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Cyperus esculentus Inhibits DNA Gyrase-B and PBP2a in Multidrug-Resistant *Escherichia coli* and *Staphylococcus aureus*

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Received: June 13, 2025; **Published:** October 01, 2025

Abstract

The global rise of antimicrobial resistance (AMR) poses a critical threat to public health, necessitating the exploration of novel therapeutic strategies against multidrug-resistant (MDR) bacterial infections. This study investigated the inhibitory potential of *Cyperus esculentus* (tiger nut) extracts against selected MDR pathogens. Phytochemicals were extracted using the maceration method, and antibacterial activity was assessed via the agar well diffusion technique. Gas Chromatography-Mass Spectrometry (GC-MS) was employed for phytochemical profiling. Identified bioactive compounds were subjected to virtual screening against key bacterial target proteins-DNA gyrase B and penicillin-binding protein 2a-using the Maestro Schrödinger Suite (2021-2). *In vitro* results revealed that the ethanolic extract exhibited notable inhibitory effects, producing zones of inhibition measuring 18 mm and 17 mm against *Escherichia coli* and *Staphylococcus aureus*, respectively. GC-MS analysis identified several active compounds, including D,L-xylitol,1-O-dodecanoyl, L-lactic acid, β-monolaurin, and dodecanoic acid pentafluorophenyl ester. Among these, D,L-xylitol,1-O-dodecanoyl demonstrated superior docking scores (-8.17 kcal/mol) compared to Levofloxacin, while dodecanoic acid pentafluorophenyl ester exhibited a more favorable binding energy (-40.32 kcal/mol) than Ceftobiprole. ADMET profiling indicated good oral bioavailability, high gastrointestinal absorption, and minimal inhibition of major CYP450 isoenzymes for most compounds. These findings suggest that *C. esculentus* phytochemicals exhibit notable antibacterial activity and favorable pharmacokinetic properties, highlighting their potential as candidates for novel therapeutics against MDR pathogens. Further investigation, including compound isolation and *in vivo* studies, is warranted to advance their development.

Keywords: *Cyperus esculentus; Multi-Drug Resistance; Escherichia coli; Staphylococcus aureus Bacteria; Phytochemicals; GC-MS Analysis; Molecular Docking; DNA Gyrase B; Penicillin-Binding Protein 2a*

Introduction

Medicinal plants have been integral to human healthcare for millennia, valued for their therapeutic, nutritional, and pharmacological properties. These plants contain diverse bioactive compounds-known as phytochemicals-that have been found to elicit distinct physiological responses in the body [1]. Many traditional herbal remedies, now increasingly supported by modern research, contain such phytochemicals, including alkaloids, saponins, tannins, and sterols, known for their antimicrobial, analgesic, and antispasmodic properties [2,3]. Consequently, herbal medications are often as effective as conventional drugs, albeit with similar risks of side effects.

Interest in plant-derived drugs has surged globally, driven by a growing preference for "green medicine" and a perception of its safety and affordability relative to synthetic alternatives [4]. This has encouraged the continuous search for new antimicrobial compounds from natural sources.

The World Health Organization (WHO) reports that approximately 60% of the global population relies on herbal medicine, and nearly 80% of people in developing countries depend almost entirely on it for their primary healthcare needs [5], underscoring their importance in public health and pharmacological research.

Among promising medicinal plants is *Cyperus esculentus*, a hardy, grass-like perennial from the family Cyperaceae that is widely cultivated in West Africa, Southern Europe, and parts of Asia. Thriving in wetland environments, it grows 1 to 3 feet tall with upright, fibrous roots and reproduces through deep, slender rhizomes that produce small, sweet, edible tubers at the tips of its subterranean stems [6,7]. Commonly referred to as tiger nuts, these tubers are known by various names across different regions, including rush nut, earth almonds, chufa, edible rush, yellow nutgrass, Zulu nut, crushed almond, and more culturally specific names such as "aya" (Hausa), "aki-Hausa" (Igbo), and "ofio" (Yoruba) in Nigeria [8,9]. Once primarily found in the Mediterranean, tiger nuts-also called "subterranean walnuts" due to their underground growth and productivity-are now extensively cultivated around the world for their nutritional, medicinal, and industrial value [7].

Traditionally, tiger nut has been used to treat various ailments, including indigestion, dysentery, and urinary tract infections. Phytochemical analyses reveal it contains bioactive compounds such as alkaloids, sterols, and tannins, which possess antimicrobial properties, supporting its traditional medicinal use [10]. Claims of its role in preventing or managing diseases such as diabetes, hyperlipidemia, prostate and colon cancers, and reproductive dysfunction have also been reported [7,11,12]. Its nutritional richness-comprising carbohydrates, fiber, vitamins (A, C, and E), essential minerals (e.g. calcium, magnesium, phosphorus), and amino acids-supports its use in dietary and therapeutic contexts [13,14]. Further pharmacological investigations have validated several of these claims. For instance, hepatoprotective effects were observed in rats pre-treated with tiger nut extract before exposure to carbon tetrachloride, with significant reductions in liver enzyme levels [15]. Additionally, *in vitro* studies demonstrated anti-sickling properties of methanol and aqueous extracts of *C. esculentus*, suggesting its therapeutic potential in managing sickle cell anemia [16]. Antioxidant [12] and reproductive health benefits have also been observed, including mitigation of lead acetate-induced testicular damage [17].

Antimicrobial resistance (AMR) in bacteria has become an escalating global concern, threatening the effectiveness of existing antibiotics and posing serious challenges to public health [2,18]. It occurs when bacterial populations evolve mechanisms that reduce or eliminate the efficacy of drugs designed to kill them or inhibit their growth. This resistance can arise naturally over time, but the process is significantly accelerated by the inappropriate use of antibiotics in clinical and agricultural settings. As resistance spreads, infections that were once easily treatable become more difficult to manage, leading to prolonged illnesses, increased healthcare costs, and greater risk of mortality [19,20,21].

In light of the diminishing effectiveness of conventional antibiotics, there is a growing urgency to explore alternative approaches to combat resistant bacterial infections. Natural sources, particularly medicinal plants, have gained attention for their potential to yield novel antimicrobial agents. These plant-derived compounds often possess complex chemical structures and multiple mechanisms of action, making them promising candidates in the search for new treatments [22]. Investigating such alternatives is essential for developing innovative therapeutic options and mitigating the global threat posed by antimicrobial resistance. This study investigates the inhibitory potential of *Cyperus esculentus* extracts against selected multidrug-resistant bacterial strains. It further employs in silico molecular docking analyses to evaluate the interactions between identified phytochemicals and key resistance-associated targets.

Methods

Test organisms and standardization of inoculum

The bacterial strains used in this study-multidrug-resistant *Escherichia coli, Staphylococcus aureus, Bacillus cereus,* and *Acinetobacter baumannii*-were obtained from the Department of Microbiology laboratory at our institution. To standardize the inoculum, a 0.5 McFarland turbidity standard was prepared by combining 0.05 mL of 1% barium chloride dihydrate ($BaCl_2.2H_2O$) with 9.95 mL of 1% sulfuric acid (H_2SO_4), yielding a 1.0% w/v barium sulfate suspension. Eighteen-hour-old bacterial cultures were suspended in sterile saline and adjusted to match the 0.5 McFarland standard by adding saline or additional culture as necessary. The final suspension was then diluted to obtain a concentration of 10^6 CFU/mL [18].

Plant sample and preparation of extract

Fresh nuts of *Cyperus esculentus* were obtained from a local market, authenticated, and assigned the voucher specimen number PSBH-262. The nuts were washed with clean water, air-dried, and then pounded using a mortar and pestle. Extraction was carried out by maceration, using ethanol and distilled water as solvents, following the modified method of Oluyele and Oladunmoye [4]. Five hundred grams of the pulverized nuts were separately soaked in 2000 mL of each solvent in clean vessels. The mixtures were allowed to stand for seven days at room temperature with occasional shaking. Afterward, the extracts were first sieved through muslin cloth, then filtered using Whatman No. 1 filter paper, and concentrated with a rotary evaporator. The resulting extracts were weighed, labeled, stored in clean containers, and refrigerated at 4°C until further use.

Assay for antibacterial activity of the extract

The antibacterial activity of the extract was evaluated using the agar well diffusion method, as described by Oluyele and Akinyeke [23]. One milliliter of each standardized bacterial suspension was uniformly spread onto sterile, dried Mueller-Hinton agar plates (Oxoid Ltd., Basingstoke, UK). After the plates had dried, wells measuring 6 mm in diameter were created using a sterile cork borer and appropriately labeled. Each well was filled with 100 µL of the extract solution (150 mg/mL in 5% dimethyl sulfoxide; Sigma-Aldrich, Germany), while Oxacillin or Ofloxacin served as the standard control in one of the wells. Plates were allowed to stand at room temperature for 15 minutes to facilitate diffusion, followed by incubation at 37°C for 24 hours. Zones of inhibition were then measured to assess antibacterial activity.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were determined using broth tube dilution and subculture plating methods, respectively [23]. For MIC determination, the extract was serially diluted to concentrations ranging from 100 to 12.5 mg/mL, and 0.5 mL of standardized inoculum was added to each tube. Negative controls (Mueller-Hinton broth only) and positive controls (broth with test organisms) were included. Following 24 hours of incubation at 37°C, the MIC was defined as the lowest concentration that showed no visible turbidity. For MBC determination, aliquots from the MIC tube and other non-turbid tubes were subcultured onto fresh Mueller-Hinton agar plates and incubated. The MBC was recorded as the lowest concentration that resulted in no observable bacterial growth.

Gas chromatography/mass spectrometric (GC/MS) analysis of *C. esculentus* extract

The extract was analyzed using gas chromatography-mass spectrometry (GC-MS) on a Shimadzu QP2010 Ultra system equipped with a fused silica capillary column (MS-5). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. A 0.5 **µL** aliquot of the sample was injected at an inlet temperature of 250°C with a split ratio of 1:5. The oven temperature was programmed in multiple stages, ultimately reaching 300°C. The ion source was maintained at 280°C with an electron ionization energy of 70 eV. Ions in the range of m/z 40-500 were filtered using a quadrupole analyzer and detected by an electron multiplier. Data acquisition and processing were performed using Shimadzu LabSolutions software. Flame ionization detection (FID) was conducted using hydrogen at 30 mL/min and synthetic air at 300

mL/min. Compound identification was based on retention indices calculated from a homologous series of n-alkanes (C_7-C_{28}) and mass spectral comparison with the Wiley and NIST libraries. Only compounds with a similarity index of 98% or higher were reported [24].

Generation and preparation of compound library

The GCMS-identified compounds from *C. esculentus* were retrieved from the PubChem repository (https://pubchem.ncbi.nlm.nih.gov) along with the standard drugs in Structure Data File (SDF) format. These molecules were then imported into the Schrödinger workspace (Schrödinger, 2021) and prepared for in silico analysis using the LigPrep tool.

Protein preparation

The X-ray crystallographic structures of *Escherichia coli* DNA-gyrase B with PDB ID: 4ZVI (Ec-4ZVI) and *Staphylococcus aureus* penicillin binding protein 2A with PDB ID: 4CJN (Sa-4CJN), both complexed with co-crystallized ligands were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (www.rcsb.org). Missing residues, loop regions, and side-chain anomalies were resolved, followed by energy optimization using the OPLS4 force field in the Protein Preparation Wizard of the Schrödinger suite (2021). The receptor grid was generated using the Glide Grid Generation tool, targeting the co-ligand binding site. The receptor grid was generated using the Glide Grid tool at the co-ligand binding site, with Glide coordinates set to x = -12.69, y = 20.64, and z = 22.3 for Ec-4ZVI, and x = 8.95, y = -1.39, and z = -69.93 for Sa-4CJN. The prepared protein crystallographic structures and the Ramachandran residue distribution are presented in figure 1 and 2.

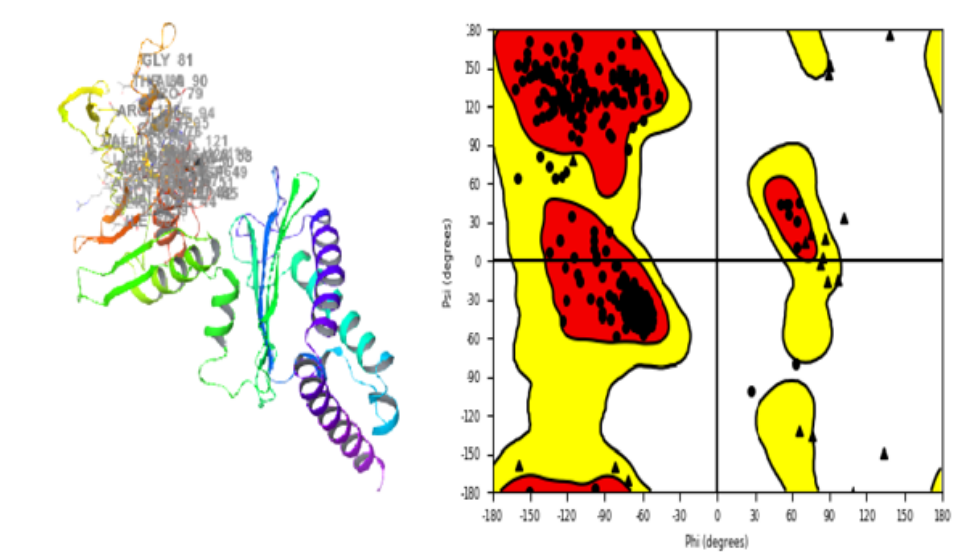

Figure 1: *The crystal structure of Ec-4ZVI and the distribution plot of Ramachandran residues.*

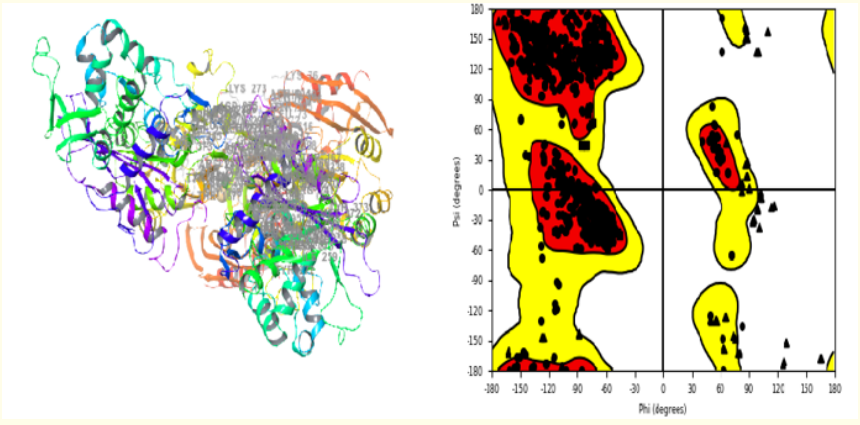

Figure 2: *The crystal structure of Sa-4CJN and the distribution plot of Ramachandran residues.*

Structure-based virtual screening

The prepared compounds from *C. esculentus* and the standard ligands were screened against Ec-4ZVI and Sa-4CJN using the extra precision (XP) GLIDE docking protocol in the Maestro Schrödinger suite (v2021). This scoring function is widely recognized for its reliability and ability to distinguish binding affinities effectively, though it requires a longer computational runtime [25].

ADME/Tox screening

The pharmacokinetic profile, drug-likeness, and toxicity of the hit compounds were evaluated using the SwissADME (http://www.swissadme.ch) and Pro-Tox II (https://tox-new.charite.de/protox II) online servers.

Results

Inhibitory activity of *C. esculentus* extracts

The ethanolic extract of *C. esculentus* exhibited the strongest antibacterial activity, with the highest inhibition observed against *Escherichia coli* (18 mm) and *Staphylococcus aureus* (17 mm) at 100 mg/ml. The aqueous extract also showed appreciable activity, with its highest effects also recorded against *S. aureus* (16 mm) and *E. coli* (15 mm), though slightly lower than those of the ethanolic extract. The results are presented in table 1 and 2.

Organism	100 mg/ml	50 mg/ml	25 mg/ml
B. cereus	15 mm	12 mm	8 mm
S. aureus	17 mm	13 mm	9 mm
A. baumannii	12 mm	0 mm	0 mm
E. coli	18 mm	15 mm	12 mm

Table 1*: Inhibitory activity of C. esculentus ethanolic extract on selected multi-drug resistant organisms.*

Organism	100 mg/ml	50 mg/ml	25 mg/ml
B. cereus	14 mm	10 mm	09 mm
S. aureus	16 mm	13 mm	11 mm
A. baumannii	14 mm	12 mm	08 mm
E. coli	15 mm	14 mm	10 mm

Table 2: *Inhibitory activity of C. esculentus aqueous extract on selected multi-drug resistant organisms.*

Phytochemical composition *C. esculentus*

Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the presence of several bioactive compounds in *C. esculentus* extract. Major constituents included 1,3-Dixane,4-(hexadecyloxy)-2-pentade (16.73%), cis-Vaccenic acid (11.40%), Propanoic acid, 2-hydroxy (10.95%), and L-lactic acid (10.92%). Other identified compounds with known antimicrobial properties included dodecanoic acid, tetradecanoic acid, stigmasterol, oleoyl chloride, and fumaric acid esters. The results are shown in table 3 and figure 3.

Compounds	Percentage (%)
2,3-Butanediol	2.19
2,3-Butylene glycol	2.25
1,3-Propanediol	1.36
L-Lactic acid	10.92
2-Hydroxy-2-methylmalonic acid	10.95
Propanoic acid, 2-hydroxy-, ethyl ester	9.05
Sulfide, sec-butyl isopropyl	1.12
2-Pyrrolidinone	1.07
Isosorbide	0.49
Dodecanoic acid	1.94
Tetradecanoic acid	1.23
Palmitoleic acid	0.36
n-Hexadecanioc acid	3.26
Cis-Vaccenic acid	11.40
Octadecanoic acid	2.01
Dodecanoic acid, pentafluorophenyl ester	2.69
Dodecanoic acid, ethenyl ester	3.15
Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	4.61
Dodecanoic anhydride	3.24
Fumaric acid, 2-heptyl isohexyl ester	0.15
d,l-Xylitol, 1-O-dodecanoyl-	0.23
Dimethyl 3-hydroxy-3-methylpentane-1,5-dioate	0.34
Neoisolongifolene, 8-chloro	0.38
Stigmasterol	0.53
Oleoyl chloride	3.18
4-(Hexadecyloxy)-2-pentadecyl-1,3-dioxane	16.73
Dodecanoic acid,2,3-dihydroxypropyl esther	4.08
beta.-Monolaurin	1.09

Table 3: *GCMS identified compounds in C. esculentus extract.*

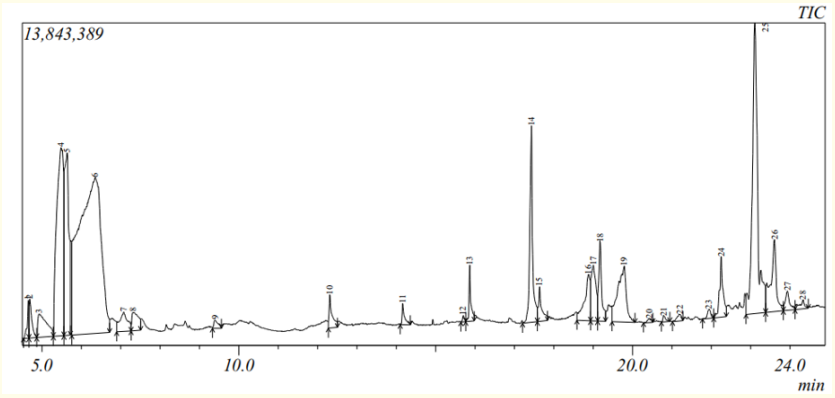

Figure 3: *GCMS chromatogram of C. esculentus extract.*

Molecular docking and MM/GBSA calculations

The molecular docking and MM/GBSA analyses of *Cyperus esculentus* bioactive compounds against *Escherichia coli* DNA gyrase B (Ec-4ZVI) and *Staphylococcus aureus* PBP2a (Sa-4CJN) demonstrated differential binding affinities and free energies (Figure 4 and 6). For Ec-4ZVI, the compound D,l-Xylitol, 1-O-dodecanoyl showed the most favorable docking score (-8.170 kcal/mol), outperforming the standard drug Levofloxacin (-4.187 kcal/mol). However, Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester had the best MM/GBSA binding free energy (-36.29 kcal/mol), surpassing Levofloxacin (-31.26 kcal/mol). Multiple compounds formed key hydrogen bonds and hydrophobic interactions at the enzyme's active site, while Levofloxacin exhibited no hydrogen bonding but showed extensive hydrophobic interactions (Table 4 and figure 5).

For Sa-4CJN, 2-Pyrrolidinone exhibited a higher docking score (-4.133 kcal/mol) than the standard drug Ceftobiprole (-4.827 kcal/mol). However, Dodecanoic acid, pentafluorophenyl ester recorded a significantly superior MM/GBSA free energy (-40.32 kcal/mol), marginally outperforming Ceftobiprole (-40.00 kcal/mol). Key binding interactions included hydrogen bonds and π-π stacking, with Ceftobiprole interacting with key residues such as TYR105 and ASN146 (Table 5 and figure 7).

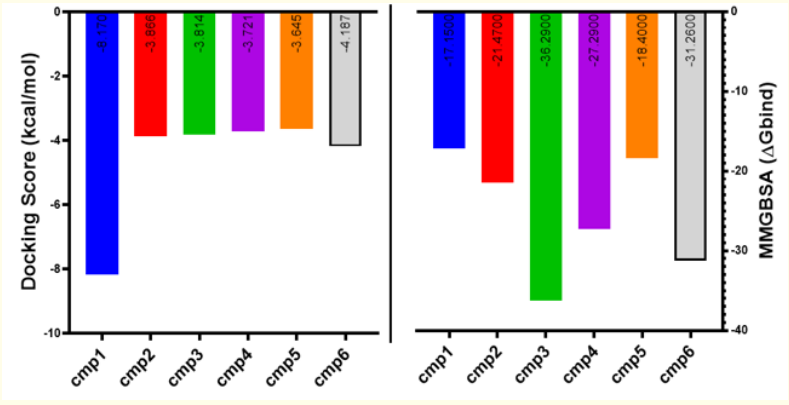

Figure 4: *Graphical representation of the binding affinity and binding free energy calculation of bioactive compounds of C. esculentus against Ec- 4ZVI.*

Legend: cmp1: D,l-Xylitol, 1-O-dodecanoyl, cmp2: Pentafluorophenyl ester, cmpd3: Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, cmpd4: beta.-Monolaurin, cmpd5: 2-Hydroxy-2-methylmalonic acid, cmpd6: Levofloxacin.

Compound Name	H-Bond	Hydrophobic interactions	Other Interactions
D,l-Xylitol, 1-O-dodecanoyl	ASN 46, ASP 73, GLY 77	ILE 78, PRO 79, ILE 94, MET 95, VAL 120, VAL 43, VAL 44, ALA 47, VAL 167, MET 166, VAL 71	None
Pentafluorophenyl ester	ASN 46	ILE 78, PRO 79, ILE 94, MET 95, VAL 120, VAL 43, VAL 167, VAL 71	None
Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	GLY 77, ARG 136	PRO 79, ILE 78, ALA 47, VAL 120, VAL 71, VAL 43, VAL 167, MET 166, ILE 59, MET 95, ILE 94, VAL 93, ALA 90	None
beta.-Monolaurin	GLY 77, GLU 50	ILE 94, MET 95, VAL 120, VAL 167, MET 166, VAL 71, ILE 59, VAL 43, VAL 44, ALA 47, PRO 79, ILE 78	None
2-Hydroxy-2-methylmalonic acid	ASP 73, THR 165, ASN 46	ILE 78, ALA 47	None
Levofloxacin	None	VAL 167, ALA 47, VAL 71, VAL 43, VAL 120, ILE 94, PRO 79, ILE 78	None

Table 4: *Hydrogen bonds and hydrophobic interactions of the top compounds of C. esculentus at the active site of Ec-4ZVI.*

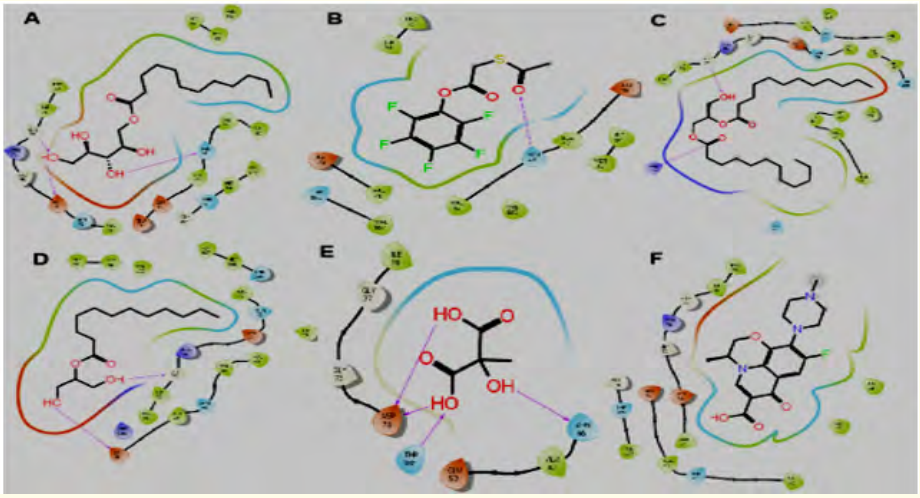

Figure 5: *2D-Molecular interactions of amino-acid residues of Ec-4ZVI with C. esculentus top compounds and standard drug. A: D,l-Xylitol, 1-O-dodecanoyl, B: Pentafluorophenyl ester, C: Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, D: beta.-Monolaurin, E: 2-Hydroxy-2-methylmalonic acid, F: Levofloxacin.*

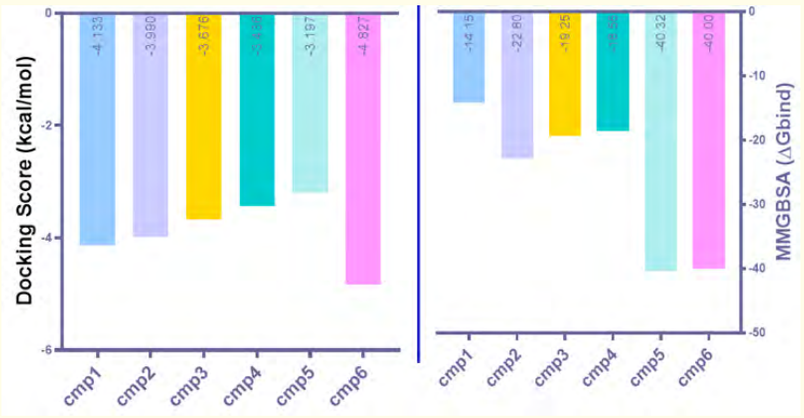

Figure 6: *Graphical representation of the binding affinity and binding free energy calculation of bioactive compounds of C. esculentus against Sa-4CJN.*

Legend: cmp1: 2-Pyrrolidinone, cmp2: Isosorbide, cmpd3: Dimethyl 3-hydroxy-3-methylpentane-1,5-dioate, cmpd4: Pentafluorophenyl ester, cmpd5: Dodecanoic acid, pentafluorophenyl ester, cmpd6: Ceftobiprole.

Compound Name	H-Bond	Hydrophobic interactions	Other Interactions
2-Pyrrolidinone	SER 72, LYS 273, GLY 296, ASN 146	TYR 105, LEU 73, TYR 297, LEU 147, ILE 144, ILE 309, VAL 302	None
Isosorbide	TYR 105	TYR 105, ILE 144, TYR 297, ILE 309	Salt-Bridge: LYS 316, LYS 273
Dimethyl 3-hydroxy-3-methyl-pentane-1,5-dioate	LYS 316, ASN 146	TYR 297, TYR 105, LEU 147, ILE 144	None
Pentafluorophenyl ester	ASN 146	TYR 105, ILE 144, TYR 297	None
Dodecanoic acid, pentafluoro-phenyl ester	ILE 309	TRP 205, ALA 310, ILE 309, ILE 144, TYR 105, TYR 297	Pi-Pi Stacking: TRP 205
Ceftobiprole	SER 306, TYR 105, ASN 146	TRP 305, ILE 309, TYR 105, TYR 297, ILE 144	Pi-Pi Stacking: TRP 297

Table 5: *Hydrogen bonds and hydrophobic interactions of the hit compounds of C. esculentus at the active site of Sa-4CJN.*

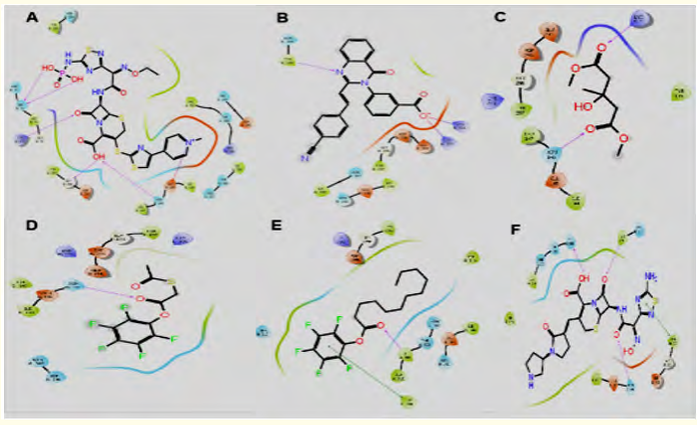

Figure 7: *2D-Molecular interactions of amino-acid residues of Sa-4CJN with C. esculentus top compounds and standard drug. Legend: A: 2-Pyrrolidinone, B: Isosorbide, C: Dimethyl 3-hydroxy-3-methylpentane-1,5-dioate, D: Pentafluorophenyl ester, E: Dodecanoic acid, pentafluorophenyl ester, F: Ceftobiprole.*

Druglikeness and ADMET profile of Top compounds from *C. esculentus*

Most of the hit phytochemicals from *C. esculentus* exhibited favorable ADMET profiles, conforming to Lipinski's Rule of Five with good gastrointestinal absorption and non-inhibition of major CYP450 isoforms (Table 6 and 7). Notably, D,l-Xylitol, 1-O-dodecanoyl and Pentafluorophenyl ester demonstrated high GI absorption and satisfactory solubility, matching or exceeding the drug-likeness of standard antibiotics.

Compound Name	MW	HBA	HBD	TPSA	iLOGP	ROV	ESOL Log S	GIA	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	BA
D,l-Xylitol, 1-O-dodecanoyl	334.45	6	4	107.22	2.83	0	-2.58	High	No	No	No	No	No	0.55
Pentafluorophenyl ester	300.2	8	0	68.67	2.36	0	-3.27	High	No	No	No	No	No	0.55
Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	456.7	5	1	72.83	6.4	1	-7.07	Low	No	No	No	No	Yes	0.55
beta.-Monolaurin	274.4	4	2	66.76	3.52	0	-3.12	High	No	No	No	Yes	No	0.55
2-Hydroxy-2-methylmalonic acid	134.09	5	3	94.83	0.09	0	0.07	High	No	No	No	No	No	0.56
Methyltartronic acid	134.09	5	3	94.83	0.09	0	0.07	High	No	No	No	No	No	0.56
Levofloxacin	361.37	6	1	75.01	2.25	0	-1.99	High	No	No	No	No	No	0.55

Table 6: *Druglikeness and ADMET profile of the top compounds from C. esculentus that inhibit Ec-4ZVI.*

Legend: MW = Molecular Weight, HBA = Hydrogen Bond Acceptor, HBD = Hydrogen Bond Donor, ROV = Rule of Five, GIA = Gastrointestinal Absorption, BA = Bioavailability.

Compound Name	MW	HBA	HBD	TPSA	iLOGP	ROV	ESOL Log S	GIA	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	BA
2-Pyrrolidinone	85.1	1	1	29.1	1.06	0	0.17	Low	No	No	No	No	No	0.55
Isosorbide	146.14	4	2	58.92	1.27	0	0.1	High	No	No	No	No	No	0.55
Dimethyl 3-hydroxy-3-methylpentane-1,5-dioate	190.19	5	1	72.83	2.07	0	-0.27	High	No	No	No	No	No	0.55
Pentafluorophenyl ester	300.2	8	0	68.67	2.36	0	-3.27	High	No	No	No	No	No	0.55

Dodecanoic acid, pentafluorophenyl ester	366.37	7	0	26.3	3.99	1	-6.12	Low	No	No	No	No	No	0.55
Ceftobiprole	534.57	10	5	256.98	3.99	1	-6.12	Low	No	No	No	No	No	0.55

Table 7: *Druglikeness and ADMET profile of the top compounds from C. esculentus that inhibit Sa-4CJN.*

Legend: MW= Molecular Weight, HBA = Hydrogen Bond Acceptor, HBD = Hydrogen Bond Donor, ROV = Rule of Five, GIA = Gastrointestinal Absorption, BA = Bioavailability.

Discussion

This study investigated the antibacterial activities of ethanolic and aqueous extracts of *Cyperus esculentus* against selected multi-drug resistant (MDR) bacterial pathogens. The results demonstrated that both extracts possess inhibitory effects, but with significant differences in potency and spectrum depending on the extraction solvent, concentration, and target organism. The ethanolic extract of *C. esculentus* exhibited stronger and broader antibacterial activity compared to the aqueous extract. At the highest concentration tested (100 mg/ml), the ethanolic extract produced inhibition zones ranging from 12 mm to 18 mm across all four MDR organisms. In contrast, the aqueous extract, while still effective, produced zones of inhibition between 14 mm and 16 mm, with slightly reduced activity against *B. cereus* and *E. coli*. Notably, *A. baumannii* showed no susceptibility to the ethanolic extract at lower concentrations (50 mg/ml and 25 mg/ml), whereas the aqueous extract maintained modest inhibitory effects even at these concentrations.

These differences can be attributed to the solvent-dependent extraction of phytochemicals. Ethanol, a polar organic solvent, is capable of dissolving a broader range of phytochemicals, including phenolics, flavonoids, alkaloids, terpenoids, and essential oils-many of which are known to possess potent antimicrobial properties. In contrast, water predominantly extracts polar compounds such as glycosides, polysaccharides, and certain organic acids, which may have comparatively milder antibacterial effects. The higher potency of the ethanolic extract thus reflects the extraction of a more diverse and potent set of antimicrobial constituents.

Notably, among the organisms tested, *E. coli* and *S. aureus* were the most susceptible to both extracts, suggesting that their cell wall structures or metabolic pathways may be particularly sensitive to the active compounds in *C. esculentus*. *E. coli*, a Gram-negative bacterium, typically exhibits resistance due to its outer membrane barrier. However, the effectiveness of both extracts suggests that certain compounds-likely amphipathic or lipid-soluble-were able to disrupt this barrier. Moreover, *B. cereus*, a spore-forming Gram-positive bacterium, showed moderate susceptibility, potentially due to its thick peptidoglycan layer which offers some protection. *A. baumannii*, one of the most challenging MDR pathogens due to its robust efflux pumps and biofilm-forming ability, exhibited limited susceptibility to the ethanolic extract but showed better response to the aqueous extract. This unusual pattern might be due to hydrophilic antimicrobial agents in the aqueous extract targeting non-membrane cellular functions or biofilm disruption mechanisms. The findings of this study are consistent with earlier research showing that various ethnomedicinal plant extracts exhibit antimicrobial activity against pathogenic microorganisms [4,26-28].

The GC-MS analysis revealed numerous bioactive compounds that can justify the observed antimicrobial activities in this study. This include fatty acids such as cis-vaccenic acid, dodecanoic acid, tetradecanoic acid, and octadecanoic. Due to their amphipathic nature and conical molecular shape, fatty acids can efficiently integrate into lipid membranes, disrupting membrane integrity, lipid packing, and the lateral pressure profile [29]. In bacterial cells, this integration increases membrane permeability, leading to leakage of intracellular contents, membrane destabilization, and ultimately, cell death. Fatty acids may also interfere with microbial metabolic pathways, hinder DNA and RNA replication, and alter the expression of virulence-associated genes [30]. Organic acids such as L-lactic acid and

2-hydroxypropanoic acid were also present in high concentrations. Organic acids can disrupt cell membrane integrity, reduce intracellular pH and inhibit key metabolic enzymes, thereby impairing bacterial growth [31]. Additionally, compound like stigmasterol, although present in smaller amounts, is reported to exert antimicrobial activity [32] through interference with bacterial membrane function and oxidative stress pathways.

The selection of Sa-4CJN (penicillin-binding protein 2a of *Staphylococcus aureus*) and Ec-4ZVI (DNA gyrase subunit B of *Escherichia coli*) as molecular docking targets was informed by the *in vitro* antibacterial screening results presented in this study, which revealed that *C. esculentus* extract exhibited the highest inhibitory activity against *S. aureus* and *E. coli.* These findings provided a strong rationale for further investigating the interactions between the extract's bioactive compounds and resistance-associated proteins in these clinically relevant pathogens. Both DNA gyrase subunit B and PBP2a represent critical therapeutic targets in the fight against multidrug-resistant Gram-negative and Gram-positive bacterial infections, respectively, making them ideal candidates for structure-based drug discovery efforts.

Ec-4ZVI, a type II topoisomerase, plays an essential role in DNA replication, transcription, and supercoiling in *Escherichia coli* [33]. It is a well-validated target of Levofloxacin, a fluoroquinolone antibiotic that stabilizes the DNA-enzyme cleavage complex, leading to bacterial cell death [34]. On the other hand, Sa-4CJN encodes penicillin-binding protein 2a (PBP2a), a key determinant of β-lactam resistance in *Staphylococcus aureus* [35]. This protein exhibits low affinity for most β-lactam antibiotics, making it a critical target for Ceftobiprole, a fifth-generation cephalosporin specifically engineered to bind PBP2a and overcome methicillin resistance [36,37]. Given the increasing failure of these antibiotics due to resistance, identifying phytocompounds capable of targeting these essential proteins offers a promising strategy for novel antimicrobial development.

The molecular docking results reveal promising inhibitory potential of *C. esculentus* phytochemicals against multidrug-resistant pathogens. The docking score of D,l-Xylitol, 1-O-dodecanoyl against Ec-4ZVI (-8.170 kcal/mol) was substantially better than Levofloxacin (-4.187 kcal/mol), indicating a stronger initial binding affinity. Similarly, its MM/GBSA score of -17.15 kcal/mol, while not the lowest, suggests moderate complex stability. In contrast, Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester exhibited the most favorable binding energy (-36.29 kcal/mol), better than Levofloxacin (-31.26 kcal/mol), pointing to a more stable ligand-enzyme complex. These results are reinforced by its formation of multiple hydrogen bonds with ARG136 and GLY77, which are vital for enzyme stabilization.

Interestingly, Levofloxacin, although a well-established fluoroquinolone with strong antibacterial efficacy, failed to form hydrogen bonds in this in silico study, suggesting that some phytochemicals may engage more directly with catalytically relevant residues. This may have implications for circumventing resistance, especially when mutations disrupt hydrogen bonding interactions critical to standard drug efficacy.

For Sa-4CJN, the docking affinity of Ceftobiprole (-4.827 kcal/mol) was superior to all phytochemicals, reaffirming its role as a potent anti-MRSA β-lactam. Nevertheless, the MM/GBSA result for Dodecanoic acid, pentafluorophenyl ester (-40.32 kcal/mol) slightly surpassed that of Ceftobiprole (-40.00 kcal/mol), suggesting comparable thermodynamic stability. This compound also formed π-π stacking with TRP205 and hydrophobic interactions with ILE309 and TYR297, mirroring the binding pattern of Ceftobiprole, which interacted with key residues like TYR105 and TRP305.

These findings are consistent with previous molecular docking studies that have identified plant-derived compounds as promising inhibitors of bacterial protein targets. Numerous investigations have demonstrated that phytochemicals-such as terpenoids, flavonoids, and fatty acid esters-can effectively bind to and inhibit essential bacterial enzymes, including DNA gyrase, β-lactamases, and penicillin-binding proteins, often rivaling the binding performance of standard antibiotics [23,38-40]. These studies highlight the therapeutic

potential of phytochemicals and reinforce the relevance of the current findings, where compounds from *C. esculentus* demonstrated competitive or superior binding energies compared to levofloxacin and ceftobiprole.

The druglikeness and ADMET evaluation further support the pharmacological potential of the top phytochemicals. While Ceftobiprole has high TPSA and low GI absorption, most phytocompounds such as Isosorbide and Pentafluorophenyl ester displayed high GI absorption, good oral bioavailability (BA ~0.55), and did not inhibit major CYP enzymes, which minimizes the risk of drug-drug interactions. These attributes point toward favorable pharmacokinetics and safety profiles.

Collectively, these findings suggest that certain *C. esculentus* phytochemicals not only match but in some aspects outperform standard antibiotics in terms of docking metrics and interaction profiles. Their multimodal interactions, favorable ADMET parameters, and potential to evade known resistance mechanisms position them as promising candidates for further *in vitro* and *in vivo* validation against resistant strains of *E. coli* and *S. aureus*.

Implications and Future Directions

This study provides evidence of the antimicrobial potential of *Cyperus esculentus*, offering scientific validation for its traditional medicinal use. The strong *in vitro* activity against *Staphylococcus aureus* and *Escherichia coli*, along with molecular docking results, highlight its relevance in addressing multidrug-resistant (MDR) infections. Notably, phytochemicals such as D,l-Xylitol, 1-O-dodecanoyl and Dodecanoic acid, pentafluorophenyl ester exhibited binding affinities and MM/GBSA free energies comparable to or exceeding those of standard antibiotics (Levofloxacin and Ceftobiprole), with stable and specific interactions at critical target sites. These compounds also demonstrated favorable drug-likeness and ADMET profiles, indicating promising pharmacokinetic behavior and safety margins.

The variation in extract efficacy also emphasizes the critical role of solvent selection in optimizing the recovery of bioactive constituents and therapeutic outcomes. The presence of multiple bioactive molecules in *C. esculentus* suggests that it may serve as a valuable source of lead compounds for antibacterial drug development.

To translate these findings into practical applications, future studies should prioritize bioassay-guided fractionation, isolation and structural characterization of active compounds, and mechanistic studies to elucidate their modes of action. Experimental validation through *in vitro* enzyme inhibition and bacterial growth assays using purified compounds is essential. Moreover, evaluating synergistic effects with existing antibiotics, cytotoxicity on mammalian cells, and *in vivo* efficacy and toxicity using appropriate infection models will be critical for assessing therapeutic potential. Finally, molecular dynamics simulations and medicinal chemistry optimization can enhance the potency, selectivity, and stability of lead compounds, advancing the development of novel treatments for MDR bacterial infections.

Funding Support

The study received no external funding.

Conflict of Interest

Author declares no conflict of interest.

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