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Chemical Composition, *In Silico* and *In Vitro* Antimutagenic Activities of Ethanolic and Aqueous Extracts of Tigernut (*Cyperus esculentus*)

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ABSTRACT: Tigernut, also known as *Cyperus esculentus*, is said to be high in nutritional and medicinal value. The purpose of this study was to determine the *C. esculentus*'s antimutagenic activity. The ethanolic and aqueous extracts of the nut were analyzed for chemical constituents, antioxidants, ultraviolet-visible, and gas chromatography-mass spectrometry using standard procedures. The extracts contained a total of 17 major compounds that were docked against human RecQ-like protein 5 (RECQL5) helicase protein. The antimutagenic property of the ethanolic extract *in vitro* was assessed using the *Allium cepa* chromosome assay. Onion bulbs were pre-treated with 200 mg/kg of ethanolic extract of *C. esculentus* for 24 h and then, grown in NaN_3 (250 μg/L) for 24 h; onion bulbs were also first exposed to NaN_3 (250 μg/L) for 24 h before treatment with 100 mg/kg and 200 mg/kg of the ethanolic extract respectively. Standard methods were used to determine the mitotic index and chromosomal aberrations. Results revealed that *C. esculentus* ethanolic extract contained flavonoids (22.47 mg/g), tannins (0.08 mg/g), alkaloids (19.71 mg/g), glycosides, phenol, and tannin and showed high scavenging activity against 2,2-diphenyl-1-picrylhydrazyland H_2O_2. Docking with RECQL5 showed good binding energies ($\triangle G > -7$) of five compounds in *C. esculentus* ethanolic extract. The *A. cepa* assay results revealed a significant ($P<0.05$) reduction in chromosomal aberrations and a higher mitotic index in groups treated with the *C. esculentus* ethanolic extract. The antimutagenic activity of *C. esculentus* ethanolic extract was attributed to its high levels of phytosterols and phenolic compounds.

Keywords: *Allium cepa* chromosome assay, antimutagenic, *Cyperus esculentus*, sodium azide

INTRODUCTION

Cancer is a group of disorders caused by mutations in the genes that control cell growth and differentiation, resulting in tissue growth dysregulation (Croce, 2008). Environmental factors that cause mutations, such as carcinogens and mutagens have been linked to the etiology of cancer because they play a role in the induction and progression of numerous diseases that affect humans including cancer (Tomasetti et al., 2017). Mutagens can be of physical origins such as ultraviolet rays, chemical origins such as reactive oxygen species, or biological origins that can induce mutations, thereby producing different breaks in DNA and the formation of base dimers (Hockberger, 2002). Sodium azide (NaN_3) is a chemical mutagen because it is an azide salt that causes plant mutations (Owais and Kleinhofs, 1988). Numerous biological assays can be used to assess the mutagenicity of various chemical compounds. Plants are commonly used in some of these assays because of their low cost, accessibility, and compatibility with other animal-based assays (Fiskesjö, 1985). Levan (1938) first proposed the use of onions (*Allium cepa*) as an effective test system for toxicity assessment. Plants are commonly used to detect the causative agents of DNA damage or agents that can protect against such effects because they are less expensive and easier to use. As a result, some species, such as onion bulbs (*A. cepa*), are used in mutagenesis studies to predict risks in higher eukaryotes such as mammals. This is because of benefits such as sensitivity to complex mixtures, distinct chromosomes (2n=16), affordability, and availability (Levan, 1938). After being exposed to a known mutagen and antimutagen, onion bulbs are examined microscopically to determine the mitotic index (MI) and the number

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of chromosomal aberrations (CA) caused by the mutagenic chemical. The MI is used to assess the cytotoxicity of a mutagen (Akinboro et al., 2011). The antimutagenicity of a compound is confirmed by a significant increase and decrease in the MI and CA after exposure to a mutagen, respectively.

Antimutagens are substances that can prevent the conversion of mutation-inducing substances into mutagenic compounds, render mutagenic compounds inactive, or inhibit the association of DNA with mutagen (Bhattacharya, 2011). Plants with antimutagenic properties could be very useful for preventing human cancer without harming normal cells (De Flora, 1998). Phytochemicals with antioxidant properties are abundant in fruits, vegetables, and plants (Luximon-Ramma et al., 2003). These plant compounds are effective in neutralizing the mutagenicity of various mutagens by removing reactive oxygen and nitrogen species, thereby ameliorating some of the resulting consequences to genetic material (Mazzio and Soliman, 2009; Akinboro et al., 2011). The antimutagenic effect of phytochemicals may be greater than flavonoids' quenching effects. Their ability to bind and activate a family of proteins known as genome guardians may be an unexplored mechanism. The RecQ-like protein 5 (RECQL5), which is found in both the cytoplasm and nucleus, is prominent among these proteins (Luong and Bernstein, 2021).

RECQL5 is a helicase that aids in the preservation of gene integrity in organisms through the processes of DNA metabolism and repair (Bachrati and Hickson, 2003). RECQL5 Knockdown has been linked to increased apoptosis in DPP-resistant A549 cells (Xia et al., 2021). Certain phytochemicals in tigernut were considered to be able to bind to and activate RECQL5.

Tigernut (*Cyperus esculentus*), as depicted in Fig. 1, is a plant that resembles a perennial grass in the Cyperaceae family and is surrounded by a fiber-like covering (Takhatajah, 1992). Tigernuts come in a variety of colors, including black, yellow, and brown (Belewu and Abodunrin, 2006). The yellow and brown varieties are widely available in Nigeria, with the yellow variety preferred over others because of its larger size, higher protein content, and lower fat and anti-nutrients content (Okafor et al., 2003). The nuts contain significant amounts of starch, dietary fiber, digestible carbohydrate (mono-, di-, and polysaccharide), and a moderate amount of minerals (Temple et al., 1990; Salem et al., 2005; Sanful, 2009). Tigernut is known to have antioxidant and antidiabetic properties (Owusu and Owusu, 2016). The oil derived from the nut has been shown to increase high-density lipoprotein cholesterol levels while decreasing low-density lipoprotein cholesterol (Belewu and Aboudurin, 2006), lowering the risk of arteriosclerosis. There is still little information on tigernut's antimutagenic potential, so this study was conducted to assess tigernut's antimutagenic potential *in silico* and *in vitro*.

MATERIALS AND METHODS

Collection and identification of the plant

The yellow variety of *C. esculentus* dried tubers (tigernuts) was purchased in the Oja Oba area of Kwara State, Nigeria. The nuts were authenticated at the Botany Department of Obafemi Awolowo University in Ile-Ife, Nigeria, and were given the identification number ILE-IFE-17678.

Plant extracts preparation

The crude extraction protocol was followed in accordance with established procedures (Feyisayo and Oluokun, 2013). A manual engine blender was used to pulverize dried tubers into powdery form. The fine powder of CE was dissolved in 1,000 mL each of 80% ethanol and water. The samples were macerated for 72 h and shaken every 6 h. The residues in the sample were removed after 72 h by filtering through filter paper into conical flasks. The filtrates were dried into a colloid form using a rotary evaporator at 45°C under low pressure inside a vacuum and stored at 4°C.

Phytochemical analysis of the ethanolic extract

***Determination of tannin content*:** In a plastic container, 500 mg of the sample was mixed with 50 mL of distilled water for 1 h using a shaker. The sample was then filtered into a 50 mL volumetric flask and adjusted to the mark. In a test tube, 5 mL of the filtrate was mixed with 2 mL of 0.1 M iron(III) chloride in 0.1 N hydrochloric acid and 0.008 M potassium ferrocyanide. Within 10 min, the absorbance was measured at 120 nm (Van Buren and

Fig. 1. Image of a tigernut (*Cyperus esculentus*).

Robinson, 1969).

***Determination of alkaloid*:** In a separating funnel, 1 mL of the extract (1 mg/mL) was mixed with 1 mL of 2 N HCl and filtered before adding 5 mL of bromocresol green solution and phosphate buffer. Drop by drop, chloroform was added to the mixture, which was then shaken before being collected in a 10 mL volumetric flask and adjusted with chloroform to the mark. Atropine solutions were prepared in known concentrations (20, 40, 60, 80, and 100 μg/mL). At 470 nm, the absorbance of the test and standard solutions were measured using a ultraviolet-visible (UV-VIS) spectrophotometer against the reagent blank. The total alkaloid content was calculated in mg of atropine equivalents/g of extract (Shamsa et al., 2008).

UV-VIS spectrum analysis

The aqueous and ethanolic extracts of tigernut were analyzed using a UV-VIS spectrophotometer (Spectroquant Pharo 300, Merck KGaA, Darmstadt, Germany) with a wavelength range of 190 to 900 nm. This was done to detect the common peaks in the extracts.

Antioxidant assay

***2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity*:** This was done exactly as described by Alhakmani et al. (2013). One mL of tigernut extract was added to a test tube containing 2 mL of 1 mM methanolic DPPH. The resulting mixture was thoroughly shaken and allowed to stand for 30 min at 37°C in an incubator. There was also a blank that did not contain either the standard or the extract. The difference in absorbance was then measured at 515 nm against gallic acid as a standard and expressed as a percentage inhibition using the formula below:

$$\text{Inhibition (\%)} = [(A_{515}\ \text{Control} - A_{515}\ \text{Sample})/A_{515}\ \text{Control}] \times 100$$

***Hydrogen peroxide (H_2O_2) scavenging activity*:** At 230 nm, the absorbance of a 40 mM H_2O_2 solution prepared in 50 mM phosphate buffer (pH 7.4) was measured. Following that, 1 mL of sample extract or standard was added to 2 mL of hydrogen peroxide solution. After allowing it to stand for 10 min, the absorbance was compared to that of a blank solution containing only phosphate buffer. H_2O_2 scavenging activity was then calculated as a percentage (Nabavi et al., 2008).

***Total antioxidant capacity*:** This was determined as described by Prieto et al. (1999). The total antioxidant capacity was then calculated using the formula below:

$$[(A_{695}\ \text{Control} - A_{695}\ \text{Sample})/A_{695}\ \text{Control}] \times 100$$

Proximate composition determination

The aqueous and ethanolic extracts of *C. esculentus* were subjected to proximate analysis in accordance with the Association of Official Analytical Chemists methods (Helrich, 1990). The nitrogen content of crude protein was calculated and expressed as (nitrogen×6.25). The carbohydrate content in percentage was calculated by subtracting the sum of all other nutrients deducted from 100.

Mineral composition analysis

The mineral content of tigernut ethanolic and aqueous extracts, including zinc, copper, magnesium, iron, nickel, and calcium, was determined using an atomic absorption spectrophotometer (PerkinElmer Inc., Waltham, MA, USA).

Gas chromatography-mass spectrometry (GC-MS) analysis

This was done with the PerkinElmer Clarus 680 system of tigernut aqueous and ethanolic extracts (PerkinElmer Inc.). At a constant movement rate of 1 mL/min, the carrier gas was unalloyed helium gas (99.99%). During the injection of 1 L of the extract, the temperature of the injector was kept at 250°C. The chemical compounds in the tigernut extract were identified by comparing their retention time (min), peak area, peak height, and mass spectral patterns to those found in the library (Linstrom et al., 2008).

***In silico* evaluation of antimutagenic activity by molecular docking**

A total of 17 major phytochemical compounds present in both the ethanolic and aqueous extracts of tigernut were docked with RECQL5 Helicase Apo form, and the binding energies were computed using AutoDock Vina (Trott and Olson, 2010).

***Protein preparation*:** The crystal structure of human RECQL5 Helicase Apo form in Protein Data Bank (PDB)

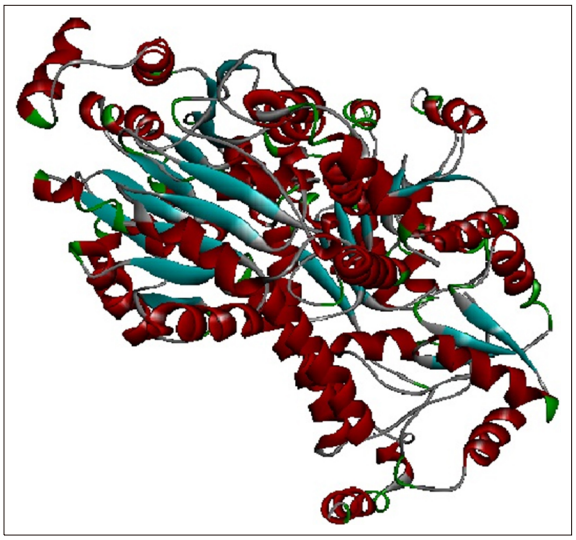

Fig. 2. Human RECQL5 protein has a three-dimensional structure (Protein Data Bank ID: 5LB8).

format (Fig. 2), which was assigned PDB ID 5LB8, was obtained from the PDB (Newman et al., 2017). PyMOL was used to analyze the active site and remove water and impurities; however, the zinc metal was retained in the structure because it has been shown to be efficient for enzymatic activity (Ren et al., 2008). Docking grid maps were created using MGLTools 1.5.4 (Center for Computational Structural Biology, La Jolla, CA, USA). The enzyme's grid box was assigned based on the amino acids at the enzyme's active site, as previously reported by Newman et al. (2017). This was then used to generate a three-dimensional grid box map with the coordinates (center_x=−14.636; center_y=6.322; center_z=−25.85; size_x=30; size_y=80; size_z=38; exhaustiveness=8). The protein was prepared for docking analysis using the deter MGLTools 1.5.4; polar hydrogens were added to the protein, along with the assignment of bond orders.

Ligand preparation: The three-dimensional configurations of 17 major phytochemicals present in tigernut aqueous and ethanolic extracts were obtained in structural data files form from PubChem (2020, http://pubchem.ncbi.nlm.nih.gov), before being converted into PDB files using PyMOL. MGLTools 1.5.4 was used to generate the ligands' pdbqt files, with the number of bonds that can be rotated set to maximum.

Molecular docking: Both the protein and the ligands that had already been modified as described earlier were used in molecular docking studies with AutoDock Vina 1.1.2 (Trott and Olson, 2010). PyMOL (The PyMOL Molecular Graphics System, version 4.40, Schrödinger LLC., New York, NY, USA) and discovery studio were used to perform docking analyses. The binding energy and interaction of the molecules were determined using the BIOVIA discovery studio visualizer (version 19, BIOVIA, San Diego, CA, USA). The ligands were docked within the RECQL5 active site. For the *in vitro* evaluation of antimutagenicity, the extract containing the compound with the highest binding energies was chosen.

Antimutagenicity assay

***Experimental design*:** The *A. cepa* assay was used to evaluate antimutagenicity, and it was done according to the Fiskesjö method (1985). Five onion bulbs ranging in size from 40 g to 60 g were purchased from a local market and sun-dried for 5 days till the layers on the outside could easily be peeled off and the root ring remained intact. Chemicals and herbicides that may have been on the bulbs were thoroughly rinsed away with tap water. The experimental grouping was done as follows:

- Control group: Onion bulbs (40 g) were placed in tap water (positive control) for 48 h.
- Mutagenicity control: Onion bulbs were grown in an aqueous solution of 250 μg/L of NaN_3 for 48 h.
- Pre-treatment group: Onion bulbs of roots with 2 to 3 cm were suspended in 200 mg/kg of tigernut ethanolic extract for 24 h before being transferred into an aqueous solution containing 250 μg/L of NaN_3.
- Post-treatment group: Onion bulbs of root with 2 to 3 cm were first suspended in an aqueous solution of 250 μg/L NaN_3 for 24 h and were then transferred into tigernut ethanolic extract solution of 100 mg/kg and 200 mg/kg, respectively, and maintained for another 24 h.

Microscopic analysis

After 48 h, the onion roots were collected and suspended in a mixture of ethanol and acetic acid (3:1, v/v) and kept for 24 h at 4°C. According to Akinboro and Bakare's method (2007), 1 N HCl was used to hydrolyze the fixed root. Following that, the roots were rinsed with distilled water before slides were prepared. Two to three cm rootlets were homogenized and smeared on a glass slide before being stained with two drops of aceto-orcein. The surplus stain was blotted with filter paper, and the stained smear was covered with a coverslip so that the stained cell did not overlap. On each slide, an oil immersion objective lens (×100) of a Nikon Eclipse E400 microscope (Nikon, Tokyo, Japan) was used to view and observe three different stages of the MI and chromosomal aberration. A total of 1,000 normal and deviant dividing cells were scored and recorded. Statistical analysis was carried out on the results of MI, the number of CA, and the percentage reduction (Akinboro et al., 2011).

The damage reduction (DR) percentage was calculated as follows:

$$\text{DR } (\%) = \frac{(A-B)}{(A-C)} \times 100$$

where A is number of CA stimulated by NaN_3, B is number of CA gotten from a mixture of NaN_3 and the extract, and C is number of CA gotten from the positive control.

The MI was calculated as follows:

$$\text{MI} = \frac{\text{Total dividing cells}}{\text{Total number of cells counted}} \times 100$$

The frequency of CA was calculated as follows:

$$\text{Frequency of CA } (\%) = \frac{\text{Average number of aberrant cells}}{\text{Total number of cells counted}} \times 100$$

Statistical analysis

The obtained results were presented as mean±standard deviation of triplicate determinations. At a significance

Table 1. Tigernut ethanolic extract was subjected to a qualitative phytochemical screening

Phytochemical	Flavonoid	Alkaloid	Tannin	Resin	Glycoside	Phenol
Tigernut extract	+	+	+	−	+	+

+, present; −, not detected.

level of $P<0.05$, one-way ANOVA was used to compare mean differences statistically.

RESULTS

Phytochemical analysis

Table 1 shows that tigernut ethanolic extract contains flavonoids, alkaloids, phenol, tannin, and glycosides but no resin. Table 2 shows the quantity of some phytochemical present in the extract, with flavonoids being the most abundant.

UV-VIS analysis

UV absorption for both the ethanolic and aqueous extracts of tigernut was measured at wavelengths ranging from 198 nm to 900 nm to cover an extensive range of values as shown in Fig. 3. Table 3 also shows the absorption bands for both the ethanolic and aqueous extracts. The UV spectrum of the ethanolic extract showed peaks at 213, 230, and 292 nm corresponding to absorbance values of 0.597, 0.562, and 3.706, respectively, while that of the aqueous extract profile showed peaks at 212, 220, and 298 nm, which correspond to absorbance values of 0.881, 0.639, and 3.634, respectively.

Table 2. *Cyperus esculentus* ethanolic extract phytochemical analysis

Phytochemical	Tigernut extract (mg/g)
Flavonoids	22.47±0.63
Alkaloid	19.71±0.75
Tannin	0.08±0.06

Values are presented as mean±SD of three determinations.

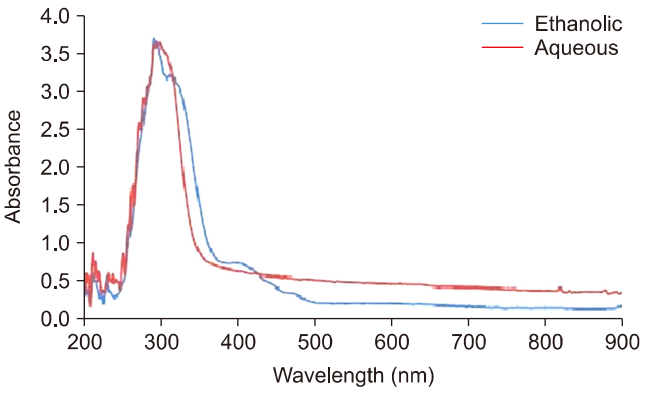

Fig. 3. Ultraviolet-visible spectra of ethanolic and aqueous tigernut extracts.

Antioxidant analysis

As shown in Table 4, the percentage of DPPH inhibition of the aqueous extract at a concentration of 0.002 g/mL was greater than that obtained for the ethanolic extract at a concentration of 0.01 g/mL, with the aqueous extract achieving 86.17%. Similarly, the total antioxidant activity of the aqueous extract was approximately 3.6 times greater than that of the ethanolic extract. However, the ethanolic extract outperformed the aqueous extract in terms of hydrogen peroxide radical scavenging activity.

Proximate composition

Table 5 shows that the aqueous extract had significantly ($P<0.05$) higher crude protein, moisture, ash, and carbohydrate content than the ethanolic extract. However, the ethanolic extract contained a significantly ($P<0.05$) higher content of crude lipid than the aqueous extract. Although the aqueous extract had a higher fiber content, the difference in crude fiber content between the two extracts was not significant.

Mineral composition

Table 6 shows that the aqueous tigernut extract has significantly ($P<0.05$) higher zinc and copper contents than the ethanolic extract. The magnesium content of the ethanolic extract was found to be higher than that of the aqueous extract, although this difference was not statistically significant ($P>0.05$).

Table 3. Ultraviolet-visible peak values of tigernut ethanolic and aqueous extracts

No	Ethanolic extract		Aqueous extract	
	Wavelength (nm)	Absorbance	Wavelength (nm)	Absorbance
1	212	0.881	213	0.597
2	220	0.639	230	0.562
3	298	3.634	292	3.706

Table 4. Antioxidant activities of tigernut ethanolic and aqueous extracts

Sample	DPPH (% inhibition)	H_2O_2 (% inhibition)	Total antioxidant activity
Ethanolic extract	76.13	61.9	22.56
Aqueous extract	86.17	48.3	82.17

Table 5. Proximate composition of ethanolic and aqueous tigernut extracts

Parameter (%)	Ethanolic tigernut extract	Aqueous tigernut extract
Crude protein	3.48±0.02[b]	6.13±0.01[a]
Crude fiber	0.007±0.01[a]	0.01±0.00[a]
Crude lipid	60.01±0.01[a]	15.99±0.04[b]
Moisture	6.0±0.00[b]	34.03±0.03[a]
Ash	0.013±0.006[b]	2.01±0.006[a]
Carbohydrate	30.49±0.01[b]	41.83±0.03[a]

Values are presented as mean±SD of triplicate determinations. Different letters (a,b) within the same row are significantly different at $P<0.05$.

Table 6. The mineral content of ethanolic and aqueous tigernut extracts

Mineral (ppm)	Ethanolic tigernut extract	Aqueous tigernut extract
Zinc	6.233±0.112[b]	10.864±1.092[a]
Copper	5.049±0.139[b]	7.793±0.034[a]
Magnesium	17.396±0.0154[ns]	15.163±28.000
Iron	6.523±0.171[b]	7.5163±0.0341[a]
Manganese	8.2474±0.001[a]	7.0448±0.0061[b]

Values are presented as mean±SD of triplicate determinations. Different letters (a,b) within the same row are significantly different at $P<0.05$. [ns]Not significant.

GC-MS analysis

Table 7 displays the phytochemical constituents identified by GC-MS in the ethanolic extract. The GC-MS revealed 103 constituents in total, but only those with a percentage abundance greater than 0.50% were presented in the table. These accounted for 90.29% of the compounds present. The major compounds present include *trans*-13-octadecenoic acid, γ-sitosterol, hexadecanoic acid ethyl ester, stigmasterol, campesterol, octadecanoic acid, vitamin E, 2,4-di-*tert*-butylphenol, 3H-[1,2,3]triazole, 3-cyclohexyl-5-(1H-pyrazol-3-yl)-4-cyclohexylamino-, and benzo[H]quinoline, 2,4-dimethyl-. The chromatogram of the ethanolic tigernut extract (Fig. 4) revealed 7 prominent peaks as 2,4-di-*tert*-butylphenol with a retention time of 11.5245 min and peak area of 2.64%, hexadecanoic acid, ethyl ester (15.7924 min, 12.68%), *trans*-13-octadecenoic acid (17.1323 min 23.69%), vitamin E (23.3177 min, 3.54%), stigmasterol (24.3456 and 24.5824 min in total, 7.58% and 0.90% in total), and γ-sitosterol (24.8654 min, 14.13%). Other smaller peaks could also be seen in the GC-chromatogram spectrum.

The phytochemical constituents in the aqueous extract are shown in Table 8. According to GC-MS, 92 constituents were present. However, only those with a percentage abundance greater than 0.50% were shown in the table, accounting for 92.23% of the total. Major compounds in the aqueous extract include propionic acid, 2-mercapto-, allyl ester; propanenitrile, 3-(methylthio)-; 2-chloroethyl methyl sulfide; 1,4-bis(trimethylsilyl)benzene; oc-

Table 7. Phytochemical components of ethanolic tigernut extract

Serial No.	Retention time (min)	Library/ID	Area (%)	Qual
1	17.1323	*trans*-13-Octadecenoic acid	23.69	90
2	24.8654	γ-Sitosterol	14.13	99
3	15.7924	Hexadecanoic acid, ethyl ester	12.68	98
4	24.3456	Stigmasterol	7.58	99
5	24.0973	Campesterol	5.87	99
6	17.2709	Octadecanoic acid	4.46	99
7	23.3177	Vitamin E	3.54	99
8	11.5245	2,4-Di-*tert*-butylphenol	2.64	97
9	24.9809	3H-[1,2,3]Triazole, 3-cyclohexyl-5-(1H-pyrazol-3-yl)-4-cyclohexylamino-	2.10	35
10	26.0494	Benzo[H]quinoline, 2,4-dimethyl-	2.08	25
11	16.5721	Behenic alcohol	1.28	94
12	25.443	Lanosterol	1.15	45
13	27.8975	Pyrido[2,3-D]pyrimidine, 4-phenyl-	1.09	25
14	22.7401	β-Tocopherol	1.02	99
15	27.4239	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	0.99	97
16	24.5824	Stigmasterol	0.90	56
17	12.2117	5-Octadecene, (E)-	0.89	98
18	15.9253	1H-1,3-Benzimidazole, 5,6-dimethyl-1-[(2,3,5,6-tetramethylphenyl)methyl]-	0.80	55
19	21.1577	1-Methoxy-3-(2-hydroxyethyl)nonane	0.63	25
20	25.2928	9,10-Methanoanthracen-11-ol, 9,10-dihydro-9,10,11-trimethyl-	0.61	53
21	21.4753	Squalene	0.60	87
22	19.2114	Diltiazem	0.52	59
23	17.4153	9-Octadecenoic acid	0.52	97
24	28.7118	1H-Indole, 5-methyl-2-phenyl-	0.51	25
Total	—	—	90.29	—

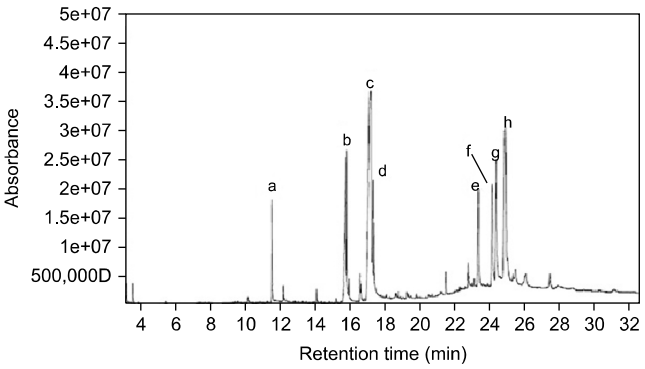

Fig. 4. Major components in the gas chromatography-mass spectrometry spectra of tigernut ethanolic extract. a, 2,4-di-*tert*-butylphenol; b, hexadecanoic acid, ethyl ester; c, *trans*-13-octadecenoic acid; d, octadecanoic acid; e, vitamin E; f, campesterol; g, stigmasterol; h, γ-sitosterol.

tadec-9-enoic acid, 1,2-bis(trimethylsilyl)benzene, furan, 2,5-dimethyl-; *p*-dioxane-2,3-diol; and 1,2,3,4-butanetetrol, [S-(R*,R*)]-, and cyclotrisiloxane, hexamethyl- (Table 8). The chromatogram of the aqueous tigernut extract (Fig. 5) portrays about 7 prominent peaks along with many small peaks indicating the presence of major compounds such as propanenitrile, 3-(methylthio)- with a retention time of 3.5373 min and peak area of 8.49%, *p*-dioxane-2,3-diol (5.0216 min, 3.10%), 1,2,3,4-butanetetrol, [S-(R*,R*)]- (5.2237 min, 3.01%), cyclotrisiloxane, hexamethyl- (5.5125 min, 2.57%), cyclotetrasiloxane, octamethyl- (7.6493 min, 1.40%), *n*-hexadecanoic acid (6.194 and 15.6366 min in total, 2.33%), octadec-9-enoic acid (4.3401 and 17.0804 min in total, 4.46%), and 1,2-bis(trimethylsilyl)benzene (4.5711 and 28.0188 min in total, 4.35%).

In silico/molecular docking studies

The binding energies obtained from the molecular docking of the major compounds in both extracts are shown in Table 9. As shown from the table, only 5 out of the 17 compounds showed a good binding affinity with the

Table 8. Phytochemical components of aqueous tigernut extract

Peak	Retention time (min)	Library/ID	Area (%)	Qual
1	3.3294	Propionic acid, 2-mercapto-, allyl ester	13.313	42
2	3.5373	Propanenitrile, 3-(methylthio)-	8.4865	7
3	3.7626	2-Chloroethyl methyl sulfide	6.7293	25
4	3.8607	1,4-Bis(trimethylsilyl)benzene	5.4717	30
5	4.2419	Butanoic acid, 3-methyl-	5.1289	53
6	4.3401	Octadec-9-enoic acid	4.4634	60
7	4.5711	1,2-Bis(trimethylsilyl)benzene	4.3453	38
8	4.7732	Furan, 2,5-dimethyl-	3.4838	22
9	5.0216	*p*-Dioxane-2,3-diol	3.1032	72
10	5.2237	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	3.0068	38
11	5.5125	Cyclotrisiloxane, hexamethyl-	2.5683	70
12	5.7839	Triethylenediamine	2.4534	32
13	5.9052	1,2-Bis(trimethylsilyl)benzene	2.3364	27
14	6.194	*n*-Hexadecanoic acid	2.3258	15
15	6.5694	2(5H)-Furanone, 5-methyl-	2.3098	35
16	6.783	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	2.2766	14
17	6.9101	Thiodiglycol	2.1097	38
18	7.0025	Benzamide, 4-ethyl-N,N-dimethyl-	1.9358	25
19	7.2566	Benzenemethanol, 3-fluoro-	1.6482	45
20	7.401	Pyrido[2,3-D]pyrimidine, 4-phenyl-	1.4234	40
21	7.6493	Cyclotetrasiloxane, octamethyl-	1.4032	32
22	7.9554	1,1,1,3,5,5,5-Heptamethyltrisiloxane	1.303	40
23	8.5329	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	1.1788	50
24	9.0585	Formaldehyde, methyl(2-propenyl)hydrazine	1.1778	38
25	9.3993	Boronic acid, ethyl-, bis(2-mercaptoethyl ester)	1.114	43
26	9.5032	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	1.0259	38
27	9.7862	1,2-Bis(trimethylsilyl)benzene	0.9234	45
28	9.9479	2-Furanmethanol	0.9226	38
29	10.3984	Pyvalic acid, 3-chlorophenyl ester	0.8639	35
30	10.6756	1H-Imidazole-4-carboxylic acid, methyl ester	0.8299	35
31	10.872	1,4-Bis(trimethylsilyl)benzene	0.8041	38
32	11.0221	Carbamic acid, N-(4-tolyl)-, 2-(3-methylpyrazol-1-yl)ethyl ester	0.622	38
33	11.1145	Benzenamine, 4-(2-phenylethenyl)-N-(3,5-dimethyl-1-pyrazolylmethyl)-	0.6137	46
34	11.2416	2(1H)-Pyrimidinone	0.5965	52
Total	—	—	92.2981	—

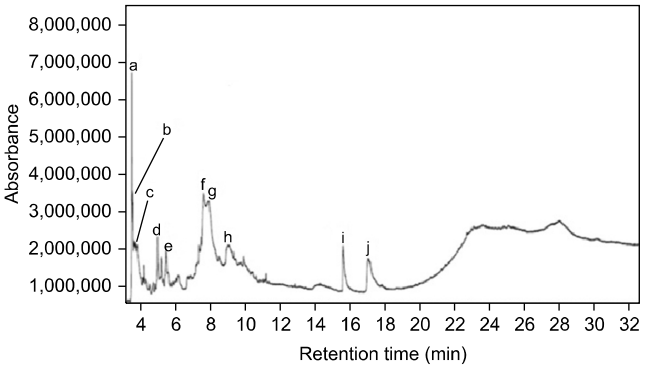

Fig. 5. Major components in the gas chromatography-mass spectrometry spectra of tigernut aqueous extract. a, propane-nitrile, 3-(methylthio); b, *p*-dioxane-2,3-diol, ethyl ester; c, 1,2,3,4-butanetetrol; d, cyclotrisiloxane, hexamethyl; e, cyclo-tetrasiloxane, octamethyl; f, *n*-hexadecanoic acid; g, bis(trime-thylsilyl)benzene; h, boronic acid, ethyl-, bis(2-mercaptoethyl ester); i, *n*-hexadecanoic acid; j, octadec-9-enoic acid.

RECQL5 protein ($\triangle G \geq 7$) and the compounds are γ-si-tosterol, benzo[H]quinoline, 2,4-dimethyl-, stigmasterol, campesterol, and vitamin E (Fig. 6), which are all pres-ent in the ethanolic extract, with the highest binding en-ergy obtained from the interaction of γ-sitosterol (−11.0 kcal/mol). Fig. 7A∼7C and 7D, 7E depict the specific interactions of the five ligands that demonstrated high binding affinity with some of the amino acid residues of the RECQL5 protein.

Antimutagenicity assay

Fig. 8 depicts the antimutagenicity assay results. When compared to the positive control, the treated groups had a significant ($P<0.05$) reduction in the total number of CA. Furthermore, all treated groups had a higher percent-age of mitotic indices than the untreated group. In the post-treatment group, DR percentages were higher than in the pre-treated group.

DISCUSSION

The UV-VIS analysis was performed to identify some of the phytoconstituents found in tigernut ethanolic and aqueous extracts. It also identifies σ-bonds, π-bonds, and lone pairs of electrons, chromophores, and aromatic rings containing chemical compounds. In the UV-VIS spectra, distinct peaks form between 200 and 400 nm, indicating the presence of unsaturated groups and numerous atoms such as sulfur, nitrogen, and oxygen (Njoku et al., 2013). The spectra for tigernut ethanolic and aqueous extracts show one major peak each at positions 292 nm and 298 nm, respectively. This demonstrates the presence of or-ganic UV light-absorbing molecules in the extract, which was further confirmed specifically using GC-MS.

The preliminary phytochemical study on the tigernut ethanolic extract revealed that it contained chemicals with medicinal uses such as alkaloids, flavonoids, tannin, and phenol but no resin. This report is also consistent with the findings of Imam et al. (2013) and Chukwuma et al. (2010). However, varying amounts of the phyto-chemicals were reported, which could be attributed to differences in soil constituents, washing away of the top-most soil layer, and climatic factors. The most abundant phytochemical in the extract was discovered to be flavo-noids. Flavonoids are the largest class of phytochemicals (Du et al., 2016). They have prominent antiviral, anti-in-flammatory, cytotoxic, antimicrobial, and antioxidant ef-

Table 9. Table displaying the binding energy and major protein residues interacting with the ligand within 4 Å

Ligand	Binding energy (kcal/mol)	Key residues interacting within 4 Å
1,2-Bis(trimethylsilyl)benzene	−4.7	PHE 169, THR 395
1,4-Bis(trimethylsilyl)benzene	−4.8	GLU 378, LYS 381
2-Chloroethyl methyl sulfide	−2.5	LYS 381, THR 196, GLU 378, GLU 131, ARG 175, ARG 269, GLU 384, TRP 165, PRO 78, ASP 172
Benzo[h]quinoline, 2,4-dimethyl-	−7.3	ARG 267, ILE 317, LYS 338, ALA 337
Butanoic acid-3-methyl	−3.8	LYS 385, ILE 317, LYS 338, GLY 342, SER 339
Campesterol	−8.6	PHE 169, PRO 171, LEU 382, THR 395
Furan-2,5 dimethyl-	−4.3	LYS 389,PRO 130, ALA 193, SER 357, THR 194,
γ-Sitosterol	−11.0	LEU 382, GLN 383, ALA 380, ILE 396, GLU 378, ILE 375
Hexadecanoic ethyl ester	−5.8	ILE 317, HIS 167, ARG 269, LYS 381
Octadec-9-enoic acid	−5.0	LYS 385, LYS 338, ILE 317, THR 395, SER 391
Octadecanoic acid	−6.1	LEU 382, PHE 169, TYR 419, ILE 317, LYS 381, GLU 384, GLU 201, GLN 164
Propane nitrile, 3-(methylthio)	−2.9	GLY 342, ILE 317, LEU 79
Propionic acid, 2-mercapto-, allyl ester	−3.4	ILE 336, ILE 317, ARG 267, ALA 337
Squalene	−5.4	LYS 385, PHE 169, ARG 386, PRO 171, LEU 382
Stigmasterol	−9.5	PRO 171, LEU 382, PHE 169, GLY 166
trans-13-Octadecenoic acid	−5.4	LEU 382, PHE 169, PRO 171, GLN 164, SER 391
Vitamin E	−7.6	PRO 171, ARG 386, LYS 385, PHE 169, THR 395, ALA 394, GLN 164, SER 391

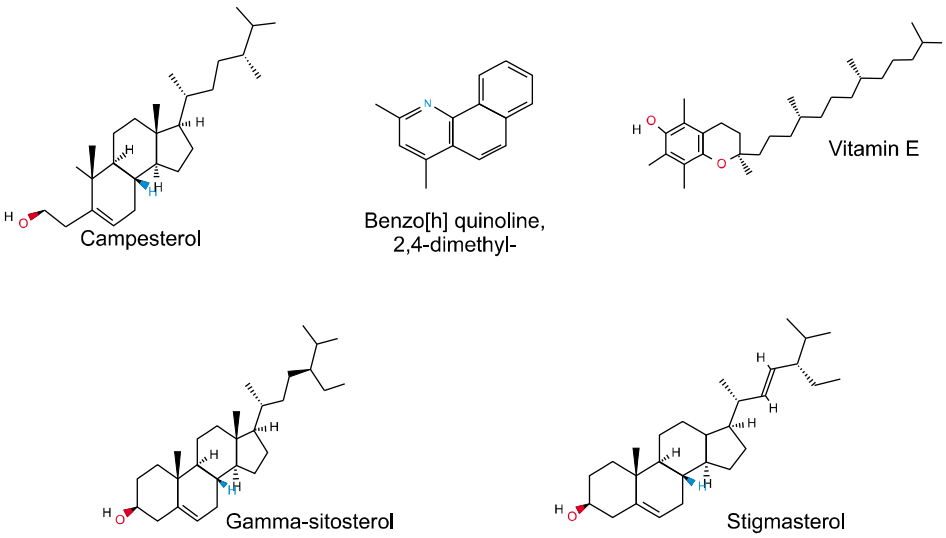

Fig. 6. Compound structures in the ethanolic extract that have a high affinity for the RECQL5 protein.

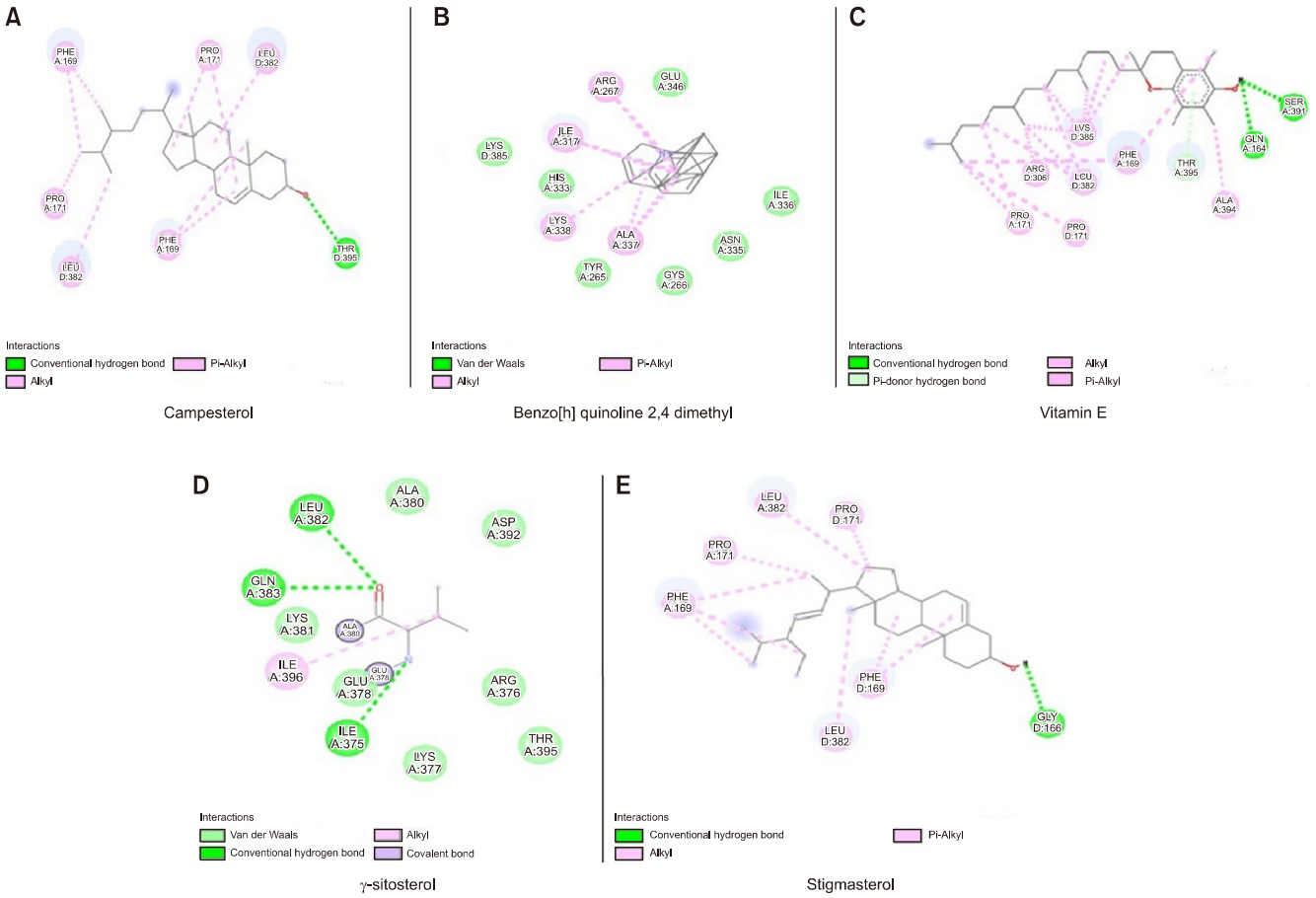

Fig. 7. Interactions between RECQL5 protein and campesterol (A), benzo[H]quinoline, 2,4-dimethyl- (B), vitamin E (C), γ-sitosterol (D), and stigmasterol (E).

fects. Like flavonoids, certain tannins and alkaloids with medicinal values have been reported (Okaka et al., 1991; Chukwuma et al., 2010).

DPPH free radicals scavenging assay determines the extract's ability to mop up free radicals, which is demonstrated by the inhibition and repair of oxidative stress in organisms. In this study, aqueous extract demonstrated a higher DPPH scavenging percentage inhibition compared to the ethanolic extract. However, the result obtained for the DPPH scavenging activity of the ethanolic extract (76.13%) in this study is still higher compared to the result obtained by Elom and Ming (2019). This could be because of a variety of factors such as climatic and geographical conditions, nature, and the type of cultivar of the tigernut used. A similar result was obtained for total antioxidant capacity, indicating a higher content of

	NC	PC	PR	PO1	PO2
MN	6	153	65	52	72
AB	3	27	24	13	10
MA	10	51	11	25	19
NB	0	5	1	0	1
BM	0	15	13	5	6
BA	0	15	10	7	5
BT	0	3	3	1	0
CBM	5	26	21	23	14
CBA	3	16	11	14	8
CBT	1	3	2	1	1
MEAN C.A	2.80^{a}	31.40^{b}	16.10^{c}	14.10^{c}	13.60^{c}
S.E	3.36	45.10	18.78	16.02	21.39
TFA (%)	0.22	2.00	1.06	0.92	0.88
MI (%)	6.25^{a}	5.77^{b}	6.55^{a}	8.19^{c}	6.95^{a}
DR (%)	-	-	48.72	55.10	56.70

Fig. 8. Heatmap indicating chromosomal aberrations and mitotic indices observed in *Allium cepa* treated with sodium azide (NaN_3) and ethanolic extract of *Cyperus esculentus* (EECE). Values with different superscripts (a–c) across row indicate statistically different result at $P<0.05$. NC, negative control (distilled water); PC, positive control (250 μg/L NaN_3); PR, pre-treatment with 200 mg/kg EECE after initial exposure to 250 μg/L NaN_3; PO1, post-treatment with 100 mg/kg EECE after initial exposure to 250 μg/L NaN_3; PO2, post-treatment with 200 mg/kg EECE after initial exposure to 250 μg/L NaN_3; SE, standard error; MN, micro nucleus; AB, anaphasic bridge; MA, multipolar anaphase; NB, nucleus bud; BM, chromosomal break in metaphase; BA, chromosomal break in anaphase; BT, chromosomal break in telophase; CBM, chromosomal bridge in metaphase; CBA, chromosomal bridge in anaphase; CBT, chromosomal bridge in telophase; CA, chromosomal aberrations; TFA, total frequency of aberrations; MI, mitotic index; DR, damage reduction.

phenolics and flavonoids in the aqueous extract as corroborated by Willis et al. (2019). The amount of phenolics in the extract that can decompose H_2O_2 to water by contributing electrons determines hydrogen peroxide scavenging activity (Bhatti et al., 2015). The ethanolic extract of tigernut demonstrated greater H_2O_2 scavenging activity, implying that the ethanolic extract has a higher amount of phenolics.

The aqueous tigernut extract had higher crude protein, ash, moisture, and carbohydrate content than the ethanolic extract in the proximate analysis. However, when compared to the results obtained by Imo et al. (2019), our study yielded lower values. This could be attributed to the loss of some of the nutrients during the process of extraction. However, the aqueous extract contains a significant amount of nutrients, particularly carbohydrates. Carbohydrates are energy-giving foods that are required for organism nutrition (Edeoga et al., 2005). Crude fats are a dense nutrient source that, when broken down, provides a lot of calories. They also serve as a form of energy storage (Potter and Hotchkiss, 1995). The ethanolic extract's high lipid content could be attributed to its higher content of phytosterols such as stigmasterol, campesterol, sitosterol, etc., as revealed in the GC-MS results.

In proximate analysis, the ash residue content is regarded as an index of mineral composition (Onwuka, 2005). The higher levels of zinc, copper, and iron in the aqueous extract corroborated the higher ash content of the extract. Copper is a vital element that is required in minute amounts and functions as a cofactor for many redox enzymes and iron metabolism, antioxidant protection, production of neuropeptides, and immune processes (Uriu-Adams and Keen, 2005). Magnesium aids in the development of strong bones and the proper functioning of certain enzymes, the nervous and the cardiovascular system. Zinc (Zn) also plays important roles in metabolism as a cofactor for some enzymes, modulator of protein configuration, and expression of genes. Magnesium is relatively present in both the aqueous and the ethanolic extracts. It is vital to the normal functioning of muscle, heart, gland secretion, and nerve transmission (Castiglione et al., 2018). Because iron is a major component of hemoglobin, it plays an important role in oxygen transport (Andreini et al., 2018). Manganese is an essential cofactor for many enzymes involved in growth and maturation, absorption, reproduction, scavenging of free radicals, generation of energy, and functioning of the immune and nervous system (Chen et al., 2018).

As shown in Tables 7 and 8, GC-MS analysis was performed to further recognize the specific compounds present in both the ethanolic and aqueous extracts. The appearance of minor peaks demonstrated that some of the compounds are only present in trace amounts and that others are by-products of the disintegration of the main compounds. The peaks with short retention times are mostly phytochemicals with small retention times that can interact with water (Satapathy et al., 2009). Table 10 displays the bioactivities of 14 prominent constituents of tigernut ethanolic extract identified using GC-MS (Kumar et al., 2010; Mondul et al., 2015; Varsha et al., 2015; Zhao et al., 2015; Kurano et al., 2018; Senyilmaz-Tiebe et al., 2018; Nna et al., 2019; Asbaghi et al., 2020; Purkait et al., 2020; Antoci et al., 2021; Maurya et al., 2022).

Helicases are enzymes that use ATP as a source of energy to unwind complementary strands of DNA. They play important roles in DNA metabolisms such as replication, recombination, transcription, and repair; all of which are necessary steps in preventing mutagenesis, which could ultimately lead to cancer (Bachrati and Hickson, 2003). The beta isoform of the RECQL5 enzyme contains a conserved Zn^{2+} binding sub-domain (residues 365～437) essential for efficient helicase activity (Ren et al., 2008). The binding conformation of two associating molecules with known chemical configurations is revealed by molecular docking. It thus provides a theoretical prediction of the most stable receptor-ligand complex's preferred conformation (Madeswaran et al., 2012). The results of this study revealed that, of the 13

Table 10. Bioactivity of 11 major components of a tigernut ethanolic extract was determined using gas chromatography-mass spectrometry

Serial No.	Compound name	Reported bioactivity	Reference
1	*trans*-13-Octadecenoic acid	Antifungal activity	Purkait et al. (2020)
2	γ-Sitosterol	Hypolipidemic, antiviral	Mauryaet al. (2022)
3	Stigmasterol	Antioxidant, antimicrobial, anticancer	Nna et al. (2019)
4	Campesterol	Antiviral, reduced apoptosis	Kurano et al. (2018)
5	Octadecanoic acid (stearic acid)	Mitofusin activity, antihypertensive activity	Senyilmaz-Tiebe et al. (2018)
6	Vitamin E	Antioxidant, anti-inflammatory	Asbaghi et al. (2020)
7	2,4-Di-*tert*-butylphenol	Antioxidant, antifungal activity	Varsha et al. (2015)
8	Benzo[h]quinoline, 2,4-dimethyl-	General antimicrobial	Antoci et al. (2021)
9	Lanosterol	Anti-cataractogenic activity	Zhao et al. (2015)
10	Hexadecanoic acid, ethyl ester	Antioxidants, hypocholesterolemic nematicide, pesticides, anti-androgenic flavor, hemolytic, 5-α reductase inhibitor	Kumar et al. (2010)
11	β-Tocopherol	Anticancer activity	Mondul et al. (2015)

compounds docked with the human RECQL5 protein, only 5 of the compounds which were identified in the ethanolic extract of tigernut showed a very strong binding affinity for the protein-based on their docking scores. This is supported by other studies that found that using an alcoholic solvent such as ethanol is preferable to other solvents such as water when extracting medicinal phytochemicals (Gberikon et al., 2015). The five compounds γ-sitosterol, campesterol, benzo[H]quinoline 2,4 dimethyl, and vitamin E are all associated with specific amino acid residues at the active site within 4 Å of the enzyme protein, which might be responsible for their strong binding affinity. The compound with the highest binding energy was γ-sitosterol (−11.0 kcal/mol). These five compounds are well-known anticancer, antioxidant, and antimicrobial agents, implying their functions.

Molecular docking enabled compounds with high binding energy values to bind to human RECQL5 protein. This suggests that some compounds present in the ethanolic extract can activate this enzyme thereby preventing the onset of mutagenesis that can lead to carcinogenesis. Thus, the antimutagenicity assay was only performed using the ethanolic extract because the compounds revealed by the GC-MS analysis of the ethanolic extract, that is, the phytosterols were the ones that showed very good binding affinities for the helicase enzyme. The results of the *in vitro* antimutagenic functional tests performed in this study confirmed the ethanolic extract's antimutagenic activity. Our findings showed that NaN_3 caused CA at the selected dosage and was cytotoxic by lowering the percentage MI. The MI can be used for evaluating cytotoxicity, whereas CA are a yardstick for determining the mutagenicity of chemicals in organisms' cells (Akinboro et al., 2011). Where NaN_3 is exposed to cells, its mutagenicity is demonstrated by the action of its by-product-L-azidoadenine, which interacts with the genetic material, thereby inducing changes in the DNA particularly base substitutions (Gulluce et al., 2010).

The pre-treatment protocol in which the bulbs were first treated with the ethanolic extract of CE and then exposed to NaN_3 resulted in a lower percentage reduction in DNA damage, whereas a much higher percentage reduction in DNA damage was observed in the post-treatment protocol in which *A. cepa* cells were first exposed to NaN_3, and then to the extract. As a result, the findings suggest that tigernut extract has antimutagenic properties. Other plants like *Origanum majorana* L. and *Ruta chalepensis* have been shown to have antimutagenic activity in the *A. cepa* chromosomal assay (Khatab and Elhaddad, 2015).

The precise mechanism by which tigernut ethanolic extract exhibits antimutagenicity is unknown. However, it can be inferred that by the time the extract's compounds percolated into the cell, particularly the phytosterols—that is, the γ-sitosterol, campesterol, stigmasterol, and vitamin E—they may have moderated the repair system of the cell by binding with a high affinity to the RECQ helicase enzyme. This is because plants have several RECQ homologs in the family of helicases, and recent studies have revealed a link between RECQ helicases of plant and their orthologs in man (Wiedemann et al., 2018). The ligands are thought to have activated the helicase enzyme in the onion plant, which acted to repair the NaN_3-induced DNA damage in the post-treatment protocol, explaining the higher percentage reduction in the CA. Phytosterols are innate plant molecules that constitute the cell membrane. They have been shown to be anticancer by inhibiting cell division, initiating tumor cell death, and modulating some of the hormones involved in tumor growth (Awad and Fink, 2000). In addition to immune-modulatory effects, they have also been revealed to act against inflammation and oxidative stress (Ogbe et al., 2015). This most likely resulted in a decrease in the frequency of mutation, implying that the extract demonstrated chemoprotection via bioantimutagenesis. Compounds in the extract may have the capaci-

ty of enhancing the efficacy of the repair system, as our findings revealed that the total number of CA in the treated groups was significantly reduced ($P<0.05$) compared to the untreated group.

Haraguchi et al. (1995) discovered that compounds in tigernut with many phenol groups in tigernut can effectively protect biological systems against diseases orchestrated by free radicals. Mutagenesis is known to be induced by the production of excess free radicals, which can have a negative effect on DNA, resulting in gene changes and chromosomal abnormalities (Degtyareva et al., 2008). In conclusion, both *in silico* and *in vitro* studies revealed that the ethanolic tigernut extract has antimutagenic activity, which could be attributed to the presence of vitamin E and other phytosterols, which have antioxidant properties and can activate repair enzymes. Because antimutagenic agents are potential anti-carcinogenic agents, compounds from tigernut extract can be isolated and used as nutraceuticals for cancer prevention. More research is needed, however, to confirm the antimutagenic activity in an *in vivo* system.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: ODO, TA. Methodology: all authors. Validation: ODO. Formal analysis: all authors. Investigation: TA, AVO. Writing—original draft preparation: TA, AVO. Writing—review and editing: ODO, TA. Supervision: ODO, TA, ATO. Project administration: ODO, TA, ATO. All authors have read and agreed to the published version of the manuscript.

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