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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF LEAVE EXTRACT OF A*maranthus spinosus*

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Abstract Identification of the constituents of *Amaranthus spinosus* was carried out using ethanol extract of the dried leaf of the plant. Microbial detection of zone of inhibition was also carried out. The extract yield from the leaves was 17.40 g while phytochemical screening indicated the presence of saponins, alkaloids, flavonoids, terpenoids and glycosides in the leaf extract. The antimicrobial assay indicated that methanolic extract of the plant were slightly active against the test isolates with the extract being more active against *Staphylococcus aureus* (15mm), *Aspergillus flavus* (15mm), *E. coli*. (13mm) and *Mucor spp* (10mm). Hence methanol extract of this plant has potent medical values.

Key words: *Phytochemical, antimicrobial, Amaranthus spinosus*, leaf extract

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1.0 Introduction

Medicinal plants are well known for their role in complementing the fight against diseases in humans (Kavitha *et al.,* 2010). Medicinal plants can be important source of previously unknown chemical substances with potential therapeutic effects (Vadnere *et al.,* 2013). In spite of modern development of sophisticated pharmaceutical chemicals for the treatment of illnesses, medicinal plants remain an important source of therapy for some diseases (Kavitha *et al.,* 2010). Due to their unique properties, a large number of plants are constantly being screened for their possible medicinal value (Ishrat *et al.,* 2011). Healing powers of herbal medicines have been realized since antiquities. About 65% of the world populations have access to local medicinal plant and the knowledge of their applications. Most medicinal plants contain good number of compounds that have diverse medicinal values (Das, 2012). One of such plants is *Amaranthus spinosus* commonly known as "spiny amaranth" it has been reported to have some pharmacological properties such as antidiabetic, antipyretic, anti-inflammatory, antioxidant and hepatoprotective etc. *Amaranthus spinosus* is useful in curing internal bleeding, diarrhea and bleeding arising from excessive menstruation (Srivastava, 2011)

2.0 Materials and Methods

Methanol sulphuric acid (H_2SO_4), dimethyl sulfoxide (DMSO), hydrochloric acid (HCl), Mayer's reagent, ferric chloride, magnesium, chloroform, ammonium solution and Fehling solution all the reagent are of analytical grade. Distilled water was used throughout the

2.1 Sample collection and preparation

Fresh leaves of *Amaranthus spinosus* were collected from Sharada vegetables garden, Sharada phase 1 Kano. The sample was identified by a botanist in the Department of Biological sciences Bayero

University Kano (as Amaranthus spinosus ;*Alaiyahu* in Hausa). The samples were air dried to a constant weight before they were grounded to a powder form. Methanol extraction of the plant's material was carried out by soaking 200 grams of the sample in 500 ml of absolute methanol and allowed to stand for 72 hours at a temperature of 37 ^{o}C. The extracts were filtered first through cotton wool, then through Whatman filter paper No.1 (125 mm) and the resultant crude extract where dried further in a desiccator.

2.2 Preparation of test reagent

2.2.1 Mayer's reagent

Mercuric chloride (1.36 g) was dissolved in 60 mL of distilled water, potassium iodide (5 g) was dissolved in 20 mL of distilled water and the two solutions were mixed. The volume of the solution was increased to 100 mL.

2.2.2 10% ferric chloride (w/v)

10 g of ferric chloride powdered was dissolved in a 100 ml volumetric flask using distilled water. The content was stirred to a homogeneous state before making it up to 100 ml mark with distilled water.

2.2.3 2% hydrochloric acid (v/v)

2 ml of concentrated hydrochloric acid was dissolved in 98 ml of distilled water to obtained 20 % HCl solution.

2.3 Phytochemical Analysis

Phytochemical screening for the presence of tannins, alkaloids, glycosides, flavonoids, and phenolic was performed using the following procedures.

2.3.1 Test for alkaloids

The extracts were evaporated to dryness and the residues were heated on a boiling water bath with 2% hydrochloric acid. It was cooled, filtered and treated with the Mayer's reagent. The treated sample was observed for the presence of yellow precipitation or turbidity (Das, 2012).

2.3.2 Test for flavonoids

1.5 ml of 50% methanol was added to 4 ml of the extracts. After warming magnesium fillings was added followed by addition of a few drops of concentrated hydrochloric acid. The presence of flavonoids was observed by a pink or red (Das, 2012).

2.3.3 Test for terpenoids

5 ml of various solvent extract was mixed in 2 ml of chloroform followed by addition of 3 ml concentrated (H_2SO_4). A layer of the reddish-brown coloration was formed at the interface thus indicating a positive result for the presence of terpenoids

2.3.4 Test for tannins

A portion of the extract was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution were added. A blue or green color indicated the presence of tannins (Fransen *et al.*, 2001).

2.3.5 Test for saponins

Saponin frothing test was adopted. A **s**mall quantity of the methanol extract was boiled, filtered and 2.5 ml of the filtrate was added to 10 ml of the distilled water in a test tube and was well shaken 2 minutes. Frothing was observed (Das, 2012).

2.3.6 Test for glycosides

In a methanol extract Fehling's reagent was added and boiled for 2 minutes. A brick red coloration indicated the presence of glycosides.

2..3.7 Test for steroid

0.5 g of solvent extract was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid A change from violet to blue or green indicated the presence of steroids.

2.4 Antimicrobial test

Microbial strains: *Staphylococcus aureus* and *Escherichia coli*, together with two pathogenic fungi (*Aspergillus flavus* and *mucor specie*) were used as microbial strains. The organisms were obtained from the Department of Microbiology, Bayero University, Kano. These bacterial and fungal strains were cultured at 37^{o}C and 28^{o}C overnight in an incubator (Memmert Germany).

2.4.1 Preparation of sensitivity discs

Whatman No. 1 filter paper discs (of 6mm in diameter) were punched out with the aid of punch and place in bijou bottles. They were then sterilized by autoclaving at 121°C for 15 minutes. The discs were allowed to cool.

2.4.2 Preparation of stock solution

0.06g of extract was dissolved in 1ml of solvent (DMSO). Half of the extract (0.5 ml) was introduce in to 50 sterile discs in Bijou bottle to make 60μg/disc concentrations 0.5ml of DMSO was added to the remaining stock solution to make up to 1ml. 0.5 ml was taken and placed into another bottle containing 50 filter paper discs and labeled 30μg/disc, 0.5 ml of DMSO was added, another 0.5 ml was taken and placed into another 50 filter paper discs and labeled 15μg/disc.

2.4.3 Disc diffusion method

The antimicrobial activity of the prepared extracts was determined by using disc diffusion method (Mitra, 2013). The inoculated extracts were then examined for inhibition zones (in mm) by zone reader, which indicated antimicrobial activity. The disc (6 mm in diameter) were impregnated with 60, 30 and 15µg/ml, sample extracts and placed in inoculated agar. Ciprofloxacin (10 µg/disc) and ketoconazole (15 µg/disc) were used as positive control for bacteria and fungi, respectively.

2.0 Results and Discussion

The results obtained from the extraction indicated that *Amaranthus spinosus* yielded gummy texture and greenish extract. 250 g of the plant leaf yielded 17.4 0g of the organic extract, which translate to 6.96 % yield. The results obtained for phytochemical analysis (Table 1) revealed the presence of medicinally active phytochemicals which included alkaloids, tannins, saponins, glycosides and flavonoids. Some of these metabolites particularly the flavonoid might have been identified as vital in antimicrobial activity of most medicinal plants (Mitra, 2013)

Table 1: Phytochemical constituent of methanol extract of *Amaranthus spinosus* leaf

Phytochemical	Test Results
Alkaloids	+
Terpenoid	+
Steroid	-
Flavonoids	+
Tannins	+
Saponins	+
Glycosides	-

****+ = Presence of the phytochemical constituent, - = absent**

The antimicrobial assay showed that methanolic extract of the plant were slightly active against the test isolates and was more active against *Staphylococcus aureus* (15 mm) and *Aspergillus Flavus* (15 mm) *E. coli* (13 mm), *Mucor spp*. (10 mm) as recorded in Table 2.

Table 2: Antimicrobial sensitivity test of *Amaranthus spinosus* against bacterial and fungal isolation

Isolates	Control (µg/disc)			
Extract (µg/disc)	60 (mm)	30 (mm)	15 (mm)	10 and 15 (mm)
Staphylococcus aureus	15	11	-	30
Escherichia coli	13	11	-	25
Aspergillus Flavus	15	11	-	2
Mucor specie	10	6	-	20

The leaf extract of *Amaranthus spinosus* showed considerable antimicrobial activities in the disc diffusion assay. Quantitative estimation of antimicrobial activity of *Amaranthus spinosus* leaf extracts against food-borne and pathogenic microorganisms are shown in Tables 2. The trend for antifungal activity was closely similar to that of antibacterial activity except that the efficacy towards fungal strain was not much effective as for bacterial strains. The current results support the earlier findings which demonstrate the presence of antimicrobial activity in leaves of *Amarathaceae* (Jiang *et al.*, 2012).

4.0 Conclusion

The demonstration of the activity against different category of pathogenic bacteria and fungi by the leave of *Amaranthus spinosus* is a scientific justification of the local application of the plant as a healthy remedy. The plant can also be used in the treatment of diarrhea and other bacterial infections.

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