Scientific paper

Biological Activities, Chemical Composition and Molecular Docking of *Urelytrum giganteum* Pilg

Abdulrahaman Mahmoud Dogara[1], Sawsan Sadiq Al-Rawi[1], Ateeq Ahmed Al-Zahrani[2*], Abubakar Abdullahi Lema[3], Aisha Abdullahi Mahmud[4], Hasan Nudin Nur Fatihah[5], Iliyasu Uwaisu[6] and Hussain Daifallah Almalki[2]

[1] *Biology Education Department, Tishk International University, Erbil, Iraq*

[2] *Chemistry Department, University College at Al-Qunfudhah, Umm Al-Qura University, Saudi Arabia*

[3] *Biological Sciences Department. College of Natural and Applied Sciences Al-Qalam University Katsina, Katsina state Nigeria*

[4] *Department of Plant Science and Biotechnology, Faculty of Life Science, Federal University, Dutsin-Ma, Katsina State, Nigeria*

[5] *Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia*

[6] *Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences Kaduna State University, Kaduna-Nigeria*

** Corresponding author: E-mail: aaalzahrani@uqu.edu.sa*

Received: 11-19-2024

Abstract

The growing interest in health-promoting food options has highlighted the need to explore new sources with unique nutritional compositions. Plants are rich sources of bioactive compounds with biological effects. *Urelytrum giganteum* Pilg is a perennial wild tufted grass that grows in dry tropical African countries. Traditionally, it has been used to treat several diseases, however, up to date, no study has been done on this plant. This study analyzed the solvent extraction of *Urelytrum giganteum* Pilg, a perennial wild tufted grass in dry tropical African countries, to determine its proximate analysis, antioxidant potential, chemical composition, antimicrobial efficacy, and molecular docking. The Soxhlet extraction method was employed, and the obtained extracts were subjected to phytochemical evaluation, DPPH radical scavenging activity, a scavenging assay of hydrogen peroxide (H_2O_2), ferric reducing antioxidant power (FRAP), disk diffusion evaluation and molecular docking studies. The phytochemicals identified included alkaloids, flavonoids, saponins, phenolic substances, tannins, carbohydrates, terpenes, and C-glycosides. The ethanol extract showed the highest activity in terms of DPPH (70.2 μg/mL), H_2O_2 (60.7 μg/mL), and alpha glycosidase (72.3 μg/mL) activities and antimicrobial activity against *Klebsiella pneumoniae* (17 mm). Most of the phytochemicals demonstrated notable inhibitory activity, with Stigmasterol showing the highest inhibition score. This underscores the potential of *U. giganteum* as a promising nominee for the pharmaceutical industry. The identified phytochemical and antioxidant compounds could be promising nutraceuticals or food additives for overall well-being.

Keywords: Antioxidants, bioactive compounds, computational modeling, GC–MS, phytochemicals, *Urelytrum giganteum*, inhibitors

1. Introduction

Plants with high nutrient quality have garnered the attention of researchers who are searching for plant-based foods as viable alternative sources of nutrition.[1] Thus, discovering a new source with exceptional high-quality nutritional value, antioxidants, and a distinctive chemical composition is vital, whether for vegetarians seeking to replace meat products or for the food and pharmaceutical industries.

Urelytrum giganteum Pilg (*U. giganteum*) is a perennial tufted grass that grows in the seasonally dry tropical biome and is only collected from the wild. Primarily native to regions such as Nigeria, the Central African Republic, Cameroon, the Congo, Zaïre, Sudan, and Uganda.[2] In Nigeria, stems are used to make mats, whereas leaves are

used as fodder.[3] Ethnobotanically, this plant has a rich history in traditional medicine, with powdered leaves being used to treat inflammation, malaria, and skin diseases.[4] Furthermore, the crude methanol extract of *U. giganteum* showed strong antimalarial activity, while the decoction of the whole plant has been applied as a remedy for headaches, as reported by Abubakar *et al.*[5] Recently, interest has increased in the potential properties of plants because of their integral role in the treatment and management of diseases. Antioxidants play crucial roles in shielding the body against diseases such as cancer, atherosclerosis, cardiovascular diseases, arthritis, and diabetes mellitus.[6]

Wild plants are rich sources of potent antioxidants, and incorporating them into diets or using them as medicinal herbs has been shown to decrease the incidence of these diseases.[7] The biological properties of plants, such as those of antioxidants, are a result of the presence of secondary metabolites, including alkaloids and polyphenolics, alkaloids, flavonoids, saponins, phenols, and tannins.[8] However, understanding the biological activities of a plant is crucial for determining its potential therapeutic effects.[9] Identifying its chemical composition can provide valuable insights into the bioactive constituents responsible for its pharmacological actions.[9] Nevertheless, determining safe and effective dosages for human use has posed a significant challenge in the healthcare system. An enhanced understanding of the pharmacokinetics associated with plants and the interactions of their active compounds with proteins in the human body is crucial for overcoming existing knowledge gaps and can yield new perspectives in the creation of safe and effective plant-based therapeutics. The recent use of molecular docking has facilitated the prediction of interactions between phytochemicals and proteins at an atomic resolution.[9] However, despite the potential benefits of this plant, little is known about the biological and chemical properties of *U. giganteum*.

There is a significant lack of data available regarding the antioxidants, chemical composition and proximate analysis of this plant. Acquiring information on its nutritional composition is crucial for establishing a baseline for utilizing these species, whether in the diet or in the pharmaceutical industry. Therefore, this is the first study to explore and elucidate the biological activities, chemical composition, proximate analysis and antimicrobial activity of *U. giganteum* via molecular docking analysis.

2. Experimental

2. 1. Chemicals and Reagents

Folin-Ciocalteu reagent (10% solution, Sigma–Aldrich, St. Louis, MO, USA), sodium carbonate (Na_2CO_3 Merck, Darmstadt, Germany), gallic acid (Sigma–Aldrich, St. Louis, MO, USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich, St. Louis, MO, USA), methanol, ethanol hexane, ethyl acetate (Merck, Darmstadt, Germany), hydrogen peroxide (H_2O_2) (Sigma–Aldrich, St. Louis, MO, USA), ascorbic acid (Sigma–Aldrich, St. Louis, MO, USA), hydrochloric acid (HCl) (Merck, Darmstadt, Germany), ferric chloride ($FeCl_3$) (Sigma–Aldrich, St. Louis, MO, USA), ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$) (Sigma–Aldrich, St. Louis, MO, USA), Mueller-Hinton agar (Sigma–Aldrich, St. Louis, MO, USA), monosodium phosphate [NaH_2PO_4, Sigma–Aldrich, St. Louis, MO, USA], and disodium phosphate [H_2O],

2. 2. Plant Collection and Herbarium Deposit

Fresh *U. giganteum* leaves were taken from the wild in Kaduna state, northern Nigeria, between May and August 2022 (Figure 1). The voucher specimens (ABU114 and FUDMA/PSB/00143) were recognized and validated by a certified botanist at Ahmadu Bello University, Zaria and Federal University Dutsin-Ma, Katsina, and were deposited in the respective herbaria for future reference. The world flora online was used to confirm the scientific name of the species https://wfoplantlist.org/.

Figure 1. *U. giganteum* from the wild.

2. 3. Extraction and Yield Percentage

The dry materials were ground into a fine uniform powder via a grinding machine, and 100 g of each powdered plant sample was weighed accurately. The powder was extracted into ethanol, hexane, ethyl acetate and aqueous forms via Soxhlet extraction.[10] The extraction byproduct was filtered through No. 2 Whatman filter paper. Crude extracts of hexane, ethyl acetate, ethanol, and aqueous plant leaves were prepared via an evaporation device (E-Z-2-Elite). The solvent pressure was set at 300, 77, 20, and 72 for the ethanol, ethyl acetate, hexane, and aqueous

extracts, respectively. The vacuum was maintained at 40 °C for each sample.[10]

Yield of extraction (%) = *B1⁄B2 × 100; thus,* B1 = weight of the crude extract, and B2 = weight of the sample.

2. 4. Quantitative Assay

The proximate analysis of the sample was performed as described previously.[11,12]

2. 4. 1. Qualitative Phytochemical Assay

A qualitative phytochemical screening was conducted on the ethanol, ethyl acetate, hexane and aqueous extracts of the leaves to identify the organic components. The ethanol extract of Simarouba glauca seeds was analyzed via chemical analysis, as reported by Sharangouda and Patil[13], Patil and Murthy[14], Fransworth[15], to identify steroids and triterpenoids, alkaloids, tannins, flavonoids, glycosides, carbohydrates, proteins, and amino acids.

2. 4. 2. Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined via the use of Folin-Ciocalteu reagent. Specifically, 150 µL of 10% Folin-Ciocalteu reagent was immersed in 30 µL of extract (1 mg/mL). After 4 minutes, 120 µL of 7.5% Na_2CO_3 was added to the mixture. The mixture was then incubated in the dark for 45 minutes before the optical density was assessed at 760 nm.[10] The findings were quantified as milligrams of gallic acid equivalent (GAE) per gram of dry weight (DW) via the gallic acid standard curve equation (Figure 2).

2. 4. 3. Total Flavonoid Content (TFC)

For approximation, methanol was added to an equal volume of a certain fixed amount of sample. A measured mixture of diluted aluminum chloride, potassium acetate, and 1.4 mL of distilled water was then added to the solution. After the mixture was sealed with aluminum foil, it was left undisturbed for thirty minutes to prevent it from contacting the light source.[16] The absorbance was measured at a wavelength of 415 nm, with quercetin used as the flavonoid standard.[16] The findings are presented as mg of quercetin equivalent (QE) per g of dry weight (DW) (Figure 3).

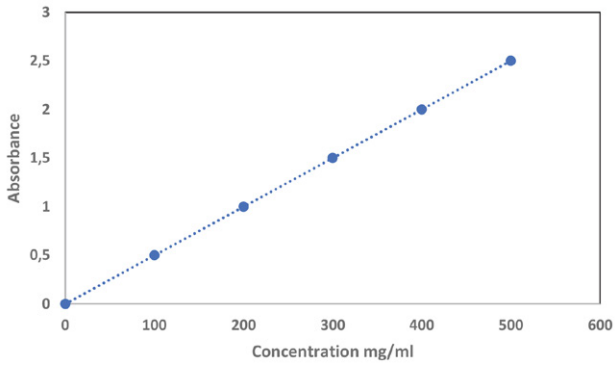

Figure 2. Standard curve for total phenolic content of *U. giganteum.*

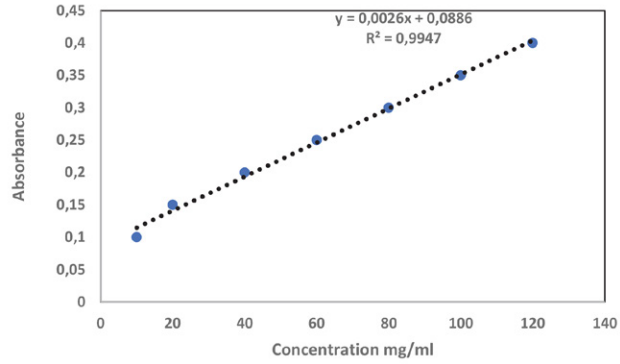

Figure 3. Standard curve for the total flavonoid content of *U. giganteum.*

2. 5. Gas Chromatography–Mass Spectrum

The analysis was carried out via an Agilent 7890 GC–MS instrument. Helium was used as the carrier gas, and the flow rate was 1 ml/min. An HP5 column with a length of 30 mm, internal diameter of 0.32 mm, film thickness of 0.25 mm, and a temperature range of –60 °C to 325 °C (350 °C) was used.[17] The GC lasted 35 minutes. The oven temperature climbed steadily from 70 °C to 280 °C at a rate of 8 °C per minute. A 4 µl sample was injected via the injector. The MS was measured at 70 eV. Compounds were identified by comparing their spectra to those of recognized chemicals from the library. Compounds were identified by comparing their spectra to those of recognized chemicals from the library. The name, molecular weight, and structure were determined.[17]

2. 6. DPPH Radical Scavenging Activity

Approximately 100 µL of each leaf extract was combined with a 0.004% w/v DPPH solution in methanol. Extracts were produced using ethanol, hexane, and aqueous solvents at concentrations ranging from 1.56 to 100 µg/mL. After 30 minutes of storage in a dark atmosphere at 37 °C, the DPPH reduction ability of the mixture was measured via the absorbance at 517 nm. The percentage of scavenging activity was calculated by comparison with a control consisting of 100 µL of methanol and 200 µL of DPPH solution. The radical scavenging activity was calculated via the equation shown below:

Inhibition (%) = *[(Co − C1)/Co] × 100*, where C1 denotes the sample absorbance and Co denotes the control absorbance.[18]

2. 7. Hydrogen Peroxide (H_2O_2) Scavenging Assay

The H_2O_2 scavenging activity of various leaf extracts was evaluated via the method proposed by Al-Owaisi *et al.*[19] A 40 mmol/L H_2O_2 solution was produced in 50 mmol/L phosphate buffer (pH 7.4), and its concentration was evaluated on the basis of its absorbance at 230 nm. After 10 min, the absorbance of an assay mixture containing 1 mL of crude extract or standard ascorbic acid solution at various concentrations (5, 10, 25, 100 µg/mL) and 2 mL of H_2O_2 was measured. This mixture was compared to a blank solution of phosphate buffer without H_2O_2. The proportion of H_2O_2 scavenged was estimated via the following formula:

H_2O_2 Scavenge (%) *= [(ODo − ODt)/ODo)] × 100,* where ODo = absorbance of control and ODt = sample absorbance.

2. 8. Ferric Reducing Antioxidant Power (FRAP) Assay

A quantity of FRAP solution was generated by combining 300 mM acetate buffer, 10 mM (2,4,6-tri (2-pyridyl)-S-triazine) TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in a 10:1:1 ratio. This mixture was heated in a water bath at 37°C for 10 minutes before use. A total of 285 µL of the working FRAP solution was added to 15 µL of each plant sample (ethanolic, aqueous, hexane, or ethyl acetate) at a concentration of 100 µg/mL. The mixture was incubated at room temperature in the dark for 30 minutes before the absorbance was measured at 539 nm. $FeSO_4{\cdot}7H_2O$ was employed as a standard at concentrations ranging from 125 to 1000 µM. The results are presented as mmol Fe^{2+} equivalents of the dried extract per gram.[18,20]

2. 9. Alpha-glucosidase Evaluation

A total of 10 µL of leaves at 100 µg/mL were mixed with 50 µL of 0.1 M phosphate buffer (pH 7.0), and 25 µL of alpha-glucosidase in 0.2 U/mL buffer was added to the well plate and incubated for 10 min at 37°C to initiate the reaction. A total volume of 25 µL of 0.5 mM 4-nitrophenyl alpha-D-glucopyranoside (pNPG) substrate was added to complete the reaction, and the mixture was incubated for another 30 min at 37°C. The reaction was terminated by adding 100 µL of 0.2 M sodium carbonate solution. Acarbose was used as a positive control.[18] The absorbance was measured at 410 nm. The percentage of inhibition was determined via the following formula: percentage of inhibition was determined as ***inhibition (%)*** *= [Control abs – sample abs)/control abs] × 100.*

2. 10. Antimicrobial Evaluation

2. 10. 1. Test Organisms

Gram-negative test organisms (*Klebsiella pneumoniae*) were procured at the Biological Sciences Department, College of Natural and Applied Sciences, Al-Qalam University, Katsina, Katsina State, Nigeria. The microbial stock cultures were streaked onto Mueller–Hinton agar plates via an inoculation loop. The plates were incubated overnight at 37 °C. The next day, they were subcultured until a new colony formed. After that, they were injected with Mueller–Hinton broth and incubated at 200 rpm overnight.

2. 10. 2. Disk Diffusion Evaluation

Microbial inoculums of 1.10^6 CFU/ml were sown on 200 µL solidified Mueller–Hinson plates. Plant extracts (ethanol, aqueous, hexane, and ethyl acetate) were infused with 20 µL at 4000 µg/mL on Whatman No. 1 filter paper discs (6 mm). By applying sterile forceps, the infused disk was placed on the plate. The plates were then incubated at 37 °C for 24 hours.[21,22]

2. 11. *In silico* Study

The antibacterial activity of the phytochemicals extracted from *U. giganteum* was evaluated through a docking study involving the bacterial protein dihydrofolate reductase (PDB: 4OR7) sourced from *K. pneumoniae* bacteria. The protein structure was obtained from the Protein Data Bank (PDB) accessible at https://www.rcsb.org/and was downloaded in pdb format. The molecular structures of the 14 phytochemicals were obtained from the PubChem search database in the form of 2D or 3D SDF files. For the docking process, the pdb files of both the proteins and phytochemicals were uploaded to the CB-Dock server.[23] The receptor and ligand input files underwent automatic optimization through the CB-Dock server, as detailed by Dakpa *et al.*[24] Following the docking process, the resulting conformations were analyzed and visualized via both the CB-Dock server and Chimera software tools. The determination of the inhibition constant (Ki) was conducted via the following equation: ***Ki*** *= exp(ΔG/RT)*, where ΔG denotes the binding energy, R signifies the universal gas constant (1.985 × 10^-3 kcal mol^-1 K^-1), and T indicates the temperature (298.15 K). The drug likeness of the phytochemicals was evaluated via the SwissADME web server to assess compliance with Lipinski's rule of five. A molecular dynamics (MD) simulation was conducted utilizing the CABS Flex 2.0 server, accessible at https://biocomp.chem.uw.edu.pl/CABSflex2, to analyze dihydrofolate reductase (in the absence of ligands) and the stigmasterol-dihydrofolate reductase complex for the purpose of determining the root mean square fluctuation (RMSF) values. The parameters employed in this simulation included a duration of 10 ns; mode set to SS2; an interval of 3; a global weight of 1.0; a total of 50 cycles; 50 cycles between trajectory frames; a simulation temperature of 1.4; and a random number generator seed of 5546.

2. 12. Statistical Analysis

The studies were completely randomized, with three replicates of each therapy. SAS (University version 9.4) was used to analyze the data. One-way repeated-measures analysis of variance (ANOVA)[21] was used, followed by a post hoc Duncan's multiple range test, to identify statistically significant differences between group means at the $p \leq 0.05$ level.

3. Results and Discussion

3. 1. Yield and Qualitative Analysis of *U. giganteum*

Previous research on other plants within the *Urelytrum* genus has also highlighted the potent antioxidant properties associated with phenolic and flavonoid compounds. Among the different extractions utilized in the present study, the ethanol extract had a significant % yield of 26%, followed by the aqueous extract, and the ethyl acetate and hexane extracts had the lowest % yields (18, 15, and 11%), as shown in Table 1. Similarly, compared with the other extraction solvents, the ethanol extract shows promising results, as it contains significant amounts of phytochemicals. The phytochemicals present in the ethanol extract are alkaloids, flavonoids, saponins, phenols, tannins, carbohydrates, terpenes and C/glycoside, as shown in Table 2.

Table 1. Extraction yield of *U. giganteum.*

S/N	Extract	Amount (g)	Yield %
1	Ethanol	0.26	26
2	Ethyl acetate	0.15	15
3	Hexane	0.11	11
4	Aqueous	0.18	18

3. 2. Proximate Analysis Composition of *U. Giganteum*

The proximate analysis of the composition of *U. giganteum* is presented in Figure 4. The results revealed that the *U. giganteum* plants contained substantial amounts of fiber (26%, 11% moisture; 11% protein; 15% fat; 62% carbohydrate; and 8% ash) (Figure 4). The amount of fiber in this plant is considered important because of its health concerns, which include decreasing the risk of disease and assisting in weight management. The recommended daily intake for adults is 30 to 40 g, but most people obtain only approximately 10 g a day.[25] According to Ioniță-Mîndrican *et al.*[26], green vegetables constitute 2–8%, fruits constitute 6–24%, and leguminous vegetables constitute 22–47% of the fiber content. These findings indicate that the *U. giganteum* plant has an equal amount of fiber to leguminous vegetables and a higher fiber content than green vegetables and fruits. The amount of fiber in *U. giganteum* is very close to the fiber content in flax seeds, which is 27%.[26] This signifies that incorporating this plant into the human diet can help support the daily need for fiber, which will improve health and wellbeing. In addition, this plant can be used as a high-fiber source in various food products, such as bakery items, pasta, cakes, etc. It can catch the interest of food companies looking to improve their products and appeal to customers. In this study, the protein content of *U. giganteum* plants was 11% protein, 15% fat, and 62% carbohydrate. This amount can constitute almost 10% of the daily protein needs of humans.[26] Surprisingly, amaranth and pseudocereal grains of high nutritional value contain 12% protein, 16.6% lipids and 65% CHO, which is remarkably close to the protein and lipid contents of our studied *U. giganteum* plants. Proteins from plant sources are considered good alternative options because of their suitability for celiac patients.[27] Proteins, which are vital macronutrients, are indispensable in human nutrition because of their complex roles. They are integral for cell growth, repair mechanisms, immune function, and the synthesis of hormones and enzymes. Proteins contribute to the main-

Table 2. Qualitative phytochemical analysis.

S/No	Constituents	Ethanol extract	Ethyl a cetate extract	Hexane Extract	Aqueous extract
1	Alkaloids	+v	+v	–v	+v
2	Flavonoids	+v	–v	–v	+v
3	Saponins	+v	–v	+v	+v
4	Phenols	+v	–v	–v	+v
5	Tannins	+v	–v	–v	+v
6	Carbohydrates	+v	+v	+v	+v
7	Steroids	–v	+v	–v	+v
8	Triterpenes	+v	+v	+v	–v
9	C/glycosides	+v	+v	+v	+v

+v = Positive; –v = Negative.

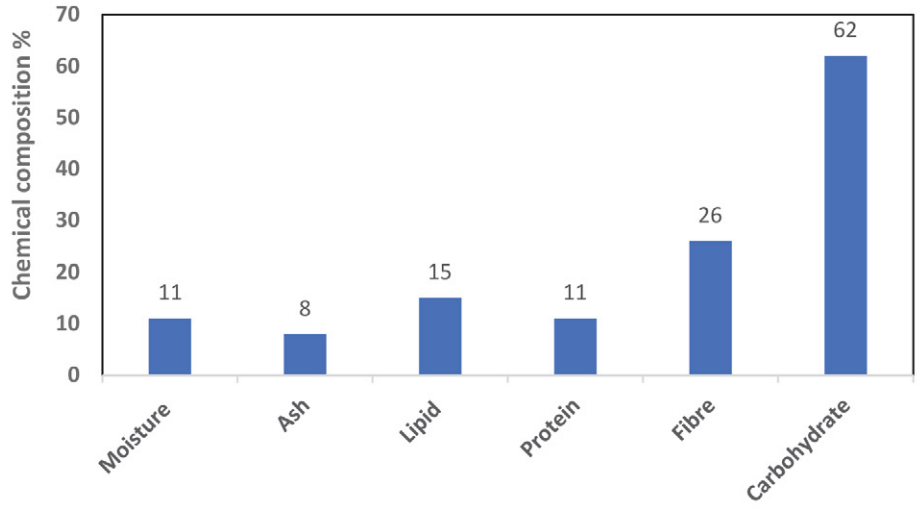

Figure 4. Proximate analysis composition of *U. giganteum.*

tenance of tissue and organ structure and can also function as energy sources when needed. On the other hand, the sufficient amount of CHO in this plant provides a good source of energy that supports body cell functions and the physical activity of the human body. These values reflect the hidden nutritional composition of *U. giganteum* plants. However, it is worth noting that this study represents the first evaluation of the nutritional composition of *U. giganteum*.

3. 3. Quantitative Phytochemical Analysis and Biological Activity

The present study demonstrated that the ethanol extract of *U. giganteum* presented the highest antioxidant activity across all the assays, including DPPH, FRAP, hydrogen peroxide H_2O_2, total phenolic content (TPC), and total flavonoid content (TFC) measurements. These findings suggest that ethanol is the most promising solvent for the extraction of phenolic compounds and other antioxidants from *U. giganteum, as* presented in Tables 2 and 3. The strong antioxidant activity of the ethanol extract may be due to its high phenolic content. Phenolic compounds are secondary metabolites that are increasingly recognized for their preventive role in degenerative diseases. They are well known for their ability to donate hydrogen atoms or electrons, diminishing free radicals and thus avoiding oxidative stress. Our results revealed that the TPC of *U. giganteum* ranged from 80-112 mg/mL (based on the extraction solvent). This result indicates that *U. giganteum* has a significant amount of TPC, which was greater than that of the positive control (77 mg/ml) (Table 3). In addition, our results revealed that the TPC in *U. giganteum* was greater than that reported in other published works on another *Urelytrum species.* In a previous study, the TPC of *U. muricatum* was found to be 0.0014 mg/mL, as reported by Aliyu *et al.*[28], whereas that of *U. giganteum* ranged from 80–112 mg/mL. This shows a significant difference in the TPC between the *Uvaria species*, which reflects the supremacy and high quality of *U. giganteum* and its possible utilization in a wide spectrum of applications. Jovanova *et al.*[29] reported that *Allium schoenoprasum*, an aromatic herbal grass used as a culinary herb that is commonly used in cooking and salad and is well known for its distinct medicinal properties, has a TPC (112 mg/mL), which is equal to the TPC of *U. giganteum*. In addition, the positive correlation between the TPC and antioxidant assays (DPPH, FRAP) underscores the role of these compounds in scavenging free radicals. Therefore, these results suggest that ethanol is the best solvent for extracting phenolic compounds and other antioxidants from *U. giganteum*.[30] α-Glucosidase is a secondary metabolite of plants that consists of phenolic acids, alkaloids, flavonoids, anthocyanins, terpenoids, and their glycosides.[31] The alpha-glucosidase content of the *U. giganteum* extracts ranged from 45–72 µg/ml, while the ethanolic extract scored the highest among all the other extracts. This result was supported by our qualitative phytochemical analysis (Table 3). The importance of this α-glucosidase content lies in its increasingly fascinating role in recent studies, which highlight its potential in protecting against chronic diseases and the positive health impacts of vegetable consumption. Moreover, there is an ongoing dedication to exploring and developing new anti-glucosidase medications from plants with enhanced safety profiles for extended treatment. Thus, the α-glucosidase of *U. giganteum* is considered important and is a new candidate for pharmaceutical drug formulation. Similarly, the ethanol extract also showed the most substantial antibacterial activity against all the bacterial strains tested (Figure 5). This broad-spectrum activity indicates that the ethanol extract contains compounds with significant antibacterial properties. The antibacterial activity observed in the ethanol extract might be a result of the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and ter-

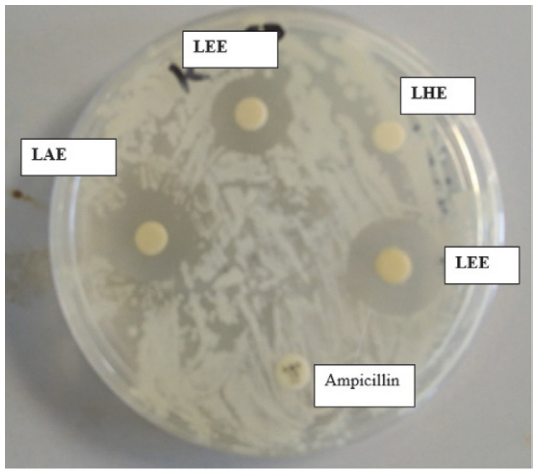

Figure 5. Inhibition of *K. pneumoniae* by different *U. giganteum* extracts via disc diffusion.
Note: LEE = leaf ethanol extract, LEA = leaf ethyl acetate extract, LHE = leaf hexane extract, LAE = leaf aqueous extract.

penoids, which are known to disrupt bacterial cell membranes, inhibit enzyme activity, or interfere with nucleic acid synthesis. The higher inhibition values for the ethanol extract highlight its effectiveness in inhibiting bacterial growth at relatively low concentrations. The current findings agree with those of Varghese *et al.*[32] and Schevenels *et al.*[33] Among the tested extracts, the ethanolic extract had the highest bioactivity in all the assays performed. It has the highest antioxidant activity and has strong DPPH, FRAP and hydrogen peroxide radical scavenging activities, which suggest that it is rich in redox-active compounds. In addition, it exhibited a strong level of α-glucosidase inhibitory activity, which might make it suitable for treating hyperglycemia and diabetes since it is rich in phenolics and flavonoids. In the antimicrobial assay, the ethanolic extract was more active than the other extracts, with the largest inhibitory zone against the organism *K. pneumoniae*. This led to its further characterization via GC-MS, after which it was subjected to molecular docking to confirm the *in silico results*. These steps are designed to provide additional insight into the molecular processes associated with observed biological activities and present a multifaceted view of its therapeutic application.

3. 4. Chemical Composition Analysis

The chemical composition of the ethanolic extract of *U. giganteum* leaves was determined via gas chromatography–mass spectrometry (GC-MS) to determine the most bioactive compounds in this plant. This ethanolic extraction of *U. giganteum* was chosen for this analysis since it has the highest antioxidant activity among all the other extraction methods. Chemical analysis of *U. giganteum* leaves resulted in the identification of 14 chemical constituents, as shown in the Supplemental Information (Table S1). The table shows that the extract contains different types of chemical classes, such as fatty acids, terpenes, phenolic compounds, sterols, flavonoids, and alkene hydrocarbons. The fatty acid group formed four compounds: 9-octadecenoic acid (Z) (omega 9), α-linolenic acid (9,12,15-octadecatrienoic acid), methyl pentadecanoic acid, (saturated), and ethyl oleate (Figure 6). Nuts, seeds, eggs, meats, and vegetable oils are sources of α-linolenic acid. Recent studies have shown that the consumption of dietary α-linolenic acid is correlated with a decreased risk of death from several diseases, including CVD, CHD and other related diseases.[34] The health benefit of α-linolenic acid is due to its role in promoting and stimulating the production of a cell signaling protein that stimulates vasculogenesis.[35] Omega-9 fatty acids, unsaturated fatty acids with a unique double bond at the 9th position, can be found in vegetable oils, nuts, seeds, fish and seafood. Omega 9 is also known for its major role in promoting health benefits and treating diseases such as inflammation, cardiovascular diseases, cancer, and neurodegenerative disease.[36] Our results of chemical identification via GC-MS are in line with the findings of prior work.[37] The author acknowledged the presence of free fatty acids and phenyl propanooids, which were identified via GC-MS.[37] Figure 4 shows the results of the quantitative GC-MS analysis of *U. giganteum*, which shows the area percentages of the identified compounds. Chemical analysis also confirmed the existence of phenolic compounds, as shown in Table 2. The GCMS identified three phenolic compounds and one flavonoid compound: phenol, 2,4-bis(1,1-dimethylethyl), 3-methoxycatechol, 5-hydroxy methylfurfural and genistein. Another group included sterols with three compounds: Megastigmatrienone, androst-2,16-diene, and stigmasterol. As steroids,

Table 3. Antioxidant and antibacterial evaluation of different extracts *of U. giganteum.*

Sample	DPPH (100 µg/mL)	H_2O_2 (100 µg/mL)	FRAP Fe2+/ (mmol/g)	TFC) (mg QE/g	TPC (mg GAE/g)	Alpha-glucosidase (µg/mL)	ZI of *K. pneumoniae* (4000 µg/mL)
LEE	70.2 ± 0.3[a]	60.7 ± 1.0[a]	15.4 ± 1.8[a]	80.2 ± 0.4[a]	112.1 ± 0.3[a]	72.3 ± 0.8[a]	17.0 ± 0.3[a]
LEA	69.8 ± 1.2[a]	76.1 ± 0.4[a]	17.1 ± 0.8[a]	68.1 ± .02[a]	98.3 ± 0.1[a]	69.1 ± 1.1[a]	11.3 ± 0.1[b]
LHE	59.9 ± 0.9[a]	43.9 ± 0.7[b]	8.9 ± 1.6[b]	65.7 ± 05[a]	88.2 ± 0.5[a]	45.6 ± 0.7[b]	NI
LAE	35.1 ± 0.5[b]	40.2 ± 0.2[b]	12 ± 0.2[b]	61.6 ± 0.4[a]	80.6 ± 0.2[a]	54.1 ± 0.2[b]	14.2 ± 0.7[b]
Ascorbic Acid/Acarbose/ Ampicillin 10 µg	92.3 ± 1.2[a]	93.5 ± 0.9[a]	ND	57.8 ±0.1[b]	77.2 ± 0.5[b]	53.9 ± 0.8[a]	NI

Note: LEE = Leaf ethanol extract, LEA = Leaf ethyl acetate extract, LHE = Leaf hexane extract, LAE = Leaf aqueous extract, ZI = Zone of inhibition, NI = No inhibition. The results are presented as the means ± SDs, and values with the same letter are not significantly different at $p \leq 0.05$.

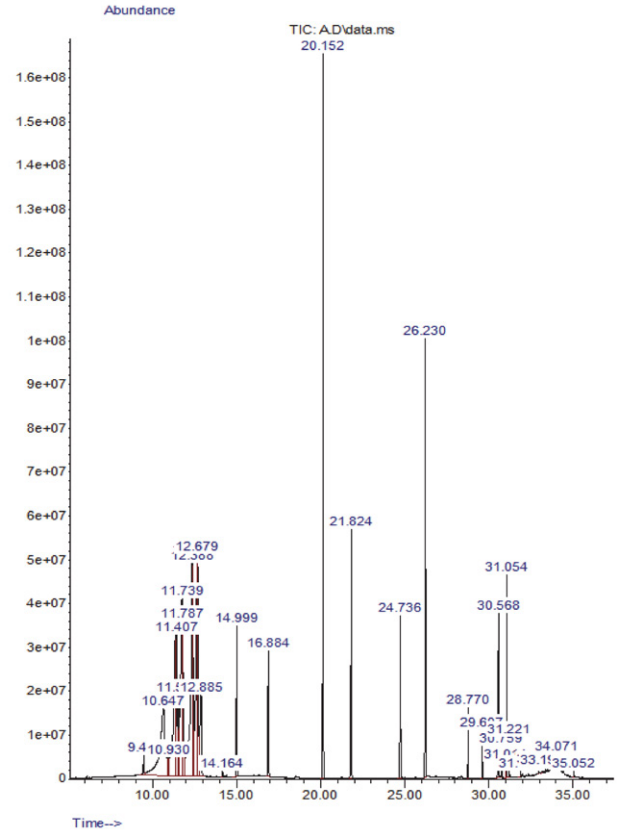

Figure 6. Chromatogram of the ethanol leaf extract of *U. giganteum*.

which are found in plants such as legumes, vegetables, fruits, vegetable oils, nuts, cereals, and seeds, plant sterols are known for their significant anticancer, anti-inflammatory, cholesterol lowering, and antioxidative activities.[38] Other compounds, such as cineole, 3-[2-(1-methyl-2-imidazolylthio)-1-oxoethyl] coumarin and the alkene hydrocarbon 1,4-cyclohexadiene, were also identified. This result indicates the richness and high quality of this healthy wild plant. These findings support its use as a medicinal plant in folk medicine in Nigeria, which promotes its use in several medicinal applications. A molecular docking study was conducted on the components listed below to determine their separate contributions to the overall biological activity of the plant extract. Understanding the underlying biological processes and designing tiny molecules logically rely on an assessment of binding behavior.

3. 5. Molecular Docking

The validation of the docking protocol in a docking study is of paramount importance, with a critical step being the removal of the cocrystallized ligand (native inhibitor) followed by its redocking into the pdb structure to verify accuracy. The CB-Dock server effectively redocked the generated ligands into the binding site of the dihydrofolate reductase enzyme. The redocked ligand exhibited perfect superimposition with the corresponding cocrystallized ligand (Figure 7). This precise alignment between the docked structure and the original pdb structure was achieved even when blind docking was employed without designating the active site. Furthermore, the ligand that was redocked was not the original native ligand; instead, the generated ligand was utilized. The phytochemicals identified in *U. giganteum* were analyzed through docking studies to predict their antibacterial efficacy against the dihydrofolate reductase enzyme, as presented in the Supplemental Information (Table S1). Most of the phytochemicals exhibited significant inhibitory activity, as indicated by binding affinity scores that were lower than −5.0 kcal/mol. A binding energy of less than −5.0 kcal/mol signifies a robust binding affinity between the ligand and the receptor protein.[39] However, some studies consider binding values of less than −6.0 kcal/mol and, in some instances, below −8.0 kcal/mol as the most common criteria for identifying potential candidates.[40] The docking analysis revealed that among the 14 phytochemicals examined, the stigmasterol compound exhibited significant binding affinities for the dihydrofolate reductase enzyme present in *K. pneumoniae* bacteria (Table 4).

Stigmasterol demonstrated maximum binding affinities and minimum binding energies relative to the other phytochemicals, achieving a score of -8.0 kcal/mol and an inhibition constant of 1.2 μM. The interactions between stigmasterol and the target protein are illustrated in Figure 8. Stigmasterol established multiple hydrophobic interactions with eleven residues, specifically ALA7, ALA19, MET20, TRP22, ASP27, LEU28, PHE31, SER49, ILE50, ARG52, and LEU54. Stigmasterol was evaluated via the SwissADME web server to predict Lipinski's rule of five. Stigmasterol conformed to Lipinski's rules, except for one violation: MLOGP (logarithm of the partition coefficient) > 4.15. Our *in silico* investigation indicated that the compound stigmasterol may possess antibacterial properties, which aligns with findings from multiple studies. The

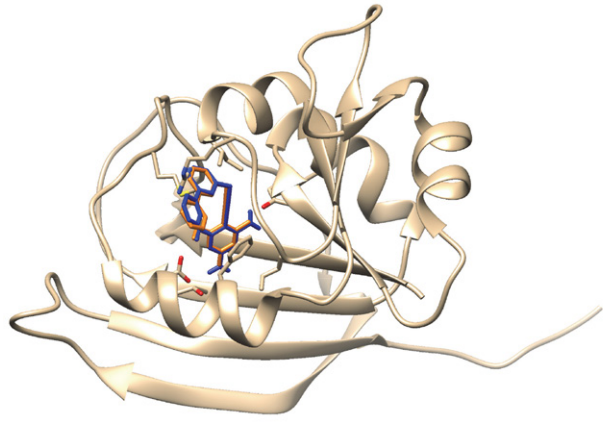

Figure 7. Validation of the docking protocol. The figure shows the redocked ligand (blue), the cocrystallized ligand (orange) and the dihydrofolate reductase enzyme (gold).

Table 4. Docking screening of the identified phytochemicals derived from *the K. pneumoniae* extract.

Compound Name	PubChem CID	Docking Score Kcal/mol	Inhibition Constant (μM)
***K. Pneumoniae* (Dihydrofolate reductase)**			
25U (co-crystalized inhibitor)	71737834	−8.4	0.65
Ethyl oleate	5363269	−5.6	71.7
5-Hydroxy methylfurfural	87289739	−4.8	293.0
Cineole	2758	−5.7	63.7
1,4 Cyclohexadiene	12343	−3.9	1346.8
3-Methoxycatechol	13622	−5.4	106.0
Androst-2,16-diene	617873	−7.5	3.0
Phenol,2,4-bis(1,1-dimethylethyl)-	7311	−5.0	208.8
Pentadecanoic acid, methyl ester	23518	−5.4	106.0
Megastigmatrienone	5375190	−6.0	38.3
Stigmasterol	5280794	−8.0	1.2
Genistein	5280961	−7.4	3.5
9-Octadecenoic acid (Z)-,Ethyl ester	5363269	−5.6	75.5
9,12,15 octadecatrienoic acid	860	−6.4	19.4
Coumarine,3-[2-(1-methyl-2-imidazolylthio)-1-oxoethyl]-	581589	−7.8	1.8

in vitro research conducted by Mailafiya *et al.*[41] demonstrated that stigmasterol exhibits significant antibacterial activity across a wide range of bacterial species, including *K. pneumoniae*, with an inhibitory concentration ranging from 6.25 μg/mL to 25 μg/mL. Furthermore, numerous studies have documented the antibacterial properties of stigmasterol.[42,43,44] Recently, the antimicrobial properties of stigmasterol derived from Piper crocatum were examined through both *in vitro* and *in silico* methods.[45] A molecular dynamics simulation of 10 nanoseconds was

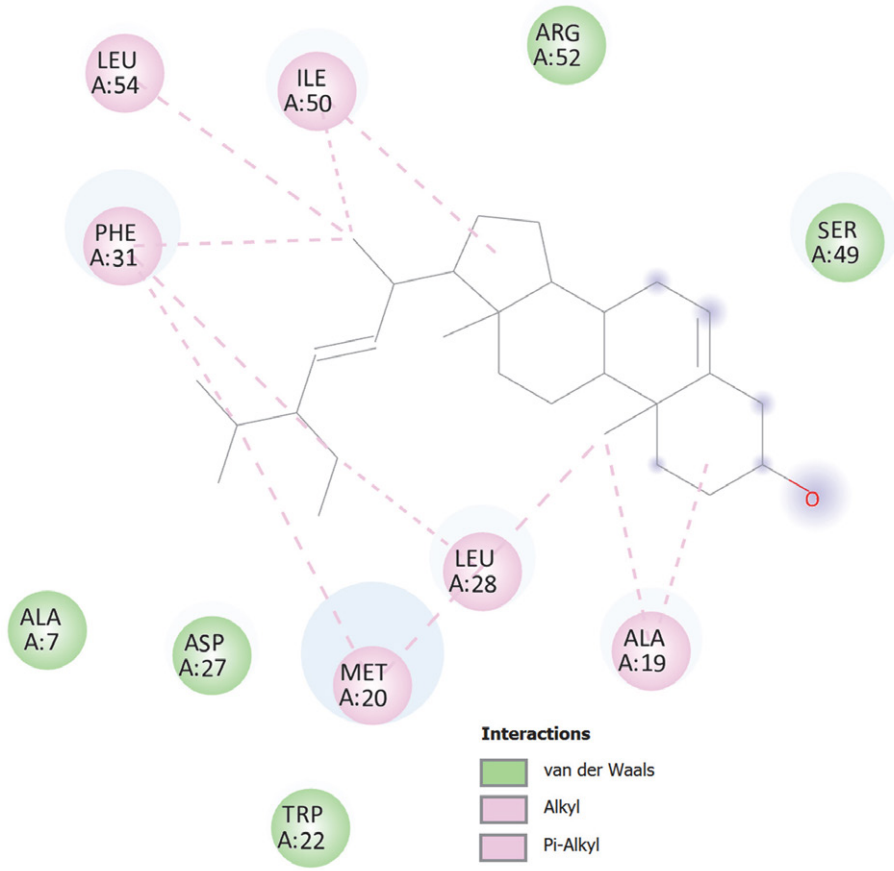

Figure 8. Stigmasterol–dihydrofolate reductase complex interactions.

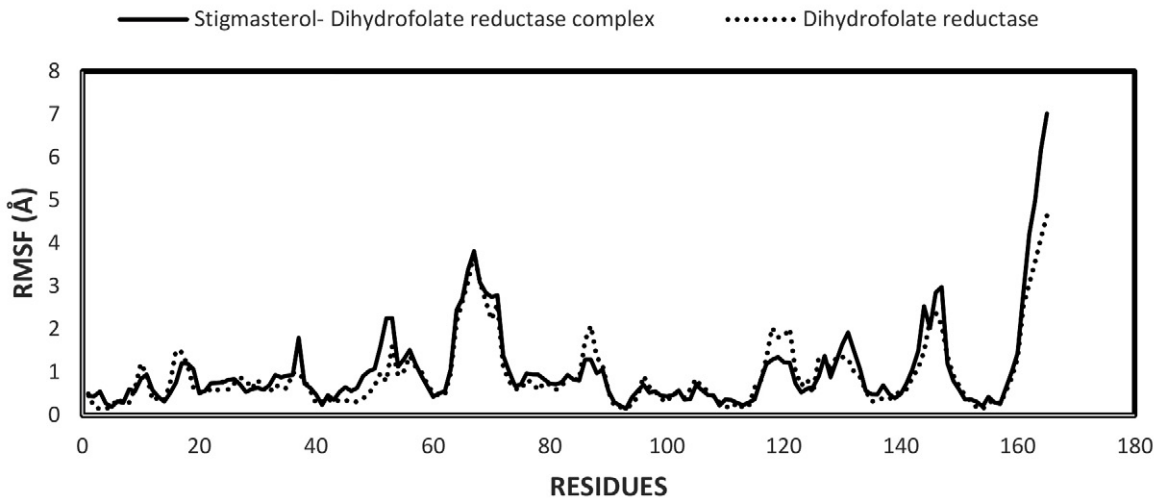

Figure 9. The RMSF plot (Å) illustrates the dihydrofolate reductase enzyme (represented by the dotted line) alongside the stigmasterol-dihydrofolate reductase complex (depicted by the solid line). The amino RMSF plots are derived from the backbone Cα atoms.

performed via the CABS-flex 2.0 server to assess the root mean square fluctuation (RMSF) values for the stigmasterol-dihydrofolate reductase complex, as illustrated in Figure 9. The atomic fluctuations observed during the simulations offer valuable insights into the flexibility and stability of different protein residues. Elevated RMSF values for specific residues indicate greater flexibility of the corresponding amino acids, whereas lower fluctuations imply more constrained movements throughout the molecular dynamics simulation. The fluctuations observed in the complex were determined to be within an acceptable range of 1-3 Å, suggesting that stigmasterol does not affect the binding of the dihydrofolate reductase enzyme. The current work combines *in vitro* and *in silico* techniques to assess the biological activities of *U. giganteum* for its therapeutic value. The ethanol extract was determined to be the most efficient solvent for the antioxidant, α-glucosidase inhibitory and antimicrobial activities of the extracts, which were further confirmed by GC/MS analysis, which revealed the presence of various phytochemicals. Experimental assays complemented with molecular docking proved that key compounds, such as stigmasterol, are significant and biologically relevant. In doing so, this research expands the pharmacological knowledge of natural products to encompass this relatively uninvestigated wild grass while simultaneously offering established procedures for duplication. These findings provide a clearer perspective on the future of *U. giganteum* application in pharmaceutical industries.

4. Conclusion

This is the first study to highlight the significant antioxidant and antibacterial potential of the ethanol extract of *U. giganteum*, making it a promising candidate for further research and development in natural health products. Owing to its nutritional composition, this plant can be incorporated into the human diet or as a source for many supplemented products. It is rich in fiber and various bioactive chemical compounds in addition to vital phytochemical contents. Docking analysis indicated that the stigmasterol compound could serve as a natural antibacterial agent because of its potent inhibitory effect on the dihydrofolate reductase enzyme present in *K. pneumoniae*. Numerous studies have confirmed that extracts from various plants exhibit significant antibacterial activity, which is attributed to the presence of stigmasterol. Our research suggests that the obvious inhibitory effect of the ethanol extract from *U. gigantea* on *K. pneumoniae* may be associated with the stigmasterol compound. Owing to its nutritional and chemical properties, *U. giganteum* has several health benefits. In addition, it could be promoted as a good candidate in the drug formation and pharmaceutical industries. However, additional studies on their biological and pharmacological properties are needed to fully understand their molecular mechanisms of action and the role of their active compounds to fully understand the scope of their applications and ensure their safety and effective use.

Authors contributions: All the authors contributed to the study conception and design. Abdulrahaman Mahmoud Dogara, Sawsan S. Al-Rawi, Abubakar Abdullahi Lema Lema, Aisha Abdullahi Mahmud and Hasan Nudin Nur Fatihah performed the *in vitro* study. Ateeq Ahmed Al-Zahrani and Ahmad Hussain D Almalki performed the *in silico* study. All the authors read and approved the final manuscript.

Data availability: All data generated or analyzed during this study are included.

Declarations

Competing interest: The authors have no relevant financial or nonfinancial interests to disclose.

Ethics approval: The University Ethical Board confirmed that no ethical approval was needed.

5. References

1. A.M. Dogara, *Future J. Pharm. Sci.* **2023**, *9*, 16. **DOI:**10.1186/s43094-023-00467-3
2. W.D. Clayton, *Kew Bull.* **1978**, 767–771. **DOI:**10.2307/4109770
3. M.M. Nura, Y. Shehu, M.S. Uwaisu, *Kaduna J. Appl. Sci.* **2001**, *5*, 75–87.
4. M.D. Abdulrahman, S.W. Bradosty, S.W. Hamad, M.T. Ibrahim, A.A. Lema, N. Sunusi, M. Usman, I. Ashiru, N.B. Ahmad, N. Wada, *Ethnobot. Res. Appl.* **2022**, *23*, 1–18. **DOI:**10.32859/era.23.33.1-18
5. U.S. Abubakar, K.M. Yusuf, K.G.T. Abdu, S.R. Saidu, G.A. Jamila, A. Fatima, *Res. J. Pharmacogn*. **2017**, *4*, 29–39.
6. D. Okello, Y. Chung, H. Kim, J. Lee, E. Rahmat, R. Komakech, Y. Kim, F. Omujal, Y. Kang, *Evid. Based Complement. Alternat. Med*. **2021**. *2021*, 1–11. **DOI:**10.1155/2021/9917810
7. R. Teterovska, I. Sile, A. Paulausks, L. Kovalcuka, R. Koka, B. Mauriņa, D. Bandere, *Plants*. **2023**, *12*, 4108. **DOI:**10.3390/plants12244108
8. A. Roy, A. Khan, I. Ahmad, S. Alghamdi, B.S. Rajab, A.O. Babalghith, M.Y. Alshahrani, S. Islam, and M.R. Islam. BioMed Research International. **2022**, *2022*, 1–9. **DOI:**10.1155/2022/5445291
9. S.S. Al-Rawi, A.H. Ibrahim, H.J. Ahmed, Z.O. Khudhur, *Saudi Pharm. J.* **2024**, *32*, 1–26. **DOI:**10.1016/j.jsps.2024.102067
10. A.M. Dogara, M.T. Ibrahim, A.A. Mahmud, M.D. Danladi, A.A. Lema, M. Usman, A.S. Tahir, K. Tabti, *J. Umm Al-Qura Univ. Appl. Sci*. **2024**, 1–14. **DOI:**10.1007/s43994-024-00160-y
11. A.B. Aborisade, A. Adetutu, and A.O. Owoade, *Clin. Med. Res*. **2017**, *6*, 209–214. **DOI:**10.11648/j.cmr.20170606.16
12. F. Afroze, M.T. Hossain, *World J. Pharm. Sci.* **2015**, *4*, 140–51.
13. P.S. Sharangouda, S. Patil, *J. Adv. Pharmacol. Toxicol.* **2007**, *49*, 305–312. **DOI:**10.1484/J.BPM.2.305776
14. S. Patil, K. Murthy, *Asian J. Biol. Life Sc*. **2020**. 9, 379–385. **DOI:**10.5530/ajbls.2020.9.57
15. N.R. Farnsworth, *J. Pharm. Sci*. **1966,** *55*, 225–276. **DOI:**10.1002/jps.2600550302
16. A.K. Yunusa, N. Abdullahi, A. Rilwan, A. R. Abdulkadir, M. A. Dandago, *Annu. Rev. Food Sci. Technol.* **2018**, *19*, 1–6.
17. R. Dandekar, B. Fegade, V. Bhaskar, *J. Pharmacogn. Phytochem*. **2015**, *4*, 148–154.
18. M.D. Abdulrahman, N.F. Hasan Nudin, M.M. Khandaker, A.M. Ali, N. Mat, *Intl. J. Agric. Biol.* **2009**, *22*, 1–8. **DOI:** 10.17957/IJAB/15.1214
19. M. Al-Owaisi, N. Al-Hadiwi, S.A. Khan, *Asian Pac. J. Trop. Biomed.* **2014**, *4*, 964–970. **DOI:**10.12980/APJTB.4.201414B295
20. A.K. Yunusa, Z.U.B. Nouruddeen, S. A. Adam, M. Usman, A. M. Dogara, *Croat. J. Food Sci. Technol.* **2023,** *15*, 130–140. **DOI:**10.17508/CJFST.2023.15.2.01
21. A.M. Dogara, A.A. Al-Zahrani, S.W. Bradosty, S.W. Hamad, S.H. Bapir, T.K. Anwar, *AIMS Biophys*. **2024**, *11*, 255–280. **DOI:**10.3934/biophy.2024015
22. M. Abdulrahman, *Plant Sci. Today*. **2021**, *8*, 410–415. **DOI:**10.14719/pst.2021.8.2.1113
23. Y. Liu, X. Yang, J. Gan, S. Chen, Z. Xiao, Y. Cao, *Nucleic Acids Res*. **2022**, 159–164. **DOI:**10.1093/nar/gkac394
24. G. Dakpa, K.S. Kumar, J. Nelen, H. Pérez-Sánchez, S.Y. Wang, *Sci. Rep*. **2023,** *13*, 1–16. **DOI:**10.1038/s41598-023-44476-x
25. N.N.N. Ab Rahman, M.M.B. Nama, S.S. Al-Rawi, A. H. Ibrahim, M. O. Ab Kadir, *Procedia Food Sci.* **2011,** *1*, 1940–1945. **DOI:**10.1016/j.profoo.2011.09.285
26. C.B. Ioniță-Mîndrican, K. Ziani, M. Mititelu, E. Oprea, S.M. Neacșu, E. Moroșan, D.E. Dumitrescu, A. C. Roșca, D. Drăgănescu, C. Negrei, *Nutrients*. **2022**, *14*, 1–29. **DOI:**10.3390/nu14132641
27. U.S. Weisz, P. Eisner, S. Bader-Mittermaier, R. Osen, *Curr. Opin Food Sci*. **2020**, *32*, 156–162. **DOI:**10.1016/j.cofs.2020.08.003
28. M. Aliyu, A. Abdullahi, A. Ugya, *Eur.. J. Biomed. Pharm. Sc*i. **2017**, *4*, 577–585.
29. B. Jovanova, S. Kulevanova, and T. Kadifkova Panovska, *Agric. Conspec Sci.* **2019,** *84*, 51–58.
30. T.M. Cruz, A. dos Santos Lima, A.O. Silva, N. Mohammadi, L. Zhang, L. Azevedo, M.B. Marques, D. Granato, *Food Chem.* **2024,** *440*, 138281. **DOI:**10.1016/j.foodchem.2023.138281
31. S.T. Assefa, E.Y. Yang, S.Y. Chae, M. Song, J. Lee, M.C. Cho, S. Jang, *Plants*. **2019**, *9*, 1–17. **DOI:**10.3390/plants9010002
32. A.E. Varghese, *Int. J. Adv. Res. Pharm. Bio. Sci.* **2013**, *2*, 31–40.
33. F.T. Schevenels, S. Jadsadajerm, R. Lekphrom, N. Yodsin, T. Suebrasri, T. Senawong, A. Wisetsai, *Nat. Prod. Res*. **2024**, 1–10. **DOI:**10.1080/14786419.2024.2324370
34. S. Naghshi, D. Aune, J. Beyene, S. Mobarak, M. Asadi, O. Sadeghi, *Bmj.* **2021,** 1–19. **DOI:**10.1136/bmj.n2213
35. A.H. Ibrahim, H. Li, S.S. Al-Rawi, A.S.A. Majid, O.A. Al-Habib, X. Xia, A.M.A. Majid, D. Ji, *Am. J. Transl. Res.* **2017**, *9*, 49–6836.
36. M.A. Farag, M.Z. Gad, *J. Genet. Eng. Biotechnol.* **2022**, *20*, 48–67. **DOI:**10.1186/s43141-022-00329-0
37. A.M. Dogara, S.W. Hamad, H.A. Hama, S.W. Bradosty, S. Kayfi, S.S. Al-Rawi, A.A. Lema, *Adv. Pharmacol. Pharm. Sci.* **2022**, *2022*, 1–15. **DOI:**10.1155/2022/3837965
38. T.G. Albuquerque, M.A. Nunes, S.M. Bessada, H.S. Costa, *Anal. Food.* **2020**, 609–656. **DOI:**10.1016/B978-0-12-813266-1.00014-0
39. Y. Wang, L. Hua, G. Chen, Z. Li, Z. Chen, *TMR Pharmacol. Res.* **2023**, *3*, 1–9. **DOI:**10.53388/PR202303010
40. L. Ivanova, M. Karelson, *Molecules.* **2022,** *27*, 1–20. **DOI:**10.3390/molecules27249041
41. M.M. Mailafiya, A.J. Yusuf, M.I. Abdullahi, G.A. Aleku, I.A. Ibrahim, M. Yahaya, H. Abubakar, A. Sanusi, H.W. Adamu, C.O. Alebiosu, *J. Med. Plants. Econ. Dev.* **2018,** *2*, 1–5.

DOI:10.4102/jomped.v2i1.38
42. S. Woldeyes, L. Adane, Y. Tariku, D. Muleta, T. Begashaw, *Nat. Prod. Chem. Res.* **2012**, *1*, 1–8. **DOI:**10.4172/2329-6836.1000101
43. A. Sen, P. Dhavan, K.K. Shukla, S. Singh, G. Tejovathi, *Sci. Secure J. Biotechnol.* **2012**, *1*, 9–13.
44. S. Saeidnia, A. Manayi, A.R. Gohari, M. Abdollahi, *J. Diabetes Metab. Disord.* **2014**, *13*, 1–5. **DOI:**10.9734/EJMP/2014/7764
45. S. Lestari, D. Kurnia, T. Mayanti, L. Heliawati, *J. Chem.* **2024,** *2024*, 1–11. **DOI:**10.1155/2024/2935516

Povzetek

Vse večje zanimanje za živila, ki spodbujajo zdravje, je okrepilo potrebo po raziskovanju novih virov z edinstveno prehransko sestavo. Rastline so bogat viri bioaktivnih spojin z biološkimi učinki. *Urelytrum giganteum* Pilg je večletna divje rastoča trava, ki raste v suhih tropskih afriških državah. Tradicionalno se uporablja za zdravljenje več bolezni, vendar do zdaj o tej rastlini ni bila opravljena nobena študija. V tej študiji je bila analizirana ekstrakcija *Urelytrum giganteum* Pilg s topilom, da bi določili njeno proksimalno analizo, antioksidativni potencial, kemično sestavo, protimikrobno učinkovitost in molekularno sidranje. Uporabljena je bila Soxletova ekstrakcijska metoda, pridobljeni ekstrakti pa so bili podvrženi fitokemičnemu vrednotenju, merjenju aktivnosti odstranjevanja radikalov DPPH, testu odstranjevanja vodikovega peroksida (H_2O_2), železovi reduktivni antioksidativni moči (FRAP), vrednotenju difuzije iz diska in študiji molekularnega sidranja. Med ugotovljenimi fitokemikalijami so bili alkaloidi, flavonoidi, saponini, fenolne snovi, tanini, ogljikovi hidrati, terpeni in C-glikozidi. Etanolni ekstrakt je pokazal največjo aktivnost pri testih DPPH (70,2 μg/ml), H_2O_2 (60,7 μg/ml) in alfa glikozidazi (72,3 μg/ml) ter protimikrobno aktivnost proti *Klebsiella pneumoniae* (17 mm). Večina fitokemikalij je pokazala opazno inhibitorno aktivnost, pri čemer je imel stigmasterol najvišjo stopnjo inhibicije. To poudarja potencial *U. giganteum* kot obetavnega kandidata za farmacevtsko industrijo. Identificirane fitokemične in antioksidativne spojine bi lahko bile obetavni nutracevtiki ali aditivi za živila za splošno dobro počutje.