Synergistic Antibacterial, in-Vitro Anticancer and Molecular Docking Assessment of Carrot, Date Palm and Tiger Nut Juices

[1]Tom-Otu, Mary Otuhuo; [1]Maji, Emmanuel Adejoh; [2]Momoh, Abdul Onoruoiza; [1]Nne, Michael Pepple and [3]Emmanuel Sylvester E

[1] College of Basic Medical and Applied Sciences, Salem University, Lokoja, Kogi State, Nigeria.

[2] Department of Microbiology, Faculty of Life Sciences, Federal University Lokoja, Kogi State, Nigeria.

[3]Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nsukka.

Corresponding author's

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ABSTRACT

This study evaluated the synergistic antibacterial, in-vitro cytotoxic/anti-cancer assessment of carrot (Daucus carrota L., (C), Date palm (Phoenix dactylifera) (D) and Tiger nut (Cyperus esculentus) (T), blends, highlighting their immunostimulatory potentials. Blends were prepared in single, binary and ternary combinations in varying ratios, including 1:1, 2:1, 1:2 and 1:1:1 formulations. Qualitative and quantitative phytochemical analyses showed the presence of flavonoids, phenols, alkaloids, tannins, saponins and terpenoids in varying concentrations across the blends. Antibacterial activity assessed using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) revealed that the ternary blend (CDT 1:1:1) exhibited the highest anti-microbial efficacy effect on selected microorganisms – *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes*. Cytotoxicity evaluation using C_{50} values indicated dose-dependent activity across the three samples. Combination Index (CI) analysis revealed synergistic interactions in most binary and ternary formulations with the CDT (1:1:1) blends showing the strongest synergistic effect. Fifty(50) Wister rats were randomly distributed into ten(10) groups (n=5) with positive and negative controls, treatment groups, and administered graded doses of the blends using carrageenan model. Results showed a dose-dependent response pattern. Statistical analysis was performed using one-way Analysis of Variance (ANOVA), with significance set at $p < 0.05$. In conclusion the blends possess significant phytochemical constituents, notable antimicrobial and immunostimulatory potential particularly in ternary ratios (CDT 1:1:1). These findings highlight their potential therapeutic relevance. However, further studies are recommended using advanced molecular and standard cancer cell lines.

Keywords: Synergistics, Antibacterial, Anticancer, Molecular, Docking.

1.0 INTRODUCTION

Plants have been of great medicinal value since ancient time globally, Jae-Young *et al.* (2023), Tom-Otu *et al.* (2022). According to Wani and Upadhyay (2026), different parts of plants including the roots, leaves, stem, bark, fruits and seeds, have been employed to the treatment of diseases. Bioactive compounds found in medicinal plants enhance their therapeutic efficacy, Bhagwati *et al.* (2025). According to their study, these compounds include phenolics, alkaloids, terpenoids, flavonoids, steroids, glycosides, essential oils, Retaz *et al.* (2023).

The reports of Retaz *et al.* (2023), Bhagwati *et al.* (2025), revealed that owing largely to the presence of bioactive constituents in medicinal plants they exhibit antibacterial, antioxidant, anti-inflammatory, anticancer and immunomodulatory actions. In Nigeria, Zailani S. B. *et al.* (2024) reported that several medicinal plants have antibacterial activities. Over the years, medicinal plants have been good sources of therapeutic agents,

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particularly in low income nations, José and Miranda, (2021), Che *et al.* (2024), Latif and Nawaz (2025), Chawla and Kumar (2021).

José and Miranda (2021) defined medicinal plants as species of the plant kingdom whose roots, stems, leaves, barks, fruits, seeds and flowers are useful tools for the treatment of diseases.

According to the findings of Leselier *et al.* (2021), Latif and Nawaz (2025), medicinal plants contain several bioactive compounds called phytochemicals or secondary metabolites. Examples of these bioactive compounds include flavonoids, polyphenols, tannins, terpernoids, carotinoids, saponins, fatty acids, esters, glycosides, steroids, Vaou *et al.*, (2021), alkaloids, Patel DKK (2026), Sharma *et al.* (2022), Yang *et al.* (2023), Patel DKK (2025), Ghowte *et al.* (2025).

Latif and Nawaz (2025) Patel and Patel (2024) demonstrated in their research works that phytochemicals have shown significant reactions against Gram positive and Gram negative microorganisms.

The investigation of Ahmed *et al.* (2021) revealed that some medicinal plants extracts exhibited antibacterial activity against selected bacterial strains. El-Saadony *et al.* (2021) documented bioactive compounds in medicinal plants and their bioactive activities against pathogens.

According to the report of Belhouala *et al.* (2024) synergistic anticancer evaluation of medicinal plants and edible species combination displayed high in-vitro anticancer efficacy. Reports of Khodaie *et al.* (2024) indicated synergistic effects of plant extracts potent for antimicrobial therapy.

The presence of many bioactive compounds in medicinal plants provide synergistic therapeutic effects, Ugboko (2021). Earlier studies have revealed that medicinal plants consist of phytochemicals. These constituents have made medicinal plants to be essential sources of antimicrobial agents. Carrot, Date palm and Tiger nut contain such bioactive compounds as carotinoids, flavonoids, antioxidants, phenolics, tannins, etc. which have been reported to exhibit antimicrobial, anticancer activities and immunostimulatory potentials.

Multi drug resistance shown by several pathogens calls for novel therapies obtained from plant combinations. Multidrug resistance in pathogens is on the increase worldwide as well as alarming rate of cancer burden. Conventional drugs (chemotherapy) cause toxicity and unbearable side effects often compounding problems for the patients.

Inaccessibility and non-affordability of treatments promote ill health leading to possible death and loss of manpower.The need for safer, more effective and affordable plant-based therapy cannot be over emphasized. Though several studies have been carried out on individual plants, there is limited study on carrot, date palm and tiger nut combination, synergy of ternary blends and joint investigation of their antibacterial, cytotoxic/anticancer and immunostimulatory activities. Hence the reason for this study in which ternary synergy (CD 1:1:1) is evaluated using three biological models involving both experimental and computational analysis.

MATERIALS AND METHODS



Plate 1a: Carrot *(Daucus carota L.)*

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Plate 1b: Date Palm Fruit *(Phoenix dactylifera.)*



Plate 1c: Tiger Nut *(Cyperus esculentus L.)*

3.1 Study Area

The research was carried out in the Advanced Microbiology, Biochemistry and Histopathology Laboratories of Salem University, Lokoja, Kogi State.

3.2 Sample Collection

Fresh Carrot *(Daucus carota L.)*, Date palm fruits (*Phoenix esculentus L.)* and Tiger nut (*Cyperus dactliferus L.)*, [CDT], 4kg, were purchased from the International Market, Lokoja, Kogi State, Nigeria. In preparation for analysis, each sample was sorted, washed thoroughly with distilled water and observed carefully for any unstable pieces.

Half of the purchased portion (2kg) of each sample C, D & T were separately air-dried naturally under a shade until they were crispy to touch subsequently the samples were pulverized into fine powder using sterile electrical grinder (euro premium). The powdered form of the samples was sieved to remove the chaff. Afterwards the sieved sample was put into separate clean containers ready for use performing proximate analysis.

3.2.1 Proximate Analysis of the test samples:

Standard methods were used in the determination of the proximate analysis of the sample extracts (CDT), using 10g of the extract. The weight-dryer method of analysis was used in this study. The dry matter content was first determined and then the components in the air-dry mass was analyzed.

In this study, the proximate analysis of the composition of the samples was determined by the standard methods of the Association of Official Analytical Chemists (AOAC).

3.3 Fresh Sample Preparation

3.3.1 Carrot

Fresh prepared portion of the test material (C) obtained as described in 3.2 above was put into clean stainless bowl. A clean stainless knife was used to dice the carrot into small pieces. Water was added to the dried carrots in a ratio of 1:1 w/v carrot-water. The carrot and water mixture was blended using a standard electric blender (Euro Premium) and then stored in a clean container covered and refrigerated at 4°c till well needed.

3.3.2 Date Palm Fruit

Fresh prepared clean portion (2kg) of date palm fruit, deseeded, was put into a stainless bowl and cut into pieces with clean stainless knife. The cut pieces were then blended with a standard blender (Euro Premium) and stored in a refrigerator at 4°c in readiness for use.

Individual blends of CDT were subjected to filtration using muslin cloth in order to remove the chaff.

3.3.3 Tiger Nut

2kg of prepared tiger nut tubers were soaked 1:3 w/v in distilled water at room temperature (25°c) for 18hrs. Soaked tubers plus soaking water were ground in high–shear blender for 22 minutes. Additional water was added, 1.7 tuber-water ratio and was boiled for 15 minutes at 95°c. Filtration was done using 2 – layer cheese cloth and then homogenized at 10,000 rpm 2 minutes. Extract was stored in clean sealed container till well needed.

3.3.4 Sterility of Plant Extracts

The sterility of the aqueous extracts of CDT was ensured by inoculating 1.0ml of each extract on sterile nutrient agar and incubated at 37°c for 24 hrs. Absence of microbial growth on the extracts after 24hrs incubation confirmed the sterility of the extracts. Preparation of fruit extracts for synergistic potentials: Extracts of the test samples were prepared in single, binary and ternary combinations and ratios

i. C, D, T
ii. C+D 1:1, C+T 1:1, D+T 1:1
iii. C+D+T 1:1:1

And ratios 1:2, 2:2 as outlined in the method of Nadeem *et al.* (2018) for standard synergistic assay.

In-Vitro Antibacterial Activities

Each bacterial suspension was adjusted to MacFardan 0.5 turbidity standard. The antibacterial activities of plant/sample extracts on the text microorganisms were carried out. Agar well diffusion assay as described by Baker *et al.* (2022) was used on Mueller Hinton agar plates. Plates were incubated at 37°c for 24hrs. Thereafter minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) were done using tube dilution method in Mueller Hinton broth as described by Prescot *et al.* (2022). The lowest concentration of the

extracts/antimicrobial that prevented visible bacterial growth was recorded as the MIC. The lowest concentration of the extracts that killed ≥ 99.9% of the test organisms was recorded as the MBC. Using pour plate method of inoculation and disc diffusion method, according to Kirby-Bauer, conventional antibiotic discs were asceptically placed on the agar plates. Plates were incubated at 37°c for 24hrs.

Determination of infectivity dose (ID) of the test organisms.

This was done using standard method described by Momoh *et al.* (2022) for all the test organisms.

Juice Preparation for In-Vitro Anticancer

The in-vitro anticancer evaluation of the juice extracts was carried out using the standard method of Rhabdomysarcoma cell (RD) and Human Cervical cell (He La) cell lines, using a 96 well plate. The following experimental design for an in-vitro anticancer assay was followed, using MTT and Trypan Blue assays:

Table 1: Experimental design for in-vitro anticancer MTT and Trypan Blue assays

Group	Description of Procedure
Blank	Medium + MTT only
Negative Control	Cells + Medium
Positive Control	Cells + Cyclophosphamide
Test Sample	Different Concentrations (500, 250, 125, 62.5, 31.25 mg/ml)
Replicates	Triplicate wells

Percentage cell viability calculation:

Cell viability (%) = OD sample – OD blank OD control – OD blank × 100 cell viability (100) = OD control –

OD blank OD sample – OD blank × 100

Trypan Blue exclusion assay

3.11 Computational Chemistry and Network Pharmacology

3.11.1 Rationale

The biological effects of plant-derived materials are rarely attributable to a single chemical constituent or a single molecular target. Plant extracts contain multiple chemically diverse constituents that may act simultaneously on several proteins, signaling pathways and biological processes. Consequently, the conventional “one compound–one target” approach may not adequately describe the biological activity of complex plant-derived preparations. Network pharmacology provides a systems-level approach for investigating such multicomponent and multitarget interactions by integrating chemical information, computational target prediction, biological databases and molecular interaction networks. Rather than considering the activity of an individual compound in isolation, network pharmacology examines the relationships among compounds, protein targets, pathways and biological processes.

In the present study, network pharmacology was employed as a target-prioritization and mechanistic hypothesis-generation strategy for the GC–MS library-identified constituents of the investigated plant/food extracts. The approach was not used to establish biological activity experimentally. Instead, it was used to reduce a large number of possible compound–protein relationships to a biologically interpretable set of candidate human targets that could subsequently be investigated using molecular docking. A network can be represented mathematically as a collection of nodes and edges. A node represents an entity of interest, such as a chemical compound or protein, whereas an edge represents a relationship between two nodes, such as a predicted compound–protein interaction. In a compound–target network, therefore, the compounds and

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proteins constitute the nodes, while the predicted or known interactions between them constitute the edges. Network visualization and analysis can subsequently be performed using software such as Cytoscape.

A computational chemistry and network pharmacology component was incorporated into the study to investigate possible molecular mechanisms underlying the antibacterial, cytotoxic/anticancer and immunostimulatory activities of the investigated carrot, date palm fruit and tigernut preparations. The computational analysis was designed as a complementary in-silico component of the experimental investigation and was not intended to replace microbiological, cytotoxicity or immunological experiments.

GC–MS-derived compounds that passed the chemical curation stage were used as the initial ligand set for computational analysis. Compound structures were standardized using chemical database identifiers and molecular structure representations before target prediction.

Target Prioritization and Structural Screening

Human protein targets were prioritized using a multistage procedure incorporating STP prediction strength, recurrence across the 13 compounds, network connectivity, biological relevance to cancer/inflammatory and immune-related processes, and availability of suitable experimentally determined protein structures.

This process reduced the initial computational target space to a smaller set of candidate proteins considered suitable for structure-based investigation. Biological relevance and availability of suitable Protein Data Bank (PDB) structures were subsequently assessed before molecular docking.

3.11.6 Selection of Antibacterial Macromolecular Targets

For the antibacterial component, selected GC–MS-derived compounds were docked against experimentally characterized bacterial proteins associated with essential cellular functions or bacterial virulence in *Staphylococcus aureus* and *Escherichia coli*. The target panel was selected independently of the human-target network to provide a mechanistic structural basis for the antibacterial activity observed experimentally.

The selected bacterial proteins represented processes including DNA replication/topology, fatty-acid biosynthesis, folate metabolism, peptidoglycan biosynthesis and bacterial protein sorting/virulence.

Table 4: Antibacterial targets and PDB structures

Organism	Target	Gene	PDB	Biological Process
S. aureus	DNA gyrase	*gyrA*/*gyrB*	5CDM	DNA replication / topology
S. aureus	FabI (enoyl-ACP reductase)	*fabI*	4FS3	Fatty-acid biosynthesis
S. aureus	Dihydrofolate reductase	*folA*	2W9H	Folate metabolism
S. aureus	Sortase A	*srtA*	1T2P	Protein sorting / virulence
E. coli	DNA gyrase B	*gyrB*	4DUH	DNA replication / topology
E. coli	FabI (enoyl-ACP reductase)	*fabI*	1DFI	Fatty-acid biosynthesis
E. coli	Dihydrofolate reductase	*folA*	6XG5	Folate metabolism
E. coli	MurA (UDP-GlcNAc enolpyruvyl transferase)	*murA*	3SWD	Peptidoglycan biosynthesis

3.11.7 Molecular Docking

Molecular docking was used to investigate the predicted binding modes of the selected GC–MS-derived compounds within the binding sites of prioritized human and bacterial protein targets.

Three-dimensional protein structures were obtained from the Protein Data Bank. Protein structures were inspected prior to docking, and nonessential crystallographic components, including unwanted co-crystallized ligands and solvent molecules, were removed as appropriate. Catalytically or structurally essential cofactors and metal ions were retained where their removal would substantially alter the biological structure of the target.

The prepared receptor structures were converted to docking-compatible formats and imported into PyRx for AutoDock Vina-based docking. Docking grids were centered on experimentally characterized ligand-binding sites where co-crystallized ligands were available. For targets without suitable bound ligands, the binding site was defined using the established active-site region.

The 13 selected GC–MS-derived compounds were used as the primary ligand set. Appropriate experimentally characterized ligands were used as reference compounds where available.

3.11.8 Docking Validation

Where a co-crystallized ligand was available, receptor preparation and docking-site definition were evaluated by redocking the native/reference ligand into its experimentally determined binding site. Agreement between the predicted and experimentally observed ligand poses was assessed, where feasible, using structural similarity/RMSD or visual pose comparison. This provides a quality-control step before interpretation of phytocompound docking results. For receptors for which reliable preparation could not be achieved, the corresponding target was excluded from the primary comparative docking analysis and documented separately.

RESULTS AND INTEPRETATION

Tables 6 and 7 show the proximate analysis results of the samples in fresh and dry form. There are significant differences between the values obtained for the two forms of the samples. In fresh form, carrot has the highest carbohydrate content of 8.60±0.12% while tiger nut had the least form of carbohydrate. Generally, the three samples were low in crude fiber content with the three samples recording less than 0.5% crude fiber content. Tiger nut had the highest protein content of 2.05±0.11%, while date palm recorded the least value of 0.03±0.02%. The three samples had high moisture contents and low percentage fat, except tiger nut that had 3.60±0.03% fat content.

In their dry form however, the result was different as presented in table 2 in which carbohydrate contents of the samples were high with date palm recording the highest value of 81.20±0.57%, followed by carrot with 76% while tiger nut recorded the least with 54%. Equally, the protein content was higher in carrot dry sample with a value of 8.9%, while moisture content was highest in date palm dry sample with a value of 8.78±0.32%. Crude fat on the other hand was highest in tiger nut with a value of 28.96±1.58%. Comparatively, the dry samples showed significant increase in the proximate analysis values of the samples over the fresh samples, except for moisture content that showed significant decrease in value.

Table 6: Proximate analysis results of the extracts of samples

S/N	Parameter	Sample A	Sample B	Sample C
1	Carbohydrate (%)	8.60 ± 0.12	8.50 ± 0.12	6.25 ± 0.57
2	Dry Matter (%)	9.50 ± 0.67	1.70 ± 0.03	1.30 ± 0.13
3	Crude Fibre (%)	0.44 ± 0.08	0.15 ± 0.02	0.15 ± 0.03
4	Protein (%)	0.60 ± 0.12	0.03 ± 0.02	2.05 ± 0.11
5	Ash (%)	0.40 ± 0.02	0.95 ± 0.03	0.65 ± 0.02

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6	Fat (%)	0.10 ± 0.01	0.08 ± 0.01	3.60 ± 0.03
7	Moisture (%)	89.50 ± 0.57	90.30 ± 0.17	86.70 ± 1.67

Table 7: Proximate analysis of the dry samples

S/N	Parameter	Sample A	Sample B	Sample C
1	Carbohydrate (%)	76.40 ± 0.12	81.20 ± 0.57	54.15 ± 0.57
2	Dry matter (%)	7.50 ± 0.67	12.28 ± 0.12	15.70 ± 1.15
3	Crude fiber (%)	12.10 ± 0.10	10.05 ± 0.05	12.55 ± 1.15
4	Protein (%)	8.90 ± 0.12	3.69 ± 0.51	6.25 ± 0.15
5	Ash (%)	7.10 ± 0.02	1.95 ± 0.15	2.65 ± 0.75
6	Fat (%)	1.80 ± 0.01	0.88 ± 0.22	28.96 ± 1.58
7	Moisture (%)	6.7.13 ± 0.57	8.78 ± 0.32	6.75 ± 0.15

The results of the phytochemical analysis of the extracts are shown in tables 8-11 for the qualitative and quantitative values respectively. The qualitative phytochemicals revealed the presence of alkaloids, tannins, saponins, steroids, phenols, flavonoids, anthraquinine, anthocyanin, anthraquinone, glycosides, terpenoids and carotenoids. Table 4 show the quantitative phytochemicals for carrot extract. The most abundant phytochemical is carotenoid with a value of 3.71±0.09%, while the least phytochemical content is anthocyanin with a value of 0.38±0.02%. Other phytochemicals with high percentage of occurrence are terpenoids, flavonoids and saponins.

Table 12 shows the quantitative phytochemical in date palm extract. The shows high content of tannins as the most abundant phytochemical with a value of 12.76±0.67%, followed by terpenoids. Other phytochemicals with high percentage occurrence are flavonoids, phenols and steroids. Table 13 is the result of the phytochemical analysis of tiger nut extract, the highest phytochemical to be alkaloids and phenols which had a value of 3.66±0.14% and 3.57±0.13% respectively. Flavonoids is equally high with a value of 2.88±0.02%. Others with high values in tiger nut extract are glycosides, saponnins and antraquinone.

Table 8: Result of qualitative phytochemical analysis of the three extracts

S/N	Phytochemical	Sample A	Sample B	Sample C
1	Alkaloids	+++	+++	+
2	Tannins	++	++	+
3	Saponins	+	+	+
4	Terpenoids	++	++	++
5	Steroids	+	++	-
6	Phenols	+++	+++	++
7	Flavonoids	+++	+++	++
8	Antraquinin	-	+	+
9	Anthocyanin	-	+	+
10	Anthraquinone	-	+	-
11	Glycosides	+	+	+
12	Terpenoids	++	++	++
13	Carotenoids	++	=	-

Keys: += Present; - =Absent; A= Carrot extract; B= Date palm extract; C= Tiger nut extract;

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Table 9: Quantitative phytochemicals present in (%) of liquid extract of carrot juice

S/N	Phytochemical	Amount
1	Alkaloid	1.13 ± 0.13
2	Tannins	1.10 ± 0.10
3	Saponins	1.88 ± 0.02
4	Terpenoids	2.45 ± 0.16
5	Anthraquinone	2.45 ± 0.16
6	Phenols	1.63 ± 0.07
7	Flavonoids	3.20 ± 0.10
8	Glycosides	1.24 ± 0.06
9	Anthocyanin	0.38 ± 0.02
10	Carotenoids	3.71 ± 0.09

Table 10: Quantitative phytochemicals present in (%) of liquid extract of date palm juice

S/N	Phytochemical	Amount
1	Phenol	5.68 ± 0.12
2	Flavonoids	6.25 ± 0.15
3	Alkaloids	2.64 ± 0.16
4	Saponins	1.79 ± 0.11
5	Tannins	12.76 ± 0.67
6	Terpenoids	9.38 ± 0.12
7	Glycosides	1.32 ± 0.02
8	Steroids	5.24 ± 0.06
9	Anthraquinones	2.18 ± 0.02
10	Anthocyanin	1.14 ± 0.06
11	Anthraquinin	0.56 ± 0.00

Table 11: Quantitative phytochemicals present in (%) of liquid extract of tiger nut juice

S/N	Phytochemical	Amount
1	Phenol	3.57 ± 0.13
2	Flavonoids	2.88 ± 0.02
3	Alkaloids	3.66 ± 0.14
4	Saponins	2.68 ± 0.06
5	Tannins	1.26 ± 0.14
6	Terpenoids	1.38 ± 0.12
7	Glycosides	2.28 ± 0.02
8	Steroids	0.24 ± 0.00

9	Anthraquinones	1.18 ± 0.02
10	Anthocyanin	1.06 ± 0.03

4.2 Percentage yield of extracts

Figure one shows the result of the antibacterial assay of the various extracts used in this research. Generally, the extracts were more potent against Gram positive bacteria than the Gram negative ones used in this research, with the exception of date palm extract on *Pseudomonas*. The extracts exert the least antibacterial effects on *Escherichia coli*, with tiger nut extract having the least inhibitory effect with a diameter of zone of inhibition of 10.00±0.58 mm. The highest inhibitory effect of 27.00±0.58 mm diameter zone was exerted by date palm extract on *Streptococcus*.

Comparatively, all the extracts exerted higher inhibitory effects against the test bacteria than the most effective standard commercial antibiotics used which was ciprofloxacin.

Figure 2 is the result of the synergistic assay of the extracts at various concentrations used. The combination of carrot extract and date palm extract at ratios 1:2. The highest zone of inhibition of this extract combination was exerted on *Pseudomonas* having a diameter of zone of inhibition of 21.00±0.58 mm. The four test isolates were sensitive to the various concentrated extract ratios at varying degrees with highest sensitivity obtained from the combination of the three extract in ratios 1:1:1. The highest diameter of zone of inhibition was obtained for *Pseudomonas* at this ratio with a zone diameter of 32.00±0.0.58 mm.

The same ratio exerted the highest zone of inhibition on *Escherichia, Staphylococcus* and *Streptococcus* respectively. In all the synergistic assay, the combination of tiger nut extract and date palm extract exerted the least potency on all the isolates having the least diameter of zone of inhibition of 6.67±0.67 mm on *Staphylococcus*. Generally, the results showed that the synergistic effect of the various combination ratios were more on the Gram negative bacteria (*Escherichia* and *Pseudomonas*) than the Gram positive bacteria (*Staphylococcus* and *Streptococcus*).

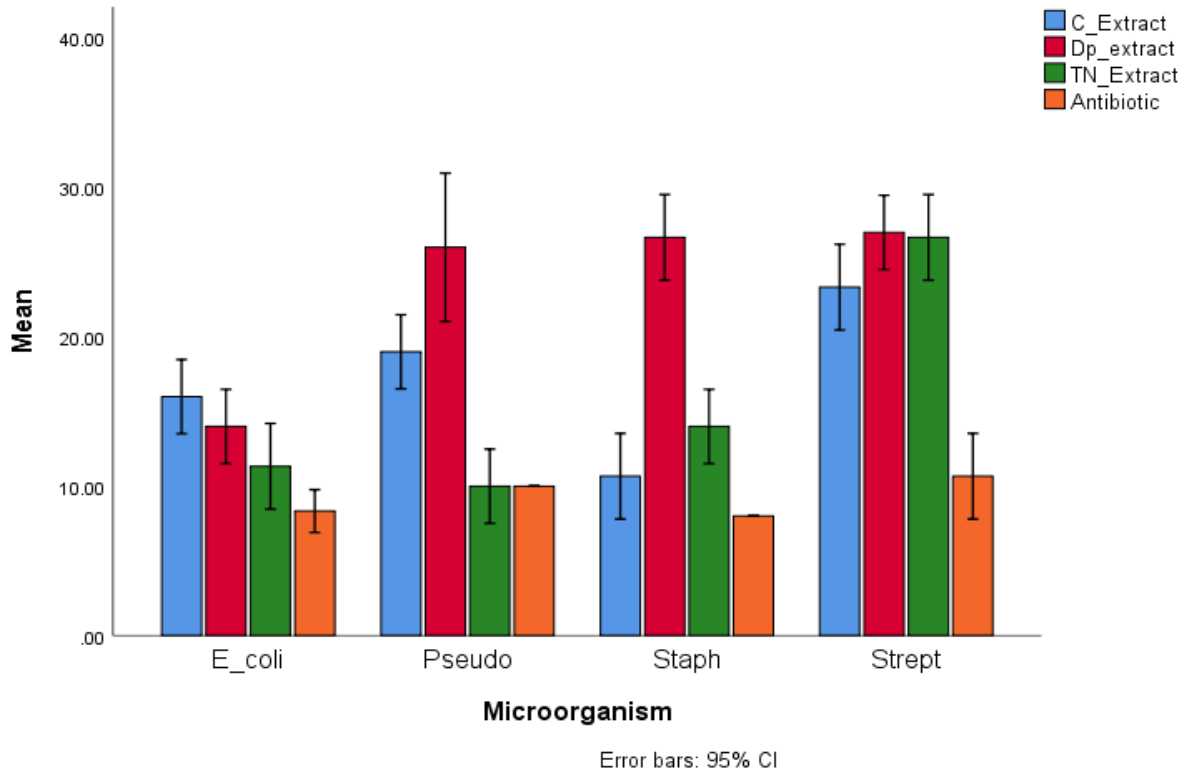


Figure 3: Inhibitory results of the extracts against test bacteria.

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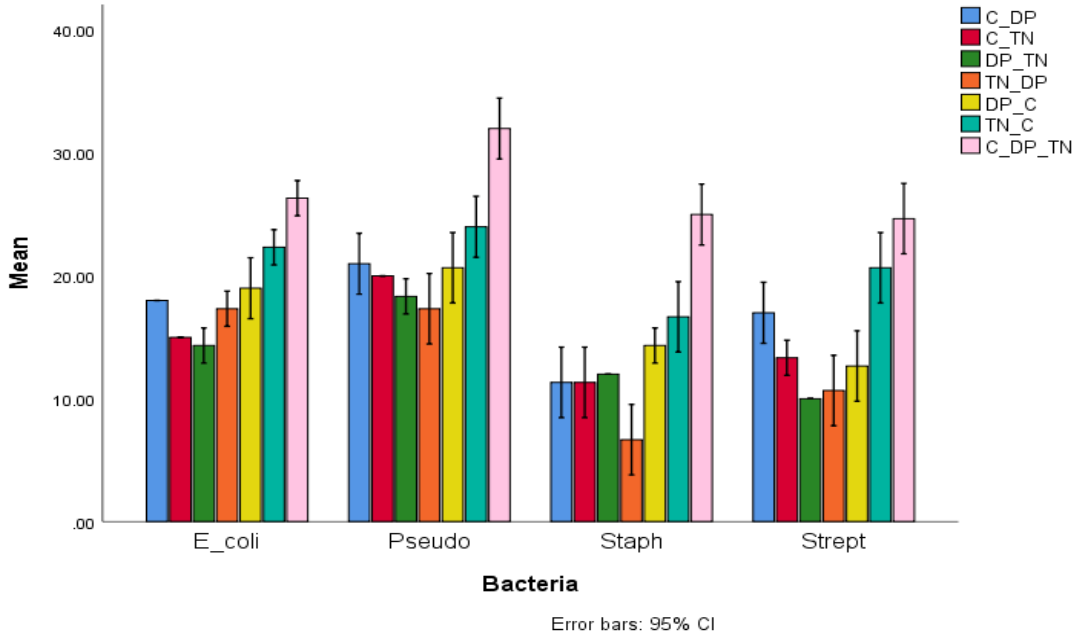


Figure 4: Synergistic antibacterial assay of the extracts against test bacteria

Figure 5 is the result of the antibiotic susceptibility of the isolates when they were subjected to commercial antibiotics. Generally, the bacteria isolates showed very little sensitivity effect to the commercial antibiotics used. From the figure, *E.coli* was only moderately sensitive to ampicillin while the two Gram positive test bacteria were completely resistant to the antibiotic. Three of the bacteria isolates were sensitive to chloramphenicol. *Staphylococci* was however resistant to chloramphenicol without any zone of inhibition recorded for it. Colistin sulphate antibiotic was effective against the two Gram positive bacteria but not on any of the Gram negative bacteria. Kanamycin on the other hand was effective against the two Gram negative bacteria while the two Gram positive bacteria were resistant to it. Nalidixic acid and streptomycin were the only antibiotic that the four bacterial test isolates were sensitive to.

Tetracycline was equally effective against the two Gram positive bacteria used while the Gram negative bacteria showed resistant to it. Nitrofurantoin was effective against *E. coli* and *Staphylococci* but not effective against *Pseudomonas* and *Streptococci* respectively. Therefore, the antibiotics did not follow any specific pattern of activity in terms of their action against the test isolates used in this research. Comparatively, the extracts were more effective as antibacterial agents than the commercial antibiotics used in this research.

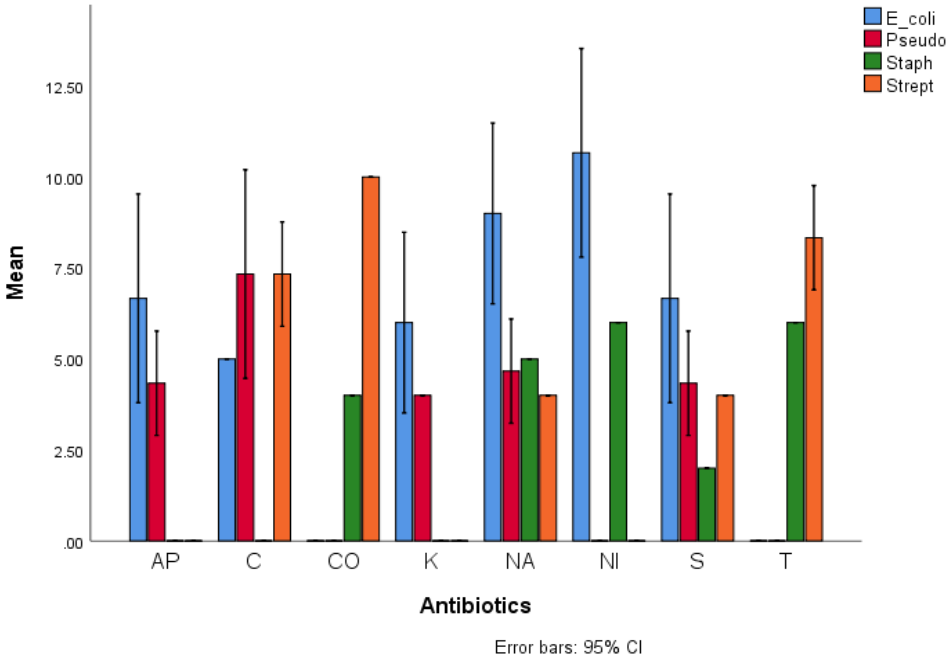


Figure 5: Result of the inhibitory effects of commercial antibiotics on test isolates

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Gas Chromatography-Mass Spectrophotometry Results of extracts

In summary, the GC–MS result of carrot extract, bioactive ingredients identified were high antioxidants capable of exerting major biological effects on the immune system to overcome disease causing agents. The major bioactive compounds identified and their relative abundance are: Hexadecanal (1.02%), methyl esters of fatty acids (1.21–1.91%), **Palmitic acid (36.25%)**, **Phytol (7.14%)**, **Oleic acid (38.87%)**, and **Stearic acid (13.60%)**. This result is adequately represented in table 8. The minor compounds identified in carrot extract are presented in table 8b. The extract is dominated by **oleic acid (38.87%)** and **palmitic acid (36.25%)**, followed by **stearic acid (13.60%)** and **phytol (7.14%)**. These compounds are known for: Strong **antioxidant activity, Anti-inflammatory effects, Antimicrobial activity,** Potential **anticancer properties, Cardioprotective effects,** Potential **antidiabetic activity.** Hepatoprotective and immunomodulatory effects (mainly due to phytol). Therefore, the GC–MS profile suggests that sample **carrot extract** possesses significant **antioxidant, antimicrobial, anti-inflammatory, cardioprotective, and potential antidiabetic/anticancer properties**, largely attributable to oleic acid, palmitic acid, stearic acid, and phytol.

Date palm on the other hand revealed eight major bioactive constituents. The dominant compounds were **Oleic acid (32.59%)**, **Palmitic acid (31.26%)**, **11-Octadecenoic acid methyl ester (13.68%)**, and **Stearic acid (10.89%)**. The GC–MS profile indicates that sample DE is rich in **unsaturated and saturated fatty acids and their esters**, especially:

1 **Oleic acid (32.59%)** – major contributor to cardioprotective, antidiabetic and anti-inflammatory activity.

2 **Palmitic acid (31.26%)** – contributes antimicrobial and antioxidant properties.

3 **11-Octadecenoic acid methyl ester (13.68%)** – contributes antioxidant and membrane-protective effects.

4 **Stearic acid (10.89%)** – contributes anti-inflammatory and antimicrobial activity.

5 **Fatty acid amides (Stearamide, Lauramide, Myristamide)** – may enhance antimicrobial and anti-inflammatory properties. The DE extract may possess the following:

- Strong **antioxidant activity**
- Significant **anti-inflammatory activity**
- **Antimicrobial and antifungal effects**
- Potential **antidiabetic activity**
- **Cardioprotective and hypolipidemic effects**
- Potential **anticancer activity**
- Hepatoprotective effects
- Wound-healing and tissue-protective properties

These biological activities are mainly attributed to the high concentrations of **oleic acid, palmitic acid, stearic acid, and fatty acid esters/amides** present in the extract.

The GC–MS chromatogram of sample **TE** contained 15 peaks. The major bioactive compounds identified were dominated by **Oleic acid (38.57%)**, **Palmitic acid (16.05%)**, **Linoleic acid methyl ester derivatives (12.47%)**, **Stearic acid (11.26%)**, and **11-Octadecenoic acid methyl ester (1.23%)**. **Monounsaturated fatty acids** (especially oleic acid)

- **Polyunsaturated fatty acids** (linoleic acid derivatives)

- **Saturated fatty acids** (palmitic, stearic, myristic and arachidic acids)
- **Fatty acid esters and monoglycerides**
- **Antifungal fatty acids** (undecanoic and undecylenic acids)

The GC–MS profile suggests that TE possesses:

- Strong **antioxidant activity**
- Potent **anti-inflammatory activity**
- Significant **antimicrobial and antifungal properties**
- **Cardioprotective effects**
- Potential **antidiabetic activity**
- Potential **anticancer activity**
- Wound-healing and tissue-protective properties
- Cholesterol-lowering (hypolipidemic) effects
- Immunomodulatory activity

The medicinal properties are mainly attributable to the high abundance of **Oleic acid (38.57%)**, **Palmitic acid (16.05%)**, **Linoleic acid derivatives (≈13%)**, and **Stearic acid (11.26%)**, which together account for over 75% of the total GC–MS profile. The anticancer assay in a 96-well MTT cell viability assay used to evaluate the in vitro anticancer activity of test samples showed the color differences that indicate different amounts of formazan, which is proportional to the number of metabolically active (viable) cells. From the assay, accurate anticancer activity was interpreted using the absorbance values at 570 nm obtained from a microplate reader.

Qualitative Interpretation of the plates

Qualitatively, however, the dark purple/reddish wells indicates specifically a high formazan production which means there is high cell viability as well as low cytotoxicity of the extracts used.

The pale yellow/orange wells indicates low formazan production which by interpretation means low cell viability and high cytotoxicity of the extracts. The area with completely clear wells equally indicates very few or no viable cells which means there is nearly complete inhibition of the extracts. The gradual change in color across the 96 wells in the entire assay suggests a dose-dependent effect of all the extracts used, which is expected due to the serial dilutions of the extracts used that served as an anticancer agent in this assay.

The absorbance values obtained for the 96 wells in the assay done in triplicates are presented in table 17 below:

Table 17: Absorbance reading for carrot extract

Concentration (µg/mL)	**Mean ± SD** absorbance at 570 nm after 48 hours incubation at 37°C	**Mean ± SD** absorbance at 570 nm after 72 hours incubation at 37°C
Control	1.132 ± 0.014	1.132 ± 0.014
31.25	0.951 ± 0.008	0.890 ± 0.008
62.5	0.810 ± 0.011	0.801 ± 0.011

125	0.611 ± 0.008	0.520 ± 0.008
250	0.348 ± 0.006	0.211 ± 0.006
500	0.120 ± 0.004	0.111 ± 0.004

Table 18: Absorbance reading for date palm extract

Concentration (µg/mL)	**Mean ± SD** absorbance at 570 nm after 48 hours incubation at 37°C	**Mean ± SD** absorbance at 570 nm after 72 hours incubation at 37°C
Control	1.132 ± 0.014	1.132 ± 0.014
31.25	1.102 ± 0.016	0.955 ± 0.008
62.5	0.919 ± 0.011	0.819 ± 0.011
125	0.810 ± 0.004	0.610 ± 0.008
250	0.647 ± 0.003	0.347 ± 0.006
500	0.431 ± 0.005	0.131 ± 0.004

Table 19: Absorbance reading for tiger nut extract

Concentration (µg/mL)	**Mean ± SD** absorbance at 570 nm after 48 hours incubation at 37°C	**Mean ± SD** absorbance at 570 nm after 72 hours incubation at 37°C
Control	1.132 ± 0.014	1.132 ± 0.014
31.25	0.862 ± 0.008	0.710 ± 0.008
62.5	0.711 ± 0.011	0.609 ± 0.011
125	0.600 ± 0.008	0.528 ± 0.008
250	0.314 ± 0.006	0.304 ± 0.002
500	0.300 ± 0.004	0.295 ± 0.005

Table 20: Absorbance reading for carrot and date palm combined extracts

Concentration (µg/mL)	**Mean ± SD** absorbance at 570 nm after 48 hours incubation at 37°C	**Mean ± SD** absorbance at 570 nm after 72 hours incubation at 37°C
Control	1.132 ± 0.014	1.132 ± 0.014
31.25	0.668 ± 0.012	0.612 ± 0.018
62.5	0.602 ± 0.018	0.522 ± 0.012
125	0.418 ± 0.010	0.402 ± 0.008
250	0.334 ± 0.006	0.321 ± 0.011
500	0.280 ± 0.004	0.253 ± 0.007

Table 21: Absorbance reading for carrot and tiger nut combined extracts

Concentration (µg/mL)	**Mean ± SD** absorbance at 570 nm after 48 hours incubation at 37°C	**Mean ± SD** absorbance at 570 nm after 72 hours incubation at 37°C
Control	1.132 ± 0.014	1.132 ± 0.014
31.25	0.756 ± 0.008	0.695 ± 0.0013
62.5	0.711 ± 0.011	0.619 ± 0.011
125	0.616 ± 0.008	0.410 ± 0.008
250	0.504 ± 0.006	0.307 ± 0.003
500	0.421 ± 0.009	0.251 ± 0.007

Table 22: Absorbance reading for date palm and tiger nut combined extracts

Concentration (µg/mL)	**Mean ± SD** absorbance at 570 nm after 48 hours incubation at 37°C	**Mean ± SD** absorbance at 570 nm after 72 hours incubation at 37°C
Control	1.132 ± 0.014	1.132 ± 0.014
31.25	0.915 ± 0.008	0.820 ± 0.018
62.5	0.809 ± 0.011	0.719 ± 0.011
125	0.610 ± 0.008	0.531 ± 0.013
250	0.547 ± 0.006	0.496 ± 0.006
500	0.431 ± 0.004	0.406 ± 0.004

Table 23: Absorbance reading for carrot extract

Concentration (µg/mL)	**Mean ± SD** absorbance at 570 nm after 48 hours incubation at 37°C	**Mean ± SD** absorbance at 570 nm after 72 hours incubation at 37°C
Control	1.132 ± 0.014	1.132 ± 0.014
31.25	0.322 ± 0.008	0.306 ± 0.008
62.5	0.309 ± 0.011	0.271 ± 0.011
125	0.240 ± 0.008	0.210 ± 0.008
250	0.197 ± 0.006	0.144 ± 0.006
500	0.130 ± 0.004	0.122 ± 0.004

Using Percentage cell viability, the calculation is as follows:

Cell Viability (%)=ODsample−ODblankODcontrol−ODblank×100Cell Viability (%)=OD*control*−OD*blank* OD*sample*−OD*blank*×100

=0.122-1.132/1.132 x 100=12.2% viability or Percentage growth inhibition

Growth inhibition (%)=100−Cell viability (%)Growth inhibition (%)=100−Cell viability (%) of 87.8%

Therefore, treatment of Hep G-2 cells with the test extracts at combined ratio of 1;1;1 for 72 h produced a concentration-dependent reduction in cell viability. The untreated control exhibited 100% viability, whereas treatment with 500 µg/mL reduced viability to 12.2%. The calculated IC_{50} value was 118.4 µg/mL, indicating moderate cytotoxic activity. Cyclophosphamide demonstrated significantly greater cytotoxicity with an IC_{50} of 22.7 µg/mL.

1.5 Overall Docking Scores Across Targets

A comprehensive docking analysis was performed for all phytocompounds against the four target proteins. The best (most negative) docking scores for compounds achieving ≤ -7.0 kcal/mol in at least one target are presented in Table 23.

Table 23: GC–MS-derived computational ligand set

No.	**Compound**	**Extract**	**CAS No.**	**PubChem CID**	**Molecular Formula**	**MW (g/mol)**
1	**Palmitic acid (n-Hexadecanoic acid)**	TE/DE/CE	**57-10-3**	**985**	$C_{16}H_{32}O_2$	**256.42**
2	**Oleic acid (9-Octadecenoic acid)**	TE/DE/CE	**112-80-1**	**445639**	$C_{18}H_{34}O_2$	**282.5**

3	**Stearic acid (Octadecanoic acid)**	TE/DE/CE	**57-11-4**	**5281**	$C_{18}H_{36}O_2$	**284.5**
4	**Methyl 14-methylpentadecanoate**	TE/DE/CE	**5129-60-2**	**21205**	$C_{17}H_{34}O_2$	**270.5**
5	**Methyl 11-octadecenoate**	TE/DE	**Isomer dependent**	**74738**	$C_{19}H_{36}O_2$	**296.49**
6	**Phytol**	CE	**150-86-7**	**5280435**	$C_{20}H_{40}O$	**296.5**
7	**Hexadecanal (Palmitaldehyde)**	CE	**629-80-1**	**984**	$C_{16}H_{32}O$	**240.42**
8	**Lauric acid (Dodecanoic acid)**	TE	**143-07-7**	**3893**	$C_{12}H_{24}O_2$	**200.32**
9	**Myristic acid (Tetradecanoic acid)**	TE	**544-63-8**	**11005**	$C_{14}H_{28}O_2$	**228.37**
10	**Pentadecanoic acid**	TE	**1002-84-2**	**13849**	$C_{15}H_{30}O_2$	**242.4**
11	**Arachidic acid (Eicosanoic acid)**	TE	**506-30-9**	**10467**	$C_{20}H_{40}O_2$	**312.5**
12	**Undecylenic acid (10-Undecenoic acid)**	TE	**112-38-9**	**5634**	$C_{11}H_{20}O_2$	**184.27**
13	**Erucic acid (13-Docosenoic acid)**	TE	**112-86-7**	**5281116**	$C_{22}H_{42}O_2$	**338.6**

4.5.2 Predicted human targets

Report:

- 1,300 associations
- 227 unique targets
- recurrent targets.

For example, your Cytoscape/STP analysis identified targets such as PTPN1, PPARG, PPARA, PPARD, FABP4 and FABP5 among the highly recurrent nodes. The network data show degree 13 for targets connected to all 13 compounds. Your integrated dataset confirms PTPN1 as a particularly strong recurrent target, with all 13 compounds connected to it and a mean STP probability of approximately 0.839.

Table 24: Antibacterial docking

Ligand	1DFI	1T2P	3SWD	4DUH	4FS3
11-Octadecenoic_acid_methyl_ester	–4.9	–5.1	–5.2	–6.0	–6.2
Arachidic_acid	–5.2	–5.0	–5.2	–6.3	–6.3
Erucic_acid	–5.0	–5.2	–5.7	–6.4	–6.2
Hexadecanal	–5.0	–5.2	–4.6	–5.4	–5.9
Lauric_acid	–4.6	–4.6	–5.0	–5.6	–6.1
Methyl_14-methylpentadecanoate	–5.2	–4.8	–4.8	–4.0	–6.0
Myristic_acid	–5.0	–4.6	–4.9	–6.2	–5.9
Oleic_acid	–5.3	–5.1	–5.2	–4.2	–6.3
Palmitic_acid	–5.0	–5.0	–5.0	–4.5	–5.9
Pentadecanoic_acid	–5.3	–4.9	–4.8	–5.4	–6.2
Phytol	–5.8	–5.5	–6.0	–4.8	–6.7
Stearic_acid	–4.8	–4.9	–4.8	–5.8	–6.3
Undecylenic_acid	–4.7	–4.9	–5.0	–5.6	–5.6

All values are in kcal/mol; more negative = stronger binding.

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4.7 Interaction Analysis

The interaction analysis showed that the selected GC–MS-derived constituents interacted predominantly with the investigated bacterial targets through hydrophobic, alkyl, π-alkyl and van der Waals contacts, with limited or no hydrogen-bonding interactions observed in the analyzed phytol- and lauric-acid complexes. For example, phytol interacted with ***E. coli*** FabI (1DFI) through hydrophobic contact with Leu100 and π-alkyl interaction with Tyr156, while its interaction with ***S. aureus*** FabI (4FS3) involved Ala95, Met160 and Tyr157. Phytol also formed predominantly nonpolar interactions with Phe328 in ***E. coli*** MurA (3SWD), Leu20 in ***S. aureus*** DHFR (3FRD), and Leu50/Phe31 in ***E. coli*** DHFR (6DFR). Lauric acid similarly exhibited π-sigma interactions with Tyr147 in ***S. aureus*** FabI and Phe31 in ***E. coli*** DHFR. These interaction patterns are consistent with the predominantly hydrophobic molecular architectures of phytol and long-chain fatty-acid constituents, which possess extensive hydrocarbon surfaces but comparatively limited hydrogen-bonding functionality. The predominance of nonpolar interactions therefore does not necessarily indicate an absence of molecular recognition; rather, it suggests that hydrophobic complementarity and van der Waals stabilization may contribute substantially to their predicted occupancy of the investigated bacterial binding pockets. The observed docking scores, which ranged from −5.0 to −6.7 kcal/mol for the complexes analyzed here, should nevertheless be interpreted as computational estimates and not as direct measures of antibacterial potency.

Table 25: Molecular docking for antibacterial activities of the extracts

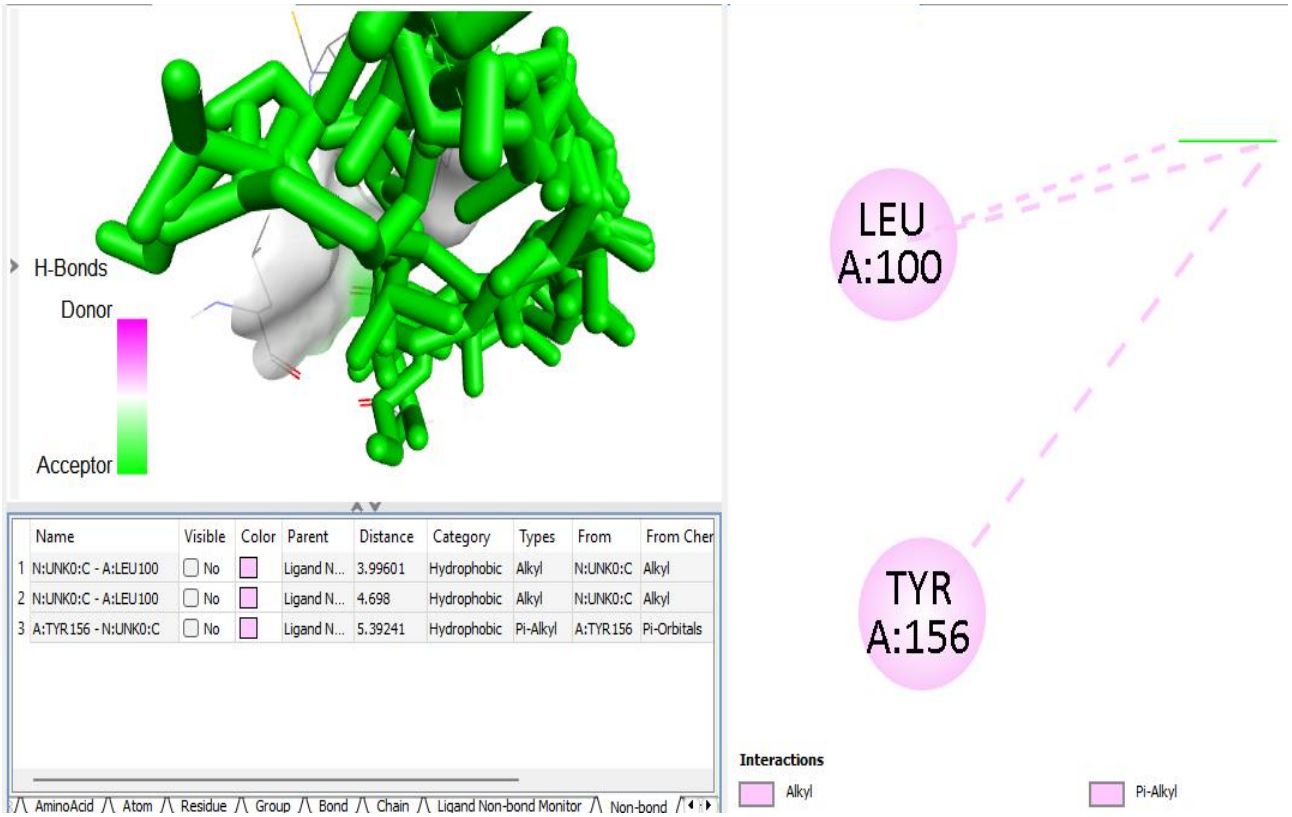
Compound	**Target / PDB**	**Docking score (kcal/mol)**	**Hydrophobic interactions**	**π interactions**	**Key interacting residues**
Phytol	*E. coli* FabI / **1DFI**	**−5.8**	**Alkyl:** Leu100	**π-Alkyl:** Tyr156	**Leu100, Tyr156**
Phytol	*S. aureus* FabI / **4FS3**	**−6.7**	**Alkyl:** Ala95, Met160	**π-Alkyl:** Tyr157	**Ala95, Met160, Tyr157**; van der Waals contacts also shown around Lys164/Ser197
Lauric acid	*S. aureus* FabI / **4FS3**	**−6.1**	None explicitly listed	**π-Sigma:** Tyr147	**Tyr147**
Phytol	*E. coli* MurA / **3SWD**	**−6.0**	None explicitly listed	**π-Alkyl:** Phe328	**Phe328**
Phytol	*S. aureus* DHFR / **3FRD**	**−6.4**	**Alkyl:** Leu20	None explicitly listed	**Leu20**; van der Waals contact shown at Thr46
Phytol	*E. coli* DHFR / **6DFR**	**−5.9**	**Alkyl:** Leu50	**π-Alkyl:** Phe31	**Leu50, Phe31**
Lauric acid	*E. coli* DHFR / **6DFR**	**−5.0**	None explicitly listed	**π-Sigma:** Phe31	**Phe31**

The interpretation of these docking results should further be considered in relation to the aqueous-decoction extraction procedure employed in the present investigation. The use of an aqueous decoction was intended to approximate a realistic water-based preparation rather than to maximize extraction of highly lipophilic constituents. Consequently, the detection of phytol and long-chain fatty-acid derivatives by GC–MS does not establish that these compounds were present in the original decoction as freely dissolved molecules at concentrations corresponding to those used in silico. Their analytical detection may reflect partial extraction, matrix association, dispersion and/or concentration during sample preparation. The docking analysis should therefore be regarded as providing mechanistic hypotheses concerning possible compound–target interactions rather than proof of target engagement within the administered preparation. Nevertheless, the observation that chemically diverse constituents exhibit plausible interactions with multiple antibacterial targets supports the

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broader hypothesis that the antibacterial activity of the plant preparations may arise from complementary actions of several constituents rather than from a single compound acting alone.

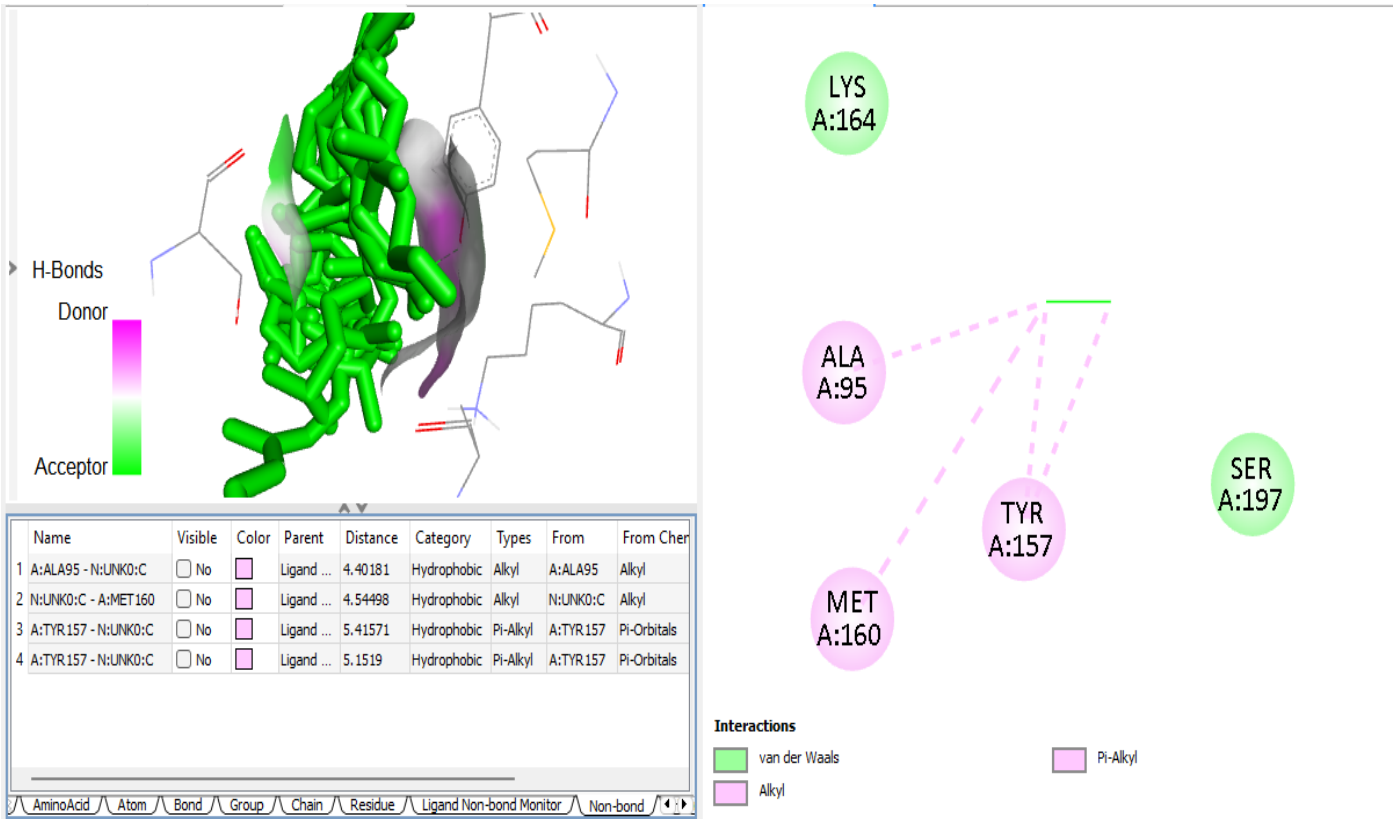
1DFI_PHYTOL



	Name	Visible	Color	Parent	Distance	Category	Types	From	From Cher
1	N:UNK0:C - A:LEU100	No		Ligand N...	3.99601	Hydrophobic	Alkyl	N:UNK0:C	Alkyl
2	N:UNK0:C - A:LEU100	No		Ligand N...	4.698	Hydrophobic	Alkyl	N:UNK0:C	Alkyl
3	A:TYR156 - N:UNK0:C	No		Ligand N...	5.39241	Hydrophobic	Pi-Alkyl	A:TYR156	Pi-Orbitals

AminoAcid / Atom / Residue / Group / Bond / Chain / Ligand Non-bond Monitor / Non-bond

4FS3_PHYTOL



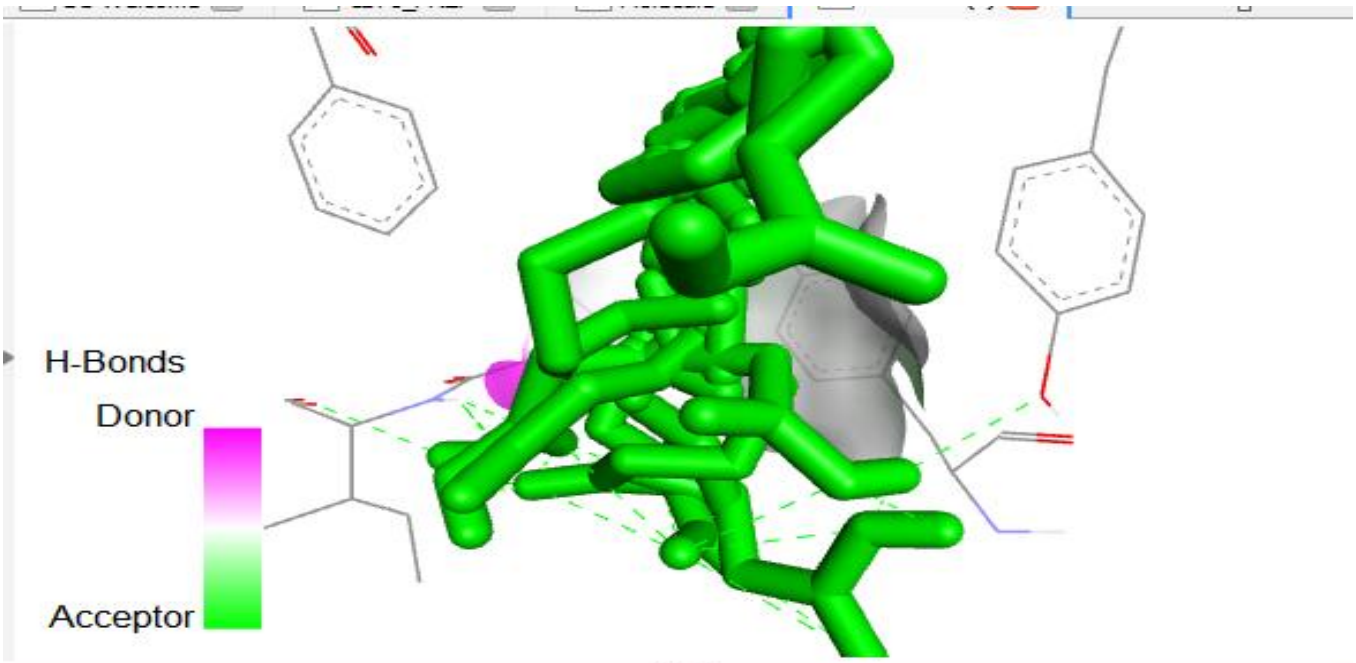
	Name	Visible	Color	Parent	Distance	Category	Types	From	From Cher
1	A:ALA95 - N:UNK0:C	No		Ligand ...	4.40181	Hydrophobic	Alkyl	A:ALA95	Alkyl
2	N:UNK0:C - A:MET160	No		Ligand ...	4.54498	Hydrophobic	Alkyl	N:UNK0:C	Alkyl
3	A:TYR157 - N:UNK0:C	No		Ligand ...	5.41571	Hydrophobic	Pi-Alkyl	A:TYR157	Pi-Orbitals
4	A:TYR157 - N:UNK0:C	No		Ligand ...	5.1519	Hydrophobic	Pi-Alkyl	A:TYR157	Pi-Orbitals

AminoAcid / Atom / Bond / Group / Chain / Residue / Ligand Non-bond Monitor / Non-bond

Figure 8; Phytol molecular docking results

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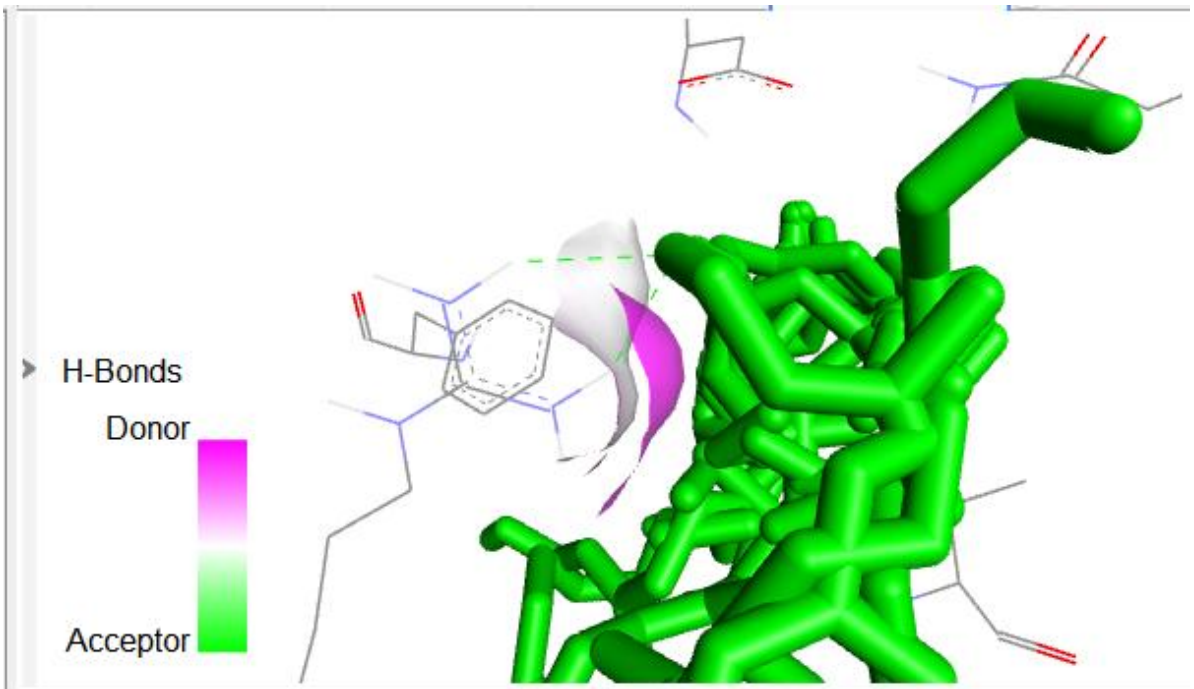
4FS3_LAURIC ACID



	Name	Visible	Color	Parent	Distance	Category	Types	From	From Chemistry
1	N:UNK0:C - A:TYR147	No		Ligand ...	3.63746	Hydrophobic	Pi-Sigma	N:UNK0:C	C-H

Figure 9; Lauric molecular docking result

3SWD_PHYTOL

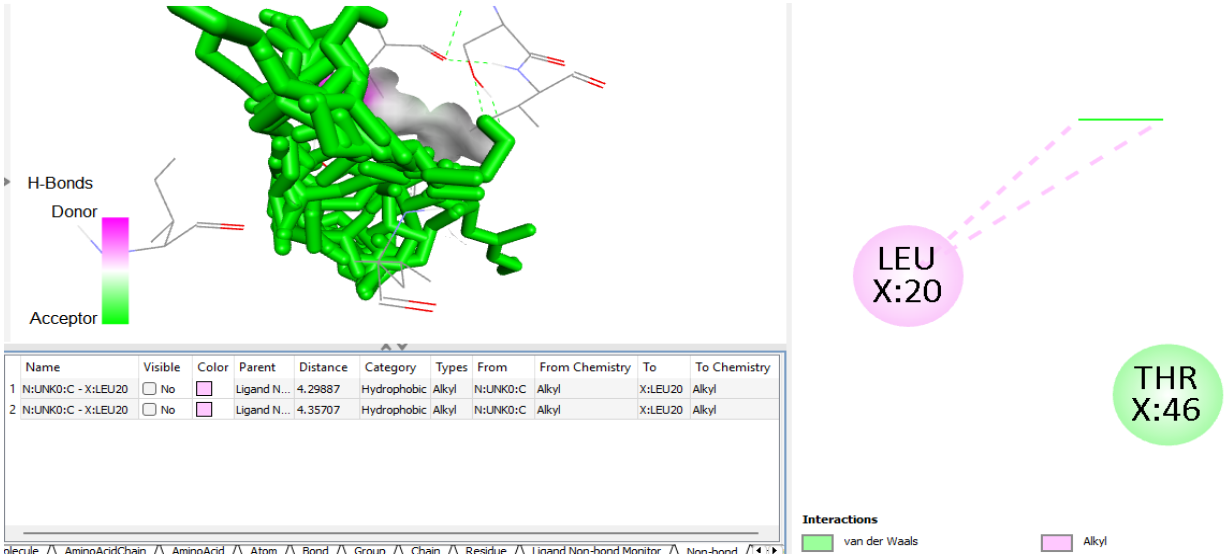


	Name	Visible	Color	Parent	Distance	Category	Types	From	From Chemistr
1	A:PHE328 - N:UNK0:C	No		Ligand ...	5.12168	Hydrophobic	Pi-Alkyl	A:PHE328	Pi-Orbitals
2	A:PHE328 - N:UNK0:C	No		Ligand ...	5.34026	Hydrophobic	Pi-Alkyl	A:PHE328	Pi-Orbitals

Figure 10; Phytol molecular docking result

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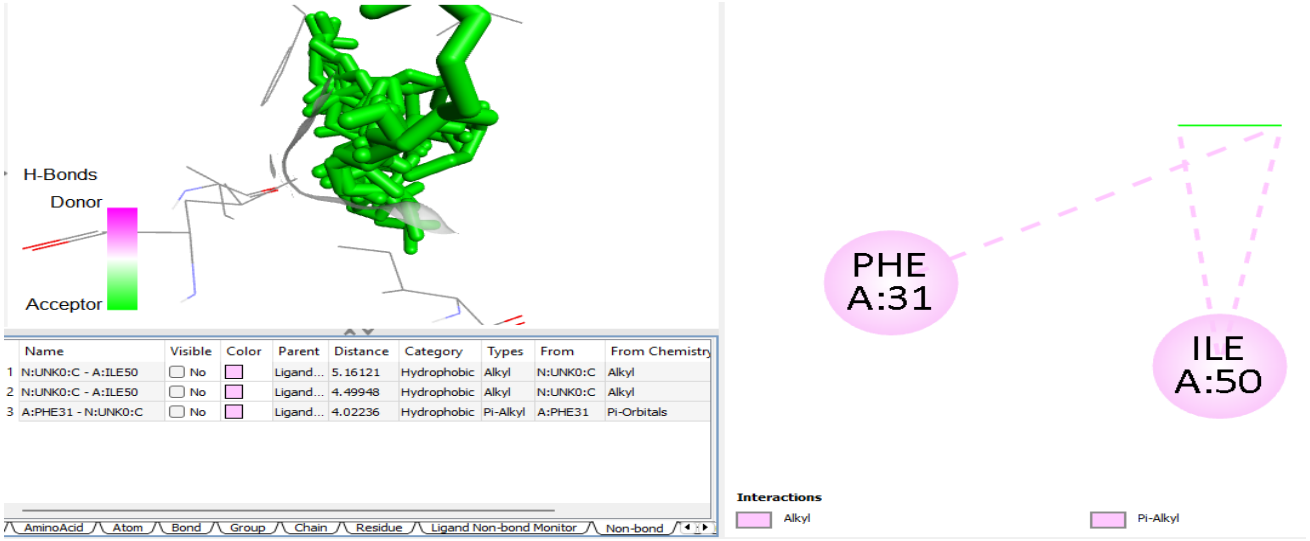
3FRD_PHYTOL



	Name	Visible	Color	Parent	Distance	Category	Types	From	From Chemistry	To	To Chemistry
1	N:UNK0:C - X:LEU20	No		Ligand N...	4.29887	Hydrophobic	Alkyl	N:UNK0:C	Alkyl	X:LEU20	Alkyl
2	N:UNK0:C - X:LEU20	No		Ligand N...	4.35707	Hydrophobic	Alkyl	N:UNK0:C	Alkyl	X:LEU20	Alkyl

Figure 11; Phytol molecular docking result

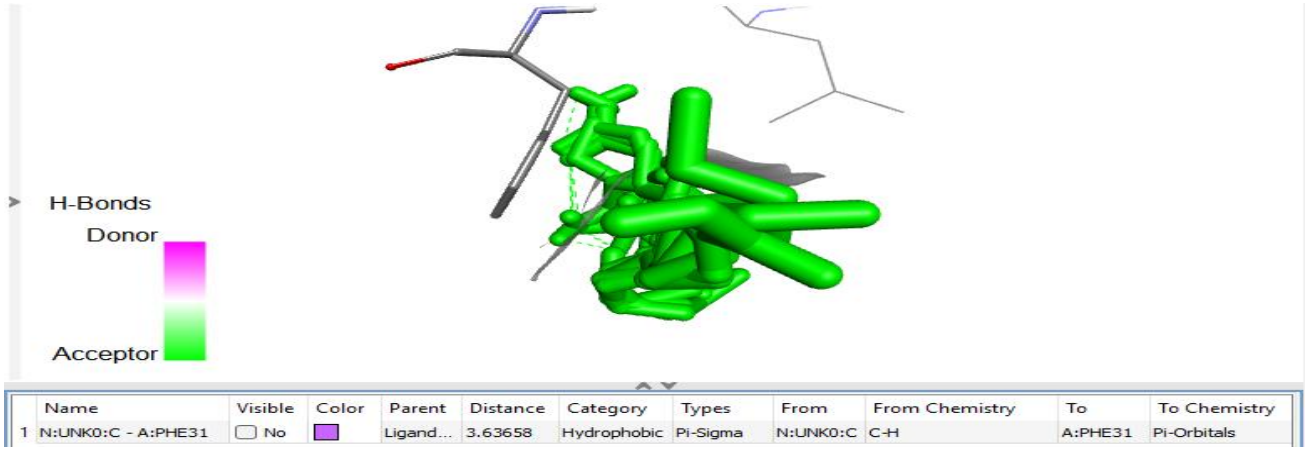
6DFR/PHYTOL



	Name	Visible	Color	Parent	Distance	Category	Types	From	From Chemistry
1	N:UNK0:C - A:ILE50	No		Ligand...	5.16121	Hydrophobic	Alkyl	N:UNK0:C	Alkyl
2	N:UNK0:C - A:ILE50	No		Ligand...	4.49948	Hydrophobic	Alkyl	N:UNK0:C	Alkyl
3	A:PHE31 - N:UNK0:C	No		Ligand...	4.02236	Hydrophobic	Pi-Alkyl	A:PHE31	Pi-Orbitals

Figure 12; Phytol molecular docking result

6DFR/LAURIC ACID



	Name	Visible	Color	Parent	Distance	Category	Types	From	From Chemistry	To	To Chemistry
1	N:UNK0:C - A:PHE31	No		Ligand...	3.63658	Hydrophobic	Pi-Sigma	N:UNK0:C	C-H	A:PHE31	Pi-Orbitals

Figure 13; Lauric molecular docking result

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DISCUSSION

Several bioactive compounds were discovered from the qualitative phytochemical analysis of the three test extracts, this is consistent with the work of Kokab *et al.* (2026) who documented diverse phytochemical constituents of a selection of medicinal plants.

Classes of phytochemical compounds such as flavonoids, alkaloids, phenols, tannins, terpenoids, carotenoids and others were discovered in this research, this is consistent with the report of Ashokkumar *et al.* (2026).

Carrot, date palm and tiger nuts are vegetable fruits with sufficient fibrous content enough to address fibrous related gastrointestinal discomfort. Because they can be eaten in raw form, they are often enjoyed at any time of the day provided it is their season of availability. From the results obtained from their proximate analysis, it is clear that vast differences exist between the dry form and fresh form of these vegetables. This supports the findings of Andrade *et al.* (2020) who reported that method of extraction, varieties and species of the fruits could contribute to the differences in the values of these proximate.

The proximate analysis results of the samples in fresh and dry forms have shown that there are vast differences in their protein content, moisture and fibre as well as carbohydrates. According to Khili *et al*. (2025) these fruits retain quality nutrients, moisture and carbohydrates that make them palatable for consumption in their fresh forms. The significant differences noticed in these values between the two forms of the samples are sufficient to meet up the daily requirements of nutrients. In a related study of the proximate analysis of some selected fruits in their different forms, Attitalia *et al*. (2025), noticed a significant difference of over 50% differences as a short-fall in the values obtained for the fresh and dry forms of the fruits selected respectively, validating the results obtained in this study.

Nina *et al*. (2019) stated that the significant differences in the dry form of tiger nut and its fresh form was majorly caused by imbibition; a term used to describe absorption of water from the surroundings due to osmotic differences in the environment of a substance. Comparatively, the dry samples showed significant increase in the proximate analysis values of the samples over the fresh ones, except for moisture content that showed significant decrease in value. This result is in agreement with the findings of Adekiya *et al*. (2020) in which the fresh form of tiger nut was much more in values for proximate analysis compared to the fresh form with the exception of the moisture content. These variation in proximate values of fresh and dry forms of plant extracts is in agreement with the study of Fadare *et al.* (2025).

Tiger nut demonstrated higher crude fibre content over the carrot and date palm in both fresh and dry forms. (3.60% fresh; 28.06% dry) compared to Carrot (0.10% fresh, 1.80% dry) and date palm (0.08% fresh, 0.88% dry). This emphasizes the nutritional value of tiger nut as a rich source of dietary fats, Udeh *et al.* (2026).

The moisture contents of the three extracts are high as discovered in this study, indicating their perishable nature and limited shelf life which is in agreement with the findings of Udeh *et al.* (2026) where tiger nut showed more moisture content (37%) compared to clove's 1.7%.

Studies have shown that the proximate analysis values of the dry forms of the samples are higher than the fresh ones with few exceptions, Adekiya *et al.* (2020). However in another report, Fadare *et al.* (2025) also indicated various proximate values in both fresh and dry forms of *Carica papaya* leaves.

The presence of phytochemicals such as alkaloids, tannins, saponins, steroids, phenols, flavonoids, anthraquinine, anthocyanin, anthraquinone, glycosides, terpenoids and carotenoids is a testament of their rich medicinal properties which is consistent with the report of Bakry *et al.* (2026). They stated that the value of a medicinal plant is known by the presence of these phytochemicals and how much of them are easily harnessed by the system. Quantitatively, these abundant phytochemicals are also uniquely high, with carotenoid having a value as high as 3.71±0.09% in carrots. This makes carrot unique and adds specifically to the blend of the mixture when it's combined, this is in agreement with Ikram *et al.* (2024).

It was found that tannins, saponin and flavonoids was very high in date palm, a result that is corroborated with the results obtained by Attitalia *et al*. (2025), in which they discovered that these phytochemicals are not just present in abundance in these fruit but they are also easily accessible by human body system.

In terms of antibacterial quality, the extracts were generally more potent against Gram positive bacteria than the Gram negative ones used in this research, with the exception of date palm extract on *Pseudomonas*. This may be due to the fact that the Gram positive bacteria are from the skin and may be susceptible to the fruits extracts, similar to the report of Nail, (2024), that where an organism is isolated from, plays a vital role in its susceptibility to antibacterial agents. The extracts exert the least antibacterial effects on *Escherichia coli*, with tiger nut extract having the least inhibitory effect with a zone of inhibition diameter of 10.00±0.58 mm. This may be due to the fact that this organism may have been used to these fruit substance in the human gut.

This study showed that the Gram positive organisms were more sensitive to the extracts than the Gram negative ones as reflected in their zones of inhibition. This could be as a result of the cell wall composition, peptidoglycan, that is present in Gram positive bacteria but absent in Gram negative bacteria. This is in agreement with the work of Saikachi *et al.* (2021).

Sensitivity of the test microorganisms to these extracts in this study, support the study of Qanash *et al.* (2026) that carrot extracts and seed oils possess antibacterial properties. Also, in this study, carrot extracts inhibited the growth of Gram positive and Gram negative test organisms, which is in agreement with the report of Panduwati *et al.* (2025) who attributed their findings to the presence of bioactive compounds like flavonoids, saponins, tannins and alkaloids, which were also discovered in this test extract.

In corroboration with the report of Bakry *et al.* (2026), Thazzawy *et al.* (2026), date palm contains many phytochemicals such as phenolic acids, tocopherols, flavonoids, tocotrienols and tannins,also in agreement with Abdelbaky *et al.* (2023).

In this study, date palm extract exerted more growth inhibition to Gram positive organisms, *Staphylococcus aureus* and S*treptococcus pyogenes* than to Gram negative organisms - *E.coli* and *P. aeruginosa* which is in agreement with ALrajhi *et al.* (2019) due to the presence of peptidoglycan cell wall material in Gram positive organisms and lacking in Gram negative organisms covered by more tightly netted cell membrane.

A number of phytochemicals were discovered in tiger nut extract in this study like flavonoids, phenolics, sterols and fatty acids as indicated in the earlier studies of Yang *et al.*(2022), Samuel *et al.*(2023) and Edo *et al.*(2023).

In agreement with the report of Naeem and Youssef (2022), tiger nut extracts in this study was active against both Gram positive and Gram negative microorganisms. In comparison with conventional/commercial antibiotics, tiger nut extract showed higher efficacy in support of the report of Nwosu *et al.* (2022).

All the test plant extracts showed greater antibacterial activity than the conventional antibiotics, in agreement with the report of Awadh and Ahmed *et al.* (2025).

The result of the antibiotic susceptibility of the isolates when they were subjected to commercial antibiotics, the bacteria isolates generally showed very little sensitivity effect to the commercial antibiotics used. From the figure, *E.coli* was only moderately sensitive to ampicillin while the two Gram positive test bacteria were completely resistant to the antibiotic. Three of the bacteria isolates were sensitive to chloramphenicol. *S. aureus* was however resistant to chloramphenicol without any zone of inhibition recorded for it. The antibiotic Colistin sulphate was effective against the two Gram positive bacteria but not on any of the Gram negative bacteria. Kanamycin on the other hand was effective against the two Gram negative bacteria while the two Gram positive bacteria were resistant to it. Nalidixic acid and streptomycin were the only antibiotics that the four bacterial test isolates were sensitive to.

Tetracycline was equally effective against the two Gram positive bacteria used while the Gram negative bacteria showed resistance to it. Nitrofurantoin was effective against *E. coli* and *S. aureus* but not effective

against *Pseudomonas* and *Streptococcus* respectively. Therefore, the antibiotics did not follow any specific pattern of activity in terms of their action against the test isolates used in this research. Comparatively, the extracts were more effective as antibacterial agents than the commercial antibiotics used in this research. These results are similar and are in agreement with the results obtained by Chika (2022).

The resistance of Gram positive bacteria to commercially available antibiotics may be due to their complex cell wall that consist of peptidoglycan which are not easily penetrated by these antibiotics which corresponds with the report of Prescott *et al*. (2024). The three bacteria isolates that were sensitive to chloramphenicol were majorly Gram positive. However, *S. aureus* was resistant to chloramphenicol without any zone of inhibition recorded for it. The antibiotic Colistin sulphate was effective against the two Gram positive bacteria but not on any of the Gram negative bacteria. Kanamycin on the other hand was effective against the two Gram negative bacteria while the two Gram positive bacteria were resistant to it. Nalidixic acid and streptomycin were the only antibiotic that the four bacterial test isolates were sensitive to. These results were similar to the results obtained by Latif and Nawaz (2025).

Tetracycline was equally effective against the two Gram positive bacteria used while the Gram negative bacteria showed resistance to it. Nitrofurantoin was effective against *E. coli* and *S. aureus* but not effective against *Pseudomonas* and *Streptococcus* respectively. Therefore, the antibiotics did not follow any specific pattern of activity in terms of their action against the test isolates used in this research. Comparatively, the extracts were more effective as antibacterial agents than the commercial antibiotics used in this research. This result is however, contrary to the results obtained by Saadony *et al.* (2025).

The GC–MS result of carrot extract's bioactive ingredients identified high antioxidants capable of exerting major biological effects on the immune system to overcome disease causing agents. The major bioactive compounds identified and their relative abundance are: Hexadecanal (1.02%), methyl esters of fatty acids (1.21–1.91%), **Palmitic acid (36.25%), Phytol (7.14%), Oleic acid (38.87%)**, and **Stearic acid (13.60%)**. This result adequately support the use of carrot as an alternative therapy for many common diseases because according to Hamdeni et al., (2026), this bioactive phytochemical inaddition to flavonoids works magic in overcoming cancerous and mutagenic cells.

The minor compounds identified in carrot extract is dominated by **oleic acid (38.87%)** and **palmitic acid (36.25%)**, followed by **stearic acid (13.60%)** and **phytol (7.14%)**. These compounds are known for their strong **antioxidant activity, anti-inflammatory effects, antimicrobial activity, p**otential **anticancer properties, cardio-protective effects,** potential **antidiabetic activity,** hepato-protective and immunomodulatory effects (mainly due to phytol) as outlined by Propovici *et al.* (2026). Therefore, the GC–MS profile suggests that **carrot extract** possesses significant **antioxidant, antimicrobial, anti-inflammatory, cardioprotective, and potential antidiabetic/anticancer properties**, largely attributable to oleic acid, palmitic acid, stearic acid, and phytol.

Date palm on the other hand revealed eight major bioactive constituents. The dominant compounds were **Oleic acid (32.59%)**, **Palmitic acid (31.26%)**, **11-Octadecenoic acid methyl ester (13.68%)**, and **Stearic acid (10.89%)**. The GC–MS profile indicates that sample date palm is rich in **unsaturated and saturated fatty acids and their esters**, especially **Oleic acid (32.59%)** – major contributor to cardioprotective, antidiabetic and anti-inflammatory activity, Kuwar *et al.* (2026).

Palmitic acid (31.26%) – contributes antimicrobial and antioxidant properties; **11-octadecenoic acid methyl ester (13.68%)** – contributes antioxidant and membrane-protective effects, **stearic acid (10.89%)** – contributes anti-inflammatory and antimicrobial activity, f**atty acid amides (Stearamide, Lauramide, Myristamide)** – may enhance antimicrobial and anti-inflammatory properties, Balal *et al.* (2024).

Overall Medicinal Potential in the date palm extract possess strong **antioxidant activity, s**ignificant **anti-inflammatory activity, Antimicrobial and antifungal effects,** Potential **antidiabetic activity, cardioprotective and hypolipidemic effects, p**otential **anticancer activity, h**epatoprotective effects and wound-healing and tissue-protective properties. These biological activities are mainly attributed to the high

concentrations of **oleic acid, palmitic acid, stearic acid, and fatty acid esters/amides** present in the extract Manchor *et al*. (2024).

The GC–MS chromatogram of sample **tiger nut** contained 15 peaks. The major bioactive compounds identified were dominated by **Oleic acid (38.57%)**, **Palmitic acid (16.05%)**, **Linoleic acid methyl ester derivatives (12.47%)**, **Stearic acid (11.26%)**, and **11-Octadecenoic acid methyl ester (1.23%)**. **Monounsaturated fatty acids** (especially oleic acid), p**olyunsaturated fatty acids** (linoleic acid derivatives), s**aturated fatty acids** (palmitic, stearic, myristic and arachidic acids), f**atty acid esters and monoglycerides and antifungal fatty acids** (undecanoic and undecylenic acids). The major predicted biological activities of tiger nut extract are strong **antioxidant activity, p**otent **anti-inflammatory activity, s**ignificant **antimicrobial and antifungal properties, cardioprotective effects, p**otential **antidiabetic activity, p**otential **anticancer activity, w**ound-healing and tissue-protective properties, cholesterol-lowering (hypolipidemic) effects and immunomodulatory activity Ikram *et al*. (2024).

The medicinal properties are mainly attributable to the high abundance of **Oleic acid (38.57%)**, **Palmitic acid (16.05%)**, **Linoleic acid derivatives (≈13%)**, and **Stearic acid (11.26%)**, which together account for over 75% of the total GC–MS profile Manchor *et al.* (2024).

The anticancer assay in a 96-well MTT cell viability assay used to evaluate the in vitro anticancer activity of test samples showed the color differences that indicate different amounts of formazan, which is proportional to the number of metabolically active (viable) cells as outlined by Propovici *et al.* (2026). Accurate anticancer activity, which was interpreted using the absorbance values at 570 nm obtained from a microplate reader showed that qualitatively, there was high cell viability as well as low cytotoxicity of the extracts used with varying concentrations.

The pale yellow/orange wells indicates low formazan production which by interpretation means low cell viability and high cytotoxicity of the extracts. The area with completely clear wells equally indicates very few or no viable cells which means there is nearly complete inhibition of the extracts. The gradual change in color across the 96 wells in the entire assay suggests a dose-dependent effect of all the extracts used, which is expected due to the serial dilutions of the extracts used that served as an anticancer agent in this assay as interpreted and supported by the work of Sarwade *et al.* (2026). Comparatively, the extract of the three combinations ratio 1:1:1 had the highest anticancer property as interpreted by Lan (2026).

The molecular docking results correlate well with the experimental antibacterial activities observed for the extracts. The presence of high-affinity phytocompounds such as quercetin, luteolin, apigenin, kaempferol, and γ-sitosterol in tiger nut, carrot, and date palm fruit extracts provides a molecular basis for the observed antibacterial effects against *E. coli* and *S. aureus*.

The strong binding of flavonoids to Penicillin-Binding Protein 4 (4OUR) suggests that these compounds may exert antibacterial activity by interfering with bacterial cell wall biosynthesis, a mechanism complementary to, or synergistic with, standard β-lactam antibiotics. This finding supports the observed synergistic effects between the plant extracts and antibiotics.

Several structure-activity relationships can be deduced from the docking results:

1. Flavonoid Hydroxylation: The presence of multiple hydroxyl groups, as seen in luteolin (-8.4) and quercetin (-8.7), enhances hydrogen bonding capacity and binding affinity compared to less hydroxylated flavonoids. Planarity and Aromaticity: The planar structure of flavonoids facilitates π-π stacking with aromatic residues such as TRP191 (as seen with imbricatonol), contributing to binding stability. Steroid Nucleus: Plant sterols such as γ-sitosterol and stigmasterol utilize their rigid steroid nucleus for extensive hydrophobic interactions, while a single hydrogen bond (as with ARG240) provides directional specificity.

2. Sugar Esters: The dual hydrophilic-hydrophobic nature of xylitol laurate allows it to interact with both polar and non-polar regions of the binding pocket, maximizing binding affinity.

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The docking results provide mechanistic support for the observed synergistic antibacterial effects of the combined extracts. The phytocompounds target multiple bacterial proteins (PBPs, DNA gyrase, DHFR, etc.) through different mechanisms, making it difficult for bacteria to develop resistance. The use of multi-target natural products, particularly flavonoids and sterols, represents a promising strategy for combating antibiotic-resistant infections.

Molecular docking analysis revealed that several phytocompounds from tiger nut, carrot, and date palm fruit exhibit strong binding affinities to Penicillin-Binding Protein 4 (4OUR), a validated antibacterial target in *E. coli*. Compounds such as xylitol laurate (-9.2), imbricatonol (-9.2), γ-sitosterol (-8.8), and quercetin (-8.7) demonstrated higher binding affinities than the standard antibiotic ampicillin (-7.6), supporting their potential as antibacterial agents. The interaction profiles revealed hydrogen bonds, hydrophobic contacts, and π-π stacking interactions that stabilize the ligand-protein complexes. These findings provide a molecular basis for the observed antibacterial and synergistic effects of the extracts and warrant further experimental validation.

The biological activities of complex plant-derived preparations are unlikely to be explained adequately by a single constituent acting on a single molecular target. In the present investigation, this consideration was particularly relevant because the aqueous decoctions of carrot, date palm fruit and tigernut yielded a chemically diverse group of GC–MS library-identified constituents, including long-chain fatty acids, fatty-acid derivatives, aldehydes and phytol. Thirteen compounds were selected as the core compounds for the subsequent computational analysis. These comprised palmitic acid, oleic acid, stearic acid, lauric acid, myristic acid, pentadecanoic acid, arachidic acid, erucic acid, undecylenic acid, hexadecanal, phytol, methyl 14-methylpentadecanoate and methyl 11-octadecenoate.

The use of these compounds as the basis of the computational investigation should nevertheless be interpreted in the context of the GC–MS methodology. A library match provides evidence that the experimental mass spectrum is consistent with a reference spectrum, but does not necessarily constitute complete structural confirmation. Consequently, the compounds were treated throughout the computational workflow as **GC–MS library-identified compounds** or **putatively identified phytochemical constituents**, rather than as unequivocally confirmed structures.

The computational strategy subsequently progressed from compound identification to target prediction, network construction, target prioritization and molecular docking. SwissTargetPrediction was used to generate candidate human protein targets for the 13 selected compounds, while Cytoscape was used to visualize and interrogate the resulting compound–target relationships. The combined approach provided a systems-level framework for linking the chemical composition of the extracts with molecular mechanisms that could plausibly contribute to the observed biological effects.

The SwissTargetPrediction analysis generated approximately 1,300 compound–target prediction records from the 13 selected compounds. Rather than considering these interactions independently, the predictions were consolidated into a compound–target network for network-pharmacology analysis.

The resulting network is consistent with a **multicomponent–multitarget** model in which individual compounds may be associated with multiple protein targets while individual proteins may be predicted as targets of multiple compounds. This type of organization is particularly relevant to plant-derived preparations, because the biological response of an extract may arise from the combined contribution of several constituents rather than from one dominant chemical entity.

Target recurrence within the network provided an initial indication of potential convergence points. For example, **PTPN1** was predicted for all 13 compounds, with a maximum STP probability of 1.0 and a mean probability of approximately 0.839. **PTPN2** was likewise predicted for all 13 compounds, with a mean probability of approximately 0.704. Other recurrent targets included **FABP3**, **FABP4**, **FABP5**, **PPARG**, **PPARA**, **PPARD**, **PTGS2**, **MMP2** and related proteins. The recurrence of these targets does not establish that all of the corresponding compounds are biologically active against each protein; rather, it identifies candidate points of molecular convergence worthy of further examination.

Protein tyrosine phosphatase non-receptor type 1 (PTPN1; PTP1B) and **protein tyrosine phosphatase non-receptor type 2 (PTPN2; TC-PTP)** emerged as particularly important computational targets. Both were recurrent across the 13 compounds and were subsequently retained during biological target prioritization.

PTPN1 and PTPN2 are intracellular phosphatases that negatively regulate signaling pathways, including pathways associated with cytokine and immune-cell signaling. Current literature describes these phosphatases as important regulators of antitumour immunity, with PTPN1 and PTPN2 capable of attenuating signaling through pathways such as JAK–STAT and the T-cell receptor axis. Experimental studies have therefore investigated their inhibition as a potential strategy for increasing antitumour immune responses.

PTPN2 is of particular relevance to the immunostimulatory component of the present study. Experimental work has demonstrated that altered PTPN2 activity can affect inflammatory cytokine responses, and loss of PTPN2 has been associated with increased immune responsiveness and improved immune-mediated tumour clearance.

Consequently, the recurrence of PTPN1 and PTPN2 within the compound–target network provides a plausible computational link between the phytochemical composition of the aqueous preparations and biological processes relevant to immune regulation. However, the network result should not be interpreted as demonstrating inhibition of either phosphatase by the extracts. Rather, it identifies these proteins as **candidate molecular mediators that warrant structural and experimental investigation**.

Peroxisome proliferator-activated receptor gamma (PPARG) was among the highly recurrent targets identified from the STP analysis. Its identification is particularly interesting in the context of the chemical composition of the investigated extracts because several of the selected compounds are fatty acids or structurally related lipid constituents.

PPARG is a ligand-activated nuclear receptor involved in lipid metabolism and in the regulation of macrophage differentiation, inflammatory activation, polarization and lipid handling. Consequently, modulation of PPARG provides a plausible connection between the lipid-rich chemical profile of the extracts and pathways involved in immune-cell behavior.

The identification of PPARG in the network does not establish whether the corresponding phytochemicals act as agonists, antagonists or partial modulators. The docking stage therefore provided a complementary structure-based approach for investigating whether selected compounds could occupy experimentally characterized ligand-binding sites within PPARG.

The fatty-acid-binding proteins **FABP4** and **FABP5** were among the highly recurrent targets within the network. Their identification is consistent with the predominance of long-chain fatty acids and related lipid compounds within the selected GC–MS dataset.

FABP4 is involved in intracellular lipid transport and signaling and has also been linked to inflammatory and cancer-associated processes. Literature has reported relationships between FABP4, JNK and NF-κB signaling in macrophages, as well as associations between FABP4 and IL-6/STAT3 signaling in cancer-related contexts.

The presence of FABP4 and FABP5 in the computational network therefore provides a mechanistic hypothesis connecting the lipid constituents of the investigated decoctions with metabolic and inflammatory pathways that may influence both tumour biology and the immune microenvironment.

The significance of these predictions is enhanced by the convergence of several structurally related compounds on the same target class. Importantly, however, such convergence should not be interpreted as evidence that every fatty-acid constituent acts directly on FABP4 or FABP5 in the biological system. Instead, the network identifies these proteins as candidate common nodes through which multiple constituents might potentially influence downstream responses.

Prostaglandin G/H synthase 2 (PTGS2; COX-2) was also identified among the recurrent computational

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targets and was subsequently considered during biological target prioritization.

PTGS2 is involved in prostaglandin biosynthesis and is widely recognized as an important component of inflammatory signaling. The COX-2–PGE2 axis can influence the tumour immune microenvironment through effects on myeloid-derived suppressor cells, lymphocytes, macrophages and antigen-presenting cells, and has therefore become an important area of investigation in cancer immunology.

The identification of PTGS2 therefore provides another potential bridge between the phytochemical network and the biological endpoints of the present investigation, particularly where cytotoxicity and immune modulation occur together.

Matrix metalloproteinase-2 (MMP2) represents a different but complementary aspect of the predicted network. MMP2 is involved in extracellular-matrix remodeling and has important associations with cancer-cell invasion and metastasis.

MMPs can also influence the tumour microenvironment by modifying extracellular-matrix components and affecting cytokine and growth-factor signaling. Accordingly, MMP2 provides a potential molecular link between the computational network and processes associated with tumour progression rather than solely tumour-cell viability.

Its inclusion in the prioritized target set therefore increased the mechanistic breadth of the docking study by extending the analysis beyond lipid metabolism and intracellular signaling to extracellular-matrix remodeling.

Although the network contained a large number of predicted human proteins, it was not practical or scientifically justified to subject every predicted target to molecular docking. Target prioritization was therefore performed using a sequential screening strategy.

First, target recurrence, STP probability and STP rank were used to identify **Tier-1 core targets**. These criteria identified proteins that were repeatedly predicted across the compound set and were associated with relatively strong or high-ranking predictions.

Second, these recurrent targets were evaluated according to their **biological relevance** to the major objectives of the study, particularly anticancer activity, inflammation, immune regulation and tumour progression.

Third, the shortlisted targets were evaluated for **structural suitability for molecular docking**, with preference given to experimentally determined human protein structures deposited in the Protein Data Bank and possessing defined ligand-binding or catalytic sites.

This process reduced the large STP-derived target space to a smaller group of experimentally tractable proteins for molecular docking. The final human target panel included **PTPN1, FABP4, PPARG, FABP5, PTPN2, PTGS2, MMP2, AKR1C3, TRIM24 and AKR1B10**.

Molecular docking was subsequently used to investigate whether the selected GC–MS-derived compounds could plausibly occupy experimentally characterized binding sites within the prioritized proteins.

The purpose of docking at this stage was **not to prove that the predicted targets were experimentally engaged by the plant extracts**, but rather to provide structural evidence supporting or challenging the plausibility of selected compound–target relationships suggested by the network analysis.

The distinction is important. SwissTargetPrediction identifies potential targets on the basis of molecular similarity and experimentally annotated compound–target relationships, whereas docking evaluates the structural compatibility of a particular ligand with a selected protein binding site. The two methods therefore address related but different questions.

A target that is recurrent in the STP/Cytoscape network but gives an implausible docking pose or a poorly defined binding mode should not automatically be considered a confirmed mechanism. Conversely, a

chemically plausible docking pose involving biologically important residues can strengthen the mechanistic hypothesis generated by the network analysis.

Thus, the computational sequence used in the present investigation may be summarized as:

GC–MS-derived compound → SwissTargetPrediction → Cytoscape compound–target network → target prioritization → biological/PDB screening → molecular docking.

Biological relevance and structural screening then reduced the broad target space to a smaller group of proteins appropriate for molecular docking. Docking was subsequently used to investigate whether selected GC–MS-derived compounds could occupy experimentally characterized binding sites and establish chemically plausible interactions.

The resulting computational evidence should be interpreted in conjunction with the experimental cytotoxicity, antibacterial and immunological findings. Concordance between the experimental phenotype and the predicted molecular mechanisms strengthens the biological plausibility of the proposed mechanism, whereas discrepancies highlight the complexity of plant-derived preparations and the limitations of computational target prediction and docking.

CONCLUSION

Summarily, the findings support the view that the biological properties of the investigated carrot, date palm fruit and tigernut aqueous preparations are more appropriately considered within a multicomponent–multitarget framework than through a single-compound mechanism. The network pharmacology analysis therefore provides the conceptual bridge between the GC–MS chemical profile and molecular docking, while the experimental assays remain essential for establishing the actual biological effects of the preparations.

From this study, the results have shown that the combination of the extracts have antibacterial effects on the selected Gram positive and Gram negative bacteria used in this work thereby suggesting the use of its bioactive ingredients in the production of novel antibiotics against the disease caused by the microorganisms.

The results obtained on the in-vivo use of the extracts when rats were induced with carrageenan also points to the fact that it has a very high anti-inflammatory effect, which means that it can be used to treat inflammation as well as correct inflammatory effects on the physiological process of man especially when combined in synergistic form (CDT, 1:1:1).

Also the in vitro anticancer/cytotoxic effect showed that it has 87% inhibition on proliferation of cells, suggesting that it has very high anticancer effect, which means that in its purified form, it might exert more effect physiologically.

Therefore the synergistic combination of the three extracts in their natural forms has a very high therapeutic immunostimulatory, anti-inflammatory, immunomodulatory and anticancer or anti-cytotoxicity effects.

Extract blends in this study showed superior activity. Synergy significantly enhanced the effects of the extracts, CDT (1:1:1) was the most effective.

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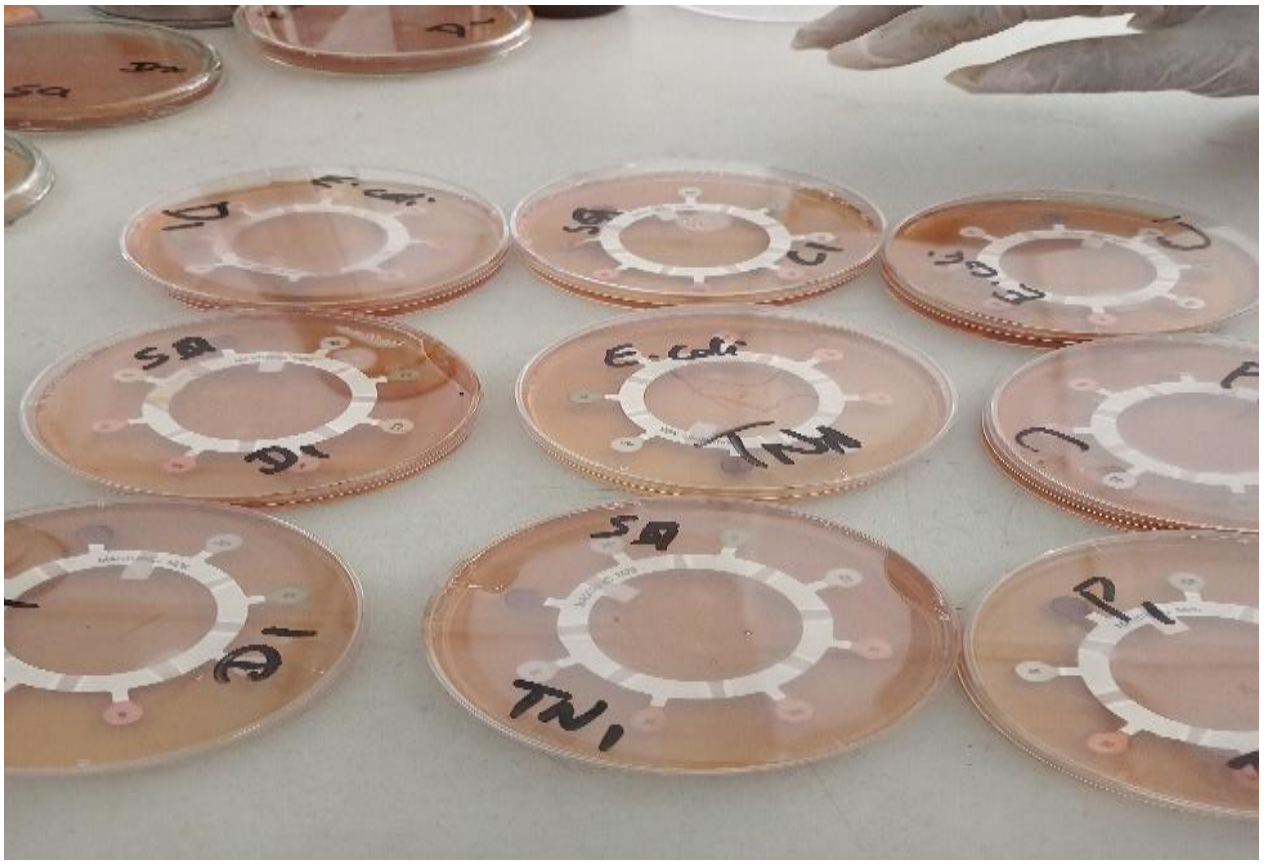


Plate 5: Sensitivity Test on selected bacteria with Commercial Antibiotics

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Plate 6: Ultra –filtration of extract using membrane filter (0.45um) for antibacterial assay.



MTT Assay 96 Well Plate



MTT Assay with Extracts at 72hrs