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# ANTIMICROBIAL ACTIVITY OF *Manihot esculanta* ROOT METHANOLIC AND AQUEOUS EXTRACTS

**[1]Shama I. Y. Adam, [1]Wasma A. A. Ahmed, [1]Esra, O.M., [1] Nazik,E. Y., [1]Eiman.F. A, Shaimaa. A. A and [2]Warda S. Abdelgadir**

[1]Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, Al Neelain, University, P.O. Box 12702, Khartoum, Sudan.

[2]Food Research Centre, P.O. Box 213, Khartoum North, Sudan

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***Correspondence for Author**
**Warda S. Abdelgadir**
Food Research Centre, P. O.Box 213, Khartoum North, Sudan.

## ABSTRACT

An experiment was conducted to assess the antimicrobial activity of methanolic and aqueous extract of *Manihot esculanta* root. Dried ground powdered bulb and peel of *M. esculanta* was evaluated against four Gram –ve bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonae and Salmonella typhimurium*), one Gram +ve bacteria ( *Staphylococcus aureus*) and one yeast (*Candida albicans*) using the agar well diffusion method. At all concentrations (100, 50, 25 and 10%), the bulb methanolic extract exhibited antibacterial activity against all the test pathogenic bacteria with varying length of inhibition zones which were concentration dependant. *K. pneumonae* was found to be the most sensitive bacteria even to the peel methanolic extract at 100 and 50 %. *C. albicans* was found to be sensitive to both methanolic of the peel at concentration of 100% and aqueous extract of the bulb at 100 and 50%. These results were comparable with those produced by gentamicin (10µg) a reference antibiotic and nystatin (10mg/ml) a reference antifungal.

**Keywords:** Manihot esculenta**,** Antimicrobial activity, Staph. aureus, E. coli, Ps. aeruginosa,S. typhimurium, C. albicans.

## INTRODUCTION

*Manihot esculanta*, also known as cassava, is a tuberous woody shrub of the Euphorbiaceae (spurge) family. In Africa Asia and Latin America, millions of people depend on cassava as a

major staple food for its edible starchy tuberous root and in Sudan the tuber is known as Bafra and is widely spread in the Southern part of Sudan. Cassava is the third largest source of food carbohydrates in the tropics, after rice and maize (Claude and Denis, 1990). It is one of the most drought-tolerant crops, capable of growing on marginal soils. Nigeria is the world's largest producer of cassava, while Thailand is the largest exporting country of dried cassava (FAO, 1995).

The raw cassava tuber contains alkaloids, flavonoids, tannins, reducing sugars and anthocyanosides, but do not contain cardiac glycosides, anthraquinone, phlobatinnins and saponins (Bahekar and Kale 2013).The plant also contains various antioxidant like â-carotene, and vitamin A (Fasuyi, 2005; Okeke and Iweala, 2007). In addition, the root is a good source of calcium, phosphorus and iron and an appreciable amount of vitamin C and a nutritionally significant quantity of riboflavin, thiamine and niacin (Bradbury and Holloway, 1988). The water content for cassava is in the range of 60.3% to 87.1% (Padonou *et al*., 2005). Although extremely low (1-3%), cassava protein quality is relatively good in terms of essential amino acids (Katz and Weaver 2003). About 50% of the crude protein in the roots consists of whole protein and the other 50% is free amino acids (predominantly glutamic and aspartic acids) and non-protein components such as nitrite, nitrate and cyanogenic compounds (Zvinavashe *et al*., 2011).

Cassava is infamous as a food crop because it can be toxic to consume due to the availability of free and bound cyanogenic glucosides (linamarin and lotaustralin). These are converted to cyanide in the presence of linamarase, a naturally occurring enzyme in cassava. Cyanogenic glucosides can be found throughout the plant and in all varieties of cassava (Katz and Weaver 2003).

In African countries, many species of *Manihot esculenta* were reported to be antiseptic, demulcent, diuretic and is a folk remedy for abscesses, boils, conjunctivitis, diarrhea, dysentery, flu, hernia, inflammation, snakebite, sores, spasm, swellings of testicles (James, 1983). Medicinal plants have become the focus of intense study in terms of validation of their traditional uses through the determination of their actual pharmacological effects. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection fighting strategies to control microbial infections (Bhaskarwar, 2008). The number of studies have been conducted in different countries to prove such efficiencies (Salihu and Garba , 2008).

The present study was conducted to evaluate the antimicrobial activity of methanolic and aqueous extracts of *Manihot esculenta* different parts, bulb and peel.

## MATERIALS AND METHODS

### Plant material

The *M. esculent* roots were purchased from a central market in Khartoum (Fig.1.) and authenticated by the scientists at the Aromatic and Medicinal Plants Research Institute, Khartoum, Sudan. The roots were cleaned, washed and peeled. The bulbs were cut into pieces and together with the peel were shade dried. Then the samples (bulb and peel) were turned into powder using an electric grinder.

**Fig. 1 *Manihot esculanta* tubers**

### Standard microorganisms

The microorganism used in the present study were kindly provided by the scientists at Khartoum National Health Laboratory and designated as follows:

*Staphylococcus aureus* - ATCC/25923

*Escherichia coli* - ATCC/27853

*Klebsiella pneumonae*- ATCC/3565

*Pseudomonas aeruginosa* - ATCC/2785

*Salmonella typhimurium*- NCTC/25953

NCTC National Collection Type Culture, London, UK.

ATCC American Type Culture Collection, Manassas, AV, USA.

**Standard antibiotics**

Gentamicin (Roussel Laboratories Ltd, England)

Nystatin (Sigma Chemical Company, USA

**Methods**

**Preparation of crude extracts**

This was carried out using the soxhlet extraction technique according to Kaushik *et al*., (2007). A quantity of 300 g, of bulb and peel were accurately weighed and suspended into a 500 ml -capacity conical flasks. Extraction was done by using petroleum ether (60 –80°C) and methanol (99.8%). These extracts were concentrated for further studies at reduced temperature and pressure in a rotary evaporator.

The plant residues were further extracted with distilled water over night at room temperature (25-30°C), filtered through WhatmanNo.1 filter paper and freeze dried for further use. Stock solutions of the extracts were prepared by reconstituting 5 mg of each extract in 5 ml of dimethyl sulphoroxide (DMSO), in a container to make 100% concentration, then 10, 15 and 50% dilutions were prepared.

**Preparation of media:** Bacterial strains were maintained on Mueller Hinton agar, and fungi on Potato dextrose agar. Nutrient broth for liquid media and the peptone water for the serial dilution, all were prepared according to the manufacturer instructions.

**Antimicrobial activity tests:** Antimicrobial activity of the crude extracts was determined by Agar-well diffusion assay methods.

**Agar-well diffusion method:** Under sterile condition swabs were dipped into the broth culture of the organism. Gently squeezed against the inside of the tube to remove excess fluid; Mueller-Hinton agar plates and potato dextrose agar plates were streaked in three direction to insure complete spread of the organisms then the plates were left (5min) to stand. Three equidistant wells of 10 mms in diameter made on the agar using a sterile cutter. The wells were then labeled with the code numbers of the test crude extracts and controls. 0.1ml of dimethyl sulphoroxide (DMSO) in one well as the negative control and 0.1ml of standard antibiotics (Gentamicin for bacteria, Nystatin for fungi) were added in two wells as positive control. 0.1ml of each extracts were added in three well. The treated plates were stored for at least 1 hour to allow diffusion of the extracts into the agar while arresting the growth of the

test microbes. The plates were then incubated for 24 hours at 37°C for bacteria and 48 hours at 28°C for molds. Antimicrobial activity was determined by measuring the diameters of zones of inhibition using a ruler after three replicates, averages and the mean values were tabulated.

**Evaluation of antimicrobial activity of Gentamicin and Nystatin**

This was estimated by agar well diffusion method (Kingsbury and Wagner, 1990). 1 ml of 24 hr of incubated broth culture was distributed in to Mueller –Hinton agar. Gentamicin (Abtech Biological Ltd, England) at concentration of 10µg was used as reference drug and distributed in the centre of Mueller –Hinton agar and the plates were kept for 1 hr at room temperature. The plates were then incubated in the upright position at 37°C for 18 hr. After incubation, the diameters of inhibition zones were recorded.

**General characteristics of standard organisms**

The bacteria used in this study were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella pneumonae* and the fungus *Candida albican*.

***Staphylococcus aureus***

Gram-positive, non-motile, non- spore forming, aerobic, facultatively anaerobic organism, 0.5 mm in diameter. It occurs singly, in pairs or irregular and many of the strain form a golden yellow pigment on colony of good growth on ordinary media. Catalse and urease positive, oxidase and indol negative.

***Escherichia coli***

Gram-negative rod, 1.1-1.5 mm wide and 2.0-6.0 mm long with rounded ends and shape varying from coccoid to rod, motile, aerobic, facultatively anaerobic oxidase and urease negative, citrate cannot be used as a sole carbon, most strain are fermenters of methyl red positive, catalase and indol positive and VP negative.

***Pseudomonas aeruginosa***

Gram-negative bacillus, non- spore forming, non- capsulated, motile by one or two polar flagella aerobic, facultatively anaerobic, grow on a wide variety of culture media, catalase positive.

***Salmonella typhimurium:*** This bacterium is Gram-negative, straight rod, aerobic, facultative anaerobic, motile with peritriehus flagella, non acid and gas formation from glucose and mannitol, formation of $H_2S$, indole, urease and (VP) negative.

***Klebsiella pneumonae:*** Gram-negative, non-motile straight rods, 0.3-1.0×0.06-6.0 µm, arranged singly, in pairs or in short chain; often surrounded by a capsule, facultatively anaerobic, having both a respiratory and a fermentative metabolism.

***Candida ablicans:*** It is yeast that is part of the normal body flora and can be found on the skin, mouth, vagina and intestines. Under normal circumstances, *C. albicans* lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis, which may also occur in the blood and the genital tract.

## RESULTS

### Extract yield of *M. esculenta* bulb and peel

The 300 g of *Manihot esculanta* bulb and peel powders yielded 9.2 and 4.3 when extracted by methanol and 13.6 and 8.3 when extracted by water respectively (Table 1).

### Antimicrobial activity of the methanolic extract

The results showed that the antimicrobial activity of the bulb and peel extracts increased with increased concentration of the extract. The antimicrobial results of the methanolic extract of the bulb and the peel are presented in Table 1. The methanolic extract of the bulb showed growth inhibition to all tested bacteria with varying degrees of inhibition zones. *K. pneumonae* was the most sensitive organism showing the highest inhibition zones of 31 (Fig 2) and 15 mm in the highest (100%) and lowest (10%) extract concentrations respectively, followed by *Staph. aureus* with an inhibition zone of 20 mm at 100% (Fig 3). The activity of this extract against *C. albicans* was weak showing an inhibition zone only at the concentration of 100% of 10.5 mm indicating the resistance to *C. albicans* to the methanolic extract. The concentrations of 25 and 10 % of the methanolic extract of the peel were not active against the test organisms including the yeast which resisted even the concentration 50%. Again *Klebsiella pneumonae* was the most sensitive to the peel methanolic extract showing the biggest inhibition zone (23.5 mm) (Fig 4).

**Evaluation of antimicrobial activity of aqueous extracts of of *M. esculenta* bulb and peel:** The results of antimicrobial activity of aqueous extracts of *M. esculenta* against the test

organisms are presented in Table 3. *E. coli* and *Ps. aeruginosa* resisted all concentrations of the aqueous extracts of the bulb and the peel. At 100 and 50 % the aqueous extract of the bulb was active against *Staph. aureus, K. pneumonae , S. typhi.* and *C. albicans* (Fig 5) which was the only sensitive test organism to the aqueous extract of the peel at 100% causing an inhibition zone in the range of 10.5 mm.

**Table1. Extracts yield and inhibition zones (mm) of methanolic extracts of *M. esculenta* bulb, and peel**

| Sample | Yield (%) | Extract conc. (%) | Mean diameter of inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | ***Staph. .aureus*** | ***E. coli*** | ***Ps. aeruginoa*** | ***K. pneumonae*** | ***S. typhimurium*** | ***C. albicans*** |
| *M. esculenta* bulb | 9.2 | 100 | 20 | 16.5 | 17 | 31 | 15 | 10.5 |
| | | 50 | 17.5 | 13 | 12.5 | 25 | 12 | R |
| | | 25 | 13.5 | 11.5 | 9.5 | 16.5 | 10.5 | R |
| | | 10 | 9.5 | 7.5 | 8.5 | 15 | 8.5 | R |
| *M. esculenta* peel | 4.3 | 100 | 9.5 | 14.5 | 15 | 23.5 | 12 | 16 |
| | | 50 | 7.5 | 10 | 11.5 | 16 | 8.5 | R |
| | | 25 | R | R | R | R | R | R |
| | | 10 | R | R | R | R | R | R |

**R= Resistant = No inhibition zone.**

**Table. 2. Extract yieldand inhibition zones (mm) of aqueous extract of *M. esculenta* bulb and peel**

| ***Sample*** | **Yield (%)** | **Extract conc. (%)** | **Mean diameter of inhibition zone (mm)** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | ***Staph .aureus*** | ***E. coli*** | ***Ps. aeruginoa*** | ***K.pneumonae*** | ***S. typhimurium*** | ***C. albicans*** |
| *M. esculenta* bulb | 13.6 | 100 | 11 | R | R | 16 | 17 | 19.5 |
| | | 50 | 8.5 | R | R | 14 | 9.5 | 16 |
| | | 25 | R | R | R | R | R | R |
| | | 10 | R | R | R | R | R | R |
| *M. esculenta* peel | 8.3 | 100 | R | R | R | R | R | 10.5 |
| | | 50 | R | R | R | R | R | R |
| | | 25 | R | R | R | R | R | R |
| | | 10 | R | R | R | R | R | R |

**R= Resistant = No inhibition zone.**

**Gentamicin and Nystatin:** The results shown in Table 4. indicated that gentamicin had antibacterial activity against the test bacteria with inhibition zones *S. aureus*, (23 mm), *E.*

*coli*, (21 mm), *Ps. aeruginosa*, (23 mm) and *S. typhi*, (20.5 mm) and that nystatin had activity against *C. albicans* at (31.5 mm) inhibition zone.

**Table 3: Antimicrobial activity of Gentamicin and Nystain against standard organisms.**

| Antibiotic | Conc. | Inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **S. typhimurium** | ***Ps. aeruginoasa*** | ***E. coli*** | ***Staph. auerus*** | ***K.Pneumonia*** | ***C. albicans*** |
| Gentamicin | 10 µg/ml | 20.5 | 23 | 23 | 23 | 23 | R |
| Nystain | 10 mg/ml | R | R | R | R | R | 31.5 |

R = Resistant = No inhibition zone.

Conc. = concentration

**Fig. 2. Inhibition zone (31 mm) produced by 100% methanolic extract of the bulb against *K. pneumonae.***

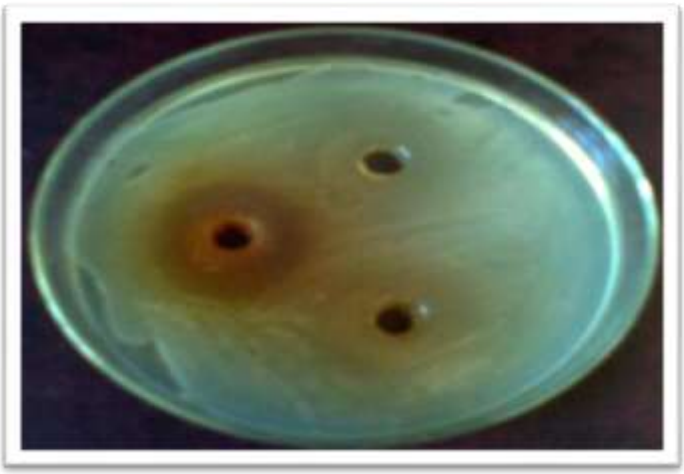

**Fig. 3. Inhibition zone (20 mm) of 100% methanolic extract of *M. esculent* bulb against *Staph. aureus***

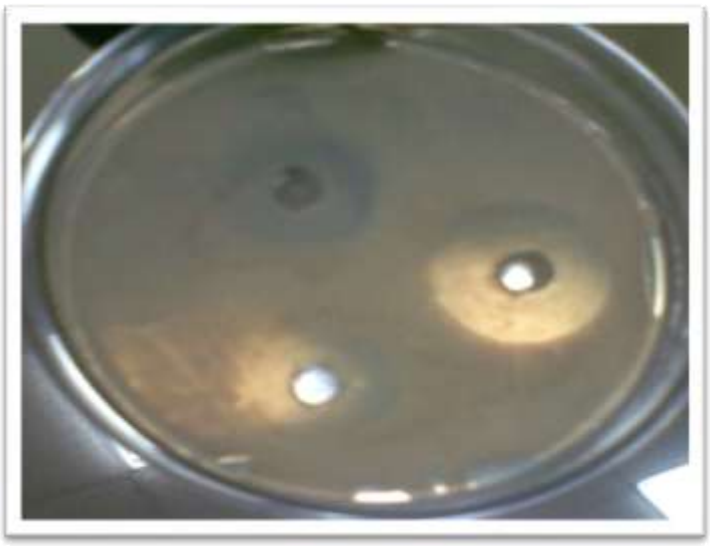

**Fig. 4. Inhibition zone (23.5 mm) produced by methanolic extract (100 %) of peel of *M. esculenta* against *K. pneumoneae***

**Fig. 5. Inhibition zone (19.5) produced by 100% aqueous extract of the bulb of *M. esculenta* against *C. albicans***

## DISCUSSION

Traditional medicines play an important role in the treatment of millions of people and have been the main source of drugs in many countries (1). The medicinal properties of these plants are due to presence of different chemical substances or active ingredients which are accumulated in different parts of the plant (Koochak *et al.,* 2010)). The plant extracts contain such a wide range of these substances including flavonoides, tannins, terpenoids, glycosides and alkaloids which can act as antimicrobial agents and sources of other natural compounds

important for other biological functions (Dwivedi *et al.*, 2011)). The presence of more bioactive phytochemical compounds in *M. esculanta* may increase the antimicrobial activity of the extract, since it is known that the active compounds produced by plants inhibit the life process of microbes especially the disease causing ones (Luc and Amold 2005; Abiy *et al.*, 2005). The combined effects of these compounds produce a synergistic effect thus increasing the antibacterial efficiency of *M. esculanta* methanolic extract. Flavonoides and tannins have also been found to be responsible for the antibacterial activity of many Indian and Nigerian plants (Ahmad and Beg 2003; Ogundare and Olorunfemi 2007).

In this study, the antimicrobial activity of *M. esculanta* bulb and peel methanolic and aqueous extracts has been compared with that of Gentamicin, a well known aminoglycoside antibiotic, and Nystatin, a popular antifungal drug (Table 3). The antibacterial activity of the methanolic extract of the bulb at 100 and 50% and of the peel at 100% concentrations against *K. pneumonae* excelled that of gentamicin at 10 µg and that of the peel was almost equivalent to 100% concentration against *Staph. aureus*. This may justify the traditional uses of the plant in the treatment of bacterial infections.

The bulb and peel methanolic and water extracts has moderate to weak anti-yeast activity against *C. albicans* however the aqueous extract at 100% showed the strongest anti-yeast activity than the other concentrations. It was reported that many plant extracts very effective against yeast like cardamom seed (Kubo *et al.*, 1991), anise seed (Kanauchi *et al.*, 1999) and Atung fruit extracts (Moniharapon and Hashinaga, 2004).

Antimicrobial activity may involve complex mechanisms such as inhibition of cell wall, nucleic acid and protein synthesis and inhibition of nucleic acid metabolism (Oyaizu *et al.*, 2003).

It was concluded that, at all extract concentrations the methanolic extract of *M. esculanta* bulb was effective against all tested pathogenic bacteria. Weak antifungal activity of the methanolic extract of the bulb and the aqueous extract of the peel at 100% was exerted against *C. albicans*.

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