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Original Article

Cheminformatic and *in vitro* Bioprospection of *Capsicum Annuum* L. Metabolites as DNA Gyrase B Inhibitors

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ABSTRACT

Introduction: *Capsicum* species are known in food and trado-medicinal uses for maladies management due their rich content of phytochemicals, but with little work done on *in silico* bioprospection of its volatilome. **Objectives**: This study targeted chemometric profiling, virtual bioprospection of potential lead metabolites in 2 *Capsicum annuum* L. fruit variants' (green and red) to identify lead gyrase B inhibitors (GBIs) and provide new mechanistic insights. **Methods**: Metabolites were profiled using Gas Chromatography-Mass Spectrometry (GC-MS), and quantitative phytochemical assays. Extracts antioxidant (DPPH, ABTS, FRAP) and antibacterial (susceptibility testing) activities were also determined. *In silico* [docking, pharmacokinetics, DFT] analyses were used to identify and predict chemical features of potential lead GBIs key to extracts molecular mechanism of action. **Results**: Mass spectral analysis identified hydrocarbons, fatty acid and other derivatives. Quantitative phytochemical analysis showed flavonoids, cardiac glycosides and alkaloids. The green *C. annuum* extract had better antioxidative action, while extracts of both green and red variant showed similar antimicrobial profiles against resistant bacterial pathogens. *In silico* highest docking scores were observed for [1-Ethyloctyl) cyclohexane (-6.6 kcal/mol)] and dibutyl phthalate (-6.4 kcal/mol). All lead GBIs had desirable pharmacokinetics in line with the Lipinski rule of 5, and chemical reactivity properties. **Conclusion**: *In silico* and *in vitro* methods combination provided robust metabolomic profiling. The identified lead *C. annuum*-based natural GBIs contribute to the bioactivity profile and molecular mechanism of action of fractions. The study provided a first-hand report on natural GBIs derivable from *Capsicum* fruits which could be exploited in formulations for non-food and pharmaceutical applications.

Keywords: *Capsicum annuum*, Phytochemicals, Antioxidant activity, Antimicrobial activity, Gyrase B, Computational analysis.

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INTRODUCTION

Globally, and since the emergence of COVID-19, there has been a heightened sensitization on the need to exploit natural plant products or secondary metabolites to bridge the increased incidence of emerging and re-emerging infection.[1] Moreso, close to eighty percent of the world's populace have shown dependency on natural remedies to tackle diseases. However, most fall within the African continent and developing countries.[2] Medicinal plants have been utilized for centuries as natural remedies for human disorders and infections and is closely associated with their content of biologically active components, which show varied activities such as antioxidant and antimicrobial activities. Plant secondary metabolites are inherent in various plant parts and are usually extracted using inorganic or organic solvents with varying polarity index. Again, it is known that plants processing and pre-processing techniques are key in maintaining the pharmacological properties for various health benefits, and as such should be carefully chosen.[3]

A plant that has been the subject of continued research is the *Capsicum* family of peppers. The bell peppers (*Capsicum annuum* Linn) within the family Solanaceae, are a group of nutrient and phytochemical dense food species easily grown worldwide, and available in different variants. Variants may be differentiated by colour (yellow, green, red), size, shape, and taste (sweet, spicy, or hot).[4] Once ripened, the taste and phytochemical content of peppers are determined by harvest time, storage conditions and practices, as well as their environment of growth.[5]

Studies on members of the *Capsicum* genus continue to collate information on the biochemical, chemical, and botanical fingerprint features on grown *Capsicum* variants, and facilitate identification of new metabolites from superior breeds.[6] *Capsicum* extracts have shown effectiveness against yeast species such as *Pichia*, *Candida*, and *Kluveromyces* cells, with antifungal action attributed to the presence a bioactive thionin compound, as well as their rich content of vitamins (A and C), tannins, flavonoids, polyphenols.[7] These chemical products have extensive food industry applications (additives and preservatives) and show an array of biological activities. Pharmacological effects associated with *Capsicum* peppers include cholesterol reduction, anti-obesogenic and antidiabetic[8], and anti-inflammatory properties.—[9] Natural *Capsicum* products like corneleic acid, and flouram C-glucoside, and alkaloids and glycosides also demonstrate pharmacological activities.[10] However, despite its rich content of bioactive natural products, little work has been done using computational workflows [molecular docking, pharmacokinetic, density functional theory (DFT)] in bioprospecting the plant's metabolites to provide new insights into potential action mechanisms of *Capsicum* extracts, and for the identification of lead bioactive and 'drug-like' moieties, compared to those focused on known *Capsicum* associated capsaicinoid).[11,12]

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In addition, plant metabolites also serve as fingerprints for the identification and comparison of plant species, since compound profile of plants vary with geographical location.[13] Typically, plant metabolites identification is usually best achieved with a combination of analytical techniques for a robust coverage of their natural products, and metabolomic workflows usually combine chromatographic [liquid (LC) or gas (GC)] separation with mass spectrometry (MS).[14] The GC-MS characterizes thermostable/volatile metabolites and their derivative reactions and produces reproducible fragment patterns under stable electron ionisation energy condition to enhance metabolites detection and identification.[15] In addition, while certain small molecules are key in inhibiting bacteria, antibiotics (clorobiocin, novobiocin) used as DNA gyrase B inhibitors have been shown to produce adverse side effects and are no longer effective against bacterial species such as Staphylococci.[16] Hence the global ongoing scientific interests focused on identifying new plant-related natural molecules with antimicrobial potential. Furthermore, given the function of gyrase B in bacterial DNA supercoiling, replication, transcription, and bacterial survival and growth, as well as it being a recognised target in the search for novel antibacterial medications[17], it was chosen as a druggable target for our cheminformatic study.

In view of the foregoing and need for continued research to expand on current scientific knowledge on *Capsicum* species metabolites' profile for enhanced industry uses, this study aimed to compare the metabolomic profiles (using GC-MS, quantitative phytochemical assays) of two *Capsicum annuum* Linn. variants, assess the bioactivities of derived fractions, followed by *in silico* bioprospection of metabolites to identify potential lead compounds central to extracts' antibacterial action mechanism and which possess DNA gyrase B inhibiting activity. The technical workflow for this study is depicted in the graphical abstract.

MATERIALS AND METHODS

Chemicals, reagents and media

The chemicals and reagents used were purchased from Merck (Modderfontein, South Africa) as analytical grade, while Oxoid (Hampshire, United Kingdom) culture media products were used.

Plant sample and culture collections

Capsicum annuum (Linn.) red and green fruits were purchased from the Akure Metropolis main market (Oja Oba), Nigeria, and identity confirmed in the Flora of West Tropical Africa collections[18], and prior to laboratory processing. All test bacterial cultures used in assays were typed multidrug resistant food-borne pathogens, and obtained from the Department of Microbiology, FUTA, Nigeria. Microbial culture stocks were kept on slants at 4°C, and fresh, overnight broth cultures prepared for use in assays. Bacterial cultures used included *Bacillus pumillus*, *Staphylococcus gallinarum*, *Staphylococcus equorum*, *Bacillus amyloliquefaciens* and *Bacillus cereus*.

Extraction of green and red bell peppers

The fruits were first washed to remove debris, then subjected to a distilled water wash, and thereafter cut into smaller pieces. The collected plant materials were then oven-dried to constant weight at 40°C and milled into fine powder using laboratory grade blender (Haden, CaterWize, South Africa) and kept refrigerated in sealed glass containers prior to further analysis. Then, about 5 g of plant powder were measured into appropriately labelled flasks containing 50 mL of 50% ethanol and shaken at 60 rpm, 25°C for 48 h. Thereafter, filtration with Whatman paper (No. 1) was used to separate extract filtrates from residue, and the filtrate extraction solvent was evaporated in an evaporator (SolventVamp 5L, Oman) at 60 rpm and 45°C. Extracts were thereafter reconstituted prior to use in related analysis.[19]

Metabolomic profiling of *Capsicum* extracts

GC-MS analysis of extracts. Extracts were filtered to remove impurities prior to analysis on Agilent Technologies (MS 5975C, GC-7890A). Instrument parameters were 50°C initial temperature (2 min), then 150°C (1 min) at per minute 4°C increments to a temperature of 250°C for about 3 min (per minute ramp of 8°C), 36 cm/s velocity. Ionisation source temperature kept stable at 250°C, helium carrier gas flow rate (1 mL/min) at 196 kPa pressure, and 70 eV ionization voltage, with injection of samples in split mode (10:1) and scan range set at 30-450 *m/z*. Mass spectra and line transfer line done at 280°C. The library of mass spectra (NIST 11) was used in low weight, thermostable volatilome identification.

C. annuum fractions quantitative phytochemical analysis

The quantitative assays included determination of total flavonoid, tannin, cardiac glycoside, and alkaloid contents.[20]

Antioxidant assays

2,2-Diphenyl-1-picrylhydrazyl (DPPH)

This assay was done using 50 µL extract which was added to 0.1 mmol/L of freshly prepared DPPH solution. The mixture was stirred, incubated in the dark (30) min, and absorbance read at 517 nm. Methanol (80%), DPPH in methanol and ascorbic acid served as blank, negative, and positive (ascorbic acid) controls, respectively. This was followed by calculation of percentage of DPPH scavenged/inhibited.[21]

$$I\ (\%) = [(A_o - A_{1)}] / A_o] * 100 \qquad (1)$$

[I% = % inhibition, A_o = absorbance of negative control, A_1 = absorbance of the extract/standard]

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) test

The generation of $ABTS^{+}$ radical was done (7 mM ABTS plus 2.45 mM potassium persulfate mixed in water). The reaction was left to stand in the dark at 25°C 12-16 h prior to use in assay. Methanol was used to dilute the radical to a of 0.70 absorbance measured at 734 nm. Then, to extract aliquots of 5 µL, 3.995 mL of the diluted radical solution was added, mixed, and absorbance taken (at 734 nm) after 30 min. The assay was replicated, and extracts scavenging activity determined.[22]

The ferric reducing antioxidant power (FRAP) test

The preparation of a new batch of FRAP reagent was done and incubated for 15 min at 37°C prior to use. Then the reagent (2.85 mL) was added to 150 µL of plant extract, and mixture left to incubate in the dark for about 30 min. Then absorbance of the coloured complex formed was read at 593 nm. Ascorbate served as positive control. The assay was replicated and results shown as µM (Fe (II)/g dry mass.[23]

Antibacterial activity assays

Antibacterial susceptibility - disc diffusion method

Antimicrobial susceptibility test was carried out using the disc diffusion method.[24] A standardized 100 µL bacterial broth (at 0.5 McFarland) for each bacterium was spread under aseptic conditions on appropriately labelled Petri plates (for each extract and bacterium) containing solidified Mueller Hinton agar (MHA). Then, earlier prepared dried discs separately impregnated with respective extracts were aseptically transferred onto respectively labelled and inoculated plates with sterile forceps. Following adequate diffusion, plates were inverted and placed in an incubator set at 37°C for 24 h. The diameter of inhibition zone was then recorded in 'mm'.

Minimum inhibitory concentration (MIC) assay

The microbroth dilution method was used in MIC determination. A 0.5 McFarland standard of each test bacterium (20 µL) was used in wells inoculation following prior dispense of sterile broth and serial dilution of extracts (250 to 15.6 mg/mL) to a final well volume of 100 µL. Alongside, positive (growth) and negative controls were prepared in separate wells. The set-up was incubated for 24 h at 37°C. The assay was done in triplicate, and the lowest concentration inhibiting bacterial growth was reported as the MIC (mg/mL), following plating of aliquots from treatment wells to ensure accurate determinations.

Computational analyses

Molecular docking

For molecular docking, the 3D structure of the DNA gyrase B protein (key in bacterial transcription and replication, and an established target for antibacterial medications whose inhibition induces bacterial cell death) (PDB ID: 1KZN) was downloaded in 'pdb' format from the protein data bank (PDB) (https://www.rcsb.org) and optimized in UCSF Chimera 1.17.2. Site-directed grid coordinates used are X:20.2452, Y:21.7360, Z:37.6791 (27.2392 Å /34.0163 Å /40.1484Å dimensions). The 3D structures of *Capsicum* metabolites and reference broad spectrum antibiotic (levofloxacin, known bacterial DNA synthesis inhibitor) were obtained in 'sdf' format from PubChem (https://www.pubchem. ncbi.nlm.nih.gov) and optimized for site directed docking using the PyRx (software 0.9.5). Molecular docking was validated at RMSD 0.5 Å, and formed complexes were thereafter visualized in Discovery Studio (DS) 2021 to derive added information on interactions between the target and metabolites (ligands).[25]

Pharmacokinetics properties determination

In predicting the pharmacokinetic properties of lead compounds with the highest binding affinities [absorption, distribution, metabolism, excretion (ADME)] the SwissADME platform (http://swissadme.ch/index.php) was used. The same open-source platform predicts metabolites *in vivo*, oral, drug-like nature and conformance with the Lipinski rule of 5 (Ro5) [mole weight ≤ 500 g/mol; bond donors ≤ 5, bond acceptors ≤ 10, and lipophilicity (log P) ≤ 5].[26]

Density function theory (DFT) analysis

In order to derive added information on lead compounds chemical reactivities and electronic structures, the density functional theory (DFT) analysis was used. The top compounds were optimized using the DFT/B3LYP/631G basis set in the Gaussian 16 software.[27] This was followed by outcome visualization in GaussView version 6.0.16 and conceptual chemical descriptors calculation [softness, hardness, ionization energy (I), energy gap, electron affinities (E), global electrophilicity and electronegativity indices] using the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) values, and Koopman's theorem correlating I and E.[28,29]

Data analysis

Data was analysed in Microsoft Excel using STATA Version 12, with descriptive means and standard deviations computed at a set significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

The GC-MS profile of *C. annuum* extracts

The GC-MS chromatographic profiles of the metabolites of hydroalcoholic fractions of both green and red *C. annuum* peppers are depicted in Figures 1a and 1b, while the list of metabolites identified are shown in Tables 1 (green variant) and 2 (red variant). The analysis showed a rich content of fatty acids and their derivatives (pentadecanoic, hexadecanoic, 11-octadecenoic acid, nonanoic and heptanoic acids, among others), heterocyclic acetal (1,3-dioxolane), hydrocarbons and their derivatives, organofluorine metabolites (trifluoroacetic acid, pentadecyl ester), and aromatic dicarboxylic acids (phthalic or 1,2-benzenedicarboxylic acid) and esters. Most of the volatile bioactive compounds identified in the green *Capsicum* had higher peak intensities compared to the red *Capsicum* variant. More volatiles were also identified in the green compared to the red pepper variety. While oleic fatty acid was specific to the red variety, the green variety showed a wider array of volatiles including carboxylic fatty acids and esters. Hexadecenoic acid was also present in both varieties. Plant metabolites identified from *Aristolochia* species like ethyl esters and hexadecanoic acid have been shown to have antioxidant, hypocholesterolemic, and flavour properties.[30,31] Although earlier studies had detected the presence of gondoic acid and other fatty acids, the reports were from non-African climates.[32,33] A major variation in metabolome profile existed between both *Capsicum* variants. This observation may not be extricated from factors such as storage conditions and environmental exposure. Likewise, location of growth, stage of maturity, and genotype are additional determinants of plant species metabolites' profile.[34,35]

In line with our study, the presence of hydrocarbons, glycosides, and organic acids from *Capsicum* accessions in germplasm banks in Brazil has been reported.[36] In another study of over 25 *Capsicum* fruits, higher amounts of alcohols, hydrocarbons and esters were identified relative to ketones, acids, sugars, and aldehydes.[37] Trovato *et al.*[38] also reported over two hundred metabolites from 3 chili pepper variants. In their work ketones and acids were predominant in *C. annuum*, and aldehydes and esters in *Capsicum baccatum* and *C. chinense* variants, respectively. This led to the suggestion that the detection of high-level aroma 'marker' metabolites could serve as a parametric tool for the differentiation of *Capsicum* pepper species. Such properties give plant species within the *Capsicum* genus a significant commercial relevance.[39]

Quantitative phytochemical constituent of *C. annuum* extracts

Within the extracts of both *Capsicum* variants, the presence of tannins, cardiac glycosides, and alkaloids were demonstrated, while flavonoids were only detected the green bell peppers. Interestingly, while there were higher levels of alkaloids and tannins with no presence of flavonoids in the red variant following quantitative analysis, the green pepper variant showed high amounts of flavonoids, as well as relatively good content of tannin, alkaloid, and glycosides (Fig. 2). The presence of cardiac glycosides in plants have been associated with the management of heart and vascular conditions. It acts directly by upregulating both smooth and heart muscles contractions. Cardiac glycosides also show favourably indirect impact on vascular and heart resistance and capacitance, thus affecting the electrical activities of the cardiovascular system.[40] Tannins are key bioactive metabolites in plant and medicine, which also find use as clarifiers and antioxidants in the beverage industry, also in wine and foods clarification, and textile dye application. Tannins are also reported to have strong antitumour and antimicrobial properties.[41] Phenols and flavonoids are a group of versatile metabolites that have been linked to antioxidant, antitumor and cardiovascular effects in the body. Bioactivities such as antiviral, antitumour, antiulcerogenic, antiallergy and anti-inflammatory responses are also associated with flavonoids. Phytosterols also find use as raw materials for creation of various products in the animal feed, cosmetic and pharmaceutical industries. Alkaloids are mainly plant sourced and show a wide range of bioactivities, for example, antihyperglycemic and antimicrobial.[42] Overall, the identified compounds may possess potential for further industrial exploitation.

Table 1: Bioactive natural products of green *C. annuum* extract.

Peak	Compound name	Peak area%	Height%	Retention Time (R_t)	Molecular weight	Formula
1	Methyl cyclooctane	0.77	0.95	3.913	126	C9H18
2	Acetohydroxamic acid	0.55	0.55	5.459	75	C2H5NO2
3	3-ethylpentanoic acid	2.89	1.78	5.823	146	C7H14O3
4	1-fluorononane	0.98	1.46	6.328	146	C9H19F
5	2-propoxy-succinic acid	1.25	1.91	6.668	204	C9H16O5
6	1-nitro-2,3-epoxy-propane	1.14	1.04	7.460	103	C3H5NO3
7	Diethyl butanedioate	0.60	0.80	7.603	190	C8H14O5
8	2-isopropyl-1,3-dioxolane	0.50	0.98	7.737	116	C6H12O2
9	1-dodecene	1.18	2.90	8.954	168	C12H24
10	Hexane	1.36	1.58	10.280	142	C10H22
11	9-methyldecanoic acid	7.11	4.19	10.686	186	C11H22O2
12	2-tridecene	2.68	5.42	11.409	182	C13H26
13	3-methyl-tridecanoic acid	0.58	1.32	12.984	228	C14H28O2
14	1-dodecene	2.90	6.56	13.686	168	C12H24
15	6,10-Dimethylundecan-2-one	0.67	1.22	14.482	198	C13H26O
16	(1-ethyloctyl) cyclohexane, (1-butylhexyl) cyclohexane	0.36	0.64	14.748	224	C16H32
17	7-pentadecanone	0.56	0.87	14.922	226	C15H30O
18	Dibutyl phthalate	6.80	7.64	15.167	278	C16H22O4
19	Heptadecanoic acid	2.52	4.02	15.717	270	C17H34O2
20	Trifluoroacetic acid, pentadecyl ester	2.71	4.05	16.840	324	C17H31F3O2
21	Hexadecanoic acid	5.44	3.53	17.102	256	C16H32O4
22	Methyl linoleate	2.72	4.59	18.932	294	C19H34O2
23	16-octadecenoic acid, methyl ester	4.26	6.16	18.996	296	C19H36O2
24	Octadecanoic acid	1.15	1.98	19.352	284	C18H36O2
25	Z-10-pentadecenol	32.33	11.91	20.284	226	C15H30O
26	2-butyl-1-octanol	0.84	1.62	21.105	186	C12H26O
27	1-Heptadecanol	0.96	1.80	22.558	256	C17H36O
28	10-undecenal	4.72	6.05	23.580	168	C11H20O
29	Diethylhexyl phthalate	7.21	10.93	24.307	390	C24H38O4
30	E, E-1,9,17-docasatriene	2.23	1.53	25.722	304	C22H40

Table 2: Bioactive components and natural products of red *C. annuum* extract.

Peak	Compound name	Area %	Height %	Retention Time (R_t)	Molecular weight	Formula
1	6-oxa-bicyclo	0.83	0.64	3.651	98	C5H6O2
2	3-undecene	1.68	1.78	3.908	154	C11H22
3	3-Hydroxybutanoic acid	0.73	0.82	4.611	104	C4H8O3
4	1-[Aminomethyl(methyl)amino] Ethanol	0.30	0.50	7.356	104	C4H12N2O
5	2-Dimethyl(isopropyl) Silyloxypentadecane	0.35	0.62	7.960	328	C20H44OSi
6	1-dodecanol	0.65	1.51	10.178	186	C12H26O
7	Nonanoic acid	0.33	0.98	10.681	158	C9H18O2
8	Cis-4-tridecene	0.70	1.74	11.405	182	C13H26
9	Tropaldehyde	0.79	1.25	12.192	150	C9H10O2
10	Neoundecanoic acid	1.38	2.25	12.983	186	C11H22O2
11	1-dodecene	0.96	2.31	13.681	168	C12H24
12	1,2-benzenedicarboxylic acid/Dibutyl phthalate	0.90	1.64	15.118	278	C16H22O4
13	Heptadecanoic acid	7.14	13.31	15.720	270	C17H34O2
14	Pentafluoropropionic acid	0.63	1.20	16.826	332	C15H25F5O2
15	Hexadecanoic acid/Palmitic acid	3.78	2.64	17.041	256	C16H32O2
16	1-Tetracosanol	0.86	1.53	18.796	354	C24H50O
17	Methyl linoleate	4.40	9.52	18.926	294	C19H34O2
18	11-octadecanoic acid methyl ester	1.91	11.43	18.991	296	C19H36O2
19	Octadecanoic acid, Methyl stearate	1.94	4.52	19.345	298	C19H38O2
20	Oleic acid	31.77	11.91	20.154	282	C18H34O2
21	2,3-Dihydroxypropyl hexadecanoate	3.74	3.72	21.692	330	C19H38O4
22	E, E-1,9,17-Docasatriene	6.45	7.32	23.565	304	C22H40
23	Paullinic/Gondoic acid	4.90	3.61	24.102	310	C20H38O2
24	Diethylhexyl phthalate	3.47	4.89	24.269	390	C24H38O4
25	10-undecenal	16.42	8.35	25.725	168	C11H20O

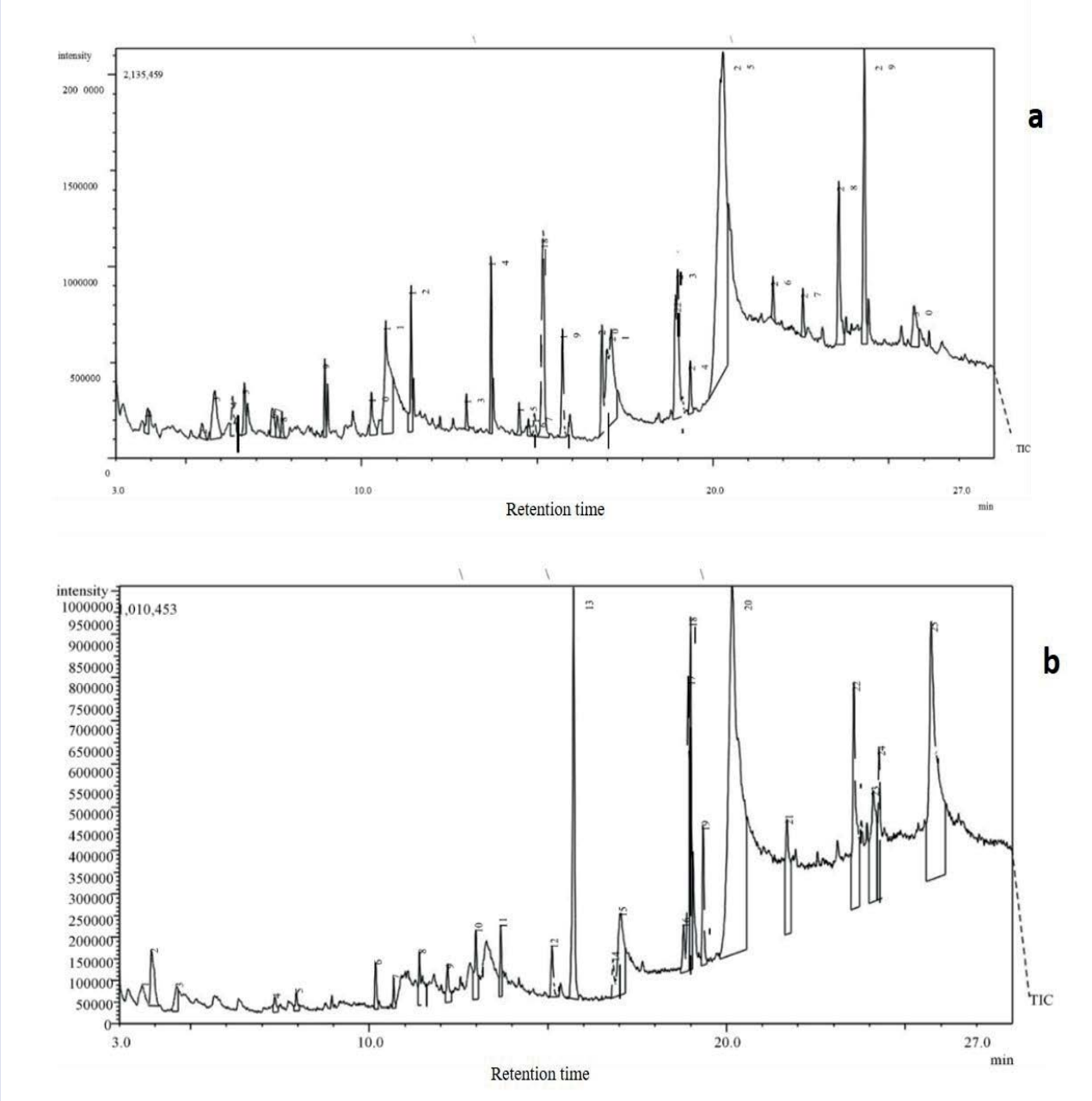

Figure 1: The GC-MS chromatographic profile of (a) green and (b) red *C. annuum* extracts.

Antioxidant activity of *C. annuum* extracts

The green bell pepper extract showed significantly different antioxidant activity across DPPH and FRAP values (84.27%, and 2.13, respectively), when compared to extracts derived from the red *Capsicum* variant (81.88%, and 1.36). Nonetheless, the standard antibiotic performed better than both extracts (Table 3). This may be attributed to the presence of flavonoids (Fig. 2) detected in only green bell pepper extracts. Nevertheless, both extracts showed good antioxidant activity linked to the presence of bioactive molecules acting in synergy.[21] An earlier study had also linked higher antioxidant activity of green peppers to higher levels of ascorbic acid, *Capsicum* exclusive carotenoids (capsorubin, cryptocapsin, capsantine) and phenols (quercetin, capsaicinoids, luteolin).[43] Devgan *et al.*[44] also demonstrated the antioxidant properties and possible health benefits of peppers for the human body. The hydroethanol solvent polarity may have also facilitated the extraction of polar and non-polar compounds with potent antioxidant activity. The antioxidant action of grafted *Capsicum* ethanol fractions has been reported to be dependent on pepper type, colour, concentration, and type of bioactive compounds, and difference in coloured pepper bioactivity outcomes could be due to the mechanism of action of each compound expressed in fractions. Factors like harvesting season and cultivar type have been reported to also causes variation in biological activities like antioxidant potential.[45] A correlation between plant phenol content and antioxidant activity in bell peppers variants has also been shown.[46] Similar observations have been made from other plant species like celeries where a high phenol and flavonoid content resulted in high antioxidant activity.[47] In line with our study, Prabakaran *et al.* (2017)[48] record highest antioxidant action from bell pepper ethanol extract and related it to the fruits high content of flavonoids and phenols, with both compound group activities facilitated by the possession of hydroxyl (OH) moieties in the aromatic ring of their chemical structures.[49]

Table 3: Antioxidant activity of *C. annuum* ethanol extracts.

Antioxidants	Green bell pepper	Red bell pepper	Ascorbate
DPPH (%)	84.27±0.12[b]	81.88±0.15[c]	91.01±0.05[a]
ABTS (mMol/g)	0.11±0.02[b]	0.18±0.03[a]	0.04±0.01[c]
FRAP	2.13±0.01[a]	1.36±0.00[b]	1.01±0.01[c]

[a,b]Values are presented as mean ± SD of replicates, and different superscripts along rows indicate significant difference ($p < 0.05$); DPPH -2, 2′-diphenyl-1-picrylhydrazyl, ABTS -2,2′-azinobis3-ethylbenzthiazoline-6-sulfonic acid, FRAP - Ferric reducing antioxidant power.

Table 4: Antibacterial activity of green and red *C. annuum* ethanol extracts.

Inhibition zones (mm)					Extract MICs
Microorganism	600 mg/mL (24 mg)	700 mg/mL (28 mg)	900 mg/mL (36 mg)	Levofloxacin (20 µg)	mg/mL
Green *Capsicum* extract					
Bacillus pumillus	6.9±0.1	8.4±0.2	15.1±0.1	23.7±0.0	125
Staphylococcus gallinarum	6.8±0.2	8.5±0.2	12.6±0.2	20.2±0.1	62.5
Staphylococcus equorum	8.1±0.2	10.0±0.1	14.5±0.1	15.1±0.1	62.5
Bacillus amyloliquefaciens	8.4±0.1	12.7±0.0	16.3±0.1	27.3±0.1	62.5
Bacillus cereus	6.7±0.1	10.9±0.2	14.5±0.2	23.7±0.1	125
Red *Capsicum* extract					
Bacillus pumilus	5.3±0.1	6.5±0.1	10.9±0.1		125
Staphylococcus gallinarum	6.2±0.1	8.9±0.1	14.5±0.0		62.5
Staphylococcus equorum	6.1±0.2	8.1±0.1	12.6±0.1		125
Bacillus amyloliquefaciens	6.4±0.1	8.5±0.0	12.4±0.1		125
Bacillus cereus	6.5±0.1	8.2±0.1	14.6±0.1		62.5

[a-d]Values are presented as mean ± SD of triplicates. Levofloxacin used as positive control.

Table 5: Bond interactions of lead *C. annuum* metabolites with target active site residues.

Target	Ligand	Docking score (kcal/mol)	Total Bonds	Conventional Hydrogen Bonds	Other significant bonds	Unfavourable bonds
DNA gyrase (1KZN)	(1-Ethyloctyl) cyclohexane	-6.6	11	-	Pi alkyl (Ala47, Ile78, Pro79), van der Waals (8)	-
	Dibutyl phthalate	-6.4	15	1 (Asn46)	Pi alkyl (Val43, Ala47, Val71, Ile78, Pro79, Ile90, Val167), Pi anion (Glu50), van der Waals (6)	-
	(1-Butylhexyl) cyclohexane	-5.9	13	-	Pi alkyl (Ala47, Ile78, Ile90), van der Waals (10)	-
	Diethylhexyl phthalate	-5.9	20	1 (Asn46)	Pi alkyl (Val43, Ala47, Ile78, Pro79, Ile90), van der Waals (14)	-
	Oleic acid	-5.8	18	3 (His95, Ala96, Ser121)	Pi alkyl (Ala47, Val71, Ile78, Ile90), van der Waals (11)	1 (Ser121)
	Levofloxacin	-7.7	11	3 (Arg76, Arg136, Thr165)	Pi alkyl (Ile78), Pi anion (Glu50), Amide-pi-stacked (Asn46). Halogen (Glu50, Gly77), van der Waals (3)	-

Antibacterial activity of *C. annuum* extracts

The antimicrobial zones of inhibition of obtained for extracts derived from both *Capsicum* variants are as shown in Table 4, with levofloxacin used as the standard antibiotic and positive control. *Bacillus pumilus* and *Staphylococcus gallinarum* showed the least antibacterial susceptibility to red and green *Capsicum* extracts, respectively. While susceptibility pattern and MIC varied across resistant bacterial species exposed to both extracts, susceptibility to extracts was concentration-dependent. Ahmad *et al.*[35] also reported that some *Capsicum* varieties showed better antimicrobial action over other variants and showed favourable inhibition of *Pseudomonas aeruginosa* (green bell pepper extracts of Saudi Arabian origin had highest inhibition zone of 17 mm), *Staphylococcus aureus* and *Escherichia coli* (0 to 13 mm across all pepper variant extracts). The broad-spectrum activity of *Capsicum* fractions is closely linked to their wide phytochemical group range and indicates that the plant's metabolome is worthy of further exploitation for discovery of existing and new moieties for various expanded industrial applications. For example, carboxylic acids and derivatives have reported pharmacological effects including anti-inflammatory, analgesic, and cell anti-proliferative activities.[50] *Capsicum* fruits could thereby have extended pharmaceutical uses as prodrugs, and beyond food and trado-medical applications, given their rich content of bioactive metabolites.

Computer-aided screening and determinations

Molecular docking of *Capsicum* metabolites against the protein target is shown in table 5, with depiction of binding affinity or docking scores of top 5 compounds and reference antibiotic, as well as their key bond interactions at the protein active site. Highest docking scores were recorded for (1-Ethyloctyl) cyclohexane (-6.6 kcal/mol) and dibutyl phthalate (-6.4 kcal/mol) metabolites relative to the standard (−7.7 kcal/mol). Metabolites with lower docking scores demonstrate enhanced orientation and fit at the DNA gyrase target binding site. Docking scores provide key information needed to select prospective compounds from a milieu and enhance the prediction of their binding affinities (interaction strength) to target(s) under investigation.[51] Dibutyl phthalate, oleic acid and diethylhexyl phthalate also had the

Table 6: Pharmacokinetic properties of the lead *Capsicum* compounds.

Ligand	Mol. W. (≤ 500 g/mol)	HBD (≤ 5)	HBA (≤ 10)	Lipophilicity (Log P ≤ 5)	Bioavailability (water solubility)	GIT absorption	BBB permeability	Lipinski violations
(1-Ethyloctyl) cyclohexane	224.43	0	0	5.96	0.55	Low	No	Yes; 1 violation: MLOGP>4.15
Dibutyl phthalate	278.34	0	4	3.69	0.55	High	No	Yes; 0 violation
(1-Butylhexyl) cyclohexane	224.43	0	0	5.96	0.55	Low	No	Yes; 1 violation: MLOGP>4.15
Diethylhexyl phthalate	390.56	0	4	6.17	0.55	High	No	Yes; 1 violation: MLOGP>4.15
Oleic acid	282.5	1	2	5.71	0.85	High	No	Yes; 1 violation: MLOGP>4.15
Levofloxacin	361.4	1	6	1.15	0.55	High	No	Yes; 0 violation

Mol. W =molecular weight; HBD = hydrogen bond donors; HBA = hydrogen bond acceptors; Log P = partition coefficient; GIT = gastrointestinal tract; BBB = blood-brain barrier.

Table 7: The chemical reactivity profile of the top 4 *Capsicum* compounds.

Descriptor	(1-Ethyloctyl) cyclohexane	Dibutyl phthalate	(1-Butylhexyl) cyclohexane
LUMO	2.142	-0.069	2.082
HOMO	-7.552	-0.261	-7.578
Energy Gap (ΔE)	9.693	0.192	9.660
Ionization Energy (I)	7.552	0.261	7.578
Electron Affinity (A)	-2.142	0.069	-2.082
Hardness (*H*)	4.846	0.096	4.830
Softness (*S*)	0.206	10.434	0.207
Electronegativity	2.705	0.165	2.748
Chemical Potential	-2.705	-0.165	-2.748
Global Electrophilicity (ωeV)	0.755	0.106	0.782

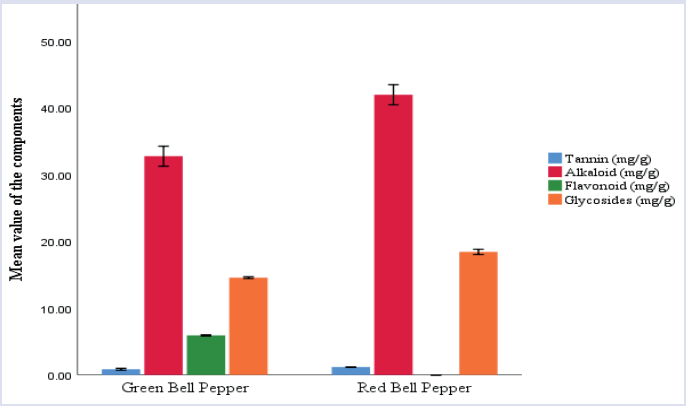

Figure 2: Quantitative phytochemical constituents of *C. annuum* extracts.

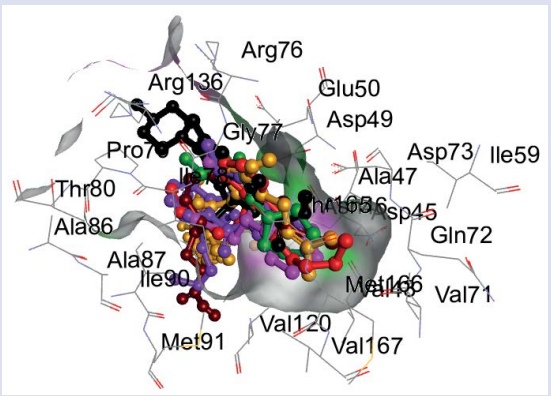

Figure 3: Superimposition of lead Capsicum compounds on co-crystal structure of gyrase B (RMSD: 0.5Å). Green = (1-Ethyloctyl) cyclohexane; Red = Dibutyl phthalate; Pink = (1-Butylhexyl) cyclohexane; Purple = Diethylhexylphthalate; Brown = Oleic acid; Black = Levofloxacin; Orange = Native inhibitor (clorobiocin).

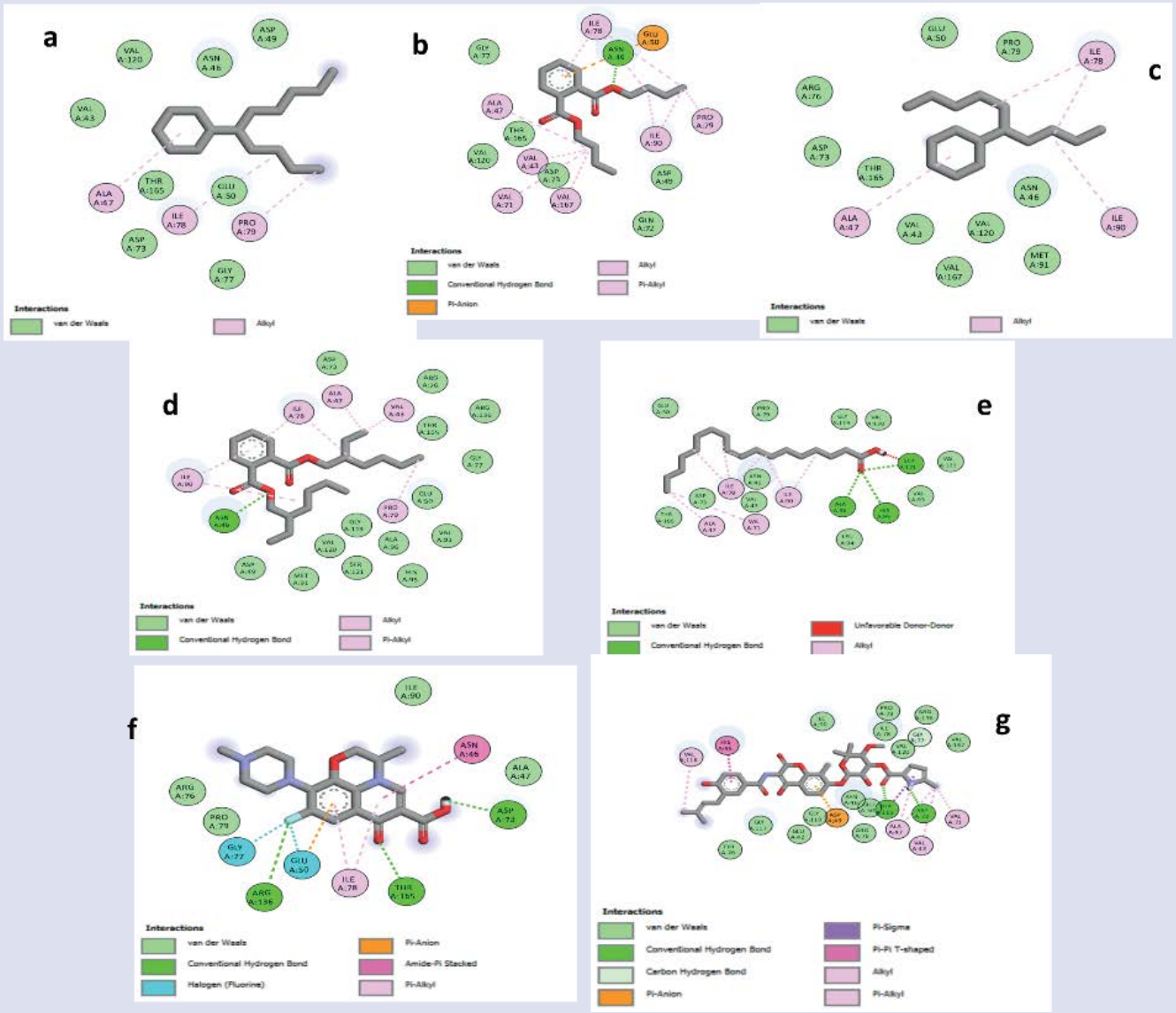

Figure 4: 2D docking interactions of top Capsicum compounds and standard.
a. (1-Ethyloctyl) cyclohexane, b. Dibutyl phthalate, c. (1-Butylhexyl) cyclohexane,
d. Diethylhexylphthalate, e. Oleic acid, f. Levofloxacin, g. Native clorobiocin inhibitor

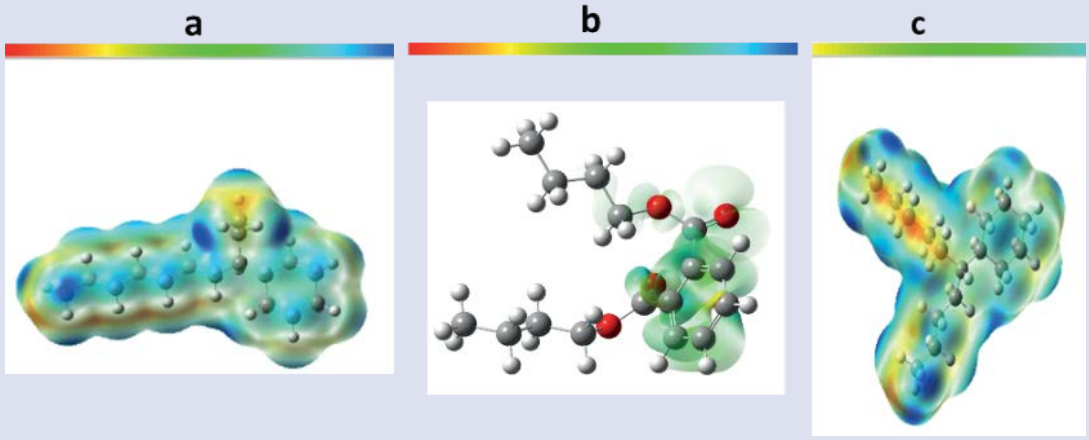

Figure 5: Electrostatic potential (ESP) mapped molecular surface of the top three compounds. In each ESP range: (a) (1-Ethyloctyl) cyclohexane, (b) Dibutyl phthalate, and (c) (1-Butylhexyl) cyclohexane.

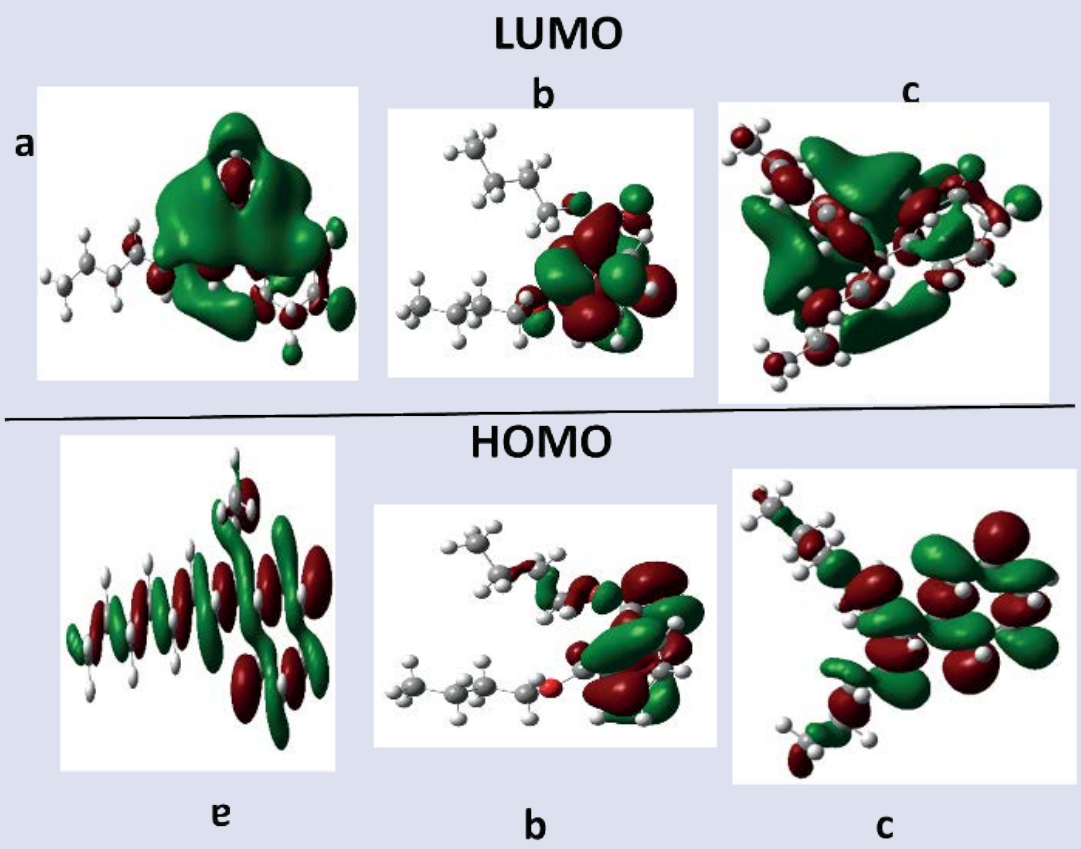

Figure 6: Frontier orbitals of the top three compounds (a) (1-Ethyloctyl) cyclohexane, (b) Dibutyl phthalate, and (c) (1-Butylhexyl) cyclohexane.

highest number of total bonds and presence of hydrogen bonds in all. The presence of an unfavourable bond in oleic acid may contributed to its low docking score. Also, similar hydrophobic and hydrophilic amino acid residue interactions were observed across metabolites and standard. Although the reference standard showed the highest number of conventional hydrogen bonds and lowest binding affinity score for the target, higher binding scores obtained for the *Capsicum* metabolites may be attributed to the presence of 0 to 1 H bonds, unfavourable interactions and length of interacting bond (a key determinant of atomic, inter- and intra-molecular bond attractions/affinity). Hydrogen bonds contribute to stabilizing the ligand-protein complexes formed by strengthening intermolecular binding.[25] The key interacting amino acids in the bound ligand-protein complexes include Asn46, Ala47, Glu50. and Val43, Ile78, Ile90 (essential amino acids). Molecular docking which was validated at a root mean square deviation (RMSD) of 0.5 Å by superimposition of compounds and reference at protein active site is shown in figure 3, and 2D amino acid interactions of the top 3 metabolites depicted in figure 4. This was done to enhance precision, accuracy and validity of the docking process, with RMSD values ≤ 2.0 Å depicting a good docking process.[52] Also, these lead compounds with DNA gyrase inhibiting activity could be responsible for the synergistic mechanism of antibacterial action reported in this study. As such, the goal to identify potential lead metabolites with drug-like, gyrase B inhibiting attribute was achieved.

The lead compounds' pharmacokinetic attributes are shown in table 6, with all compounds falling within the Lipinski's rule of 5 (Ro5) with 0 to 1 violation only, thus depicting their desirable drug-like, pharmacokinetics, and physicochemical characteristics for oral medications development.[26] In addition, most of the Capsicum metabolites and standard showed good bioavailability scores (0.55) which is required in drug dose calculations. When the bioavailability score is high, for example, as observed for oleic acid it means more of the metabolite is needed to evince optimal therapeutic effect at the target site.[53] Again, both metabolites and standard could not permeate the blood-brain barrier (BBB). Compounds predicted to permeate the BBB are reported to possess an added benefit in preventing influx of toxic molecules above 400g/mol in weight.[54] Of the metabolites, only dibutyl phthalate, oleic acid and diethylhexyl phthalate, as well as levofloxacin showed a high absorption rate in the gut, a key consideration when designing oral medicines.[55] All metabolites, except dibutyl phthalate, showed violation in log P (values ≥ 5), as such only dibutyl phthalate had good membrane absorption and permeability features due to better lipophilicity score.[56] Pharmacokinetic screening mimic *in vivo* biomolecule interactions to reduce failure rate during clinical trial and medication development stages.[57]

Using DFT computations, the molecular electrostatic potential (MEP) of lead metabolites was done (Figure 5). The electrostatic potential of regions is depicted as blue (positive, promotes electrophilicity), green (neutral, zero potential), and red, orange or yellow (negative, electron dense, promotes nucleophilicity).[58] The MEP regions mapping show identified best fit sites which are central to the interactions that occur within (intra-) and across (inter-) molecules.[59] The structures of the lead compounds were optimized and utilized in the derivation of dihedral angles, bond angles and lengths (Figure 6). The LUMO, HOMO, energy gap (ΔE) and other calculations on the top three lead compounds are depicted in table 7. While all leads showed good reactivity profiles, dibutyl phthalate and (1-Butylhexyl) cyclohexane showed the least energy gap and hardness, highest softness, electronegativity and global electrophilicity values. These compounds showed higher levels of intramolecular charge transfer from their donor occupied states

to the unoccupied acceptor groups.[60,61] The HOMO is an energy level associated with the potential to donate electrons and is proportional to the ionization energy of a molecule. On the other hand, the LUMO speaks to the electron accepting or energy withdrawing groups. The electron affinity and ionization energy parameters depict a molecule's ability to accept electrons, and energy requirement for separation of electrons from the atom, respectively. In both cases, lower values show high reactivity, and vice versa for higher values. A compound's reactivity and stability are also depicted by its energy gap, and lower gaps show increased softness and reactivity.[58] High softness and low hardness values are also desirable attributes for a highly reactive molecules.[62] Also, high electrophilicity values suggest an electrophilic compound, while reactive nucleophilic compounds would have lower values, and some lead compounds had higher electrophilicity values which were consistent with electronegativity scores. These features attest to the lead molecules modulatory potentials against the target enzyme to achieve inhibition of the test bacteria. Overall, although the reference performed better than the lead compounds in binding or inhibiting DNA gyrase activity, our aim to identify potential lead *Capsicum*-based molecules that could be key to the demonstrated extracts' bioactivities and mechanism of antibacterial action (either singly or synergistically) was achieved. The lead *Capsicum* compounds could be further derivatized to optimize their pharmacological action range and for development into enhanced biologically active nano-encapsulated forms to enhance stability and bioactivity, for added non-food (pharmaceutical) related industry applications. Also, following derivatization, an assessment of derived products' toxicity should also be done.

CONCLUSION

In summary, this study has shown that *Capsicum* bell peppers are rich in potential bioactive molecules with antioxidant and antibacterial capacities, as well as good drug-likeness (DNA gyrase B inhibition), pharmacokinetic, physicochemical and chemical reactivity attributes. The lead phytochemical compounds identified may hold key functions in human health. The study gives a first-hand added scientific backing to the mechanism of action and efficacy of *Capsicum annuum* fruits in infections therapy associated with bacterial pathogens, as well as proof for the use of the plant in food and ethnomedicinal practices. The *C. annuum* fruits and extracts may also serve as dependable sources of lead natural antioxidant and antibacterial compounds which could be optimized for enhanced application in the non-food, pharmaceutical industry.

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CONFLICTS OF INTEREST

None to declare.

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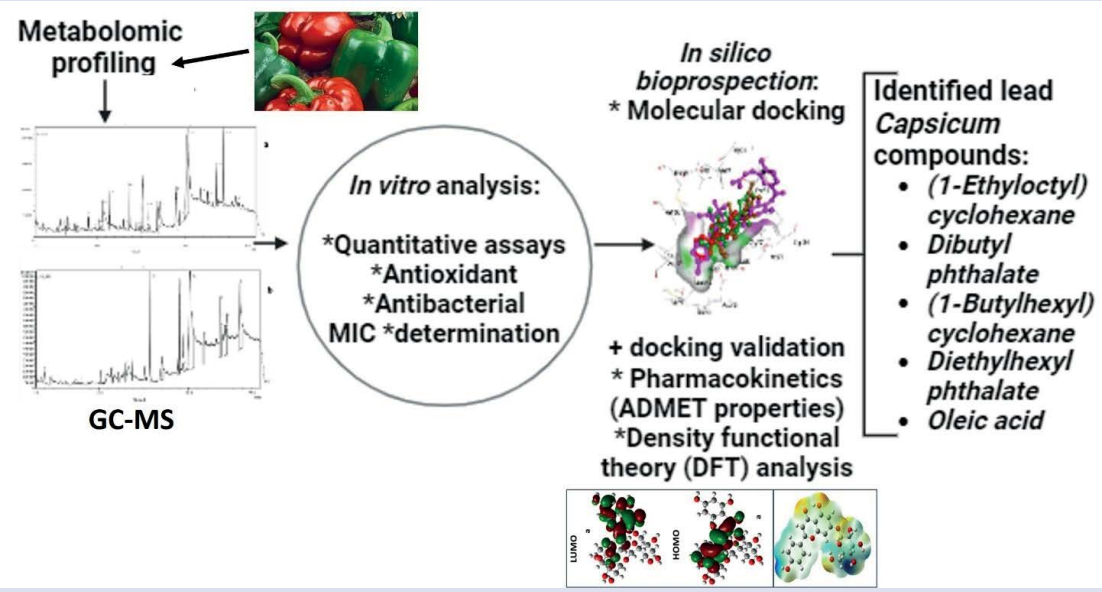
GRAPHICAL ABSTRACT



HIGHLIGHTS

- The metabolomic fingerprints of two *Capsicum* fruit variants are presented
- *Capsicum annuum* fractions possess significant biological activities *in vitro*
- Lead gyrase B inhibitors; oleic acid, cyclohexanes, phthalates detected *in silico*
- Fractions action mechanism; due to lead natural products binding, 'drug-likeness'
- *C. annuum* metabolites array could have added applications in industry

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