox, dysentery, dysurea, fever, hysteria, malaria, mania, tonsillitis and vomiting. Recent studies showed antidiabetic property of the plant. In many parts of Africa, it is in nutritional deficiency disorders and various other diseases [6]. The usage of herbal preparations for the management of diseases have been gaining momentum since the turn of the century and there have been concern about the safety of some of the plants used in the folkloric medicine, thus this study was carried out to determine the antibacterial and cytotoxic activities of *Amaranthus spinosus* extract on selected human pathogens and brine shrimp respectively.

2. MATERIALS AND METHODS

2.1 Plant collection, identification and preparation

Fresh *Amaranthus spinosus* whole plants were harvested from the compound of Rufus Giwa Polytechnic, Owo, Ondo State Southwest Nigeria. The plant material was identified at the herbarium section of the Department of Crop Production Technology and a voucher specimen (HX-1308Wx) was deposited. The authenticated plant materials were washed and cleaned thoroughly with tap water and then air-dried under shade. The dried samples were then ground into coarse powder with the aid of a mechanical grinder and were stored in clean air- tight containers, and kept in a cool, dry place until required for use. One hundred gram (100 g) of the powdered sample was soaked in 300 ml of ethanol for 72 hr with intermittent stirring using sterile spatula. The plant extracts were then filtered through Whatman No 1. Filter paper into bijou bottles and then dried using rotary evaporator at a temperature of 50°C to yield crude extracts. Different concentrations of the extracts were prepared by diluting 0.10g, 0.20g, 0.30g, 0.40g and 0.50g of the extracts in 100ml of 0.01% Tween-20 to obtain concentrations of 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml and 50mg/ml respectively [7].

2.2 Test microorganisms

The bacteria used in the research were five clinical isolates (*Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Salmonella typhi*) obtained from the Federal Medical Center, Owo, Nigeria

2.3 Reagents /chemicals

All reagents and chemicals were of analytical grade and were obtained from the Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria.

2.4 Experimental Design

2.4.1 In vitro antibacterial susceptibility test

The extracts obtained from the test plants were screened against the test bacteria by agar well diffusion method. A 25ml aliquot of Mueller-Hinton agar (MHA, Lab Oratorios Britania, Argentinia) was poured into each Petri dish. When the agar solidified, the pathogenic test organisms were inoculated on the surface the plates (1×10^6 *cfu*/ml) using a sterile glass spreader. Subsequently, the surface of the agar was punched with 6mm diameter cork borer into wells and a portion of 50µl of each of the extract concentrations was filled into the wells. Control wells containing the same volume of 30% Dimethyl sulphoside (DMSO) served as negative control, while Chloramphenicol (50µg) was used as positive control for the plates respectively and the plates were incubated at 37^0C for 24 h. Each experiment was carried out in triplicate and the diameter of the zones of inhibition was then measured in millimeters.

2.4.2 Minimum inhibitory concentration (MIC)

The MIC of the plants extracts were determined by double dilution broth method. Twofold serial dilutions of the extracts were prepared in Mueller-Hilton broth to achieve a decreasing concentrations ranging from the least concentration that produced clear zone of inhibition. All tubes with the controls were labeled accordingly. Each dilution was seeded with 1ml of standardized inoculums (1.0×10^6 *cfu*/ml) and incubated at 37^0C for 24 hr. A tube containing only seeded broth (i.e. without plant extract) was used as the positive control while the un-inoculated tube was used as negative control. The lowest concentration of each extract that showed a clear of inhibition was when compared with the controls was considered as the MIC [8].

2.5 Determination of cytotoxic effect of plant extracts

The brine shrimp (*Artemia salina*) lethality bioassay was carried out according to the method described by [9] and modified by Opawale et al. [8]. Brine shrimp eggs were hatched in salt water prepared by dissolving 38g of salt in 1 liter of distilled water to mimic sea water. Then, it was filtered and put in shallow container. A transparent plastic divider with tiny pores was used to separate the container into separate compartments. Brine shrimp eggs were placed in one side of the compartment while the other compartment was illuminated artificially. After 48h, nauplii were collected by using pipette from the lightened side. Samples were then prepared by dissolving 20mg each of the extracts in 2mls of DMSO from where further diluted concentrations of 1000, 100, 10 and 1 ppm were prepared. A 4ml portion of the artificial sea water was added into each test tube and 20 shrimps were transferred into it. This was followed by the addition of 1ml of each of the test extracts and of previously prepared concentrations and maintained under illumination at room temperature. Survivors were

counted with the aid of magnifying glass after 24h. The percentage mortality was calculated using Abbot's formula and the LC_{50} was also determined [10].

2.6 Data Analysis

Data were presented as mean±standard error of mean (SEM). Significant difference between different groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 10 version 25.0 software. The significance was determined at the level of $p< 0.05$.

3. RESULTS AND DISCUSSION

Medicinal plants are rich in secondary metabolites which are potential sources of drugs and of therapeutic importance. The evaluation of various plants according to their traditional uses and medical values based on their therapeutic efficacy lead to the discovery of newer and recent.

Table1: Antimicrobial activity of aqueous extract of *Amaranthus spinosus* on selected pathogens

Conc. (mg/ml) Organisms	10	20	30	40	50	DMSO(30%)	Chl(100µg/ml)
S. aureus	0.00±0.00^{a}	0.00±0.00^{a}	4.67±0.00*^{b}	7.00±0.02*^{c}	11.00±0.08^{d}	0.00±0.00^{a}	28.33±0.01^{e}
E. coli	0.00±0.00^{a}	2.33±0.00^{b}	6.33±0.06^{c}	11.33±0.17^{d}	14.33±0.04^{e}	0.00±0.00^{a}	26.00±0.05^{f}
E. faecalis	0.00±0.00^{a}	5.67±0.02*^{b}	8.00±0.11^{c}	11.33±0.05^{d}	13.67±0.01^{d}	0.00±0.00^{a}	31.00±0.18*^{e}
K. pneumoniae	0.00±0.00^{a}	3.67±0.01^{b}	7.33±0.16^{c}	10.00±0.02^{d}	12.33±0.02^{d}	0.00±0.00^{a}	25.00±0.10^{e}
S. typhi	0.00±0.00^{a}	0.00±0.00^{a}	2.33±0.00*^{b}	4.67±0.05*^{c}	7.33±0.08*^{d}	0.00±0.00^{a}	23.33±0.00^{e}

Values are Mean±S.E.M (mm), Values followed by different alphabet along the rows and * along columns are significantly different at $p\leq0.05$. drugs for treating various ailments. This facts forms the basis for the development of new drugs from various plant sources. Pharmaceutical studies have also accepted the value of medicinal plants as potential source of bioactive compounds and serve as lead compounds in antimicrobial discovery [11].

The results of the antibacterial activities screening of *A. spinosus* extracts is presented in Table 1. The inhibitory activity was concentration dependent since higher activities correlates with increase in concentration. None of the test bacteria were susceptible to the extracts at the lowest concentration used (10 mg/ml) whereas at the highest concentration used (50mg/ml), there were appreciable activity of the extracts against the test organisms with *E. coli* recording the highest zone of inhibition (14.33mm) against the extract while *S. typhi* (7.33mm) recorded the least zone of inhibition. These inhibition zones are significantly lower than that of the control antibiotics used. These results are in line with the observation of Maiyo et al. [12] who reported antimicrobial activity of *A. spinosus* against *E. coli* (14mm), *Pseudomonas* (13mm), *Staphylococcus* (10mm), *Paracoccus* (9mm), and *Klebsiella* (15mm) at similar concentrations. In another study, Ishrat et al. [13] reported that *A. spinosus* extracts showed good antibacterial activity against both gram positive and gram negative bacteria with average zone of inhibition 8-15mm.

Table 2: Minimum inhibitory concentration of *Amaranthus spinosus* extract on selected pathogens (mg/ml)

Organism	MIC
S. aureus	25
E. coli	20
E. faecalis	15
K. pneumoniae	20
S. typhi	30

The demonstration of antimicrobial activity against both Gram positive and Gram negative bacteria suggests that the plant may possess some antimicrobial agent with potential wide spectrum activity and may explain why the plant is used in the management of various disease conditions in traditional medical practices in Nigeria. The susceptibility of *Staphylococcus aureus* which is known to have a very high ability to acquire resistance to antibiotics to the plant extracts suggest that the plant could be further purified to produce very useful antibiotic agents. The minimum inhibitory concentration (MIC) has been described as the least concentration of the extracts that inhibit growth of test organisms [14]. It is employed as a diagnostic tool because it helps in confirming resistance of microorganisms to antimicrobial agents. The MIC obtained in this

study ranged between 15 mg/ml and 30 mg/ml (Table 2) which are encouraging for crude extracts and it suggests that potent antimicrobials may be developed from the plant when purified.

Table 3: Percentage mortality of brine shrimps at different concentrations of the extracts of *Amaranthus spinosus*

Dosage (ppm)	Initial larvae	No. of survivors	No. of deaths	% mortality
1000	20	7	13	65
100	20	11	9	45
10	20	17	3	15
1	20	20	0	0
LC_{50}				**472.18**

In the cytotoxicity assay, brine shrimp lethality test was adopted which was based on the ability of tested samples to kill laboratory cultured brine shrimp (*Artemia salina*) nauplii. The assay is considered a useful tool for preliminary assessment of toxicity since the brine shrimp is highly sensitive to a variety of chemical substances and it represents a rapid in-exposure and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumour properties [13].

Percentage lethality of brine shrimp at four different concentrations (1, 10, 100, 1000 ppm/ml) of *A. spinosus* extracts showed that higher concentration of extract leads to higher mortality of the shrimps as 0, 15, 45 and 65 % mortality was recorded at the respective concentration. This observation corroborates the reports of Joshua et al. [15]. The LC_{50} value of obtained for this plant extract against the brine shrimp naupli was 472.18 ppm which falls within the range of moderately toxic compounds. Acoording to Moshi et al. [16] LC_{50} values higher than 1000 ppm/ml are not significant while those within the range of 0-100 ppm/ml are considered to be very toxic. Earlier reports on brine shrimps assay that are already available [13, 16-17] suggests that plant extracts with LC_{50} values of 20mg/ml have a likelihood of yielding anticancer compounds. Our result is at variance with the report of Ishrat et al. [13] who determine the cytotoxicity activities of *A. spinosus* extracts and Vincristine Sulphate was used as positive control. The LC_{50} values of standard vincristine sulphate, chloroform, n-hexane and ethyl acetate extract they obtained were 7.55µg/ml, 18.15 µg/ml, 29.51µg/ml and 18.15µg/ml respectively.

4. CONCLUSION

Based on the foregoing, it may beconcluded that Amaranthus spinosus contain bioactive substances which exhibited some level of antibacterial activity against both gram negative and gram positive organisms and cytotoxic effect on brine shrimps with moderate toxicity level. Therefore, the therapeutic potential of the plant as claimed in the folkloric African traditional medicinal practice is justified.

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