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Antimicrobial Properties of Tiger nut (*Cyperus esculentus*) extract on some Clinical Isolates

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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 study was aimed to evaluate the antibacterial efficacy of different solvent extracts (ethanol, hot water, cold water, hydrochloric acid, acetic acid, and isopropyl alcohol), to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Bacterial isolates (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Morganella morganii*), were obtained from a tertiary health institution (Irrua Specialist Teaching Hospital, (ISTH), Irrua) and they were identified using standard microbiology techniques. Tiger nut extracts were prepared using standard extraction procedures and disc diffusion method was used to test for antibacterial activity. It was observed that aqueous extracts (hot and cold water) exhibited greater antibacterial activity compared to ethanol extracts. *Staphylococcus aureus*, *Escherichia coli*, and *Morganella morganii* showed notable zones of inhibition while *Pseudomonas aeruginosa* was resistant to all extracts. The susceptible organisms showed MIC values ranging from 50 to 100 mg/mL while bactericidal effects (MBC values) were observed at higher concentrations. This result suggests that the antimicrobial activity of tiger nut extracts may be attributed to the presence of phenolic compounds. In conclusion, tiger nut extracts possess moderate antibacterial activity, particularly in aqueous forms, which can 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

1. 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 (Ekeanyanwu & Ononogbu, 2010). 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 (Webster & MacDonald, 2001). In Africa, tiger nut is cultivated more in Ivory Coast, Ghana, Mali, Niger, Nigeria, Senegal and Togo where they are consumed uncooked as a side dish (Omode *et al.*, 1995). It is known as one of the nutritional crops used to as addition to diets, because 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 (Martinez, 2003), and also a good source of iron and calcium for growth and development.

Tiger nut (*Cyperus esculentus*), commonly referred to as "Aya" in Northern Nigeria, is believed to possess strong antimicrobial properties against a broad spectrum of infections. Beyond its nutritional benefits, the tuber has long been employed by traditional healers and users of herbal medicine for various therapeutic purposes. Food nutraceuticals and antimicrobials have added high health values as they present compounds in foods that hinder microbial growth or kill microorganisms (Saleh & Otaibi, 2013). Tiger nut has also been reported as healthy and it helps in preventing heart attacks thrombosis and promotes blood circulation. Due to its high glucose content, it aids in cancer prevention and also reduces the risk of colon cancer (Abdu, 2011). 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 (77.08mg/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 known for their health benefits and nutritive value (Jamishidi *et al.*, 2018). Also, *Cyperus esculentus* contain alkaloid, resins, tannins, sterols and saponins (Chukwuma *et al.*, 2010). Furthermore, *Cyperus esculentus* was reported to contain high content of fiber, protein, sugar, oleic acid, glucose, phosphorous, potassium, and vitamin C and E (Sánchez-Zapata *et al.*, 2012).

Antibiotic resistance can be described 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 very effective, are now less effective and potent because bacteria have become more resistant to them. Tiger nut (*Cyperus esculentus*) is widely consumed in Nigeria and in various other parts of West and East Africa (Abaejoh *et al.*, 2006). It can eaten raw or dried, can be used as hog feed or pressed for its juice for consumption (Belewu & Abodunrin, 2008). Tiger nuts have high nutritious starch content, dietary fibre and carbohydrate. They are rich in sucrose, fat and protein with an approximate value of 17.4-20.0%, 25.5%, and 8.0% respectively. It also contains mineral elements such as sodium, calcium, potassium, magnesium, zinc and traces of copper (Chukwuma *et al.*, 2010).

The increase in antimicrobial resistance has become a great threat in the current treatment of common infections (Sanofi, 2020). The most remarkable is the fast-spreading multi-resistant bacteria species which cause different kinds of human and animal infections (Gray & Michaela, 2020). The World Health Organization recently identified and acknowledged antimicrobial resistance and rated it as one of the top ten global public health threats in the world (WHO, 2020).

Antimicrobial resistance has direct and severe consequences on both morbidity and mortality rates of humans and animals (Dadgostar, 2019). 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 (Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), 2019). According to the final report and recommendations of O'Neill, (2016) 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 (O'Neill, 2016). Looking further into recent yearly accounts, about 33, 000deaths with 1.1 billion Euros health care expenditures are recorded for European countries (OECD, 2019), while more than 35,000 lives have been lost in the United States (CDC, 2019). 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. 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 (World Health Assembly (WHA), 2014), 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 (Anyanwu & Okoye, 2017). 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 (Jamishidi *et al.*, 2018). 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 (Jamishidi *et al.*, 2018). Phytochemicals 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 (Phillipson, 2001). 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 in managing conditions like 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.

In the quest and ongoing research for 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 (Abaejoh *et al.*, 2006; Belewu & Belewu, 2007). In ayurvedic medicine tiger nuts are used in the treatment of flatulence, diarrhoea, dysentery, debility and indigestion (Okwu, 2004; Turesson *et al.*, 2010; Sánchez-Zapata *et al.*, 2012). 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.

Some studies have examined the chemical composition of the essential oil of *Cyperus* species which include; *C. rotundus* (Sonwa & König, 2001; Aghassi *et al.*, 2013), *C. difformis* L. and *C. arenarius* (Feizbakhsh *et al.*, 2012). In addition, comparative analyses of the chemical compositions of the three Egyptian *Cyperus* species; *C. articulatus*, *C. esculentus* and *C. papyrus* was carried out by Hassanein *et al.*, (2014). This study showed assessed the antimicrobial properties of Tiger nut (*Cyperus esculentus*) on some selected organisms.

2. 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, Iruakpen, Emaudo, Ujoelen, Ihumudumu, Illeh, Uke, Uhiele, Ujemen, Ukpenu, Idua, Ukhur, Egoro, Emehi, Igor and Idumebo (Tenebe *et al.*, 2023). From statistics, 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, rainy and dry seasons. The rainy season spans between April to October with peak in August, average rainfall ranging 150cm to 250cm. The dry season occurs between November to March with cold harmattan between December and January, average temperature of about 25°C (National Population Commission of Nigeria (NPCN), 2012; Iyevhobu *et al.*, 2026).

Materials and Equipment

The materials and equipment employed in this study included a hot air oven, incubator, electric weighing balance (Galenkamp), aluminium foil, cotton wool, 10ml pipettes, 250ml and 500ml conical flasks, a 100ml measuring cylinder, wire loop, Bunsen burner, nutrient agar, MacConkey agar, sterile distilled water, beakers, test tubes, sterile borer, separating funnel, Whatman No. 2 filter paper, ethanol, methanol, ethyl acetate, magnetic stirrer, 1% hydrochloric acid, 5% acetic acid, isopropyl alcohol, filter paper, and a filtering funnel.

Source of Tiger nut

Fresh tiger nuts (*Cyperus esculentus* L.) were procured from Ekpoma market, Esan West Local Government Area, Edo State, Nigeria, and authenticated by a taxonomist in the Department of Botany, Ambrose Alli University, Ekpoma. The samples were transported in sealed plastic bags to the Medical Microbiology Laboratory, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Preparation of Successive Extracts

The *Cyperus esculentus* purchased were washed with tap water and then rinsed 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). The prepared extracts were filtered using fine linen material 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 for final use by using soxhlet apparatus and then collected and stored in a vial for further use. The extraction procedure proceeded as follows:

- i. Approximately 500g of tiger nut tubers were weighed and dried in a drying cabinet at 50°C for five days.
- ii. The dried material was ground into powder using an electric grinding machine.
- iii. Ten grams of the powdered sample were weighed into each of four separate 250ml conical flasks, to which 100ml of the respective extraction solvents (1% HCl, 5% acetic acid, water, and isopropyl alcohol) were added.
- iv. The flasks were placed on a mechanical shaker and left to extract overnight.
- v. Each extract was filtered into a sterile conical flask using Whatman No. 1 filter paper.
- vi. The resulting filtrates were retained for experimental use, while the residual deposits were discarded (Orhue & Momoh, 2013).
- vii. The yield of each extract was recorded, and the extracts were stored in a refrigerator at 4°C until required. Serial dilution was subsequently employed to prepare tiger nut extract concentrations of 100%, 50%, 25%, and 12.5%.

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 collection. The extract was filtered using a sterile muslin cloth thereafter the extract was obtained, air-dried and stored below ambient temperature until required (Harborne, 1984). Same was done for hot water but for hot water, it was boiled and mix after boiling the water.

Determination of Purity of Extract

Using a 4mm wire loop, a loopful of the extract solution was streaked onto nutrient agar and incubated at 37°C for 24 hours. The absence of microbial growth following overnight incubation was taken as confirmation of the extract's purity. A 1-in-2 dilution of the extract solution was then prepared, followed by a subsequent 1-in-20 serial dilution, carried out according to the following protocol:

Protocol

- i. A 1-in-2 dilution of the extract solution was prepared across five test tubes.
- ii. Using a 50ml dropper pipette, a 1-in-100 dilution of an overnight broth culture of the test organism was added to each test tube.
- iii. Controls were included alongside the test samples: one tube containing 1ml of plain broth (C1), and another containing 1ml of extract solution (C2), both incubated under identical conditions.

Determination of Extract Concentration

The specific gravity (S.G.) of each diluent/solvent — 1% HCl, 5% acetic acid, water, and isopropyl alcohol (IPA) — was determined using the following procedure:

- i. The weight, W1 of the clean dried empty specific gravity, S. G bottle was taken.
- ii. Specific gravity bottle was filled with the diluents and the stopper was placed.
- iii. The overflowed fluid was cleared off from the body of the bottle with a filter paper.
- iv. The weight W2 of the diluents with the S.G bottle was taken.
- v. 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 test organisms used in this study were isolated from clinical samples obtained from patients attending Irrua Specialist Teaching Hospital (ISTH), located in Irrua, Esan Central Local Government Area, Edo State. Analysis was carried out at the Microbiology Laboratory, Department of Medical Microbiology, Faculty of Medical Laboratory Science, College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State. The organisms isolated for the purpose of this study comprised *Staphylococcus aureus*, *Klebsiella* species, *Escherichia coli*, and *Salmonella* species.

The various isolates recovered — including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus* species, *Proteus* species, *Pseudomonas* species, *Escherichia coli*, and *Salmonella* species — were sub-cultured in peptone water for 8 hours prior to antibiogram and extract testing.

Identification of Test Organisms

All isolates were characterised on the basis of colonial morphology, Gram-staining reaction, biochemical profiling, and sugar fermentation patterns. Colonial features assessed included size, shape, elevation, opacity, edge, colour, haemolytic activity, and fermentation properties. Gram staining and biochemical testing were subsequently performed on cultures exhibiting growth, in accordance with standard laboratory procedures.

Antibiotic Sensitivity Testing

The antibacterial potential of the tiger nut extracts was assessed primarily through the disc diffusion assay, as described by Akinjogunla (2016). Clinical isolates were sub-cultured and incubated at 37°C for 24 hours prior to testing. Antimicrobial discs were prepared in-house by punching 6mm-diameter discs from good-quality Whatman No. 1 filter paper, which were then sterilised by autoclaving for 15 minutes and stored in corked test tubes until use.

Mueller Hinton Agar (Oxoid, UK) was prepared and dispensed into Petri dishes to serve as the growth medium, after which the sub-cultured isolates were seeded onto the solidified agar surface. Pre-sterilised filter paper discs were soaked in the various extract concentrations for 30 minutes and left to air-dry before being aseptically transferred onto the seeded plates.

Prior to disc application, the bacterial isolates were inoculated into peptone water and incubated for 8 hours, then sub-cultured onto nutrient agar; excess broth was drained off and the plates allowed to dry. Using sterile forceps, the prepared discs were impregnated with 0.01ml of the respective extract concentrations (25mg/ml, 125mg/ml, and 250mg/ml) and positioned equidistantly on the inoculated agar plates. Two locally prepared reference discs were included alongside the test discs: gentamicin (20µg/disc) as the positive control, and dimethyl sulfoxide (DMSO) as the negative control.

The seeded plates were left undisturbed for 30 minutes before being incubated upright at 37°C for 24 hours. Zones of inhibition (ZI) were subsequently measured in millimetres using a calibrated ruler.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the tube dilution method described by Cowan and Steel (1965), representing the lowest concentration of aqueous extract capable of inhibiting the growth of the bacterial isolates.

- i. Approximately 0.5ml of prepared nutrient broth was dispensed into test tubes 2 through 10, while 0.5ml of the extract was added to tubes 1 and 2.
- ii. Serial dilutions were then carried out, producing a progressively decreasing concentration of the aqueous extract across the tubes.
- iii. The extract was diluted sequentially from tube 3 through to tube 9, with 0.5ml discarded from the final tube. Thereafter, 0.5ml of the test bacterial isolates was introduced into tubes 2 through 10.
- iv. Tube 1, containing nutrient broth and the test organism only, served as the control (Nester *et al.*, 2004).
Note: This procedure was applied to all bacterial isolates that had demonstrated susceptibility to the extracts, as indicated by a zone of inhibition greater than 10mm.
- v. The tubes were thoroughly mixed and incubated at 37°C for 24 hours, after which they were examined for visible turbidity, an indicator of bacterial growth.
- vi. The MIC was recorded as the lowest concentration of tiger nut (*Cyperus esculentus*) extract at which no visible growth occurred (Cheesbrough, 2000).

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of the tiger nut extract was determined using a slightly modified approach. Samples from the MIC assay were sub-cultured onto freshly prepared nutrient agar and incubated at 37°C for 48 hours. Specifically, plates containing freshly solidified nutrient agar were seeded with inoculum drawn from the MIC tubes that showed no turbidity, and these were incubated at 37°C for a further 24 hours. The lowest extract concentration from the MIC series that yielded no bacterial growth on sub-culture was recorded as the MBC.

Data Analysis

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

3. 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 presents 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 an MBC of 0mg/mL. Table 5 presents 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. The corresponding MBC results for the cold-water extract, shown in Table 6, indicate that *Escherichia coli* and *Morganella morganii* each recorded an MBC of 100mg/mL. *Staphylococcus aureus*, however, continued to exhibit significant growth throughout, resulting in an MBC of 0mg/mL. Table 7 presents 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)									MIC (mg/mL)
	100	50	25	12.3	6.25	3.125	0.781	0.391	0.195	
<i>Staphylococcus aureus</i>	-	+	+	+	+	+	+	+	+	100
<i>Escherichia coli</i>	-	+	+	+	+	+	+	+	+	100
<i>Morganella morganii</i>	-	-	+	+	+	+	+	+	+	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	
<i>Staphylococcus aureus</i>	+	+	+	+	+	+	+	+	+	0
<i>Escherichia coli</i>	+	+	+	+	+	+	+	+	+	0
<i>Morganella morganii</i>	-	+	+	+	+	+	+	+	+	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	
<i>Staphylococcus aureus</i>	-	+	+	+	+	+	+	+	+	100
<i>Escherichia coli</i>	-	-	+	+	+	+	+	+	+	50
<i>Morganella morganii</i>	-	-	+	+	+	+	+	+	+	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	
<i>Staphylococcus aureus</i>	+	+	+	+	+	+	+	+	+	0
<i>Escherichia coli</i>	-	+	+	+	+	+	+	+	+	100
<i>Morganella morganii</i>	-	+	+	+	+	+	+	+	+	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	
<i>Staphylococcus aureus</i>	-	+	+	+	+	+	+	+	+	100
<i>Escherichia coli</i>	+	+	+	+	+	+	+	+	+	0
<i>Morganella morganii</i>	+	+	+	+	+	+	+	+	+	0

Key: - = No turbidity; + = Turbidity

4. DISCUSSION

Tiger nut (*Cyperus esculentus*) 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.

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. *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. 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. 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). The efficiency of these extracts against the bacteria could be because of the phenolic composition (Baydar *et al.*, 2004; Vaquero *et al.*, 2007). The inhibitory effect of phenol in compounds could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation (Scalbert, 1991). 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 Ariekpar & Alade (2020) and Prakash & Ragavan (2009), which showed that ethanolic extract of *Cyperus esculentus* has antibacterial activity against the bacterial isolates tested. 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. 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 (Nweze *et al.*, 2004; Nweze *et al.*, 2005; Nweze & Eze, 2009; Nweze & Onyishi, 2010; Anejionwu *et al.*, 2011; Anejionu *et al.*, 2012; Anikwe *et al.*, 2017; Eze *et al.*, 2017; Akaihe *et al.*, 2019; Nweze & Okafor, 2010).

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.

5. CONCLUSION

In conclusion, 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. More research 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 potent and effective contribution towards the treatment and healing of humans and act as a source of research focus for novel drug compounds.

Tiger nut is cheap, unexploited, and eaten without much knowledge of its benefits; hence we recommend an urgent need for awareness campaigns on its health and nutritional benefits. There is scanty information on tiger nut metabolites bioavailability, this area needs to be explored through various approaches including in vivo studies. Promoting tiger nut consumption will play a role in the health, nutrition and economy of some developing countries where this tuber is grown.

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Author Contributions:

All authors participated in the entire study process.

Ethical Approval

In this article, as per the plant regulations followed in the Department of Medical Microbiology, Faculty of Medical Laboratory Science, College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria; the authors observed the Antimicrobial Properties of Tiger nut (*Cyperus esculentus*) extract. The ethical guidelines for plants & plant materials are followed in the study for observation, identification & experimentation. This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that they have no conflicts of interest, competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Data and materials availability

All data associated with this study will be available upon reasonable request to the corresponding author.

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