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Research Article

ASSESSMENT OF CYTOTOXICITY, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF THE LEAF EXTRACTS OF ABELMOSCHUS ESCULENTUS CULTIVATED IN NORTHERN NIGERIA

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ABSTRACT

Background: Okra (*Abelmochus esculentus*) belongs to Malvaceae family and is widely cultivated in Africa, India, America, and Brazil for its fibrous fruits containing round, white seeds. This study was carried out to assess the cytotoxicity, antioxidant and antimicrobial activities of the leaf extracts of Abelmoschus esculentus cultivated in north-western, Nigeria.

Methods: The plant sample was collected, washed, identified, grounded into powder and extracted using solvent extractor with ethanol as solvent, and this was followed by fractionation using three more solvents of different polarities (starting with n-hexane, ethyl acetate, and then methanol). The cytotoxicity of the extracts was assessed using Brine Shrimp Lethality assay; Antioxidant activity of the extracts was studied using DPPH radical scavenging assay method, while antimicrobial activity was determined using agar well diffusion method.

Results: The cytotoxicity results showed n-hexane having highest activity (LC50 394.499 µg/ml), followed by methanol extract (LC50 538.098 µg/ml), ethyl acetate extract (LC50 2106.499 µg/ml), and then crude extract (LC50 5634.091 µg/ml) respectively. The results also revealed that the extracts possessed significant antioxidant activity, with crude extract showing highest activity (IC50 = 74.229 µg/ml), followed by methanol extract (IC50 = 83.396 µg/ml), ethyl acetate extract (IC50 = 126.159 µg/ml), and then n-hexane extract (IC50 = 138.936 µg/ml). The extracts revealed remarkable antimicrobial activity and were even more noticeable in antibacterial result at higher concentrations with ethyl acetate extract showing largest zone of inhibitions, followed by n-hexane extract, crude extract, and then methanol extract respectively.

Conclusion: From the findings of this research study we have concluded that the leaf extracts Abelmoschus esculentus possesses antimicrobial and antioxidant activities and is also less toxic.

Keywords: Abelmoschus esculentus, Antimicrobial Activities, Cytotoxicity, Radical Scavenging, Solvents

INTRODUCTION

The significance of medicinal plants to human health cannot be overemphasized. This is because they contain bioactive phytochemicals that have specifically crucial physiological impact on human body systems [1]. The most essential of these bioactive constituents of plants are alkaloids, saponins, tannins, flavonoids, and phenolic compounds. Some of these medicinal plants are used as medicines while others play a great role as food spices [2]. Owing to the prevalence of many common drug-resistant strains and the more recent emergence of strains with reduced antibiotic resistance, there is exist spectrum of incurable infection. Because of these instances, there is a need to search for new methods that can combat infection with safer therapeutic agents than what is already known [3].

Abelmoschus esculentus belongs to the family of Malvaceae which is a warm-seasonal herbaceous vegetable crop grown in tropical and subtropical countries [4, 5]. In developing countries like Nigeria, Okra proves noticeable among other fruits and vegetables on account of its remarkable medicinal, nutritional, exportability, and adaptability applications [6, 7]. Okra fruits are a good source of mucilage, fats, fibers, minerals, ascorbic acid, carotene, vitamins, proteins, and carbohydrates [8, 9]. Okra provides good source of calcium and other body building minerals that contributes to healthy living. Okra fruit provide numerous health benefits which are useful in treating cardiovascular disease, digestive disease, genitor urinary disorders, coronary heart diseases, diabetes, and chronic dysentery [5]. In northern-western region of Nigeria Okra is commonly used almost by the majority of the population as medicines, food spices and other things especially in soup-making, but unfortunately the scientific studies on the cytotoxicity, antioxidant and antimicrobial activities of the plant are scanty in the literature. So this research is done to fill this gap.

MATERIAL AND METHODS

Instruments and Reagents

Apparatus (glass wares) used in this study were thoroughly washed with detergent solution and distilled water and were dried thoroughly before use. These materials were sterilized in a portable laboratory autoclave at 1210C for 15 minutes [10].

Sample Collection and Preparation

The fresh and healthy leaf samples of okra were collected from Katsina State, Nigeria. The plant specimen was identified by the department of plant biology, Bayero University, Kano, Nigeria. The leaf samples of the plant were washed with distilled water to remove debris and contaminants, shade-dried and grounded into powder using mortar and pestle [10-11].

Extraction of the plant sample

The extraction of the plant was carried out using standard procedure. A 100 g of the plant's powdered material was extracted using sohlet extractor for 9 hours at a reduced temperature. The extract was filtered using Whatman filter paper and dried under fan. This was labeled as crude extract (AE001). The crude extract was fractionated using n-hexane and labeled as AE002. The crude extract was further fractionated using ethyl acetate which was labeled as AE003. The crude was finally extracted with methanol and labeled AE004 respectively [10].

Brine Shrimp Lethality Essay

Clean test tubes were taken and labeled. The plant extract of 10 mg was weighed by an analytical balance. Then stock solution was prepared by dissolving 10 mg of plant extract in 1 ml of sea water. Concentrations of 300 µg/ml, 100 µg/ml, and 10 µg/ml were prepared from the stock solution using serial dilution method and put in a glass vials. Two drops of DMSO were added into each glass vial (36 glass vials and 1 control) and then 10 brine shrimp larvae were counted and put into each glass vials and 3 ml of distlled water was piured in each sample. These were allowed to stand for 24 hours under constant aeration and at room temperature and adequate light source. The number of dead nauplii was counted and after the 24 hours [12].

Antioxidant Screening

Free radical scavenging activity of the fractions of Abelmoschus esculentus in this study was screened by 2, 2 - diphenyl -1-picryl hydrazyl (DPPH). A solution of 0.1mM of the DPPH was prepared by dissolving 39.4 g of DPPH in 100 ml methanol. The concentrations of the extracts were made by dissolving 5 mg of each extract in 5ml of ethanol (5 mg/5 ml = 1 mg/ml) to create 1000 µg/ml stock solution. Concentrations 1000 µg/ml, 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, and 10 µg/ml were prepared from the stock solution using serial dilution method. The mixture of sample and DPPH was put in a 96 well plate and incubated for 30 minutes. Absorbance was then measured at 517 nm by using spectrophotometer (UV-VIS Shimadzu). Reference standard compound used was Ascorbic acid, and the experiment was done in triplicates. Mixture of methanol and DPPH without the sample was taken as negative control. The IC_{50} values of the fractions and the standard were calculated from their % inhibitions using IC_{50} software (IC_{50}.kt). The percent DPPH scavenging effect was calculated by the use of the formula below [13]:

$$\%\ inhibition = \frac{A_0 - A_1}{A_0} \times 100$$

Where A0 is the absorbance of negative control, and A1 is the absorbance of the sample

Antimicrobial Activity Test

Collection of pathogenic organism

The test Organisms consisting of two bacteria (Staphylococus aureus and Escherichia coli) and two fungi (Aspergillus fumigatus, and Mucov spp) were obtained from Microbiology unit, Aminu Kano Teaching Hospital and preserved in Microbiology Department Bayero, University, Kano, Nigeria [10].

Standardization of the Inoculum

A suspension of the test bacteria was made by emulsifying loop full of colony into test tube containing normal saline solution. Inoculum density of the bacterial suspension was adjusted to that of 0.5 ml McFarland standard [14].

Preparation of the Stock Solution

Stock solution was prepared by mixing 16 mg of each extract in 2 ml of dimethyl sulfoxide (DMSO) to give 8 mg/ml. Concentrations of 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, and 10 µg/ml of the extract and fractions were prepared using the standard dilution formula (C1V1 = C2V2). Gentamycin (20 µg/ml) and Nystatin (50 µg/ml) were used as standards for bacterial and fungi respectively. Negative

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control was a solvent of dilution (Dimethyl Sulfoxide, DMSO) [15].

Bioassay Procedure

After sterilization, the media were allowed to cool at 40 to 450C and poured into petri-dishes. The petri-dishes were labeled with culture name and the fractions to be used. A sterile cotton-wool swab (swab stick) was dipped into the standardized bacterial and fungal suspension to seed the entire surface of the agar media. By the use of sterile Cork borer, 5 wells (6 mm in diameter) were stroke at the center of the each agar medium of each petri-dish. The wells created were then filled with the prepared fractions and incubated at 370C for 24 hours. The zones of inhibition, (in millimeters) of each fraction at different concentrations were measured using a ruler [16].

RESULTS

Brine Shrimp Lethality Assay

Table 1: Brine Shrimp lethality assay of the extracts

Extracts	Conc. (µg/ml)	T1 (24h) Mortality	T2 (24h) Mortality	T3 (24h) Mortality	Total Mortality	LC_{50} (µg/ml)
AE001	1000	4	3	5	12	5634.091
	100	2	4	4	10	
	10	1	3	2	6	
AE002	1000	7	3	8	18	394.499
	100	5	3	3	11	
	10	1	2	0	3	
AE003	1000	4	3	6	13	2106.499
	100	2	5	3	10	
	10	2	2	1	5	
AE004	1000	8	2	6	16	538.098
	100	6	2	4	12	
	10	2	3	1	6	

Antimicrobial activity

Table 2: Antibacterial zones of inhibition of the extracts in millimeters

Key = Value of Gentamycin control was 15.60 mm

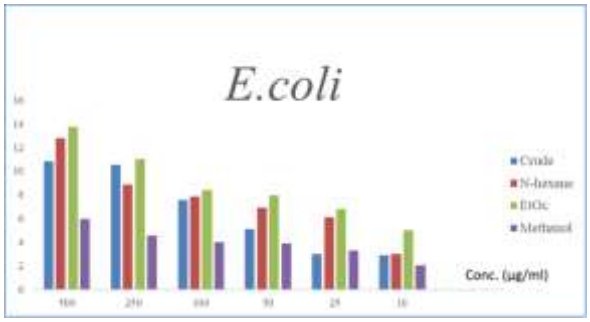

Fig 1: Graph of zone of inhibition against concentration of E. coli bacteria

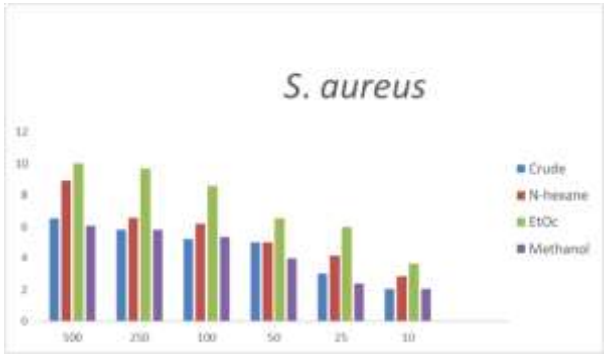

Fig 2: Graph of zone of inhibition against concentration of S. aureus bacteria.

Table 3: Antifungal activity of the extracts in millimeters

Extracts/ Fractions	Fungi	Conc. (µg/ml) 10	25	50	100	250	500	Control (mm)
AE001 (mm)	*A. Fumigatus*	1.66	2.90	3.15	4.22	7.87	8.58	16.40
	Mucov Spp	0.88	2.10	2.54	3.63	5.21	6.70	17.95
AE002 (mm)	*A. Fumigatus*	0.15	1.50	1.90	2.52	2.70	3.93	16.40
	Mucov Spp	0.10	0.88	1.25	1.78	2.00	2.85	17.95
AE003 (mm)	*A. Fumigatus*	0.55	1.32	2.86	4.00	7.85	7.90	16.40
	Mucov Spp	0.65	1.90	2.00	4.16	6.79	7.45	17.95
AE004 (mm)	*A. Fumigatus*	0.30	1.47	1.95	3.34	4.90	5.53	16.40
	Mucov Spp	0.15	1.00	1.17	3.51	3.78	4.00	17.95

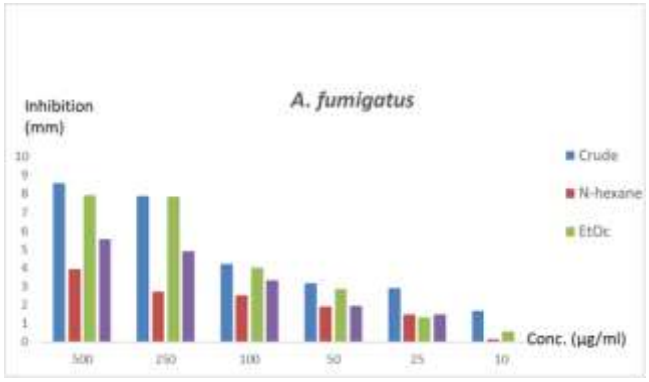

Fig 3: Graph of zone of inhibition of the extracts against concentration of A. fumigatus

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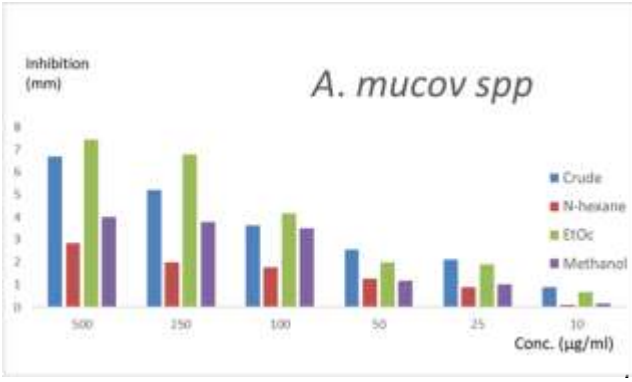

Fig 4: Graph of zone of inhibition of the extracts against concentration of Mucov spp

Antioxidant activity.

Table 4: Absorbance of the standards and extracts in percentage (%) and their respective Inhibition Concentrations (IC50) in microgram per mil (µg/ml)

Conc. (µg/ml)	1000	500	250	100	50	25	10	IC_{50} (µg/ml)
Vit. C (mm)	97.41	97.21	97.02	96.08	96.93	72.28	39.76	9.442
AE001 (mm)	92.33	82.11	68.05	50.15	35.86	31.77	25.09	74.229
AE002 (mm)	92.46	62.93	44.07	34.66	33.37	32.76	26.04	138.936
AE003(mm)	78.59	67.16	56.12	40.24	34.01	32.75	27.77	126.159
AE004 (mm)	92.65	72.02	54.01	45.48	39.22	37.09	29.26	83.396

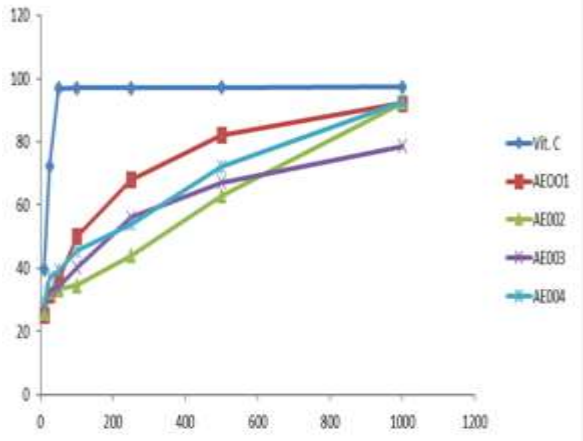

Fig 5: Graph of absorbance against concentration of the standard and the extracts

DISCUSSION

Brine Shrimp lethality assay is an important tool for the preliminary cytotoxicity assay of the plant extracts based on the ability to kill a laboratory cultured larvae (nauplii). It is a simple and inexpensive bioassay used for testing the efficacy of the phytochemical present in the plant extracts. Table 1 contains the results of cytotoxicity study of the plant extracts. The results shows that n-hexane shows highest activity with an LC50 394.499 µg/ml, followed by methanol extract (LC50 538.098 µg/ml), ethyl acetate (LC50 2106.499 µg/ml), and crude extract (LC50 5634.091 µg/ml) respectively.

Antibacterial activity of a molecule is completely associated with the compounds that provincially kill bacteria or slow down their growth rate without being extensively toxic to nearby tissues. The results of antibacterial activity of the extracts against bacterial isolates were contained in table 2 and figures 1, 2 and 5 respectively. The results showed that the leaf extracts of Abelmoschus esculentus have significant antibacterial activity especially at higher concentrations. The results showed that Escherichia coli exhibit greater microbial resistance than the Staphyloccocus aureus for all the fractions and extract except for methanol extract. On the other hand, ethyl acetate extract exhibits greater antibacterial activity (larger zone of inhibition), followed by n-hexane extract, crude extract, and then methanol extract respectively

The result of antifungal activity of Okra leaf extracts was presented in table 3 and figures 3, 4 and 5 respectively. The result showed moderate antifungal activity with crude extract showing highest activity (larger zone of inhibition), followed by ethyl acetate extract, methanol extract and then the least was n-hexane extract respectively. Aspergillus fumigatus showed greater resistance than the Mucov Spp in this study.

Antioxidant compounds are molecules that fight free radicals in the body system. These free radicals, when too much in the

body, are linked to multiple diseases, including diabetes, heart disease, and cancer. The body has its own antioxidant defenses to keep free radicals in check, while more antioxidant compounds are consumed from plants by humans. The table 4 showed that the extracts of okra leaves have antioxidant activity, with crude extract showing highest antioxidant activity (lowest IC50, = 74.229 µg/ml), followed by methanol fraction (IC50 = 83.396 µg/ml), ethyl acetate extract (IC50 = 126.159 µg/ml), and then n-hexane extract ((IC50 = 138.936 µg/ml) respectively.

CONCLUTION

From the present study, it was found that extract and fractions of Abelmochus esculentus possessed good antioxidant and antibacterial effects with minimal cytotoxicity. The findings can be also an indicator that Okra leaf extracts may contain phytochemicals such as polyphenols that may have powerful biological effects in the body.

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