JJBS

Volume 15, Number 4,December 2022
ISSN 1995-6673
Pages 603 – 609
https://doi.org/10.54319/jjbs/150408

Jordan Journal of Biological Sciences

Nutritional and Endophytic Composition of Edible Tubers of Tiger nut (*Cyperus esculentus* L.)

Omotayo O. Oyedara[1,2,*], Abdulfatai B. Rufai[3], Gideon O. Okunlola[3], Folasade M. Adeyemi[1]

[1]*Department of Microbiology, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria;* [2]*Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, 66450, Nuevo León, Mexico;* [3]*Department of Plant Biology, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria*

Received: July 6, 2021; Revised: January 22, 2022; Accepted: January 31, 2022

Abstract

In this study, different parameters including phytochemical, nutritional, anti-nutritional compositions, and endophytic microbial populations that can play roles in the health benefits of tiger nut (*Cyperus esculentus* L.) tubers were determined in tiger nut tuber samples obtained from three different local markets situated in Osogbo, Ile-Ife, and Ado-Ekiti in the Southwestern part of Nigeria. Tannins (127.33-620.1 GAE/100 g), alkaloids (0.5-18%), flavonoids (4.5-9.5%), terpenoid, and cardiac glycoside were present in all the samples. The most and least abundant elements in the tiger nuts were Potassium (0.255-0.345%) and Copper (0.0007-0.0009%), respectively. The anti-nutrients found in the tiger nut tubers were oxalate (0.42-1.08 mg/100 g), tannin (0.42-0.98 mg/100 g), and phytic acid (0.56-1.64%). By molecular techniques, *Lactococcus lactis*, *Bacillus cereus*, *Enterobacter cloacae*, *Bacillus licheniformis*, *Bacillus aryabhattai*, and *Enterobacter roggenkampii* were identified as the endophytic bacteria in the tiger nut tubers, while the endophytic fungi isolated were *Saccharomyces cerevisiae* and *Candida tropicalis*. The presence of reportedly pharmacologically active phytochemicals, essential elements, endophytic bacterial and fungal strains with probiotic potentials, and negligible amounts of anti-nutrients in the study tiger nut samples supports the nutritional and health benefits of tiger nut tubers.

Keywords: Tiger nut tuber, phytochemicals, anti-nutrients, elements, endophytes

1. Introduction

Tiger nut (*Cyperus esculentus* L) is a sedge plant belonging to the same genus as the papyrus plant (*Cyperus papyrus* L.) of the family "Cyperaceae". Tiger nut tuber (Plate 1), also known as "Aya", "Imumu", and "Aki-hausa" among the Hausa, Yoruba, and Igbo tribes respectively in Nigeria, is either eaten raw or processed into different products such as milk, flour, bread, and beverages (Oladele and Aina, 2007; Aguilar *et al*., 2015). The consumption of tiger nut or its derived products are associated with health benefits, and reports have shown that tiger nut has medicinal and nutritional properties (Sabah *et al*., 2019; Bazine and Arslanoğlu, 2020). It is useful medically for the prevention and management of diseases such as thrombosis, heart cancer, erectile dysfunction, obesity, diabetes, and high blood pressure (Adejuyitan, 2011).

For centuries, humans have used plants for several medicinal purposes. The nutritional content, phytochemical composition, presence of beneficial and pharmacologically relevant endophytes in plants contribute to their medicinal and nutritional characteristics. Phytochemicals, for instance, contribute to plant potentials to promote good health and cure diseases (Barbieri *et al*., 2017). Furthermore, plants also contain anti-nutritional compounds that can adversely affect humans by interfering with metabolic processes such as nutrient absorption (Kunatsa *et al*., 2020). Hence, knowledge of anti-nutrients in a plant or its product can provide information on its nutritional significance.

Similar to phytochemicals, endophytic microbes defend plants against pathogens and aid their growth (Nayak *et al*., 2017; Bind and Nema, 2019; Yasser *et al*., 2020). When consuming plant materials including, fruits and leaves, animals also ingest endophytes. According to Martínez-Romero *et al*. (2020), microbes associated with plants form part of herbivores' gut microbiota.

Endophytes aid the degradation of complex plant materials such as cellulose, and antimetabolites in animals. Furthermore, endophytic *Lactobacillus plantarum* has probiotic potential to protect humans against bacterial and viral infections (Martínez-Romero *et al*., 2020).

With limited information on endophytic microbes associated with tiger nut tuber, this study examined tiger nut tubers purchased from three local markets in the South-Western part of Nigeria for the endophytic bacterial and fungal composition alongside parameters including phytochemicals, nutritional elements, anti-nutrients that can play roles in the health benefits.

* Corresponding author. e-mail: omotayo.oyedara@uniosun.edu.ng.

Plate 1. A picture of the tiger nut tubers (*Cyperus esculentus* L.)

2. Materials and methods

2.1. Sample collection

Fresh tiger nut tubers were purchased from three different local markets Ile-Ife (7.52 ^{o}N and 4.28 ^{o}E), Osogbo (7.77 ^{o}N and 4.57 ^{o}E), and Ado-Ekiti (7.62 ^{o}N and 5.22 ^{o}E) in the Southwestern part of Nigeria. The tiger nuts were then transported to the laboratory for processing and analysis. Before analysis, a taxonomist at the Forest Herbarium Ibadan (FHI), Forestry Research Institute of Nigeria, Ibadan, Nigeria, identified and confirmed the tiger nut tubers.

2.2. Phytochemical, Elemental, and Anti-nutritional analysis

The tiger nuts were qualitatively and quantitatively analyzed for the presence of terpenoids, saponins, alkaloids, steroids, flavonoids, cardiac glycosides, and tannins according to the method described by Harbone (1984). The mineral contents were analyzed using colorimetric or spectrophotometric, or titrimetric methods where applicable (AOAC, 2005). The tiger nut samples were analyzed to determine the content of sodium (Na), potassium (K), calcium (Ca), iron (Fe), magnesium (Mg), zinc (Zn), manganese (Mn), copper (Cu), phosphorus (P), total nitrogen (T.N), and organic matter (O.M). The atomic absorption spectrophotometer was used to evaluate the anti-nutritional compounds, tannin, and oxalate contents of the tiger nut samples in triplicate, according to the standard methods of AOAC, (2005). The phytate contents were estimated using the solvent extraction gravimetric method (Onwuka, 2005).

2.3. Isolation of Endophytic bacteria and fungi

The tiger nut tubers were carefully inspected visually to select ones without superficial damages. The surfaces of selected tiger nuts (100 g) were sterilized, following the process described by Afzal *et al*. (2019), with slight modifications. Briefly, tiger nuts were washed thoroughly three times with sterile distilled water and then soaked in 5% sodium hypochlorite (NaOCl) for 5 minutes. The tiger nuts were later washed twice in sterile distilled water to remove the sterilizing agent (NaOCl) and thrice in 96% ethanol to ensure the total removal of epiphytic microbes on the surface of the tiger nuts. This was then followed by rinsing the tiger nuts in sterile distilled water eight times, and the last rinse water was cultured on nutrient and potato dextrose agar to confirm the removal of the epiphytic microorganisms. The processed tiger nuts were grinded with sterile mortar and pestle, and the resulting milky extract was serially diluted in sterile phosphate buffer and inoculated on sterile nutrient and potato dextrose agar plate to isolate the endophytic bacteria and fungi, respectively. The plates were incubated at 30 ^{o}C for 24-72 h and 5 days for bacterial and fungal isolation, respectively.

2.4. Molecular Identification of Endophytic bacteria and fungi

DNA was extracted from isolated endophytic bacteria using the NIMR Biotech (Lagos, Nigeria) DNA Purification Kit according to the manufacturer's instruction, while fungal DNA was extracted using the "Bust n' Grab" method described by Harju *et al*. (2004). Molecular identification of endophytic bacterial isolates were based on the Polymerase Chain Reaction (PCR) amplification of the 16S rRNA gene fragment using universal primers; 27F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R:5′-GGTTACCTTGTTACGACTT-3′ (Chen *et al*., 2015) that amplified 1500 bp of the gene fragment. The rDNA internal transcribed spacer (ITS) region of the fungal isolates was amplified using primers sets (ITS1, 5′-TCCGTAGGTGAACCTGCGG-3′) and (ITS4, 5′-TCCTCCGCTTATTGATATGC-3′) that amplified the 600 bp of the ITS region. The PCR amplified products were sequenced by Inqaba Biotech West Africa Ltd (IITA, Ibadan, Nigeria), and the nucleotide sequences obtained were edited using FinchTV software. The edited sequences were subjected to similarity search against nucleotides in the NCBI database using BLASTn program for bacterial and fungal identification. Phylogenetic tree showing the relationship between isolated endophytes and sequences retrieved from the GenBank was inferred using the Maximum Likelihood method of the MEGA7 software (Kumar *et al*., 2016) based on the Tamura-Nei model (Tamura and Nei, 1993) with 1000 pseudoreplicates to obtain the bootstrap values.

3. Results

3.1. Phytochemical, Elemental, and Anti-nutritional analysis

The result of the qualitative analysis of the phytochemicals in the tiger nut samples is presented in Table 1. Saponins, steroids, and phenols were absent in all the samples. Meanwhile, anthraquinone was found only in samples purchased from Ado-Ekiti. The quantity of tannin in the tiger nut samples ranged between 127.33 and 620.1 (Table 2), with tiger nut samples from Ado-Ekiti having the lowest amount (127.3 GAE/100 g). The highest quantity of flavonoids was found in the tiger nut tubers from Osogbo (9.5%), followed by Ado-Ekiti (4.5%) and Ile-Ife (5%). The alkaloid contents in the tiger nut samples from Ile-Ife, Ado-Ekiti, and Osogbo were 18%, 4%, and 0.5%, respectively. The mineral composition of the tiger nut samples is presented in Table 3. While slight variations were observed in percentage of minerals; Na (0.021-0.020%), Ca (0.030-0.040%), Mg (0.101-0.112%), K (0.255-0.345%), Mn (0.001-0.0001%), Cu (0.0007-0.0009%), Fe (0.0045-0.0048%), Zn (0.0024-0.0025%), and P (0.108-0.166%) in the tiger nuts purchased from the different markets, the organic matter and total nitrogen content were low in tiger nut samples from Ile-Ife (69.48% and 3.47%, respectively) compared to tiger nuts from Ado-Ekiti (84.65% and 4.23%, respectively) and Osogbo (85.99% and 4.30%, respectively). The amount of oxalate, which is one of the anti-nutrients tested in the tiger nut sample, ranged from 1.02 to 0.42 mg/100 g, with tiger nuts from Osogbo having the highest amount followed by samples from Ile-Ife (0.88 mg/100 g) and Ado-Ekiti (Table 4). The phytic acid (1.64%) and tannin (0.98 mg/100 g) compositions were highest in Osogbo tiger nut samples. The amounts of phytic acid and tannin in the tiger nut samples purchased from the Ile-Ife market were 1.45% and 0.42 mg/100 g, respectively. Meanwhile, tiger nuts from Ado-Ekiti had 0.56% and 0.44 mg/100 g of phytic acid and tannin, respectively.

Table 1: Qualitative analysis of phytochemical compounds in tiger nut samples obtained from the three different markets.

Phytochemical	Method of analysis	Sample A (Ile-Ife)	Sample B (Ado-Ekiti)	Sample C (Osogbo)
Alkaloid	Dragenduff	+	+	++
	Mayer	+	+	++
	Wagner	+	+	++
Flavonoid	Ethyl acetate/Ammonia	+	+	+
Saponin	Frothing	-	-	-
Tannin	Ferric chloride	+	+	+
Anthraquinone	Borntrager	-	+	-
Terpenoid	Salkowski	++	+	+
Cardiac Glycoside	Keller-Killiani	+	++	+
Steroid	Liebermann-Burchard	-	-	-
Phenol	Lead acetate	-	-	-

Keys: ++: present in high concentration; +: present in trace amount; -: absent

Table 2: Quantitative analysis of phytochemical compounds in tiger nut samples obtained from the three different markets

Phytochemical	Sample A (Ile-Ife)	Sample B (Ado-Ekiti)	Sample C (Osogbo)
Tannin (GAE/100 g)	620.1	127.33	413.74
Flavonoid (%)	5	4.5	9.5
Alkaloid (%)	18	4	0.5

Keys: GAE - Gallic Acid Equivalent

Table 3: The mineral composition of tiger nut samples obtained from the three different markets

Mineral (%)	Sample A (Ile-Ife)	Sample B (Ado-Ekiti)	Sample C (Osogbo)
Sodium (Na)	0.020	0.021	0.021
Calcium (Ca)	0.030	0.040	0.030
Magnesium (Mg)	0.101	0.102	0.112
Potassium (K)	0.345	0.255	0.285
Manganese (Mn)	0.001	0.001	0.0001
Copper (Cu)	0.0007	0.0009	0.0008
Iron (Fe)	0.0048	0.0048	0.0045
Zinc (Zn)	0.0025	0.0024	0.0025
Phosphorus (P)	0.144	0.166	0.108
Organic matter	69.48	84.65	85.99
Total Nitrogen	3.47	4.23	4.30

Table 4: The anti-nutritional constituents of tiger nut samples obtained from the three different markets

Phytochemical	Sample A (Ile-Ife)	Sample B (Ado-Ekiti)	Sample C (Osogbo)
Oxalate (mg/100g)	0.88	0.42	1.02
Phytic acid (%)	1.45	0.56	1.64
Tannin (mg/100g)	0.42	0.44	0.98

3.2. Molecular Identification of Endophytic bacteria and fungi

A total of twenty-one endophytic bacteria were isolated from the tiger nut samples. However, six PCR amplicons were successfully sequenced, and based on the BLASTn search and molecular phylogenetic analysis (Figure 1), the sequences shared similarities with *Lactococcus lactis*, *Bacillus cereus*, *Enterobacter cloacae*, *Bacillus licheniformis*, *Bacillus aryabhattai*, and *Enterobacter roggenkampii* with percentage identity ranging from 90.90 to 99.80% (Table 5). Two endophytic fungi successfully identified include *Saccharomyces cerevisiae* and *Candida tropicalis* (Figure 2). The bacterial 16S rRNA and fungal ITS sequences obtained in this study were submitted to the National Center for Biotechnology Information (NCBI) GenBank database under the accession numbers MZ452337-MZ452342 for bacterial isolates and MZ42353, MZ452354, and MZ452357 for fungal isolates (Table 5).

Table 5: The BLAST hit identification of endophytic bacteria and fungi isolated from tiger nut samples

Isolate code	BLAST hit Homologue Sequence (Accession number)	Percentage Identity (%)	E-value	Accession number of deposited sequence
TG1	*Lactococcus lactis* strain IMAU98440 (MW135286.1)	90.90	0.0	MZ452337
TG2	*Bacillus cereus* strain Q21 (MH915663.1)	98.29	0.0	MZ452338
TG3	*Enterobacter cloacae* strain BY56 (MN133903.1)	99	0.0	MZ452339
TG4	*Bacillus licheniformis* strain QT445 (MT065669.1)	97.99	0.0	MZ452340
TG5	*Enterobacter roggenkampii* strain CCI9 (LC529204.1)	99.19	0.0	MZ452341
TG6	*Bacillus aryabhattai* strain 3595 (MT538470.1)	98.34	0.0	MZ452342
TGY1	*Saccharomyces cerevisiae* strain XZFM7F3 (MW710205.1)	99.75	0.0	MZ452353
TGY3	*Saccharomyces cerevisiae* strain XZFM7F3 (MW710205.1)	99.38	0.0	MZ452354
TGY4	*Candida tropicalis* isolate ZA030 (JN606259.1)	99.80	0.0	MZ452357

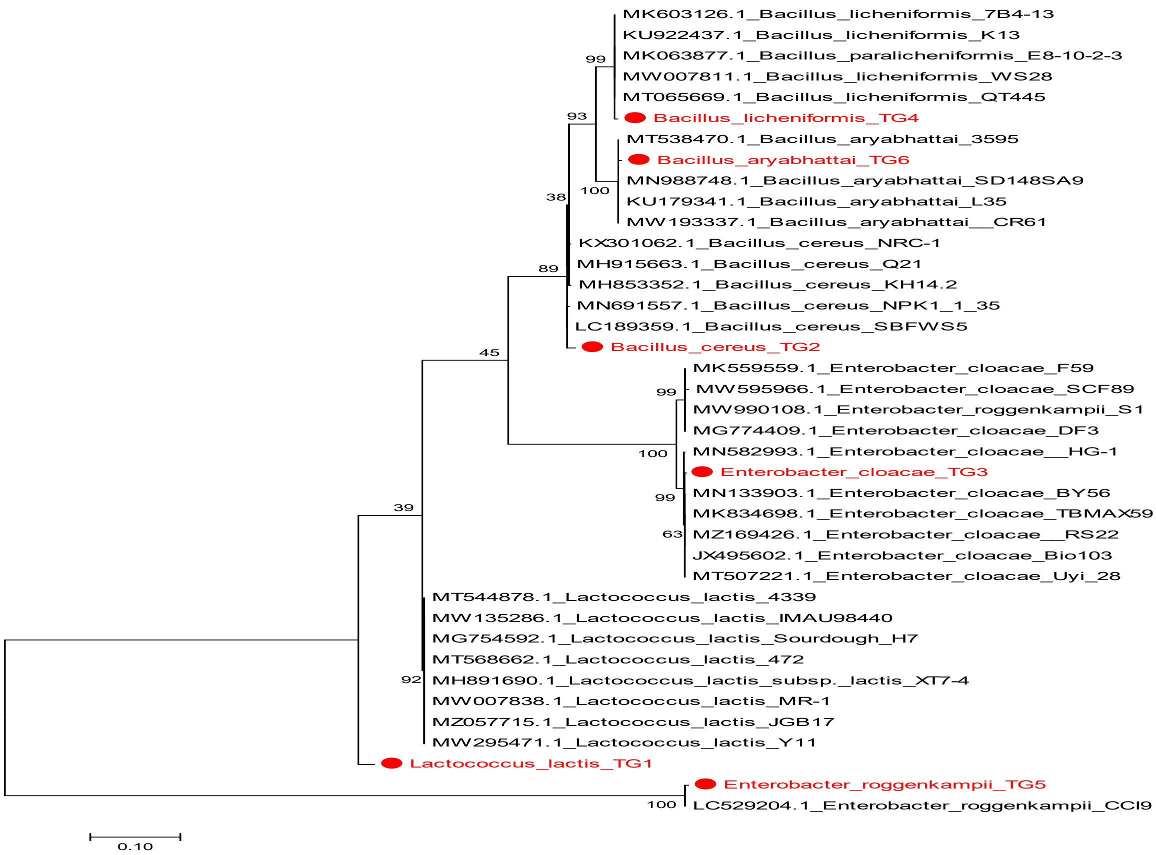

Figure 1. Molecular Phylogenetic relationship of the isolated endophytic bacteria by Maximum Likelihood method.

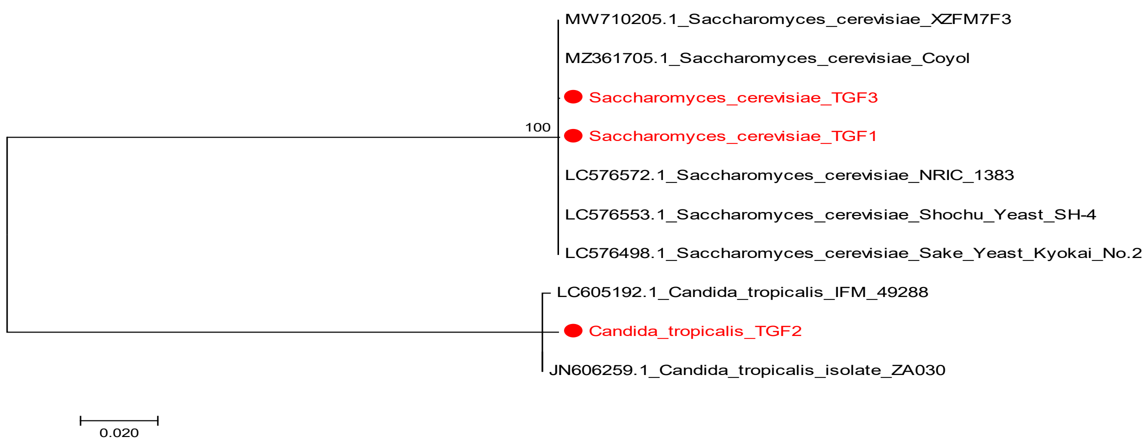

Figure 2. Molecular Phylogenetic relationship of the isolated endophytic fungi by Maximum Likelihood method.

4. Discussion

This study analyzed the properties such as phytochemical, elemental, anti-nutritional, and endophytic microbial constituents that can play roles in the beneficial importance of tiger nuts when consumed. The results obtained in this study revealed alkaloids, flavonoids, tannins, terpenoids, and cardiac glycosides as the phytochemical compounds present in the tiger nuts purchased from the three markets. Quantitatively, tiger nuts purchased from Ile-Ife had the highest quantities of tannin (620.1 GAE/100 g) and alkaloids (18%). Meanwhile, the tiger nuts bought in the Osogbo market had the highest amount of flavonoids (9.5%). The variations observed in the phytochemical components of the tiger nuts bought from the different locations could be because of some factors, which may include, the cultivation conditions, a difference in the period between harvest and purchase, period of exposure, and preservation methods (Asante *et al*., 2014; Bado *et al*., 2015). Phytochemicals reported in this study have different pharmacological properties, hence supporting the nutritional and medicinal values of tiger nut tubers. The phytochemicals have hepatoprotective, anti-sickling, aphrodisiac, antimicrobial, anti-inflammatory, and antioxidant properties (Da Silva *et al*., 2012; Rehman *et al*., 2015; Dermane *et al*., 2018; Yang *et al*., 2020). Furthermore, anthroquinone, a phytochemical compound known to have a laxative effect and relevant in the cure for constipation (Lombardi *et al*., 2020), was identified, in tiger nut samples purchased from the Ado-Ekiti market. Similar to the report of Aduwamai *et al*., (2018), saponin was not detected in any of the tiger nut tubers analyzed in this study.

The result of the mineral composition showed that the study tiger nuts were rich in organic matter. The abundance of elements in the tiger nut samples was in the following order: K > P > Mg > Ca > Na > Fe > Zn > Mn > Cu. The high amount of potassium (0.255-0.345%) and magnesium (0.101-0.112%) to sodium (0.020-0.021%) in tiger nut makes it suitable as a diet formulation for individuals with high blood pressure. Phosphorus, a component of Adenosine triphosphate (ATP) which is an energy rich molecule in the body, was the second most abundant element in the tiger nut samples. The least abundant elements in the tiger nut samples were iron (0.0045-0.0048%), zinc (0.0024-0.0025%), and copper (0.0007-0.0009%). Zinc plays a vital role in alleviating neurodegenerative disorder-related problems in the elderly (Prasad, 2014). Hence, tiger nut consumption can be a source of zinc to improve the health conditions of the elderly. Tiger nut can also be a good food supplement to prevent anemic conditions because it contains iron, a component of hemoglobin. Furthermore, Fe, Cu, Zn, and Ca present in the tiger nut samples have been reported as essential elements in male fertility and sexual function (Shinohara and Watanabe, 1996).

Anti-nutrients are substances that prevent the efficient utilization of food nutrients by the body. They are sometimes toxic, and their effects may result from their metabolic products (Okoye and Ene, 2018). Phytic acid, oxalate, and tannin were the anti-nutrients identified in the tiger nut samples. High amounts of oxalate, phytic acid, and tannin in food samples can reduce calcium absorption (Bello *et al*., 2008), cause mineral deficiencies (Phillippy *et al*., 2004), and have interaction that is detrimental to humans with dietary protein (Cirkovic Velickovic and Stanic-Vucinic, 2018), respectively. The amount of tannins (0.42-0.98 mg/100 g) observed in this study is tolerable and may not cause any adverse effects considering the recommended amount of tannins daily intake for a man, which is 560 mg/100 g (Bello *et al*., 2008). Overall, the low amount of anti-nutrients observed for the tiger nuts in this study supported its usage as a medicinal, beneficial, and functional food that can improve the health status of consumers.

This study, to our knowledge, is the first report on the endophytic bacteria associated with tiger nut tuber. The endophytic bacteria isolated include the genera *Lactococcus*, *Bacillus*, and *Enterobacter*, which have been reported as endophytes of several plants (McCulloch *et al*., 2014; Hu *et al*., 2017; Yaish, 2017; Guo *et al*., 2020; Panigrahi *et al*., 2020). *Lactococcus lactis* belongs to the group of lactic acid bacteria with the status of "generally regarded as safe" (GRAS) and probiotic potential (Oliveira *et al*., 2017). *Bacillus* species promote plant growth by producing auxins, siderophores, gibberellin, and indole acetic acid that protect plants against phytopathogens and adverse conditions, such as drought (Suhandono *et al*., 2016). Macedo-Raygoza *et al*. (2019) also reported the nitrogen-fixation and phosphorus solubilization potentials of *Enterobacter cloacae*. There are reports on the probiotics potentials of genera *Bacillus*, strains of *L. lactis*, and *E. cloacae* in human, aquatic, and poultry nutrition (Gao *et al*., 2018; Girijakumari *et al*., 2018; Yerlikaya, 2019; Zhao *et al*., 2019), hence suggesting the application of tiger nut as a probiotic functional food in these different areas.

The two species of endophytic fungi isolated in this study (*S. cerevisiae* and *C. tropicalis*) have been reported to be implicated in the spoilage of exposed tiger nut milk (Onovo and Ogaraku, 2007). Similar to *B. cereus*, *C. tropicalis* is a potential pathogen of humans. However, studies have reported *S. cerevisiae* and *B. cereus* as important endophytic microorganisms (Pennacchi *et al*., 2008; Cutting, 2011). Further work is to examine the biological activities of the endophytic bacteria and fungi isolated from the tiger nut tubers for possible beneficial applications.

5. Conclusion

This study provided information on the different parameters that can play roles in the health benefits of tiger nut, perhaps as a functional food. This study has revealed that tiger nut tubers have important pharmacologically active phytochemicals, nutritional elements, negligible amount of anti-nutrients, and endophytic bacterial and fungal strains previously reported by several authors to have probiotic potentials.

References

Adejuyitan JA. 2011. Tigernut processing: its food uses and health benefits. *Am. J. Food Technol*., **6(3)**: 197-201.

Aduwamai UH, John UI, Aminu A and Isaac UK. 2018. Influence of different processing methods on proximate and anti-nutritional value of tigernuts (*Cyperus esculentus* L.). *GSC biol. pharm. Sci*., **3(3)**: 029–034.

Afzal I, Shinwari ZK, Sikandar S and Shahzad S. 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. *Microbiol. Res.*, **221**: 36-49.

Aguilar N, Albanell E, Miñarro B, Guamis B and Capellas M. 2015. Effect of tiger nut-derived products in gluten-free batter and bread. *Food Sci. Technol*., **21(5)**: 323-331.

AOAC. 2005. The Official Method of Analysis. 17th Ed. Association of Official Analytical Chemists, Washington D.C. U.S.A.

Asante FA, Oduro I, Ellis WO and Saalia FK. 2014. Effect of Planting Period and Site on the Chemical Composition and Milk Acceptability of Tigernut (*Cyperus Esculentus* L) Tubers in Ghana. *Am. J. Food Nutri*., **2(3)**: 49-54.

Bado S, Bazongo P, Son G, Kyaw MT, Forster BP, Nielen, S, Lykke AM, Ouédraogo A and Bassolé, IHN. 2015. Physicochemical characteristics and composition of three morphotypes of *Cyperus esculentus* tubers and tuber oils. *J Anal Methods Chem.*, **2015**:1-8.

Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF and Nabavi SM. 2017. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiol. Res*., **196**: 44-68.

Bazine T and Arslanoğlu F. 2020. Tiger Nut (*Cyperus esculentus*); Morphology, Products, Uses and Health Benefits-Review. *BSJ Agri*., **3(4)**: 324-328.

Bello MO, Falade OS, Adewusi SRA and Olawore NO. 2008. Studies on the chemical compositions and anti-nutrients of some lesser known Nigeria fruits. *Afr. J. Biotechnol*., **7(21)**: 3972-3979.

Bind M and Nema S. 2019. Isolation and molecular characterization of endophytic bacteria from pigeon pea along with antimicrobial evaluation against *Fusarium udum*. *Appl Microbiol*., **5(2)**: 19.5.163.

Chen YL, Lee CC, Lin YL, Yin KM, Ho CL and Liu T. 2015. Obtaining long 16S rDNA sequences using multiple primers and its application on dioxin-containing samples. *BMC bioinform*., **16(18)**: 1-11.

Cirkovic Velickovic TD and Stanic-Vucinic DJ. 2018. The role of dietary phenolic compounds in protein digestion and processing technologies to improve their antinutritive properties. *Compr. Rev. Food Sci. Food Saf*., **17(1)**: 82-103.

Cutting SM. 2011. Bacillus probiotics. *Food Microbiol*., **28(2)**: 214-220.

Da Silva CV, Borges FM and Velozo ES. 2012. Phytochemistry of some Brazilian plants with aphrodisiac activity. *Phytochemicals-A Global Perspective of Their Role in Nutrition and Health*, 307-326.

Dermane A, Kpegba K, Metowogo K, Joppa MK, Eklu-Gadegbeku K, Aklikokou, AK and Gbeassor, M. 2018. Evaluation of the anti-sickling activity of *Newbouldia laevis* P. Beauv extracts. *Int. J. Chem. Biol. Sci*., **12(6)**: 2808-2817.

Gao X, Zhang M, Li X, Han Y, Wu F and Liu Y. 2018. Effects of a probiotic (*Bacillus licheniformis*) on the growth, immunity, and disease resistance of *Haliotis discus hannai* Ino. *Fish Shellfish Immunol*., **76**: 143-152.

Girijakumari NR, Ethiraja K and Marimuthu PN. 2018. *In vitro* and *in vivo* evaluation of probiotic properties of *Enterobacter cloacae* in Kenyi cichlid, *Maylandia lombardoi*. *Aquac Int*., **26(4)**: 959-980.

Guo DJ, Singh RK, Singh P, Li, DP, Sharma A, Xing YX., Song XP, Yang LT and Li YR. 2020. Complete Genome Sequence of *Enterobacter roggenkampii* ED5, a Nitrogen Fixing Plant Growth Promoting Endophytic Bacterium with Biocontrol and Stress Tolerance Properties, Isolated from Sugarcane Root. *Front. Microbiol*, **11**:2270.

Harbone JB, 1984. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd Ed. Chapman and Hall, London, New York, 3rd edition, 21-29.

Harju S, Fedosyuk H and Peterson KR. 2004. Rapid isolation of yeast genomic DNA: Bust n'Grab. *BMC Biotechnol*., **4(1)**: 1-8.

Hu HJ, Chen YL, Wang YF, Tan, YY, Chen SL and Yan SZ. 2017. Endophytic *Bacillus cereus* effectively controls *Meloidogyne incognita* on tomato plants through rapid rhizosphere occupation and repellent action. *Plant Dis.*, **101(3)**: 448-455.

Kumar S, Stecher G and Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol*., **33(7)**: 1870-1874.

Kunatsa Y, Chidewe C and Zvidzai CJ. 2020. Phytochemical and anti-nutrient composite from selected marginalized Zimbabwean edible insects and vegetables. *J. Agric. Food Res*., **2**: 100027.

Lombardi N, Bettiol A, Crescioli G, Maggini V, Gallo E, Sivelli F, Sofi F, Gensini GF, Vannacci A and Firenzuoli F. 2020. Association between anthraquinone laxatives and colorectal cancer: protocol for a systematic review and meta-analysis. *Syst. Rev*., **9(1)**: 1-6.

Macedo-Raygoza GM, Valdez-Salas B, Prado FM, Prieto KR, Yamaguchi LF, Kato MJ, Canto-Canché BB, Carrillo-Beltrán M, Di Mascio P, White JF, Beltrán-García MJ. 2019. *Enterobacter cloacae*, an endophyte that establishes a nutrient-transfer symbiosis with banana plants and protects against the black Sigatoka pathogen. *Front Microbiol*., **10**: 804.

Martínez-Romero E, Aguirre-Noyola JL, Bustamante-Brito R, González-Román P, Hernández-Oaxaca D, Higareda-Alvear V, Montes-Carreto LM, Martínez-Romero JC, Rosenblueth M and Servín-Garcidueñas LE. 2020. We and herbivores eat endophytes. *Microb. Biotech*., **14(4)**: 1282-99.

McCulloch JA, de Oliveira VM, de Almeida Pina AV, Pérez-Chaparro PJ, de Almeida L., de Vasconcelos JM, de Oliveira LF, da Yang, DEA, Rogez HLG., Cretenet M and Mamizuka EM. 2014. Complete genome sequence of *Lactococcus lactis* strain AI06, an endophyte of the Amazonian açaí palm. *Genome Announc*., **2(6)**: e01225-14.

Nayak S, Mukherjee A and Samanta S. 2017. Endophytic microorganisms of tropical tuber crops: Potential and perspectives. *J. Appl. Nat. Sci*., **9(2)**: 860-865.

Okoye JI and Ene GI. 2018. Effects of processing on the nutrient and anti-nutrient contents of tiger nut (*Cyperus esculentus* Lativum). *J. Food Sci. Technol*., **1(1)**: 1-7.

Oladele AK and Aina JO. 2007. Chemical composition and functional properties of flour produced from two varieties of tiger nut (*Cyperus esculentus*). *Afr. J. Biotechnol*, **6(21)**: 2473-2476

Oliveira LC, Saraiva TD, Silva WM, Pereira UP, Campos BC, Benevides LJ, Rocha FS, Figueiredo HC, Azevedo V, Soares SC. 2017. Analyses of the probiotic property and stress resistance-related genes of . *Lactococcus lactis* subsp. lactis NCDO 2118 through comparative genomics and in vitro assays. *PLoS One* 12(4): e0175116.

Onovo JC and Ogaraku AO. 2007. Studies on some microorganisms associated with exposed Tigernut milk. *J. Biol. Sci*., **7(8)**: 1548–1550.

Onwuka GI. 2005. Food Analysis and Instrumentation: Theory and Practice. Lagos, Nigeria: Naphtali print. **5**:133-135.

Panigrahi S, Mohanty S and Rath CC. 2020. Characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from

Ocimum sanctum with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. *S. Afr. J. Bot*, **134**: 17-26.

Pennacchia C, Blaiotta G, Pepe O and Villani F. 2008. Isolation of *Saccharomyces cerevisiae* strains from different food matrices and their preliminary selection for a potential use as probiotics. *J. Appl. Microbiol*., **105(6)**: 1919-1928.

Phillippy BQ, Lin M and Rasco B. 2004. Analysis of phytate in raw and cooked potatoes. *J. Food Compos. Anal*., **17(2)**: 217-226.

Prasad AS. 2014. Zinc is an antioxidant and anti-inflammatory agent: its role in human health. *Front Nutr.,* **1(14)**: 1-10.

Rehman JU, Aktar N, Khan MY, Ahmad K, Ahmad M, Sultana S and Asif HM. 2015. Phytochemical screening and hepatoprotective effect of *Alhagi maurorum Boiss* (Leguminosae) against paracetamol-induced hepatotoxicity in rabbits. *Trop. J. Pharm. Res*., **14(6)**: 1029-1034.

Sabah MS, Shaker M and Moursy I. 2019. Nutritional Value of Tiger Nut (*Cyperus esculentus* L.) Tubers and Its Products. *Environ. Sci*., **14(1)**: 301-318.

Shinohara A and Watanabe H. 1996. Role of essential trace elements on sexual function and its disorder. *Nihon rinsho. Japanese J. Clin. Med*., **54(1)**: 155-161.

Suhandono S, Kusumawardhani MK, Aditiawati P. 2016. Isolation and molecular identification of endophytic bacteria from Rambutan fruits (Nephelium lappaceum L.) cultivar Binjai. *HAYATI J. Biosci*., 23(1): 39-44.

Tamura K and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.*, **10(3)**: 512-526.

Yaish MW, 2017. Draft genome sequence of the endophytic *Bacillus aryabhattai* strain SQU-R12, identified from *Phoenix dactylifera* L. roots. *Genome Announc*., **5(32)**: e00718-17.

Yang L, Zhu Y, He Z, Zhang T, Xiao Z, Xu R. and He J. 2020. Plantanone D, a new rare methyl-flavonoid from the flowers of *Hosta plantaginea* with anti-inflammatory and antioxidant activities. *Nat. Prod. Res.*, **35(22)**: 4331-4337.

Yasser MM, Marzouk MA, El-Shafey NM and Shaban SA. 2020. Diversity and Antimicrobial Activity of Endophytic Fungi from the Medicinal Plant *Pelargonium graveolens* (geranium) in Middle Egypt. *Jordan J. Biol. Sci.*, **13(12)**: 197-205.

Yerlikaya O. 2019. Probiotic potential and biochemical and technological properties of *Lactococcus lactis* ssp. *lactis* strains isolated from raw milk and kefir grains. *J. Dairy Sci*., **102(1)**: 124-134.

Zhao Y. Zeng D. Wang H. Qing X. Sun N, Xin J, Luo M, Khalique A, Pan K, Shu G and Jing B. 2019. Dietary Probiotic *Bacillus licheniformis* H2 Enhanced Growth Performance, Morphology of Small Intestine and Liver, and Antioxidant Capacity of Broiler Chickens Against *Clostridium perfringens*–Induced Subclinical Necrotic Enteritis. *Probiotics Antimicrob Proteins*, **12**:883-895.