

Antimicrobial Properties of Tiger Nut (*Cyperus Esculentus*) Extract on Some Clinical Isolates

Iyevhobu KO^{1,2,3*}, Okparaku SO¹, Abbulimen AS^{1,3}, Obohewemu KO^{3,4}, Adeji AJ⁵, Mahmud LH⁵, Akhadelor RB⁵, Braimah MN⁶, Abdulazeez B⁵, Alabi OI⁷ and Akharo G¹

¹Department of Medical Microbiology, Faculty of Medical Laboratory Science, College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

²Department of Medical Laboratory Science, Edo State University, Iyamho, Edo State, Nigeria.

³Saint Kenny Diagnostic and Research Centre, Ekpoma, Edo State, Nigeria.

⁴Department of Interdisciplinary Research and Statistics, PENKUP Research Institute, Birmingham, United Kingdom.

⁵Department of Microbiology, School of Applied Sciences and Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria.

⁶Department of Environmental Biology, School of Applied Sciences and Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria.

⁷Department of Tourism Management Technology, School of Applied Sciences and Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria.

*Corresponding authors

Iyevhobu KO, Department of Medical Microbiology, Faculty of Medical Laboratory Science, College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Received: August 21, 2026; Accepted: September 07, 2026; Published: September 14, 2026

Abstract

Cyperus esculentus (Tiger nut) has been described as a root tuber. It is from the family Cyperaceae. *Cyperus esculentus* is a monocotyledonous plant and belongs to the family Cyperaceae. This study investigated the antimicrobial properties of tiger nut (*Cyperus esculentus*) extracts against selected pathogenic bacteria. The aim was to evaluate the antibacterial efficacy of different solvent extracts (ethanol, hot water, cold water, hydrochloric acid, acetic acid, and isopropyl alcohol) and to determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Clinical bacterial isolates, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Morganella morganii*, were obtained from Irrua Specialist Teaching Hospital and identified using standard microbiological techniques such as Gram staining and biochemical tests. Extracts of tiger nut were prepared using standard extraction procedures and tested for antibacterial activity using the disc diffusion method. The results revealed that aqueous extracts (hot and cold water) exhibited greater antibacterial activity compared to ethanol extracts. Zones of inhibition were observed notably against *Staphylococcus aureus*, *Escherichia coli*, and *Morganella morganii*, while *Pseudomonas aeruginosa* showed resistance to all extracts. The MIC values ranged from 50 to 100 mg/mL for susceptible organisms, while MBC values indicated bactericidal effects mainly at higher concentrations. The findings suggest that the antimicrobial activity of tiger nut extracts may be attributed to the presence of bioactive phytochemicals such as phenolic compounds. In conclusion, tiger nut extracts possess moderate antibacterial activity, particularly in aqueous forms, and could serve as a potential source of natural antimicrobial agents. However, further studies are recommended to isolate active compounds and evaluate their clinical applicability.

Keywords: Antimicrobial, Tiger nut, *Cyperus esculentus*, MIC, MBC

Introduction

Cyperus esculentus (Tiger nut) has been described as a root tuber. It is from the family Cyperaceae. *Cyperus esculentus* is a monocotyledonous plant and belongs to the family Cyperaceae which is made up of over 4000 species [1]. Its common names include; tiger nut, Aya, chufa sedge, yellow nut sedge and earth almond. It is a perennial grass that grows in wet areas and often

occurs as a weed especially on farmlands used for cultivation of vegetables [2]. In Africa, tiger nut is mostly cultivated in the west, Ivory Coast, Ghana, Mali, Niger, Nigeria, Senegal and Togo where they are used primarily uncooked as a side dish [3]. It has long been recognized as one of the best nutritional crops used to augment diets, since a substantial intake decreased reported cases of various health related conditions such as cardiovascular disease, diabetes, cancer, and obesity, and also ideal for children, older persons and sportsmen [4], as well as an excellent source of iron and calcium for body growth and development [5].

Citation: Iyevhobu KO, Okparaku SO, Abbulimen AS, Obohewemu KO, Adeji AJ, et al. Antimicrobial Properties of Tiger Nut (*Cyperus Esculentus*) Extract on Some Clinical Isolates. J Clin Virol Trans Res. 2026. 1(1): 1-9. DOI: doi.org/10.61440/JCTV.2026.v1.03

Tiger nuts (*Cyperus esculentus*), popularly known in Northern Nigeria as "Aya" are believed to have potent antimicrobial effects over a wide range of infections. Apart from their nutritional values, traditionalists and consumers of native medicine have used these two nut fruits for medical exploits [6]. Food nutraceuticals and antimicrobials have added high health values as they present compounds in foods that hinder microbial growth or kill microorganisms [7]. Tiger nut has also been reported as healthy and it helps in preventing heart attacks thrombosis and activates blood circulation. It helps in preventing cancer, due to high content of soluble glucose. It was also found to assist in reducing the risk of colon cancer [8], typically, 100g tiger nuts contain 386kcal. As 7% proteins, 26% fats (oils), 31% glucose. They contain 26% fiber of which 14% is non-soluble and 12% soluble. Research studies have shown that tiger nut oil has golden color, nutty taste, low acid value (0.45 mg/g), saponification value (192.88 mg/g, iodine value (77.08 mg/g and peroxide value (3.69 meq/kg) which makes it ideal for different uses. This makes the oil suitable for salad making. It produces a sweet almond-like tuber which is highly appreciated for their health benefits and nutritive value [9]. Also, *Cyperus esculentus* contain alkaloid, resins, tannins, sterols and saponins [10]. Furthermore, *Cyperus esculentus* was reported to contain high content of fiber, protein, sugar, oleic acid, glucose, phosphorous, potassium, and vitamin C and E [11].

The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal. Antibiotics like penicillin and erythromycin, which used to be one-time miracle cures, are now less effective because bacteria have become more resistant. Tiger nut (*Cyperus esculentus*) is a tuber that is consumed widely in Nigeria and in various other parts of West and East Africa [12]. It is eaten raw or roasted, used as hog feed or pressed for its juice to make a beverage [13]. Tiger nuts are valued for their highly nutritious starch content, dietary fibre and carbohydrate and are rich in sucrose (17.4-20.0%), fat (25.5%), protein (8.0%). Tiger nut is also rich in mineral elements such as sodium, calcium, potassium, magnesium, zinc and traces of copper [10].

The increase in antimicrobial resistance has become a great threat in the current treatment of common infections [14]. The most remarkable is the fast-spreading multi-resistant bacteria species which cause different kinds of human and animal infections [15]. The World Health Organization recently declared antimicrobial resistance as one of the top 10 global public health threats [16].

Antimicrobial resistance has direct and severe consequences on both morbidity and mortality rates of humans and animals [17]. In effect, healing process is delayed, surgical operations and other medical procedures are at the risk of microbial infections, with the attendant surge in treatment cost [18]. According to the final report and recommendations of Jim O'Neill on tackling drug-resistance infections globally, about 700, 000 people die annually due to infections caused by drug-resistant microorganisms. This figure could rise to 10 million per year by 2050, if no proactive measure is taken [19]. Looking further into recent yearly accounts, about 33, 000 deaths with 1.1 billion Euros health care expenditures are recorded for European countries [20], while more than 35,000 lives have been lost

in the United States [21]. Again, the United States Centre for Medicare and Medicaid Services also reported that the spending rate on drug prescriptions increases to 3.7% in 2020. Meanwhile, the national health care estimate of the United State for 2020 is projected for \$4.01 Trillion, to reach \$6.19 Trillion by 2028. This will contribute to 19.7% increase in the United States National Gross Domestic Product (GDP) when compared to 2018 account. Obviously, these negative impacts have kept spreading beyond the health sector and would frustrate the present global sustainable development agenda of the United Nations over 2030 if nothing is done now [22]. Considering the current dryness in the supply of new antibiotics to combat the list of priority pathogens and the recommendations on the Global Action Plan issued by World Health Assembly [23], there is immediate need for quality-assured and cost-effective novel antibiotics remedies. The exploration of orthodox phytomedicine and a careful choice of innovative plants extracts for this demand is essential [24]. The knowledge of medicinal herbs is as old as man, whom in search of food and shelter discovered that the plants used as food could also cure them of certain ailments. Despite this primordial insight, identification of herbs appropriate for the treatment of a specific infection is apparently elusive [9]. Before now, several studies on the importance and therapeutic uses of plants have been done, which have continued to suggest a diverse plant species with such potentials [9]. Phytocompounds have a wide range of medicinal applications including anti-proliferative and antiparasitic effects. For example, artemisinin isolated from *Artemisia annua* showed activity against malaria and was proven to be effective against multi-resistant strains of *Plasmodium falciparum* which causes malignant cerebral malaria in human [25]. Shadia et al, (2010) reported that some herbs have been used as food additives, flavouring agents and natural food preservatives such as: rosemary, garlic, pepper, ginger, thyme etc. Herbs can also be used to treat asthma, eczema, premenstrual syndrome, rheumatoid arthritis etc. The herbs are considered safe to use as an antimicrobial agent, because they have been used for long time in the local traditions and their use is associated with minimal complications [26].

In the search for novel, safe and effective antimicrobials; natural products such as fruits and nuts have been long neglected. While many scientists have recently agreed that natural products with antimicrobial properties should be deeply investigated, the clinical efficacy have not been established. *Cyperus esculentus* have long been documented for their beneficial importance as they are rich in fiber, protein and natural sugars, minerals (phosphorus, potassium), including high levels of vitamins C and E [27,28]. In ayurvedic medicine tiger nuts are used in the treatment of flatulence, diarrhoea, dysentery, debility and indigestion [5,29-31]. Tiger nut oil has been in use by locals, in the cosmetic industry, as an antioxidant due to its vitamin E content. Unpublished data and inquiries from local cosmetologists in southeast Nigeria claim that tiger nut oil slows down ageing of the body cells, enhances skin texture and elasticity by reducing formation of skin wrinkles.

There are some studies on the chemical composition of the essential oil of *Cyperus* species. Essential oils of some *Syperus* species including *C. rotundus* [32,33], *C. difformis* L. And *C. arenarius* [34] were studied regarding their chemical composition. In addition, chemical compositions of the three

Egyptian *Cyperus* species; *C. articulatus*, *C. esculentus* and *C. papyrus* were showed by comparison [35]. In this study we showed the chemical composition of essential oil of *C. fuscus* and antimicrobial activity of ethanol extracts in the first time. The objective of the study is to assess the antimicrobial properties of Tiger nut (*Cyperus esculentus*) on some selected organisms.

Materials and Methods

Study Area

This study was carried out in Ekpoma. The administrative headquarter of Esan West Local Government Area of Edo State which lies between latitude 6.45°N to 6.75°N of the Equator and longitude 6.08°E to 6.13°E of the Greenwich Meridian with altitude of about 332m above sea level. It is made up of quarters including Eguare, Iruokpen, Emaudo, Ujoelen, Ihumudumu, Illeh, Uke, Uhie, Ujemen, Ukpenu, Idua, Ukhur, Egoro, Emehi, Igor and Idumebo [36]. Ekpoma has a population of 89,628 in 1991 and 127,718 in 2006, majority of which are civil servants, traders, business men/women, transporters, farmers, teachers/lecturers and students by occupation. A university (Ambrose Alli University) is situated in this town. The main sources of water in the locality are rainfall and wells. It has 2 distinct seasons, wet and dry seasons. The wet season occurs between April and October with peak in August, average rainfall ranging 150cm to 250cm. The dry season occurs between November and March with cold harmattan between December and January, average temperature of about 25°C [37,38].

Materials and Equipments

The materials and equipments used in this study include; Hot air oven, Incubator, Electric weighing balance (galencamp), Aluminum foil, Cotton wool, 10ml pipettes, 250ml conical flask, 500ml conical flask, 100ml measuring cylinder, Wire loop, Bursen burner, Nutrient agar, Macckonkey agar, Sterile distilled water, beakers, test tubes, sterile borer, separating funnel, what man's No 2 filter paper, ethanol, methanol ethyl acetate, Magnetic stirrer, 1% HCL, 5% acetic acid, Isopropyl alcohol, Filter paper and Filtering funnel.

Source of Tiger Nut

Fresh Tiger nuts (*Cyperus esculentus* L) were purchased from Ekpoma market in Esan West Local Government Area, Edo state, Nigeria. The Tiger nuts (*Cyperus esculentus* L) were identified by a taxonomist in the Department of Botany, Ambrose Alli University, Ekpoma, Edo Sate, Nigeria. The samples in sealed plastic bags were conveyed to the Medical Microbiology Laboratory, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo Sate, Nigeria.

Preparation of Successive Extracts

The *Cyperus esculentus* purchased were washed thoroughly with tap water and then with sterile distilled water and allowed to air dry at room temperature for 72 hr. The dried *Cyperus esculentus* were grounded into fine powder using electric blender. The fine powder was stored in an air-tight container at room temperature prior to extraction.

Ten (10) grams of powdered plant materials was mixed with 100ml of various solvents (1%Hydrochloric acid, 5%Acetic acid, water and Isopropyl alcohol) and left for 72 hours with

intermittent shaking. The extracts preparations were done as previously described by Alade & Irobi (1993) [38]. The extracts were filtered and then concentrated using rotary evaporator at 40°C, and each extract was transferred into well labelled sterile glass vials and stored at 4°C before use. The plant extracts were prepared by using soxhlet apparatus collected and stored in a vial for further studies.

1. About 500g of plant parts (Tiger nut (*Cyperus esculentus*)) was weighed and dried for 5days in a drying cabinet at 50°C.
2. The dried plant was grounded to powered using electric grinding machine
3. 10grams of the powered plant was weighed into four 250ml conical flasks. 100mls of each extraction solvent (1%Hcl, 5% acetic acid, water and Isopropyl alcohol (IPA) was added to the conical flasks respectively.
4. The conical flasks were placed on mechanical shaker and allowed to extract overnight.
5. The extracts were filtered into sterile conical flasks using Whatman No 1 filter paper.
6. The filtrates were used for the experiment while the deposits were discarded [39].
7. The yield of each extract thus obtained was recorded and stored in a refrigerator at 40C till further use. Different dilutions were prepared by serial dilutions to obtain 100%, 50%, 25% and 12.5% of Tiger nut (*Cyperus esculentus*) extracts concentration.

Cold-water Extraction

Exactly 20g of the fine powered fresh Tiger nut were soaked in 100ml of distilled water for 24 hrs. The pulp obtained was left in a clean, sterile glass container and shaken at 150 rpm for 8 hrs vigorously to allow for proper extraction and it was filtered using a sterile muslin cloth after which the extract was obtained, air-dried and stored below ambient temperature until required [40]. Same was done for hot water but for hot water, it was boiled and mix after boiling the water.

Determination of Purity of Extract

With the aid of 4mm wireloop, one loop extract solution was streaked inoculated onto nutrient agar and incubated at 37°C for 24hours. The absence of growth after overnight incubation indicates purity of extract solution. A 1 in 2 dilution of the extract solution was made, followed by a 1 in 20 dilution serial dilution of the extract solution was made as follows;

Protocol

- 1 in 2 dilutions of the extract solution was made in 5 test tubes.
- With the aid of a 50ml dropper pipette, 1 in 100 dilutions of an overnight both culture of the test organism was added to each test tube
- Controls were set up as follows; test tube with 1ml of plain broth C1 and 1 tube with 1ml of extract solution (C2) was incubated along with the teet.

Determination of Extract Concentration

The specific gravity, S.G of each diluent/solvent (1%HCL, 5% acetic acid, water and Isopropylalcohol (IPA)) was determined as follows:

1. The weight, W1 of the clean dried empty specific gravity, S. G bottle was taken.

2. The specific gravity bottle was filled with the diluents and the stopper was placed.
3. The overflowed fluid was cleared off from the body of the bottle with a filter paper.
4. The weight W₂ of the diluents with the S.G bottle was taken.
5. The weight W₃, of the diluent was gotten by subtracting the weight W₁ of the empty S.G bottle from the weight W₂ of the diluent with S.G bottle i.e W₃=W₂-W₁

The S.G of the diluents was determined by the expression below.

$$\text{Specific gravity} = \frac{\text{weight of solvent + S.G bottle} - \text{weight of empty S.G bottle}}{\text{volume of S.G bottle (V)}}$$

$$\text{S.G of solvent /diluents} \frac{W_2 - W_1}{V} = \frac{W_3}{V}$$

Where V= Vol. of S.G bottle = 25ml

In the same manner above, the S.G of each extract solution was also determined. The concentration of each extract solution was determined by the expression below;

Concentration of extract solution = specific gravity of extract solution – specific gravity of plain solvent = SG₂ – SG₁.

Source of Bacterial Isolates

The different test organisms were isolated from clinical samples of patient visiting Irrua Specialist Teaching Hospital (ISTH) located in Irrua, Esan Central Local Government Area, Edo State and were analyzed using the Microbiology Laboratory, Department of Medical Microbiology, Faculty of Medical Laboratory Science, College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State. The test organisms isolated for the study include; *Staphylococcus aureus*, *Klebsiella* species, *Escherichia coli* and *Salmonella* species.

The different organism isolated; *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus* species, *Proteus* species, *Pseudomonas* species, *Escherichia coli* and *Salmonella* species were sub-cultured into peptone water for 8 hours before antibiogram extract testing.

Identification of Test Organisms

All isolates for this study were identified by their colonial morphology, Gram stain reaction, biochemical test characterization and sugar fermentation test. Also using their colonial appearances on the media which include Size, Shape, Elevation, Opacity, Edge, Colour, haemolysis and fermentation. Gram staining and biochemical tests was carried out on culture that yielded growth using standard procedures.

Antibiotic Sensitivity Testing

Disc diffusion assay as described by Akinjogunla (2016) was the key process used in evaluating the antibacterial potential of tiger nut extracts [41]. The clinical isolates used were sub-cultured and incubated at 37°C for 24hrs. The antimicrobial discs were locally prepared by punching out disc 6mm in diameter from a good quality Whatman no 1 filter paper and were sterilized for 15 minutes with autoclave and kept in corked test tubes. The antibiotic media Mueller Hinton Agar (Oxoid, UK) was prepared and poured into Petri dishes. The sub-cultured isolates were then seeded on the solidified agar. Pre sterilized filter paper disc was soaked in the various concentrations for 30 minutes and allowed to dry. The impregnated disc was transferred aseptically to the

seeded plates. The bacterial isolates were first inoculated in peptone water and incubated for 8 hours and were sub-cultured on nutrient agar, excess broth were tilted off and allowed to dry. Using a sterile forceps, the prepared sterile discs were picked and impregnated with the various extract with different concentration (25mg/ml, 125mg/ml and 250mg/ml) in 0.01ml and the disc placed on the inoculated agar plate equidistant from each other alongside with two locally prepared antibiotic disc (Gentamycin (20 g/disc)) as positive control and dimethylsulfoxide (DMSO) as negative control. The seeded plates were kept for 30 mins before incubating in an upright position for 24 hours at 37°C in an incubator and Zones of inhibition (ZI) was measured by using ruler calibrated in millimeters.

Minimum Inhibitory Concentration (MIC)

Tube dilution method by Cowan & Steel (1965) [42]. The minimum inhibitory concentration gave the lowest concentration of the aqueous extract that can inhibit the growth of the bacteria isolates.

1. About 0.5ml prepared nutrient broth was dropped into the test tube 2 to 10, 0.5ml of the extract was added to tube 1 and 2.
2. Serial dilutions were made resulting to decreasing concentration of the aqueous extract.
3. The extract in tube 3 was diluted until tube 9 from which 0.5ml was discarded. 0.5ml of the test bacteria isolates were added to tube 2 to tube 10.
4. Tube 1 which contain nutrient broth and organism serves as control tube [43].
5. The entire procedure was done for the bacteria isolates that were susceptible to the extracts with zone of inhibition above 10.
6. The tubes were thoroughly mixed and incubated at 37°C for 24hours after which they were examined for visible growth which is seen as turbidity.
7. The MICs reported as the lowest concentration of the Tiger nut (*Cyperus esculentus*) extracts that prevent visible growth [44].

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal of Tiger nut (*Cyperus esculentus*) extract was determined with little modification, by obtaining samples in the MIC assay and Sub-cultured onto freshly prepared nutrient agar medium and incubated at 37°C for 48 hours. Plates containing fresh solidified nutrient agar were seeded with inoculums from tubes with no turbidity in the MIC setup and incubated at 37°C for 24 hours. The plates with the least concentration of the extract from MIC that shows no growth was recorded as the MBC.

Data Analysis

The data generated was analyzed statistically using the mean±SD to ascertain the significance of the study.

Results

Table 1 shows the antibacterial activities of fresh tiger nut (*Cyperus esculentus*) cold water, hot water and ethanol extract against test bacteria isolates. *Staphylococcus aureus* showed no zone of inhibition from the ethanol control and ethanol extract, but showed zone of inhibition of 10mm and 9mm for cold water

and hot water extract respectively. *Escherichia coli* showed no zone of inhibition from the ethanol control and ethanol extract, but showed zone of inhibition of 17mm and 16mm for cold water and hot water extract respectively. *Pseudomonas aeruginosa* showed no zone of inhibition in all the extract. *Morganella morganii* showed no zone of inhibition on the ethanol extract, but showed zone of inhibition of 12mm on the ethanol control and, 15mm on the cold water and hot water extract.

Table 2 shows the antibacterial activities of dried tiger nut (*Cyperus esculentus*) cold water, hot water and ethanol extract against test bacteria isolates. *Staphylococcus aureus* showed no zone of inhibition on the ethanol control and cold-water extract, but showed zone of inhibition of 4mm and 12mm for ethanol extract and hot water extract respectively. *Escherichia coli* showed no zone of inhibition from the ethanol control, cold water and hot water extract, but showed zone of inhibition of 6mm for ethanol extract. *Pseudomonas aeruginosa* showed no zone of inhibition in all the extract. *Morganella morganii* showed no zone of inhibition on the hot water extract, but showed zone of inhibition of 12mm, 5mm and 1mm on the ethanol control, ethanol extract and cold-water extract respectively.

Table 3 shows the Minimum Inhibitory Concentration (MIC) of Fresh Tiger Nut Hot Water Extract against Test Organisms. *Staphylococcus aureus* and *Escherichia coli* had an MIC of

100mg/mL each while *Morganella morganii*, had MIC of 50mg/mL.

Table 4 shows the Minimum Bactericidal Concentration (MBC) of Fresh Tiger Nut Hot water extract against test organisms. It was observed that *Morganella morganii* had an MBC of 100mg/mL while *Staphylococcus aureus* and *Escherichia coli* had significant growth (0mg/mL).

Table 5 shows the Minimum Inhibitory Concentration (MIC) of Fresh Tiger Nut cold water Extract against test organisms. *Staphylococcus aureus* had an MIC of 100mg/mL each while *Escherichia coli* and *Morganella morganii* had MIC of 50mg/mL.

Table 6 shows the Minimum Bactericidal Concentration (MBC) of Fresh Tiger Nut cold water extract against test organisms. It was observed that *Escherichia coli* and *Morganella morganii* had an MBC of 100mg/mL while *Staphylococcus aureus* had significant growth with an MBC of 0mg/mL.

Table 7 shows the Minimum Inhibitory Concentration (MIC) of dried Tiger Nut Hot water extract against test organisms. *Staphylococcus aureus* had an MIC of 100mg/mL each while *Escherichia coli* and *Morganella morganii* had MIC of 0mg/mL. Therefore, no MBC was done on the extracts.

Table 1: Antibacterial Activities of Fresh Tiger Nut Extracts Against Test Bacteria Isolates

Test Organisms	Zones of inhibition (diameter in mm) Extraction type			
	Ethanol control	Ethanol (70%) Extract	Sterile dH ₂ O Cold Extract	Sterile dH ₂ O Hot Extract
<i>Staphylococcus aureus</i>	0	0	10	9
<i>Escherichia coli</i>	0	0	17	16
<i>Pseudomonas aeruginosa</i>	0	0	0	0
<i>Morganella morganii</i>	12	0	15	15

Key: 0 = No zone of inhibition

Table 2: Antibacterial Activities of Dried Tiger Nut Extracts Against Test Bacteria Isolates

Test Organisms	Zones of inhibition (diameter in mm) Extraction type			
	Ethanol control	Ethanol (70%) Extract	Sterile dH ₂ O Cold Extract	Sterile dH ₂ O Hot Extract
<i>Staphylococcus aureus</i>	0	4	0	12
<i>Escherichia coli</i>	0	6	0	0
<i>Pseudomonas aeruginosa</i>	0	0	0	0
<i>Morganella morganii</i>	12	5	1	0

Key: 0 = No zone of inhibition

Table 3: Minimum Inhibitory Concentration (MIC) of Fresh Tiger Nut Hot Water Extract Against Test Organisms

Test Organisms	Concentration of Extracts (100 – 0.195 mg/mL)									
	100	50	25	12.3	6.25	3.125	0.781	0.391	0.195	MIC (mg/mL)
Staphylococcus aureus	-	+	+	+	+	+	+	+	+	100
Escherichia coli	-	+	+	+	+	+	+	+	+	100
Morganella morganii	-	-	+	+	+	+	+	+	+	50

Key: - = No turbidity; + = Turbidity

Table 4: Minimum Bactericidal Concentration (MBC) of Fresh Tiger Nut Hot Water Extract Against Test Organisms

Test Organisms	Concentration of Extracts (100 – 0.195 mg/mL)									MBC (mg/mL)
	100	50	25	12.3	6.25	3.125	0.781	0.391	0.195	
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	0
Escherichia coli	+	+	+	+	+	+	+	+	+	0
Morganella morganii	-	+	+	+	+	+	+	+	+	100

Key: - = No turbidity; + = Turbidity

Table 5: Minimum Inhibitory Concentration (MIC) of Fresh Tiger Nut Cold Water Extract Against Test Organisms

Test Organisms	Concentration of Extracts (100 – 0.195 mg/mL)									MIC (mg/mL)
	100	50	25	12.3	6.25	3.125	0.781	0.391	0.195	
Staphylococcus aureus	-	+	+	+	+	+	+	+	+	100
Escherichia coli	-	-	+	+	+	+	+	+	+	50
Morganella morganii	-	-	+	+	+	+	+	+	+	50

Key: - = No turbidity; + = Turbidity

Table 6: Minimum Bactericidal Concentration (MBC) of Fresh Tiger Nut Cold Water Extract Against Test Organisms

Test Organisms	Concentration of Extracts (100 – 0.195 mg/mL)									MBC (mg/mL)
	100	50	25	12.3	6.25	3.125	0.781	0.391	0.195	
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	0
Escherichia coli	-	+	+	+	+	+	+	+	+	100
Morganella morganii	-	+	+	+	+	+	+	+	+	100

Key: - = No growth; + = Significant growth

Table 7: Minimum Inhibitory Concentration (MIC) of Dried Tiger Nut Hot Water Extract Against Test Organisms

Test Organisms	Concentration of Extracts (100 – 0.195 mg/mL)									MIC (mg/mL)
	100	50	25	12.3	6.25	3.125	0.781	0.391	0.195	
Staphylococcus aureus	-	+	+	+	+	+	+	+	+	100
Escherichia coli	+	+	+	+	+	+	+	+	+	0
Morganella morganii	+	+	+	+	+	+	+	+	+	0

Key: - = No turbidity; + = Turbidity

Discussion

Tiger nut (*Cyperus esculentus*) and *Irvingia gabonensis* (bush mango) have medicinal properties which have been harnessed by traditional medicine practitioners, but only a few of these properties have been proven scientifically. In this study, the result of the antibacterial activity of ethanol, hot and cold-water extracts of *Cyperus esculentus* against test bacterial isolates showed slight antibacterial activity. The result correlates with the report of Kiran et al, (2015) which stated that the aqueous extracts inhibited the test bacterial isolates at different concentrations of 10, 20, 30, 40 mg/L etc [45].

Staphylococcus aureus showed no zone of inhibition from the ethanol control and ethanol extract, but showed zone of inhibition of 10mm and 9mm for cold water and hot water extract respectively. *Escherichia coli* showed no zone of inhibition from the ethanol control and ethanol extract, but showed zone of inhibition of 17mm and 16mm for cold water and hot water extract respectively. *Pseudomonas aeruginosa* showed no zone of inhibition in all the extract. *Morganella morganii* showed no zone of inhibition on the ethanol extract, but showed zone of inhibition of 12mm on the ethanol control and, 15mm on the cold water and hot water extract. And *Staphylococcus aureus*

showed no zone of inhibition on the ethanol control and cold-water extract, but showed zone of inhibition of 4mm and 12mm for ethanol extract and hot water extract respectively. *Escherichia coli* showed no zone of inhibition from the ethanol control, cold water and hot water extract, but showed zone of inhibition of 6mm for ethanol extract. *Pseudomonas aeruginosa* showed no zone of inhibition in all the extract. *Morganella morganii* showed no zone of inhibition on the hot water extract, but showed zone of inhibition of 12mm, 5mm and 1mm on the ethanol control, ethanol extract and cold-water extract respectively. According to Junior & Zani (2000), diameter of the inhibition zone: <9 mm is inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active [46]. Thomas et al., (1996) equally suggested that the plant extract disc showing an inhibitory zone greater than (8mm) were considered as sensitive to the particular pathogen [47]. From the results obtained, Sterile dH₂O Cold fresh Tiger nut extract showed significant antimicrobial activity at higher concentrations against *Staphylococcus aureus*, *Escherichia coli* and *Morganella morganii*, Sterile dH₂O Hot fresh Tiger nut extract showed significant antimicrobial activity at higher concentrations against *Escherichia coli* and *Morganella morganii* while Sterile dH₂O Hot dried Tiger nut extract showed significant antimicrobial activity at higher concentrations against

Staphylococcus aureus based on the criteria suggested by Junior & Zani, (2000) and Thomas et al., (1996) [46,47]. On the other hand, the efficiency of these extracts against the bacteria could in part be because of their phenolic composition [48,49]. The inhibitory effect of phenolic compounds could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation [50]. However, further purification and isolation of the active compounds in these extracts are required for pharmacological evaluation because tiger nut may be a potential source of a new type of antibiotic.

The antibacterial activity of ethanolic extract of *Cyperus esculentus* showed no antibacterial effect against all the test bacterial isolates for the fresh ethanol tiger nut but showed a weak sensitivity for the dried ethanol extract. This finding contradicts the reports of Arikpar & Alade (2020) and Prakash & Ragavan (2009), which showed that ethanolic extract of *Cyperus esculentus* has antibacterial activity against the bacterial isolates tested [51,52]. The minimum inhibitory concentration (MIC) revealed that the Fresh Tiger Nut Hot Water Extract all showed good inhibitory activity with the highest MIC of 100 mg/ml against *Staphylococcus aureus* and *Escherichia coli* while *Morganella morganii* had MIC of 50 mg/ml. This observation aligns with the study of Georgios et al, (2010), that revealed interesting and promising antibacterial activity of Tiger Nut [53]. Furthermore, the the Minimum Bactericidal Concentration (MBC) of Fresh Tiger Nut Hot water extract against test organisms. It was observed that *Morganella morganii* had an MBC of 100mg/mL while *Staphylococcus aureus* and *Escherichia coli* had significant growth (0mg/mL). The findings in the study constitute further justification that many plant materials have good and proven therapeutic potentials as we have demonstrated in several other studies [54-63].

Tiger nuts are packed with vitamins and fiber, which makes them a great addition to your diet. They are helpful in treatment of cardiovascular diseases, cancer, inflammation and are even a great substitute for milk products causing lactose intolerance. They are also a valuable source of magnesium and iron, some of the most important nutrients the body needs.

Conclusion

In conclusion, it is worthy of note that the sterile dH2O Cold fresh Tiger nut extract, sterile dH2O Hot fresh Tiger nut extract and Sterile dH2O Hot dried Tiger nut extracts in this study exhibited a broad-spectrum antibacterial activity on *Staphylococcus aureus*, *Escherichia coli* and *Morganella morganii* with highest activity recorded for sterile dH2O Cold fresh Tiger nut extract. This may be attributed to its vast concentration of phytochemical compounds. Further studies should be carried out on tiger nuts for comprehensive structural elucidation of bioactive compounds in order to formulate a new drug to treat infections.

While benefits of tiger nuts are countless it is also worth noting that there are some side effects, too, mainly skin allergies, respiratory reactions and gastrointestinal issues. Those suffering from any disorder need proper diagnosis. Use tiger nuts (and other natural remedies) wisely, only with prior consent of a physician. Medicines derived from plants have made immense contribution towards the betterment of human health and act as a source of inspiration for novel drug compounds.

Although tiger nut is largely unexploited, cheap and eaten without much knowledge of its benefits, there is an urgent need for awareness campaigns on its health and nutritional benefits. There is scanty information on tigernut metabolites bioavailability, this area needs to be explored through various approaches including in vivo studies. Promoting tigernut consumption will play an imperative role in the health, nutrition and economy of many developing countries.

Funding

No grants from public, private, or nonprofit funding organisations were awarded for this study.

Dispute of Interest

No conflicts of interest are disclosed by the writers. The paper's writing and content are solely the authors' responsibility.

Acknowledgements

The management and technical staff of St. Kenny Diagnostic and Research Centre, Ekpoma, Edo State, Nigeria, are acknowledged by the authors for their outstanding support and for offering medical writing and editorial support in compliance with Good Publication Practice (GPP3) guidelines.

Data and Materials Availability

The consent for all relevant data used in this study is declared by the authors.

Contributions of the Authors

All authors participated in the entire study process.

References

1. Ekeanyanwu C, Ononogbu IC. Nutritive value of Nigerian Tiger Nut (*Cyperus esculentus* L). *Agricultural Journal*. 2010. 5: 297-302.
2. Webster M, MacDonald E. Survey of weeds in Georgia. *Weed Technology*. 2001. 15: 771-790.
3. Omode AA, Fatoki OS, Olaogun KA. Physicochemical properties of some underexploited and nonconventional oilseeds. *Journal of Agricultural and Food Chemistry*. 1995. 43: 2850-2853.
4. Martinez V. Scientific analysis of effects of tiger nut on heart diseases. In *Tiger Nut and health*. 2003. 23-32.
5. Oladele AK, Aina JO. Chemical Composition and Functional Properties of Flour Produced from Two Varieties of Tigernut (*Cyperus esculentus*). *African Journal of Biotechnology*. 2007. 6: 2473-2476.
6. Vayalil PK. Date fruits (*Phoenix dactylifera* Linn): an emerging medicinal food. *Crit Rev Food Sci Nutr*. 2012. 52: 249-271.
7. Saleh FA. Antimicrobial activity of date palm (*Phoenix dactylifera*) fruit at different ripening stages. *Journal of Food Processing & Technology*. 2013. 4: 12.
8. Abdu SB. The protective role of Ajwa date against hepatotoxicity induced by ochratoxin A. *Egyptian Journal of Natural Toxicology*. 2011. 8: 1-15.
9. Jamishidi FK, Zahra L, Hossein AK. Medicinal plants: Past history and future perspective. *Journal of HerbMed Pharmacology*. 2018. 7: 1-7.
10. Chukwuma ER, Njoku C, Ononogbu IC. Phytochemical composition and biochemical effects of tiger nut (*Cyperus*

- esculentus L.) tuber. Pakistan Journal of Nutrition. 2010. 9: 709-715.
11. Zapata ES, Fernández-López J, Pérez-Álvarez JA. Tiger nut (*Cyperus esculentus*) commercialization: Composition and applications. Comprehensive Reviews in Food Science and Food Safety. 2012. 11: 366-377.
 12. Abaejoh R, Djomdi I, Ndjouenkeu D. Characteristics of tiger nut (*Cyperus esculentus*) tubers and their performance in the production of a milky drink. Journal of Food Process Preservation. 2006. 30: 145-163.
 13. Belew MA, Abodunrin AO. Preparation of kunun from tiger nut (*Cyperus esculentus*). Pakistan Journal of Nutrition. 2008. 7: 109-111.
 14. Sanofi. Antimicrobial resistance (AMR) fact sheet. 2020.
 15. Gray DA, Michaela W. Multitarget approaches against multiresistant superbugs. ACS Infectious Diseases. 2020. 1346-1365.
 16. World Health Organization. Antimicrobial resistance. 2020. <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>
 17. Dadgostar P. Antimicrobial resistance: Implications and costs. Infection and Drug Resistance. 2019. 12: 3903-3910.
 18. Joint Programming Initiative on Antimicrobial Resistance. Strategic research and innovation agenda on antimicrobial resistance. 2019. <https://www.jpamr.eu>
 19. O'Neill J. Tackling drug-resistant infections globally: Final report and recommendations. 2016. <https://amrreview.org>
 20. Organisation for Economic Co-operation and Development (OECD). Antimicrobial resistance: Tackling the burden in the European Union. OECD Publishing. 2019.
 21. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States. 2019. <https://www.cdc.gov/drugresistance/biggest-threats.html>
 22. Interagency Coordination Group on Antimicrobial Resistance (IACG). No time to wait: Securing the future from drug-resistant infections. United Nations. 2019. <https://www.who.int/antimicrobial-resistance/interagency-coordination-group/final-report/en/>
 23. World Health Assembly. Antimicrobial resistance (WHA67.25). World Health Organization. 2014.
 24. Anyanwu MU, Okoye RC. Antimicrobial activity of Nigerian medicinal plants. Journal of Intercultural Ethnopharmacology. 2017. 6: 240-259.
 25. Phillipson JD. Phytochemistry and medicinal plants. Phytochemistry. 2001. 56: 237-243.
 26. Shadia MAA, Aeron A, Kahil T. Health benefits and risks of herbal medicine. Springer. 2010.
 27. Ejoh R, Djomdi A, Ndjouenkeu R. Characteristics of tiger nut (*Cyperus esculentus*) tubers and their performance in the production of a milky drink. Journal of Food Processing and Preservation. 2006. 30: 145-163.
 28. Belew MA, Belew KY. Comparative physicochemical evaluation of tiger nut, soybean, and coconut milk sources. International Journal of Agriculture and Biology. 2007. 5: 785-787.
 29. Okwu DE. Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. Journal of Sustainable Agriculture and the Environment. 2004. 6: 30-34.
 30. Tureson H, Marttila S, Gustavsson KE, Hofvander P, Olsson ME, et al. Oil and starch accumulation in *Cyperus esculentus*. American Journal of Botany. 2010. 97: 1884-1893.
 31. Sánchez-Zapata E, Fernández-López J, Pérez-Álvarez JA. Tiger nut (*Cyperus esculentus*) commercialization: Health aspects, composition, properties, and food applications. Comprehensive Reviews in Food Science and Food Safety. 2012. 11: 366-377.
 32. Sonwa M, König W. Chemical study of essential oil of *Cyperus rotundus*. Phytochemistry. 2001. 58: 799-810.
 33. Aghassi A, Naeemy A, Feizbakhsh A. Chemical composition of the essential oil of *Cyperus rotundus* L. from Iran. Journal of Essential Oil-Bearing Plants. 2013. 16: 382-386.
 34. Feizbakhsh A, Aghassi A, Naeemy A. Chemical constituents of essential oils of *Cyperus difformis* L. and *Cyperus arenarius* Retz from Iran. Journal of Essential Oil-Bearing Plants. 2012. 15: 48-52.
 35. Hassanein H, Nazif N, Shahat A, Hammouda F, Aboutable E, Saleh M. Chemical diversity of essential oils from *Cyperus articulatus*, *Cyperus esculentus*, and *Cyperus papyrus*. Journal of Essential Oil-Bearing Plants. 2014. 17: 251-264.
 36. Tenebe IT, Babatunde EO, Neris JB, Mikano C, Ezeudu OB, et al. Reliability of stored river water for consumption in Ekpoma, Nigeria. Journal of Water and Health. 2023. 21: 571-585.
 37. Iyevhobu KO, Obohwemu OK, Aliemhe C, Omokpo VO, Oni IV, et al. Antimicrobial effects of *Cocos nucifera* water on selected bacterial isolates. International Journal of Biomedical and Clinical Research. 2026. 6: 1-5.
 38. Alade PI, Irobi ON. Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*. Journal of Ethnopharmacology. 1993. 39: 171-174.
 39. Orhue PO, Momoh ARM. Antimicrobial activities of solvent extracts of *Carica papaya* fruit parts on selected microorganisms. International Journal of Herbs and Pharmacological Research. 2013. 2: 42-47.
 40. Harborne JB. Phytochemical methods. Chapman and Hall. 1984.
 41. Akinjogunla OJ. Quantitative microbiology (1st ed.). Foresight Press. 2016.
 42. Cowan ST, Steel KJ. Cowan and Steel's manual for the identification of medical bacteria. Cambridge University Press. 1965.
 43. Nester EW, Roberts CE, Pearsall NN, Anderson DG, Nester MT. Antimicrobial medicine. In Microbiology: A human perspective. McGraw-Hill. 2004. 447-521.
 44. Cheesbrough M. Summary of clinical and laboratory features of microorganisms. In M. Cheesbrough (Ed.), District laboratory practice in tropical countries. Cambridge University Press. 2000. 178-244
 45. Kiran B, Bhushan S, Lalitha V, Raveesha KA. Antibacterial efficiency of *Cyperus esculentus* L. tubers against bacterial species. World Journal of Pharmacy and Pharmaceutical Sciences. 2015. 6: 61-116.
 46. Junior A, Zanil C. Biological screening of Brazilian medicinal plants. Brazilian Journal of Science. 2000. 95: 367-373.
 47. Thomas ES, Shanmugam J, Rafi MM. Antibacterial activity of plants in the Zingiberaceae family. Journal of Biomedicine. 1996. 16: 15-20.
 48. Baydar NG, Ozkan G, Sagdic O. Total phenolic contents and

- antibacterial activities of grape (*Vitis vinifera* L.) extracts. *Food Control*. 2004. 15: 335-339.
49. Vaquero MR, Alberto MR, de Nadra MC. Antibacterial effect of phenolic compounds from wines. *Food Control*. 2007. 18: 93-101.
50. Scalbert A. Antimicrobial properties of tannins. *Phytochemistry*. 1991. 30: 3875-3883.
51. Ariekpar I, Alade TL. Antimicrobial activities of *Irvingia gabonensis* leaf and *Cyperus esculentus* extracts on selected isolates. *American Journal of Biomedical Science and Research*. 2020. 7.
52. Prakash R. Phytochemical observation and antibacterial activity of *Cyperus esculentus*. *Ancient Science of Life*. 2009. 28: 16-20.
53. Georgios S, Eleni M, Theodore GS, Ioanna C. Chemical analysis, antioxidant, and antimicrobial activity of three Greek cucumber (*Cucumis sativus*) cultivars. *Journal of Food Biochemistry*. 2010. 34: 61-78.
54. Nweze EI, Eze EE. Justification for the use of *Ocimum gratissimum* L. in herbal medicine and its interaction with disc antibiotics. *BMC Complementary and Alternative Medicine*. 2009. 9: 37.
55. Nweze EI, Okafor JI. Antifungal activities of medicinal plant extracts and essential oils against *Scedosporium apiospermum*. *American-Eurasian Journal of Scientific Research*. 2010. 5: 161-169.
56. Nweze EI, Onyishi MC. In vitro antimicrobial activities of methanolic and ethanolic fruit extracts of *Xylopi aethiopica* and their interactions with antibiotics. *Journal of Rural and Tropical Public Health*. 2010. 9: 1-6.
57. Nweze EI, Okafor JI, Njoku OU. Antimicrobial activities of methanolic extracts of *Trema guineensis* and *Morinda lucida* used in Nigerian herbal medicine. *Journal of Biological Research and Biotechnology*. 2004. 2: 36-39.
58. Nweze EI, Okafor JI, Njoku OU. Antifungal activities of combinations of extracts from *Morinda lucida* using decimal additive assay. *Journal of Biological Research and Biotechnology*. 2005. 3: 99-103.
59. Anejionwu MG, Nweze EI, Dibua EU, Odimegwu DC, Esimone CO. In vitro antifungal activity of the combination of *Mitracarpus scaber* and *Ocimum gratissimum* extracts and some non-steroidal anti-inflammatory drugs. *Microbiology Journal*. 2011. 1: 181-190.
60. Anejionu MG, Nweze EI, Dibua EU, Esimone CO. Efficacy of two commonly used antifungal herbs in Nigeria against clinical isolates of fungi. *Microbiology Journal*. 2012.
61. Anikwe LU, Onoja US, Onyeke CC, Nweze EI. Antimicrobial activities of four varieties of *Capsicum annum* fruits cultivated in Southeast Nigeria. *Journal of Basic Pharmacology and Toxicology*. 2017. 1: 21-26.
62. Eze H, Nweze EI. Antibacterial activities of aqueous and ethanolic leaf extracts of *Abrus precatorius* against urinary tract pathogens and *Listeria monocytogenes*. *Journal of Basic Pharmacology and Toxicology*. 2017. 1: 11-17.
63. Akaihe CL, Chidebelu PE, Nweze EI. Preliminary studies on antifungal activities of ethanol and hot water extracts of local spices against fluconazole-resistant *Candida albicans*. *Bio-Research*. 2019. 17: 1063-1070.