INTERNATIONAL JOURNAL OF FAMILY STUDIES, FOOD SCIENCE AND NUTRITION HEALTH

PRINT-ISSN: 2735-5373

VOLUME 3, ISSUE 2, 2022, 165 – 193.

ONLINE-ISSN: 2735-5381

Study the antioxidant and antimicrobial of tiger nut and the biological effects of some its products

Mai M.M. Naeem and Ghada M. Youssef
Department of Special food and nutrition, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

Corresponding author:

Correspondence to Mai M.M.Naeem Assistant professor in Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

Email: mima.mmm81@gmail.com ; mima_mmm81@yahoo.com

https://orcid.org/0000-0002-6922-1958

Abstract

Tiger nut (*Cyperus esculentus*) has high nutritive properties, therapeutic potential and health benefits; it grows mostly as a weed. This research aimed to study the nutrition value, phenolic compounds and antimicrobial activity of tiger nut and preparation of fermented and non-fermented tiger nut milk and its effect on the treatment and protection of hepatotoxicity rats induced by CCl_4. Fermented Tiger nut milk (FTNM) decreased the content of fiber and fat but increased the protein, carbohydrate, and ash contents compared with tiger nut milk (TNM). Total phenolic, total flavonoid and antioxidant activity were 211.5 mg GAE/100g, 95.7 mg QE/g and 78.4%, respectively. The major components of the phenolic compound were ρ-OH benzoic acid (57.9 mg/100g). The phenolic extract at concentration of 150 mg\ml has the highest antimicrobial activity against pathogenic microorganisms *Escherichia coli* > *Staphylococcus aureus* > *Salmonella typhimurium* > *Pseudomonas aeruginosa* >*Candida albicans* > *Aspergillus niger*. The biological experiment was done on 30 adults male Wistar weight 220±10 g. The experimental period was 6 weeks. Rats were divided into six groups of five rats in each; hepatic rats have done by injecting 0.1 ml of the prepared mixture (1:1) CCl_4: olive oil. FTNM and TNM were observed to reduce the

PRINT-ISSN: 2735-5373
VOLUME 3, ISSUE 2, 2022, 165 – 193.
ONLINE-ISSN: 2735-5381

activities of serum AST, ALT and ALP (hepatic biomarker enzymes). There were no significant differences ($p>0.05$) between the serum albumin, globulin, and total protein levels of the group of rats taken 2ml of FTNM and the negative control group, which indicates that the 2 ml of FTNM repaired damaged liver.
Keywords: *Cyperus esculentus*, microbiological activity, hepatoprotective.

Introduction

These tiger nuts (*Cyperus esculentus*) were first identified in Egypt and are also called walnut grass or yellow nuts. The origin of this plant was in southern Europe, Ghana, Nigeria, and Sierra Leone. The ancient Egyptians used it for thousands of years in embalming because it naturally contains compounds with high health benefits, which are the so-called prebiotics aimed at functional food. Therefore, TNM is one of the most promising alternative food sources **(Malashree *et al.*, 2021).**

Tiger-nut is an underutilized crop of the family Cyperaceae which produces rhizomes from the base and tubers that are spherical and look like a peanut, also known as earth-almond was purchased as dried earth-almond tubers **(Adejuyitan *et al.*, 2008 and Charity *et al.*, 2020)**.

Tiger nut is rich in minerals (phosphorous and potassium), vitamin E and C, soluble monosaccharide (glucose), and monounsaturated fatty acid (oleic acid). It is also rich in the following nutrients: starch, fats, and sugars. It contains fiber, unsaturated fat and moderate amounts of protein and is rich in phytochemicals. Hence, it has a role in the nutrition, health and economy of many countries **(Nura Abdullahi *et al.*, 2022).**

Many researchers are interested in using non-dairy products to be used as alternatives to dairy products, which led to an increase in research interest in developing probiotic products. However, these alternatives, which are called plant-based milk, did not gain market popularity. But with the increase in

awareness and the spread of nutritional education, especially with regard to the problems resulting from allergies, intolerance and high cholesterol, and with the advantages of vegetable milk in that it is free of cholesterol and the low energy resulting from the digestion and absorption of these products, the percentage of purchases made it rise. TNM is called tiger nut drink or tiger nut beverage and is also known as tiger nut milk drink **(Maduka *et al.*, 2017).**

TNM is consumed in most parts of the world and the people of the northern part of Nigeria drink it because it resembles milk. It has high nutritional value, and because it is perishable and short shelf life, its availability is limited **(Olofu *et al*., 2021).**

TNM is suitable for many ages because it is a lively and healthy drink. It protects the heart from attacks, stimulates blood circulation, and protects the colon from cancer, in addition to being suitable for people who suffer from diabetes, and its consumption leads to weight loss **(Borges *et al*., 2008 and Adejuyitan *et al*., 2009).**

TNM, also known as "Horchatade Chufa" in Spanish, is a tasty, all-natural vegetable drink or dessert that is made with water, sugar, and tiger nuts. For both young and old, it is a wonderfully nourishing energy drink **(Djomdi *et al*., 2020)**.

TNM is a nutrient-rich source of energy, protein, fiber, vegetable fat, vitamins, minerals, and several digestive enzymes like amylase, lipase, and catalase. It also contains more carbohydrates, magnesium, and iron than cow milk **(Adejuyitan, 2011).**

Tiger nuts are gluten-free, making them a healthy alternative. In addition, it contains resistant starch, this makes them a super food, especially given that their nutritional profile. Prebiotic resistant starch has the potential to be an effective treatment for or preventive measure for diabetes and obesity **(Richard and Paul, 2016).**

Tiger nuts are characterized by their high content of vitamins and the content of phenolic compounds, in addition to containing some compounds that have a strong effect as antioxidants, and this makes them useful alternatives for use in medical aspects **(Chelsea, 2021).**

Since it does not contain lactose, TNM is a better alternative for those who are lactose intolerant. People who suffer from lactose intolerance suffer from symptoms resulting from their inability to break down lactose into its primary component. Lactose is a disaccharide and it is one of the ingredients found in dairy products. Lactose intolerance is due to an increase in the concentration of lactose as a result of its not being hydrolyzed into glucose and galactose by the enzyme lactase due to its lack of presence in the small intestine (**Malashree *et al.*, 2021).**

Additionally, it has the benefit of not having sodium, lactose sugar, casein protein, gluten, or cholesterol and is therefore perfect for those who have hypertension or cannot take the lactose, gluten, or derivatives of lactose that are present in milk. Additionally, unlike soymilk or other soy products, TNM does not cause any allergy **(Anon., 2012).**

TNM's safety, nutritional value, and general acceptance are all influenced by lactic acid fermentation procedures. Pasteurization and fermentation enhanced the products' microbiological, nutritional, and chemical properties **(Sherifah *et al.*, 2014.)**

Njoku-Oji *et al.* (2019) have demonstrated that *C. esculentus* seed extract may be beneficial in the treatment of diabetes mellitus**.** The impact is influenced by the presence of vitamins, minerals, and phytochemical components.

This investigation aimed to study the nutrition value, phenolic compound, and antimicrobial activity of tiger nut and the preparation of fermented and non-fermented tiger nut milk and its effect on the treatment and protection of hepatotoxicity rats induced by CCl_4.

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PRINT-ISSN: 2735-5373
VOLUME 3, ISSUE 2, 2022, 165 – 193.
ONLINE-ISSN: 2735-5381

Materials and Methods

Materials

The tubers of tiger nut (*Cyperus esculentus*) have been obtained from the local market in Tanta city, Egypt. Obtained all chemicals from Sigma Chemical Co., Saint Louis, Missouri, USA with a purity of 96-99%. The kits used in the biochemical analysis were purchased from Gama trade Company, Cairo, Egypt.

Lactic acid bacterial cultures

Streptococcus thermophilus CH-1 was obtained from Chr. Hansen's Lab., Denmark. *Lactobacillus delbrueckii subsp. Bulgaricus* Lb-12 DRI-VAC and *Lactobacillus rhamnosus* B-445 were obtained from the Laboratory of the Northern Regional Research (NRRL), Illinois, USA.

Pathogenic microorganism

These strains are *Staphylococcus aureus* ATCC 25923, *E.coli* ATCC 25922**,** *Salmonella typhimurium* ATCC 20231**,** *Pseudomonas aeruginosa* ATCC 27853**,** *Candida albicans* CAIM -22 were bought from MIRCEN (Microbial resource Center) Ain–Shams University, Cairo, Egypt**.** *Aspergillus niger* was isolated from several sources of rotting (vegetables, fruits, grains).

Methods

Preparation of Tiger Nut Extract

The nuts were thoroughly washed and dried in a hot air oven **(**Venticell 55- ECO line, Germany**)** at 40^{o}C for 72 hrs. Then they were ground using an electric mixer till they became a fine powder **(**Braun blender JB3010, Germany). The approximately 225g of the powdered substance was soaked in 400ml ethanol (80%) for 48 hrs. with intermittent shaking **(**GFL1083, Germany). powdered material was extracted , filtered and concentrated by using a rotary evaporator **(**JE –RE-5, India**)** at 40 ^{o}C and then put into perfectly sterile glass vials and kept at 4 ^{o}C before use**.(Malann *et al* ., 2014).**

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PRINT-ISSN: 2735-5373
VOLUME 3, ISSUE 2, 2022, 165 – 193.
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Preparation of Tiger Nut Milk (TNM)

In unscheduled results, several ratios of tiger nut to water (1:1, 1:3 and 1:6) were tested for the preparation of a milk-like product from a vegetable source (tiger nut). The best ratio of tiger nut to water was 1:3, as it was accepted by more than twenty of the trained members' panelists.

One Kg of the fresh tiger nuts was blended several times with a three liter of tap water using the auto-clean blender (Braun blender JB3010, Germany), separates the milk by filtration through a clean sterile muslin cloth **(Ukwuru *et al*., 2011)** .The milk was bottled in clean screw-cap bottles then pasteurized in a water bath at 90˚C for 15 minutes, cooled to a temperature 43˚C, and stored in a refrigerator until use. **(Rita, 2009).**

Preparation of Fermented Tiger Nut Milk (FTNM)

Prepared the starter culture containing *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Lactobacillus rhamnosus* B-445 *Streptococcus thermophiles*. Pasteurized tiger nut milk inoculated with 2% v/v of starter culture at 43°C. Covered the jar and incubated at 42°C for 4 hours, the jars were placed in a refrigerator **Wakil *et al*., (2014).**

Analytical Methods

Chemical composition of tiger nut, TNM and FTNM

According to the methods outlined by **AOAC (2018),** the proximate analysis of tiger nut, TNM and FTNM including moisture, protein, fat, ash, and crude fibers, and minerals (Magnesium (Mg), potassium (K), phosphorous (P), calcium (Ca), sodium (Na) and Iron (Fe)) were determined and carbohydrates were calculated by difference.

Determination of polyphenols and flavonoids

Total phenolic compounds of tiger nut were determined colorimetrically using the modified Folin-Ciocalteu method (as gallic acid equivalent) according to the method described by **Singleton *et al.*, (1999).** Total flavonoid compounds were determined using the $AlCl_3$ method (as quercetin acid equivalent) according to the methods of **Zhishen *et al.*, (1999)**.

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PRINT-ISSN: 2735-5373
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Identification of phenolic acids compounds of tiger nut extract

Phenolic compounds of tiger nut extract were fractionated using HPLC according to the method of **Goupy *et al*. (1999).**

Antioxidant activity (DPPH radical scavenging activity)

The antioxidant activity of tiger nut extract was determined using the free radical, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent, The DPPH assay was measured according to the method reported by **Brand-Williams *et al*., (1995).**

Assessment of antimicrobial activity of tiger nut extract

Four pathogenic bacterial strains, one pathogenic yeast strain, and one pathogenic fungal strain were used to test the antimicrobial activity of ethanolic extract (50,100,150 mg/mL). This procedure was done by the disc diffusion technique according to the method reported by **Kotzekidou *et al*., (2008).**

Physiochemical characteristics of TNM and FTNM.

The pH value, titratable acidity and total soluble solids of TNM and FTNM were determined according to **AOAC (2018).**

Effect of TNM and FTNM on liver enzymes and proteins using experimental animals.

The National Research Centre's (NRC) animal house provided a total of 30 adult male Wistars weighing (220 ±10 g). Before beginning testing, animals were placed in quarantine and given seven days to adapt.

All animals were kept in environments with controlled air quality, temperature and access to feed and water. The experimental period was 6 weeks. Thereafter, they were randomized into six groups of five rats in each. The rats will be divided into two main groups as follows **:**

-The first main group G1: (n = 5) fed on the basal diet as a negative control.

-The second main group: (n = 25 hepatic rats) was fed on either the basic diet as a positive control (G2), or different concentrations of TNM, or FTNM (G3-G6). G3 - G6 were fed

by stomach tube per day (dose for rat weight). This group (25 rats) was intraperitoneally injected by using carbon tetrachloride dissolved in olive oil at a ratio of 1:1 at a concentration of 0.5 mg/kg of the rat's body weight (carbon tetrachloride was prepared by dissolving 100 mg CCl_4 in 100 ml of olive oil). Each rat was given 0.1 ml of the prepared solution intraperitoneally twice weekly over the entire period of the experiment) **(Iredale *et al.*, 1998).**Then divided into the following subgroups: G2 - Hepatic rats + basal diet (positive control), G3 - Hepatic rats + 0.24 g/1ml from TNM, G4 - Hepatic rats + 0.48 g/2ml from TNM, G5 - Hepatic rats + 0.24 g/1ml from FTNM and G6 - Hepatic rats + 0.48 g/2ml from FTNM. The dose of milk was taken for (G3, G4, G5 and G6) every day for a long period of experiment (0.24 g/1ml, 0.48 g/2ml for TNM and FTNM).

Basal Diet

The following formula was used to prepare the following basal diet which is provided by **(Reeves *et al*., 1993)** as follow: casein (140 g/kg), soybean oil (40 g/kg), vitamin mixture (10 g/kg), mineral mixture (35 g/kg), sucrose 100 g/kg and choline chloride (2.5 g/kg), the remained is corn starch.

Biochemical analyses

According to the method of **Reitnman and Frankel (1957)**, which used kits from the commercial Randox enzymes, the activities of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured. Alkaline phosphatase (ALP) activity was determined by phenolphthalein monophosphate method described by **Babson *et al.* (1966).** The protein content of serum was determined by the Biuret method **(Peters, 1968)**. Using the QCA albumin test kit which is based on the bromocresol green method the serum albumin was measured by **Johnson, (2008)**. The serum albumin levels were subtracted from the total protein levels to calculate the globulin levels **(Johnson, 2008).**

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PRINT-ISSN: 2735-5373
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Statistical analysis

ANOVA was used to analyses the data followed by Duncan's multiple range tests ($P \leq 0.05$) and carried out using SPSS computer program **(SPSS, 1997)**.

Results and Discussion

Chemical composition of Raw Tiger nut, TNM and FTNM

The proximate composition of results raw tiger nut, TNM, and FTNM is tabulated in Tables (1and 2). The results shown in Table (1) indicated that the raw tiger nut was higher in carbohydrate content (53.72 %) followed by the fat content (23.4%), while the protein and crude fiber were (7.78 % and 7.5 %, respectively) whereas, the ash was found the lowest content (2.4%). **Sabah *et al* ., (2019)** found that tiger nut tubers contained the highest proportion of carbohydrates (45.73 %) and oil (30.01 %) furthermore, the protein, ash, and crude fiber contents of tiger nut tubers were 5.08 %, 2.23 %, and 14.80 %, respectively. **El-Naggar ,(2016)** studied that the fiber content was 6.5% and this result was similar to the results of this study's measurements. **Ayat *et al*., (2021)** reported that the chemical composition of raw tuber tiger nuts was 6.00% protein, 1.70% ash, 4.30% crude fiber, and 58.23% total carbohydrates. Concerning minerals contents of the raw tiger nut, the results presented in the same Table indicated that K showed the high amounts (262.10 mg/100g), followed by Ca (244.06 mg/100g), Na (163.24 mg/100g), then P (160.04 mg/100g) and finally Mg (101.01 mg/100g). However, the lowest value was that of Fe (3.31 mg/100g).

The results of the TNM and FTNM in Table (2) showed approximately the same of the protein, fat and ash content (5.5 and 6.9%, 7.7 and 7.1%, and 1.5 and 1.9%, respectively). Concerning a dry weight basis the protein, fat and ash content were 23 and 28.4%, 32 and 29.2%, and 6.25 and 7.8%, respectively. From the results in Table (2), that based on dry weight basis, it could be noted that there was a significant increase ($P<0.05$) in the content of both protein and ash for FTNM (28.4 and 7.8%, respectively) compared to TNM (23 and 6.25%, respectively). Whereas, the content of fats, crude

fiber, and carbohydrates increases significantly ($P<0.05$) when comparing TNM (32, 8.75, and 30%, respectively) with FTNM (29.2, 7.0, and 27.6%, respectively). **Adejuyitan, (2011)** reported that fermentation increased the protein and ash contents. The increase in ash content may be due to fermentation which is one of the processing methods used to liberate the complex minerals found in the ash and make them easily accessible. **(Pranoto *et al*., 2013).**

Mineral deficiency causes many diseases such as high blood pressure, heart-related diseases, diseases resulting from malnutrition; also some minerals play vital roles, such as potassium and sodium **(Jayasinghe *et al.*, 2016 and Hoffman, 2017)**.

On the other hand, it was found that the results of the mineral content of FTNM was slightly higher than that of TNM. These result are in agree with **Day and Morawicki, (2018)** who reported increased mineral content due to dry matter loss during fermentation with microbial decomposition. **Pranoto *et al*., (2013)** found that increased iron, zinc, magnesium and calcium contents were associated with decreased phytate content in some fermented foods. In addition to the nutrients they currently contain, fermented functional foods may also have additional health benefits. Fermented products have been found to increase immunological function and have a positive effect on body health. **Kaur *et al*., (2022)**.

INTERNATIONAL JOURNAL OF FAMILY STUDIES, FOOD SCIENCE AND NUTRITION HEALTH
PRINT-ISSN: 2735-5373
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ONLINE-ISSN: 2735-5381

Table (1): Chemical composition of Tiger nut raw material (g/100g).

Parameters %	Tiger nut (Raw)
Moisture	5.2 ±0.10
Protein	7.78 ±0.15
Fat	23.4 ±0.09
Ash	2. 4 ±0.11
Crude fiber	7.5 ±0.10
Carbohydrates	53.72 ±0.10
Minerals (mg/100g)	
Mg	101.01 ±1.03
K	262.10 ±0.10
P	160.04 ±1.03
Ca	244.06 ±1.08
Na	163.24 ±0.83
Fe	3.31 ±0.97

Values are means ± SD of three measurements.

Table (2): Chemical composition of Tiger nut milk and Fermented tiger nut milk.

Parameters (%)	Tiger nut milk (TNM)	Fermented tiger nut milk (FTNM)
Moisture	$76^{a} \pm 1.01$	75.7^{b} ±0.01
Protein	$5.5^{b} \pm 0.10$	6.9^{a} ±0.01
Fat	$7.7^{a} \pm 0.10$	7.1^{b} ±0.08
Ash	$1.5^{b} \pm 0.10$	1.9^{a} ±0.11
Crude fiber	$2.10^{a} \pm 0.09$	1.7^{b} ±0.10
Carbohydrates	$7.2^{a} \pm 0.09$	$6. 7^{b}$ ±0.10
Minerals (mg/100ml)		
Mg	50.63^{b} ±0.15	57.51^{a} ±0.10
K	221.04^{b} ±1.01	231.04^{a} ±1.0
P	130.02^{b} ±1.04	133.01^{a} ±1.05
Ca	225.01^{b} ±1.04	229.02^{a} ±1.10
Na	141.05^{b} ±1.01	143.93^{a} ±1.01
Fe	3.11^{b} ±0.08	3.21^{a} ±0.10

Values are means ± SD of three measurements. Means in the same row with different letters are significantly different ($p < 0.05$).

INTERNATIONAL JOURNAL OF FAMILY STUDIES, FOOD SCIENCE AND NUTRITION HEALTH
PRINT-ISSN: 2735-5373
VOLUME 3, ISSUE 2, 2022, 165 – 193.
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Total phenolic and total flavonoid compounds content of tiger nut extract and its antioxidant activity.

The results presented in Table (3) showed the amount of total phenolic and flavonoid contents of tiger nut extract. From these results, it could be observed that the quantity of total phenolic content in tiger nut extract was 211.5 mg GAE/100g, while flavonoid contents were 95.7 mg QE/g. **Owon *et al*., (2013)** found that the total phenol compounds contents in tiger nut tubers were 197.20 mg/100g. **Oladele *et al.*, (2017)** reported that the total phenol in tiger nut extract was 169.21 mg/100g and the total flavonoid was 236 mg/100g. The variation in total phenol and flavonoid were attributed to the method of analysis used by the authors.

The antioxidant activity of tiger nut extract can be attributed to the presence of polyphenols and flavonoids. The antioxidant activity of tiger nut extract is shown in the same Table (3). The results indicated that the antioxidant activity was 78.4%. **Willis *et al.* (2019)** found that the tiger nut was roasted and extracted with 80% methanol and the free radical scavenging activity by DPPH was 58%.

Table (3): Total phenolic and total flavonoid compounds content of tiger nut extract and its antioxidant activity.

Parameters	Tiger nut extract
Total phenolic (mg GAE/100g)	211.5±0.5
Total flavonoid (mg QE/g)	95.7±0.1
DPPH radical scavenging activity (%)	78.4± 0.1

GAE: gallic acid equivalent – QE: quercetin acid equivalent.

Identification of phenolic acids compounds of tiger nut extract.

The results presented in Table (4) showed the phenolic compounds of tiger nut extract. It could be observed that the tiger nut extract had 15 phenolic compounds. The major component of the present phenolic was found to be ρ-OH benzoic acid at a concentration of 57.9 mg/100g followed by salicylic acid (38.6 mg/100g), cholchecien acid (36.7 mg/100g), then ellagic acid (24.8 mg/100g). However, other phenolic

PRINT-ISSN: 2735-5373
VOLUME 3, ISSUE 2, 2022, 165 – 193.
ONLINE-ISSN: 2735-5381

compounds were also found such as catechol, caffeic acid, ferulic acid, vanillic acid, pyrogallol, ρ - coumaric acid, catechin, protocatechuic acid, and gallic acid but in low concentrations varied between 7.71 mg/100g (catechin) to 1.34 mg/100g (caffeic acid). These results are in agreement with **Owon *et al.*, (2013)** which they found that ρ-hydroxy benzoic acid was the major phenolic compound (54.95 mg/100g) followed by salicylic acid (37.41 mg/100g); then cholchecien (35.25 mg/100g). **Halliwell, (2007)** reported that phenolic compounds may play an important role for prevent damage to the gastrointestinal tract caused by reactive species present in foods. **Queiroz *et al.*, (2019)** reported that gallic acid has a remarkable ability to inhibit peroxides resulting from fat oxidation, and it is also characterized by its low toxicity, so it is used in many food products as a powerful antioxidant.

Table (4): Identification of phenolic acids compounds of tiger nut extract.

Phenolic compounds	mg/100g
Cholchecien	36.7
Salicylic acid	38.6
Ellagic acid	24.8
Catechol	7.03
Caffeic acid	1.34
Vanillic acid	3.65
Pyrogallol	6.02
Ferulic acid	5.33
ρ - Coumaric acid	2.02
Chlorogenic acid	12.5
Catechin	7.71
Protocatechuic acid	2.33
ρ-OH Benzoic acid	57.9
Gallic acid	2.89
Caffeine	13.67

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PRINT-ISSN: 2735-5373
VOLUME 3, ISSUE 2, 2022, 165 – 193.
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Assessment of antimicrobial activity of tiger nut extract.

Four pathogenic bacterial strains, a pathogenic yeast strain, and a pathogenic fungal strain were used to study the antimicrobial effect of the three different concentrations of tiger nut phenolic extracts (50, 100, and 150 mg/ml). The results presented in Table (5) showed that the areas of inhibition increased because of the increase in the concentrations of tiger nut extract used. All used concentrations gave inhibition zones against *Salmonella typhimurium*, *Staphylococcus aureus*, and *Escherichia coli*. No inhibitory effect was detected at the level of 50 mg/ml against *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*. The maximum antimicrobial activity at a concentration of 150 mg/ml was found with *Escherichia coli* with an inhibitor of 23 mm area followed by *Staphylococcus aureus* which showed a 21 mm area inhibitor, then *Salmonella Typhimurium* which showed an 18 mm area inhibitor.

However, the other strains showed zone inhibitors varied between 9 mm (*Aspergillus niger*) to 10 mm (*Candida albicans* and *Pseudomonas aeruginosa*). The obtained results are in harmony with the results of **Ndikom and Elutade (2016)** who studied the effect of extracts of four types of organic solvents (ethanol 50%, acetone, chloroform, and petroleum ether) of tiger nut tubers as antimicrobials (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp*, *Kb. pneumoniae*. *Proteus vulgaris*) where they found The greatest activity of the 50% ethyl extract of tiger nut tubers was against *Escherichia coli, Staphylococcus aureus and Salmonella spp*, while in the case of using acetone extract, it had the greatest activity against each of *Staphylococcus aureus, Kb. pneumoniae and Proteus vulgaris*, and in the case of chloroform extract, it was found that its greatest activity was against *Staphylococcus aureus*, while when using petroleum ether extract, its greatest activity was found against *Kb. pneumonia*. **Owon *et al*., (2013)** and **Adeniyi *et al*., (2014)** found that phenolic tiger nut extracts showed significant antimicrobial activity against Gram (+) and Gram (-) bacteria and against several pathogens.

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The potential of tiger nut extracts to resist bacteria in the human body is also listed among various dietary plants that can be utilized against common bacterial infections including *salmonella* and *E. coli*. **(Richard and Paul, 2016).**

Table (5) : Antimicrobial activity of tiger nut extract.

Tiger nut extract (mg/ml)	Diameters of inhibition zones (mm) of microorganisms					
	Salmonella typhimurium	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Candida albicans***	***Aspergillus niger***
50	11	13	ND	16	ND	ND
100	14	17	7	20	6	6
150	18	21	10	23	10	9

ND: not detected.

Physiochemical characteristics of TNM and FTNM.

The results presented in Table (6) showed the physiochemical characteristics of TNM and FTNM during storage at 4^0C for 14 days. It could be noticed that the results of the pH of the TNM and FTNM were reduced from 7.3 to 5.4 and from 7.3 to 4.02, respectively. The percentage of decrease in pH was greater in the FTNM samples compared to the TNM; this decrement represents 26% and 45%, respectively. These results are in agreement with **Wakil *et al.*, (2014).** They reported that the reduction in pH could be a result of the rapid growth rate of lactic acid bacteria that break down carbohydrates during fermentation.

Table (6): Physiochemical characteristics of Tiger nut milk and Fermented tiger nut during storage at 4^0C.

Parameters	Storage periods (days)					
	Tiger nut milk (TNM)			Fermented tiger nut milk (FTNM)		
	0	**7**	**14**	**0**	**7**	**14**
pH	7.3^{a} ±0.01	6.2^{b} ±0.04	5.4^{c} ±0.05	7.3^{a} ±0.01	4.6^{d} ±0.10	4.02^{e} ±0.15
Titratable acidity (%)	0.02^{e} ±0.01	0.13^{d} ±0.02	0.31^{c} ±0.01	0.02^{e} ±0.01	0.36^{b} ±0.01	0.71^{a} ±0.01
Total solids (%)	15.5^{a} ±0.14	14.3^{b} ±0.10	11.2^{c} ±0.10	15.5^{a} ±0.14	5.49^{d} ±0.05	2.86^{e} ±0.06

Values are means ± SD of three measurements. Means in the same row with different letters are significantly different ($p< 0.05$).

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Regarding the titratable acidity, it was found that the results of the titratable acidity were very small, there was a rise in the titratable acidity with an increasing storage period. The rate of rise in titratable acidity was greater for the FTNM samples compared to the TNM samples, and this increment ranged between 0.02 (%) in zero time and 0.71(%) in FTNM. It was also noted that there is a consistency of pH results and titratable acidity results. These results are in agreement with **Maduka *et al.*, (2017)** they found an increase in titratable acidity of FTNM with mixed culture strains of lactic acid bacteria during the period of storage.

For total solids (%), a significant reduction ($p<0.05$) was noticed in total solids (%) in all studied samples at different storage periods. Furthermore, the amount of total solids was initially high (15.5%) and decreased during storage to 11.2 and 2.9 (%) for each TNM and FTNM samples, respectively after 14 days and the amount of reduction in FTNM samples increased more than TNM samples. **Ukwuru *et al.*, (2008)**. reported that the reduction of total solids may be due to the use of solids in milk by microorganisms, which transform the substrate into non-solid products. In the fermentation group that decreases the pH of TNM and increases titratable acidity, a decrease in pH could have an effect on the sensory qualities **(Komolafe and Arawande, 2010).**

Effects of TNM and FTNM on liver enzymes of rats administered CCl_4.

The main metabolic organ in the body is the liver which is considered a viable defense system against metabolic toxins and environmental toxicants**.** AST and ALT are the hepatic metabolic enzymes that were discharged into the bloodstream after the liver was damaged. The damage to the liver caused by the administration of hepatotoxic substances which is expected to be reflected in these enzymes. **(Airaodion *et al.*, 2019)**. The levels of liver enzymes of rats before and after administration of CCl_4 are shown in Table (7). From these results, it could be observed that there was a significant increase ($P\leq0.05$) in levels of liver enzymes (AST, ALT, and ALP) for the group of rats

that exposed to CCl_4 (Control +) compared to the group of rats that were not exposed to CCl_4 (Control -), after the induction of liver damage. There was a significant decrease ($P \leq 0.05$) in the levels of liver enzymes for all groups containing TNM and FTNM compared to the positive control group.

These decrements were increased with increasing the dose of two kinds of tiger nut milk from 1ml to 2ml. It was also observed that these decrements were more significant ($P \leq 0.05$) in the case of groups of rats fed on FTNM compared to groups of rats fed on TNM. The increase in the amount of the tiger nut in the feed formula is confirmed to the improvement of liver enzymes and the FTNM is more effective than TNM.

Since ALT is restricted to the liver only, unlike AST and ALP, which are also abundantly found in other body organs like the kidneys and brain, it is regarded as the most accurate indicator of hepatic damage (**Airaodion *et al*., 2019).**

Table (7): Effects of TNM and FTNM on liver enzymes of rats administered CCl_4.

Animal Groups	Level of liver enzymes (IU/L)		
	AST	**ALT**	**ALP**
G1: (Negative Control)	98^{d} ±1.001	54^{e} ±1.022	149^{e} ±6.21
G2: (Hepatic rats " Positive Control")	180^{a} ±1.110	97^{a} ±1.16	211^{a} ±1.18
G3: (Hepatic rats + 1ml from TNM)	119^{b} ±1.003	64^{b} ±1.01	$16\,1^{b}$ ±1.104
G4: (Hepatic rats +2ml TNM)	113^{c} ±1.053	61^{c} ±1.23	154.2^{c} ±0.721
G5: (Hepatic rats + 1ml FTNM)	99.1^{d} ±0.956	57^{d} ±1.06	150.4^{d} ±0.953
G6: (Hepatic rats + 2ml FTNM)	97.1^{d} ±0.945	55.1^{e} ±1.044	148.1^{e} ±0.957

Values are expressed as mean ± STD; n=5 in each group, Values with different superscripts along the same column are significantly different ($p < 0.05$). AST = Aspartate Amino Transferase, ALT = Alanine Amino Transferase, ALP = Alkaline Phosphatase.

It was demonstrated that the intraperitoneal injection of CCl_4 cause an increase in levels of ALT significantly in the positive control ($p \leq 0.05$) when compared with the negative control group. Comparing all test treatments to the positive control, the reductions were statistically significant in ALT levels ($p \leq 0.05$) the result is consistent with (**Roselló-Soto *et al*., 2019**) who

investigated the nutritive value and microbiological effect of beverages made from plant-based and lactic fermented tiger nut tubers (*Cyperus esculentus*).

The enzyme alkaline phosphatase (ALP) acts as a marker for the endoplasmic reticulum and plasma membrane of tissues. When comparing results in all test groups to the positive control group, it was found that ALP levels were significantly decreased. **Li *et al*., (2015)** and **Liu et al., (2017)** showed that probiotic (*Lactobacillus*, *Bifidobacterium*, and *Saccharomyces sp*.) supplementation reduced the signs and symptoms of CCl_4-induced hepatic damage via altering the gut microbiota.

By reestablishing healthy gut flora, enhancing liver enzymes, and treating liver disease caused by many factors like alcohol, viral infections, and metabolic problems, probiotics are considered to effectively reduce the risk of disease (**Wang *et al*., 2011 and Bang *et al*., 2014**). The result agrees with **Aswad *et al*., (2021)** demonstrating that the bacteria *Lactococcus lactis* subspecies cremoris, *Pediococcus pentosaceus*, *Lactobacillus acidophilus*, and *Lactobacillus plantarum* combine with prebiotics inulin and lactulose have a hepato-protective effect against CCl_4-induced liver toxicity in rats. Tiger nut administration could reduce the rise in serum enzyme activity by enhancing liver functions, demonstrating its protective effect through the preservation of glutathione, the main hepatocyte-protective intracellular sulfhydryl peptide (**Mehta *et al*., 1999**). In this study, TNM and FTNM decreased the activities of hepatic biomarker enzymes (AST, ALT, and ALP).

Effects of TNM and FTNM on liver proteins of rats administered CCl_4.

Another sign of liver damage caused by CCl_4 treatment is decreased levels of both total proteins and albumin in the blood of rats (**Navarro and Senior, 2006**). The levels of liver proteins of rats before and after administration of CCl_4 are shown in Table (8). A significantly decreased ($p \leq 0.05$) in the level of each albumin and total proteins was observed for the

positive control group compared to the negative control group. On the other hand, there were no significant differences ($p>0.05$) in the globulin level between the positive control group and the negative control group.

Also, from the same results, it was observed that there was a slight significant increase ($p\leq0.05$) in the level of albumin for all groups of rats containing tiger nut, and it increased with increasing dose. Also, this increment was more significant ($p\leq0.05$) in FTNM compared to TNM. While there were no significant differences ($p>0.05$) between the group of rats fed on 2ml of FTNM and the negative control group, which indicates that the FTNM with this dose (2ml) repaired the damaged liver.

Table (8): Effects of TNM and FTNM on liver proteins of rats administered CCl_4.

Animal Groups	Level of liver proteins (g/dl)		
	Albumin	**Globulin**	**Total Protein**
G1: (Negative Control)	3.80^{a} ±0.10	3.11^{a} ±0.11	6.91^{a} ±0.10
G2: (Hepatic rats " Positive Control")	3.01^{e} ±0.10	3.09^{a} ±0.11	6.10^{c} ±0.10
G3: (Hepatic rats + 1ml from TNM)	3.31^{d} ±0.11	2.60^{c} ±0.10	5.86^{d} ±0.04
G4: (Hepatic rats +2ml TNM)	3.40^{c} ±0.12	2.62^{c} ±0.10	6.05^{c} ±0.37
G5: (Hepatic rats + 1ml FTNM)	3.63^{b} ±0.07	2.91^{b} ±0.12	6.51^{b} ±0.10
G6: (Hepatic rats + 2ml FTNM)	3.77^{a} ±0.10	3.12^{a} ±0.08	6.81^{a} ±0.10

Values are expressed as mean ± STD; n=5 in each group, Values with different superscripts along the same column are significantly different ($p< 0.05$).

Concerning the levels of globulin and total proteins, the same trend was observed (significantly decreased ($p\leq0.05$)), where the levels began to gradually increase using TNM at a dose of 1ml and 2ml as well as FTNM at a dose of 1ml but this increment did not reach the level of globulin and total proteins of the negative control group which showed significant differences ($p\leq0.05$) between them and the negative control group. Also, there were no significant differences ($p>0.05$) between the group of rats fed on a diet containing 1ml of FTNM and the negative control group, which indicates that the

FTNM with this dose (2ml) repaired damaged liver. These results are in agreement with **Ihedioha *et al*., (2019**).

Chukwuma *et al.*, (2010) who found that the levels of albumin and total proteins were elevated in rats blood when given the *Cyperus esculentus* tuber aqueous extract. It has been established that protein synthesis stimulation is a hepato-protective mechanism.

Conclusion

The obtained results of this study show that tiger nut is rich in phytonutrients and its extract has antimicrobial activity against pathogenic microorganisms. In addition, fermented and non-fermented milk prepared from tiger nuts with probiotic bacteria is called a non-dairy probiotic drink. Also, it has hepatoprotective against liver damage.

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