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Indian Shot (*Canna Indica* L). Leaves Provide Valuable Insights into the Management of Inflammation and Other Associated Disorders Offering Health Benefits

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Background: Throughout history, plants have played a crucial role in advancing medicinal treatments by providing a diverse range of compounds for the development of innovative therapies. *Canna indica* L. a tropical herb of the Cannaceae family, also known as Indian shot, has a rich history of traditional use in treating ailments like inflammation, malaria, dysentery, fever, dropsy, and diarrhea.
Objective: This comprehensive research invesigates the extract preparation of *C. indica* leaves using multidisciplinary analytical approaches for this extract in order to shed light on its therapeutic potentials.
Methods: The research, an international collaboration involving researchers from Bangladesh and China, utilized GC-MS/MS analysis to identify bioactive compounds across different *C. indica* extracts. Biological assays were conducted to assess antimicrobial activity using the disc diffusion method (in vitro), cytotoxicity through the brine shrimp lethality assay (in vitro), analgesic effects via the acetic acid-induced writhing test (in vivo), and antidiarrheal activity with the castor oil-induced diarrhea model (in vivo). Molecular docking studies were performed to determine binding affinities with Epidermal Growth Factor Receptor (EGFR), Dihydrofolate Reductase (DHFR), Delta Opioid Receptor (DOR), Tumor Necrosis Factor-alpha (TNF-α), and Cyclooxygenase-2 (COX-2) receptors.
Results: The GC-MS/MS analysis identified 35, 43, 27, and 20 compounds in dichloromethane, aqueous, petroleum ether, and ethyl acetate extracts, respectively. The aqueous (AQSF) and dichloromethane (DCMSF) extracts showed notable antimicrobial activity, particularly against gram-negative bacteria. Cytotoxicity tests indicated that ethyl acetate (EASF) and dichloromethane (DCMSF) fractions were potent. Analgesic activity was highest in DCMSF, and antidiarrheal effects were dose-dependent, with DCMSF showing the greatest efficacy. Molecular docking revealed strong affinities of Ergostane-3,5,6,12,25-pentol, 25-acetate, (3.beta.,5.alpha.,6.beta.,12.beta).- for EGFR and Norgestrel for COX-2.
Conclusion: This research provides valuable insights into the bioactivity evaluation of *C. indica*, bridging the gap between its chemical composition and diverse biological effects. The findings contribute to the growing body of knowledge in natural product-based drug discovery and underscore the significance of *C. indica* as a potential source of novel therapeutic agents to treat inflammation and other disease states.

Keywords: *Canna indica*, GC-MS/MS, cytotoxicity, antimicrobial, antidiarrheal, analgesic, molecular docking

Introduction

The search for new therapeutic agents has increasingly led researchers to explore bioactive compounds derived from natural sources, which present a complex and vast network of chemical diversity across various organisms, offering an

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extensive reservoir of potentially valuable compounds with therapeutic properties.[1,2] Bioactive substances, which are defined by their capacity to influence living things biologically, have attracted a lot of attention due to their possible use in the creation of novel medications and medical treatments[3,4] The historical significance of natural products in medicine dates back to ancient civilizations, where traditional remedies were often derived from plants, fungi, and marine organisms.[5–7] In recent decades, advancements in molecular biology and analytical techniques have intensified the identification, isolation, and characterization of these bioactive compounds,[1,8,9] rejuvenating interest in natural product-based drug discovery and fostering a renewed commitment to investigating their therapeutic potential.[10–12]

Plants provide several classes of phytochemicals such as alkaloids, polyphenols ie flavonoids, phenolic acids, tannins, lignans, and terpenoids including terpenes, and carotenoids, along with glycosides, saponins, polysaccharides, and lipids.[13,14] These substances have shown promise in treating several illnesses, such as cancer,[15] diabetes mellitus,[13] inflammatory conditions,[16] and cardiovascular disease.[17] Additionally, microbial sources like fungi, bacteria, and actinomycetes are increasingly studied for their bioactive compounds, which offer diverse chemical structures and unique modes of action.[18] Notably, around half of the pharmaceutical agents approved by the FDA incorporate natural products or their derivatives.[19]

Canna indica L., a tropical herb belonging to the Cannaceae[20] family, is sometimes called Indian shot, African arrowroot or purple arrowroot,[20] features dark green leaves with purple-brown veins and edges, arranged spirally and alternately. The hermaphrodite flowers are crimson, while the green, oblong fruits are slightly echinate. Seeds start white and ripen to black with brown markings. Rhizomes have a yellowish-white interior and a brownish exterior, with a thick coating at maturity. They can be tuberous or stoloniferous, and the roots are thick, cylindrical, and creamy white.[21] Though *C. indica* is native to Mexico, the West Indies, Central America, and South America, it is also found in the Southeast United States and naturalized in Europe, sub-Saharan Africa, Southeast Asia, and Oceania.[20] Malaria, dysentery, fever, dropsy, diarrhea, and scrapes and bruises have been treated using *C. indica*.[22] The root was used to cure diaphoretic, diuretic, and dropsy[23] and seed juice for the ears. There was a belief that the flowers could heal eye conditions.[24,25]

Numerous phytochemicals, including Alkaloids, Carbohydrates, Proteins, Flavonoids, Terpenoids, Cardiac glycosides, Steroids, Tannins, Saponins, and Phlobatannins, were found in the *C. indica* flower, according to a phytochemical study.[26] The aerial component of *C. indica* contains Betulinic acid, Oleanolic acid, and Taraxer-14-en-3-one;[27] the flowers include Violaxanthin and β-Carotene; the leaves contain Hemicellulose, Furfural, and Lignin;[28] and the rhizomes contain Tricosane, 7-Heneicosyne, 6-Hydroxyicosane, 3,15-Dihydroxy-2-octadecene, 5.8-Heneicosdiyne, and Tetracosane.[29] Anthocyanins, found in the flower of *C. indica* are Cyanidin-3-O-(6”-O-α-rhamnopyranosyl)-β-glucopyranoside, Cyanidin-3-O-(6”-O-α-rhamnopyranosyl)-β-galactopyranoside, Cyanidin-3-O-β-glucopyranoside, and Cyanidin-3-O-β-galactopyranoside.[30]

Global health concerns such as diarrhea and antimicrobial resistance underscore the urgent need for novel therapeutics. Diarrhea, a significant cause of mortality in developing countries, accounted for 1.1 million deaths in 2019 alone,[31,32] with unhygienic conditions and microbial infections (eg, *Candida albicans, Escherichia coli*, and *Salmonella typhi*) contributing factors.[31,33] Consequently, there is a growing focus on plant-derived antimicrobials as alternatives to conventional antibiotics in addressing these global health challenges.[34,35] Additionally, pain, a common consequence of inflammation, arises from the body’s response to injury or infection, with NSAIDs and opioids playing a crucial role in managing pain despite their potential side effects.[36,37]

The rise in infectious diseases poses a global threat and amplifies the risk of antimicrobial resistance.[38] According to the WHO, approximately 10 million deaths are projected to be attributable to AMR by 2050.[39] While numerous synthetic drugs are available on the market to treat illnesses, they often come with significant side effects. In contrast, plant-based medicines offer more potent therapeutic benefits and fewer adverse effects.[40,41] Therefore, developing new antibacterial drugs from natural sources could be advantageous in curbing bacterial growth and mitigating antimicrobial resistance, as natural plant-based products have recently gained attention as a substantial source of novel, safe, and potent secondary bioactive metabolites with therapeutic potential.

This study aimed to characterize the phytochemical composition of *C. indica* leaf tissue using GC-MS/MS techniques. Employing in vivo and in vitro methodologies, the research explored various biological functions associated with the leaf extract. Additionally, to address the challenge of correlating phytochemical identity with biological activity, an in silico approach was employed to predict the interactions of identified compounds with biological targets, providing

preliminary insights into their potential therapeutic mechanisms. This combined in vitro, in vivo, and in silico assessment provides a comprehensive investigation aiming to bridge the gap between chemical composition and biological efficacy, thereby unveiling *C. indica*'s potential therapeutic properties.

Materials and Methods

Drugs and Chemicals

Analytical-grade medicines and substances were used in this investigation. Methanol, Petroleum ether, Dichloromethane, Ethyl-acetate, and Tween-80 were purchased from Merck (Darmstadt, Germany). Azithromycin, Amoxicillin, Ciprofloxacin, Diazepam, and Loperamide were collected from the local market, which are manufactured by Square Pharmaceuticals Ltd. or Renata Ltd. in Bangladesh.

Test Organisms

The antimicrobial assay utilized gram-positive bacteria (*Sarcina lutea, Bacillus megaterium, Staphylococcus aureus, Bacillus cereus*, and *Bacillus subtilis*) and gram-negative bacteria (*Vibrio mimicus, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi, Escherichia coli, Shigella dysenteriae*, and *Vibrio parahemolyticus*) sourced from the University of Dhaka, Bangladesh.

Plant Collection

In November 2022, leaves from mature plants of *Canna indica* L. were collected from the hill regions of Bandarban (22.1961° N, 92.2176° E), Chittagong, Bangladesh. Khandaker Kamrul Islam, Senior Scientific Officer, Bangladesh National Herbarium, located in Mirpur, Dhaka, verified the authenticity of the plant samples. The voucher specimen of the plant has been preserved in the herbarium for future reference, cataloged under the accession number DACB 66976.

Plant Extraction

The leaves of *C. indica* L. were acquired from the wild, subjected to shade drying, and pulverized using a mechanical grinder. A total of 800 g of leaf powder was accurately measured and placed into a 5 L round bottom clean flask. The powder was wholly submerged in 2.5 L of distilled methanol, which covered approximately two-thirds of the flask's volume. The mixture was left to incubate in normal laboratory conditions for 15 days, during which it was periodically shaken and stirred. Following incubation, the mixture underwent filtration, and the filtered extract was concentrated using a rotary evaporator.[42] A total of 45.56 g of slurry crude extract (around 5.695%) yielded.

The modified Kupchan Partitioning method[43] was employed for solvent-solvent partitioning. Around 30 g of crude methanol extract (CME) obtained from *C. indica* leaves was mixed with 200 mL of distilled methanol and then subjected to fractionation. This process used 400 mL (200 mL for 2 times) each of petroleum ether (PET), dichloromethane (DCM), ethyl acetate (EA), and distilled water, in that order, based on an increasing relative polarity index. After the partitioning process, rotary evaporation was utilized to yield the petroleum ether-soluble fraction (PESF, 2.35 g; 2.94 mg/g of dry weight of plant material), the dichloromethane-soluble fraction (DCMSF, 3.43 g; 4.29 mg/g of dry weight of plant material), the ethyl acetate-soluble fraction (EASF, 1.68 g; 2.10 mg/g of dry weight of plant material), and the aqueous-soluble fraction (AQSF, 1.6 g; 2.00 mg/g of dry weight of plant material).

Phytochemical Assay

Gc-Ms/Ms

Using an electronic ionization detector along with GC–MS/MS (Shimadzu, Japan; Model GC–MS TQ 8040) analysis, the bioactive chemicals from the leaves of *C. indica* plants were examined. A 50 °C fused silica capillary column (Rxi5Sil MS, 30 m, 0.25 mm ID, and 0.25 µm) was utilized. After that, the samples were collected at 250 °C in a fix split mode. Preheating the oven took one minute at 500 °C, two minutes at 200 °C, and seven minutes at 300 °C. The electron multiplier was tuned to 900 V, and the ionization voltage was increased to 70 eV. The GC-MS unknown spectra were then contrasted with those of recognized

compounds kept in the NIST or Wiley libraries.[44,45] After that, the compound names, molecular formulae, and weights were established. It took a total of 39 minutes to complete the GC-MS run.

Compound Quantification and Identification

Quantification of each component involved comparing its average peak area to the total area, allowing the determination of proportional percentages. Identification of compounds via GC–MS spectra utilized the National Institute of Standards and Technology (NIST) database, which houses approximately 62,000 patterns.[46] Unknown component spectra were compared against both the NIST database's extensive collection and known components stored therein to ensure accurate identification during the GC–MS/MS experiment.

In vitro Assay

Antimicrobial Test

The antimicrobial efficacy of four fractions derived from the methanol extract of *C. indica* was evaluated using the disc diffusion technique.[47] Sterilized filter paper discs (6 mm diameter), each containing 100 µg of DCMSF, PESF, EASF, or AQSF, were placed on nutrient agar previously inoculated with test bacteria and fungi strains. Commercial antibiotic discs (Azithromycin, Amoxicillin, and Ciprofloxacin, 30 µg/disc) served as positive controls, while blank discs served as negative controls. The plates were inverted and stored at 4°C for 24 hours to ensure even distribution, followed by incubation at 37°C for another 24 hours. Zones of inhibition, indicative of antibacterial activity, were measured in millimetres. The test was repeated 3 times for each sample, and the result was expressed as mean ± Standard Deviation (SD).

In vitro Cytotoxic Test

The study utilized brine shrimp (*Artemia salina* leach) eggs exposed to varying concentrations of a solution prepared by dissolving test samples in dimethyl sulfoxide.[48] The experimental setup involved introducing live shrimp into test tubes containing simulated seawater. After 24 hours, the mortality rates were assessed, employing a serial dilution method to generate a range of concentrations from the stock solution. Each of the fractionated extractives (test samples) and vincristine standard solution (positive control) were serially diluted, starting from a concentration of 400 µg/mL to 0.78125 µg/mL and from a concentration of 20 µg/mL to 0.039 µg/mL, respectively. The LC_{50}, representing the concentration causing 50% shrimp mortality within the given timeframe, was determined as a measure of toxicity. The mortality percentage for each dilution was computed using the formula

$$\% \text{ mortality} = (\text{Number of dead shrimp} / \text{Number of shrimps introduced}) \times 100.$$

This approach allowed for the assessment of the extract's impact on brine shrimp survival across various concentrations.

Molecular Docking Study

Software

A computational methodology was utilized to evaluate the binding affinities of compounds extracted from the leaves extract of *C. indica* towards different target proteins. The analysis involved the application of various software tools, including PyRx, PyMOL 2.3, Discovery Studio 4.5, and Swiss PDB Viewer, to perform comprehensive assessments of the molecular interactions.[49]

Ligand Preparation

The three-dimensional Structure Data File (SDF) representations of the compounds listed in the table were sought and retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/; accessed on 17 November 2023). Additionally, the 3D SDF structures for five reference compounds—Lapatinib (PubChem CID_208908), Ciprofloxacin (PubChem CID_2764), Loperamide (PubChem CID_3955), and Diclofenac (PubChem CID_3033) were acquired from various sources.[50–54] The ligand library was constructed by systematically importing both the listed compounds and the standard references into Discovery Studio 4.5. Subsequently, all compounds were optimized using the Pm6 semiempirical method, enhancing the precision of the docking process.[49]

Receptor Selection

A total of 103 compounds derived from the methanol, DCM, pet ether, and ethanol fractions of *C. indica* leaf extract underwent computerized docking analysis to investigate their potential cytotoxic, antimicrobial, hypoglycemic, anti-inflammatory, and analgesic properties. The assessment of cytotoxicity involved the utilization of the 3D crystal structure of the epidermal growth factor receptor (EGFR) [PDB ID: 1XKK],[52] retrieved from the Protein Data Bank (https://www.rcsb.org/; accessed on 19 October 2023). Similarly, the 3D structures of dihydrofolate reductase (DHFR) [PDB ID: 4M6J], human delta-opioid receptor (DOR) [PDB ID: 4RWD], Tumor necrosis factor-α (TNF-α) [PDB ID: 6VI4], and cyclooxygenase-2 (COX-2) [PDB ID: 1CX2] were obtained from the same source for the evaluation of their antimicrobial, hypoglycemic, anti-diarrheal, and analgesic activities, respectively.[50,51,53,54]

Ligand- Receptor Binding

The assessment of affinities and potential binding patterns between phytocompounds and target molecules involved a sophisticated computer-aided approach by creating ligand-protein interaction diagrams. The utilization of the PyRxAutodock Vina software, known for its advanced capabilities in molecular drug-protein linking, employed a semi-flexible modeling approach during the docking process. A literature-based selection of specific amino acids with their respective IDs was meticulously curated for individual receptors to ensure precision in target docking.

The preparation of the protein involved loading and formatting it as the necessary macromolecule, carefully ensuring that ligands are exclusively bound to the intended target. For optimal docking against these selected macromolecules, the ligands' SD files were imported and converted into pdbqt format using the Open Babel tool within the PyRxAutoDock Vina software. The definition of active amino sites within grid boxes was accomplished through grid mapping, with the specified center and dimension axes maintained according to the details provided in the accompanying Table 1. Default supportive functions were retained during this stage.

Subsequently, a comprehensive docking analysis was executed using AutoDock Vina (version 1.1.2) to determine the ligands' affinity for their respective macromolecules. The final step involved result interpretation, where BIOVIA

Table 1 Selection of Target Site and Grid Mapping of Target Receptors

Receptor	Standard	Target Binding Sites	Reference	Grid box	
EGFR	Lapatinib	Leu 718, Val 726, Ala 743, Lys 745, Met 766, Lys 775, Arg 776, Leu 777, Leu 788, Thr 790, Gln 791, Leu 792, Met 793, Gly 796, Cys 797, Leu 799, Asp 800, Arg 803, Leu 844, Thr 854, Asp 855, Phe 856	[52]	Center	x = 15.9006706175, y = 34.5810564289, z = 35.8511159995
				Dimension	x = 24.7784116406, y = 19.7826045166, z = 32.3280197617
DHFR	Ciprofloxacin	Ala 9, Ile 16, Leu 93, Ser 92, Arg9 1, Arg 77, Glu 78, Ser 76, Leu 75, Lys 54, Val 120, Ser 119, Lys 55, Thr 56, Ser 118, Gly 117	[54]	Center	x = 3.22631914693, y = −3.74047127586, z = −18.5356969616
				Dimension	x = 20.8747603874, y = 27.8891050273, z = 27.2979925831
DOR	Loperamide	A chain- Val 62, Leu 65, Gly 66, Leu 69, Val 70, Phe 72, Gly 73, Tyr 77, Pro 315, Val 316, Ala 319, Phe 325, Cys 328, Phe 329, Gln 331, Leu 332	[51]	Center	x = −54.7882736979, y = −0.0619991477186, z = 54.9119221162
				Dimension	x = 31.2223397671, y = 24.3896375603, z = 27.4247000789

(*Continued*)

Table 1 (Continued).

Receptor	Standard	Target Binding Sites	Reference	Grid box	
TNF-α	Diclofenac	A chain- Tyr 59, Tyr 119, Leu 120, Gly 121, Tyr 151; B chain- Tyr 59, Ser 60, Gln 61, Tyr 119, Leu 120, Gly 121	[53]	Center	x = −19.8668590785, y = 74.1791111098, z = 37.6236254721
				Dimension	x = 19.55913465, y = 22.2400774665, z = 15.1069916676
COX-2	Diclofenac	His 90, Gln 192, Val 349, Leu 352, Ser 353, Tyr 355, Tyr 385, Ala 516, Phe 518, Val 523, Ala 527, and Ser 530	[50]	Center	x = 23.1078401223, y = 21.112183808, z = 15.5157206145
				Dimension	x = 21.543116081, y = 18.449889569, z = 24.2116427641

Discovery Studio version 4.5 was employed to predict the most suitable 2D and 3D models, adding depth and insight to the overall analysis.

ADME/T Analysis

In the domain of computer-based drug design, there is an increasingly prevalent focus on executing comprehensive pharmacokinetic studies that delve into critical facets such as absorption, distribution, metabolism, excretion, and toxicology. Moreover, the evaluation of drug-likeness through bioavailability studies has emerged as a pivotal component in drug discovery endeavors. Integral to these analyses are ADMET studies, which play a crucial role in unravelling the pharmacological characteristics of compounds. Accessible through resources like http://biosig.unimelb.edu.au/pkcsm/prediction, these ADMET analyses provide essential insights[7].

Online platforms such as Swiss ADME (http://www.sib.swiss) have become widely adopted for predicting drug-likeness by leveraging Lipinski rules and pharmacokinetic parameters. According to Lipinski's criteria, a compound is deemed orally accessible if it satisfies specific conditions, including a molecular weight below 500 amu, fewer than 5 hydrogen bond donor sites, fewer than 10 hydrogen bond acceptor sites, and a lipophilicity value (LogP) of ≤5.[49] This set of criteria serves as a guideline for assessing the potential oral bioavailability of compounds.

In vivo Assay

Animal Preparation

The experimental animals for this study were Swiss-albino mice of both sexes, weighing between 25 and 30 grams, sourced from the Animal Resources Facility at the International Centre for Diarrheal Diseases (ICDDR, B). Prior to the commencement of the study, a one-week acclimatization period was provided in the new environment. Throughout the experimental phase, the mice were housed in a well-ventilated animal facility with a temperature of 25 °C, relative humidity maintained between 55% and 65%, and a 12-hour light/dark cycle. The mice had *ad libitum* access to standard laboratory food and drinking water. An intraperitoneal overdose of ketamine HCl (100 mg/kg) and xylazine (7.5 mg/kg) was administered to euthanize the mice at the end of the experiment, following the previously established protocol delineated by Zimmermann, 1983.[55] The "Animal Ethics Number" for the test animal models of this research is 2023–01-04/SUB/A-ERC/002 and ratified by the Animal Ethics Committee, State University of Bangladesh. All experiments undertaken were executed in strict adherence to the approved Animal Use Protocol, as sanctioned by the Ethics Committee, and in full compliance with the Guidelines established by the United States National Institutes of Health for the Care and Use of Laboratory Animals. Moreover, the directives and recommendations set forth by the Federation of European Laboratory Animal Science Associations (FELASA) were meticulously followed to minimize any discomfort or distress experienced by the laboratory subjects.[56]

In vivo Oral Toxicity Test

The mice were administered a high oral dose of 2000 mg/kg body weight of methanol-soluble crude extract obtained from *C. indica* under standard laboratory conditions using the OECD Guidelines 420 fixed-dose method.[57] After the treatment, different parameters were examined for 72 hours. The results showed no signs of allergic reactions, changes in behavior (such as drowsiness or excitability), or any instances of mortality. As a result, doses of 200 and 400 mg/kg (body weight, taken orally) were selected for the investigation.

In vivo Analgesic Test

To evaluate the peripheral analgesic effect of *C. indica* in albino mice, we conducted the acetic acid-induced writhing test[58]. Diclofenac sodium (50 mg/kg body weight) was administered intraperitoneally as a standard reference. In contrast, the treatment groups received different doses of *C. indica* extract, specifically 200 and 400 mg/kg body weight, with each type of extract being administered orally at both dosage levels to form two groups. For DCMSF, groups III and IV were created, with group III receiving 200 mg/kg and group IV receiving 400 mg/kg. For AQSF, groups V and VI were assigned, with group V administered 200 mg/kg and group VI 400 mg/kg. Similarly, groups VII and VIII were treated with PESF, where group VII received the 200 mg/kg dose and group VIII received the 400 mg/kg dose. Finally, for EASF, groups IX and X were created, with group IX receiving 200 mg/kg and group X 400 mg/kg, while the negative control group received only distilled water. Thirty minutes after the administration of these samples, glacial acetic acid (0.1 mL/30 mg body weight) was injected intraperitoneally. For each group, the number of abdominal constrictions, or writhes, was counted from 5 minutes to 10 minutes post-acetic acid injection. The percentage inhibition of writhing, indicating peripheral analgesic activity, was then calculated using the formula:

$$\%\ \text{inhibition of writhing} = (W_{control} - W_{test})/W_{control} \times 100\%.$$

where W_{test} represents the average number of writhes in the test group, and $W_{control}$ represents the average number in the control group.

Castrol Oil-Induced Diarrhea

Diarrhea was induced by administering 0.5 mL of castor oil to mice, and only those exhibiting diarrhea were included in the experiment.[56] The mice, divided into ten groups with four members each, were given different treatments. Group I received a control vehicle (distilled water with 1% Tween-80), and Group II was treated with loperamide (2 mg/kg, b.w; i.p), a standard anti-motility agent. The treatment groups were administered different quantities of *C. indica* extract, specifically 200 and 400 mg/kg body weight. Groups III and IV were provided DCMSF, V and VI received AQSF, and VII and VIII were given PESF, while groups IX and X were treated with EASF. Following the administration of test samples, each mouse received 0.5 mL of castor oil after one hour, and they were individually placed in a box with clear paper on the floor. The observational period recorded the onset of duration, weight of wet stools, total number, and the total weight of faecal yields. The percentage of diarrheal inhibition (% of defecation inhibition) was calculated using the previously mentioned equation. The animals in all groups had free access to water overnight during the study.

Statistical Analysis

GraphPad Prism 5.2 (GraphPad Software, Inc., La Jolla, CA, United States) was employed for statistical analysis, and the results were expressed as mean ± standard error (SEM). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significance levels were indicated by *$p < 0.5$, **$p < 0.01$, and ***$p < 0.001$.

Results

Phytochemicals Identified from GC–MS/MS Analysis of DCM Extract of Canna Indica

GC-MS/MS screening of the DCM extract of *C. indica* revealed the presence of 35 compounds. The peak area % of each compound was determined to represent the relative concentration of the compound. Among the identified compounds, 1-

Heptatriacontanol (13.3%), 9-Octadecenamide, (Z)- (6.44%), Eicosyl isopropyl ether (5.07%), 11-Methyltricosane (3.82%) were found to have the most prevalence (Figure 1, Table 2).

Phytochemicals Identified from GC–MS/MS Analysis of Aqueous Extract of Canna Indica

Our analysis of a methanol-based extract of *C. indica* unveiled a significant discovery-The presence of 43 distinct compounds. Notably, the most abundant compounds were Curcumenol (12.98%), Phytol (8.66%), (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one (8.3%), 1-Heptatriacontanol (3.06%), Neophytadiene(2.94%), Zederone (2.82%) (Figure 2, Table 3).

Phytochemicals Identified from GC–MS/MS Analysis of Petroleum Ether Extract of Canna Indica

Our meticulous screening of the petroleum ether fraction was instrumental in the discovery of a total of 27 compounds. Among these, Benzene, (2-iodoethyl) – (31.28%), Tetradecane (6.13%), Cyclopentene, 1-ethenyl-3-methylene-(6.05%), Hexadecane (5.56%), Epicurzerenone (5.48%), Ethylbenzene (4.69%) were found to have the most comparative concentration (Figure 3, Table 4).

Phytochemicals Identified from GC–MS/MS Analysis of Ethyl Acetate Extract of Canna Indica

Ethyl acetate extract resulted in the detection of 20 compounds and high abundance compounds were 9-Octadecenamide, (Z)- (30.57%), Sambucinol (27.37%), (-)-Spathulenol (6.92%), Biphenylene, 1,2,3,6,7,8,8a,8b-Octahydro-4,5-dimethyl- (5.02%), 1-Hydroxymethyl-7,7-dimethylbicyclo[2.2.1]heptan-2-one (4.57%) (Figure 4, Table 5).

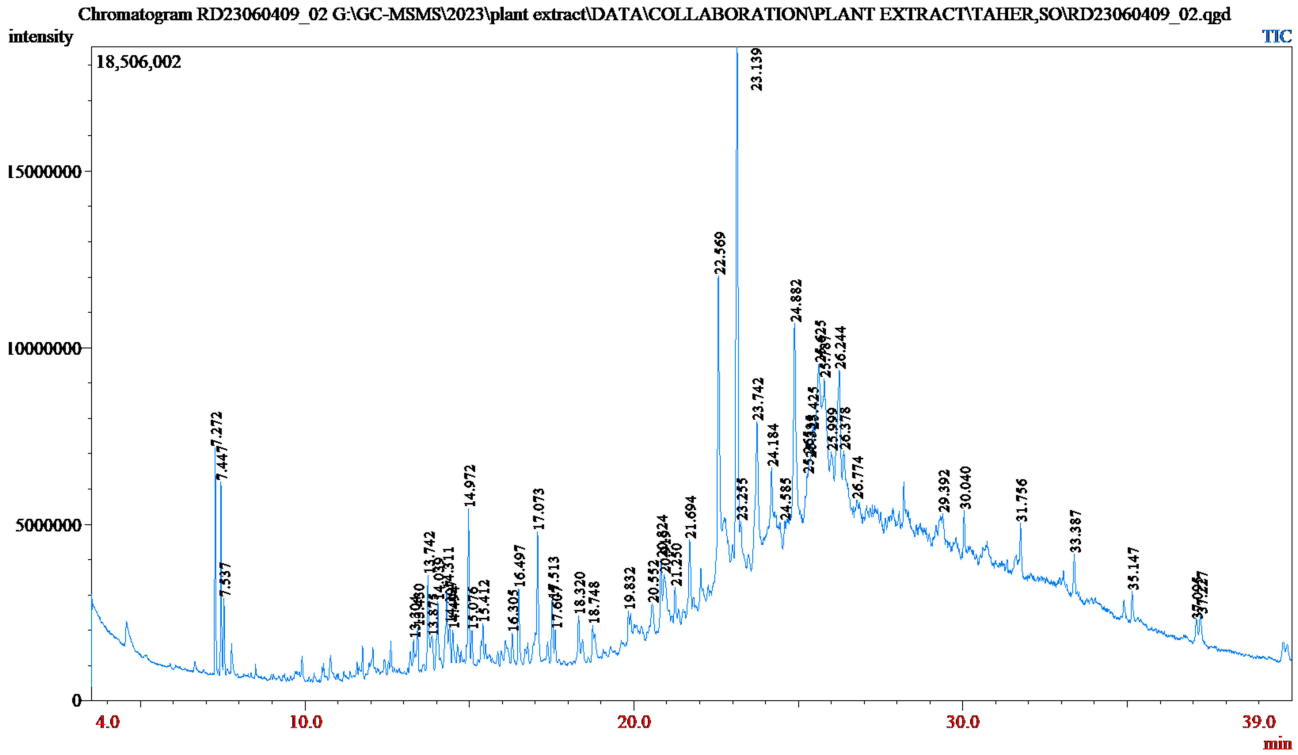
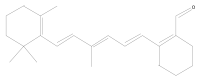
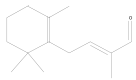
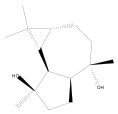
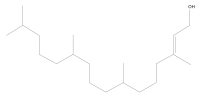
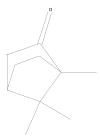
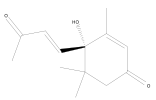


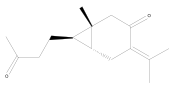
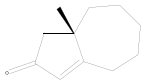
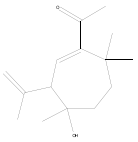
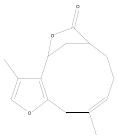
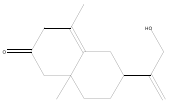
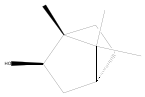
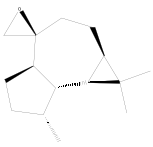
Figure 1 GC–MS/MS Chromatogram of Dichloromethane extract of *Canna indica*.

Table 2 Phytochemicals Identified from GC–MS/MS Analysis of Dichloromethane (DCM) Extract of Canna Indica

S/N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
Aldehyde						
1	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	17.51	1.23	121	78	
2	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	17.07	2.35	81	79	
Alcohol						
3	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	13.74	1.99	111	76	
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	14.31	1.71	68	87	
5	Thunbergol	16.30	0.55	137	77	
6	1-Heptatriacotanol	23.13	13.3	81	71	
7	1-Heptatriacotanol	23.74	4.45	57	76	
Ketone						
8	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	7.27	2.68	95	97	
9	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-enone	13.87	0.99	124	79	

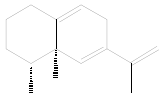
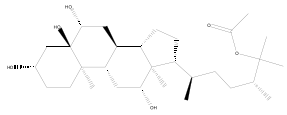
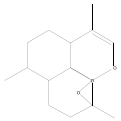
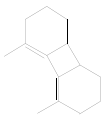
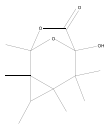
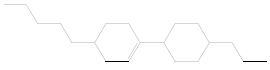
(*Continued*)

Table 2 (Continued).

S/N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
10	Curcumenone	14.49	0.67	68	91	
11	2(1H)-Azulenone, 4,5,6,7,8,8a-hexahydro-8a-methyl-, (S)-	14.97	2.7	–	–	
12	1-(4-Hydroxy-3-isopropenyl-4,7,7-trimethyl-cyclohept-1-enyl)-ethanone	20.55	0.78	124	68	
13	4,7-Methanofuro[3,2-c]oxacycloundecin-6 (4H)-one, 7,8,9,12-tetrahydro-3,11-dimethyl-	20.91	1.69	91	68	
14	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	16.49	1.38	105	80	
Terpene						
15	Isoborneol	7.44	2.53	95	95	
16	endo-Borneol	7.53	0.94	95	96	
17	Aromadendrene oxide-(2)	15.07	0.58	79	84	

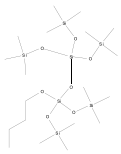
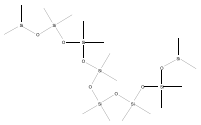
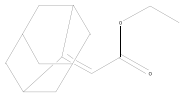
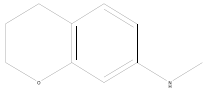
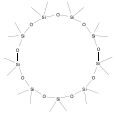
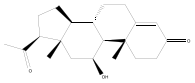
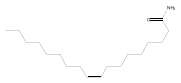
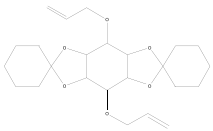
(*Continued*)

Table 2 (Continued).

S/N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
18	(4R,4aR)-4,4a-Dimethyl-6-(prop-1-en-2-yl)-1,2,3,4,4a,7-hexahydronaphthalene	14.03	0.95	159	77	
19	Ergostane-3,5,6,12,25-pentol, 25-acetate, (3.beta.,5.alpha.,6.beta.,12.beta).-	14.39	0.86	71	77	
20	Dihydroartemisinin, 6-deshydro-5-deshydroxy-3-desoxy-	20.82	1.43	124	72	
Hydrocarbon						
21	Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl-	21.69	1.61	145	70	
22	Eicosane	23.25	0.5	57	91	
23	Triacontane, 1-bromo-	25.26	1.17	57	76	
24	11-Methyltricosane	25.42	3.82	153	64	
Others						
25	1S,3R,4S,5R,6S-1-Hydroxy-2,2,3,4,5,6-hexamethyl-8-oxo-7,9-dioxatricyclo[4.2.1.0(3,5)]nonane	24.88	6.16	124	71	
26	Cyclohexene, 4-pentyl-1-(4-propylcyclohexyl)-	13.3	0.57	67	72	

(*Continued*)

Table 2 (Continued).

S/N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
27	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy)tetrasiloxane	15.41	0.54	73	73	
28	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	17.60	0.54	73	65	
29	Acetic acid, tricyclo[3.3.1.1(3,7)]decylidene-, ethyl ester	18.32	0.65	174	69	
30	(Chroman-7-yl)methylamine	18.74	0.64	163	69	
31	Cyclononasiloxane, octadecamethyl-	19.83	0.49	73	70	
32	11.beta.-Hydroxyprogesterone	21.25	0.65	124	67	
33	9-Octadecenamide, (Z)-	22.56	6.44	59	94	
34	2,3:5,6-Di-O-1-Cyclohexylieden-1,4-cyclohexandiallylether	25.33	1.35	57	60	
35	Eicosyl isopropyl ether	26.24	5.07	57	70	

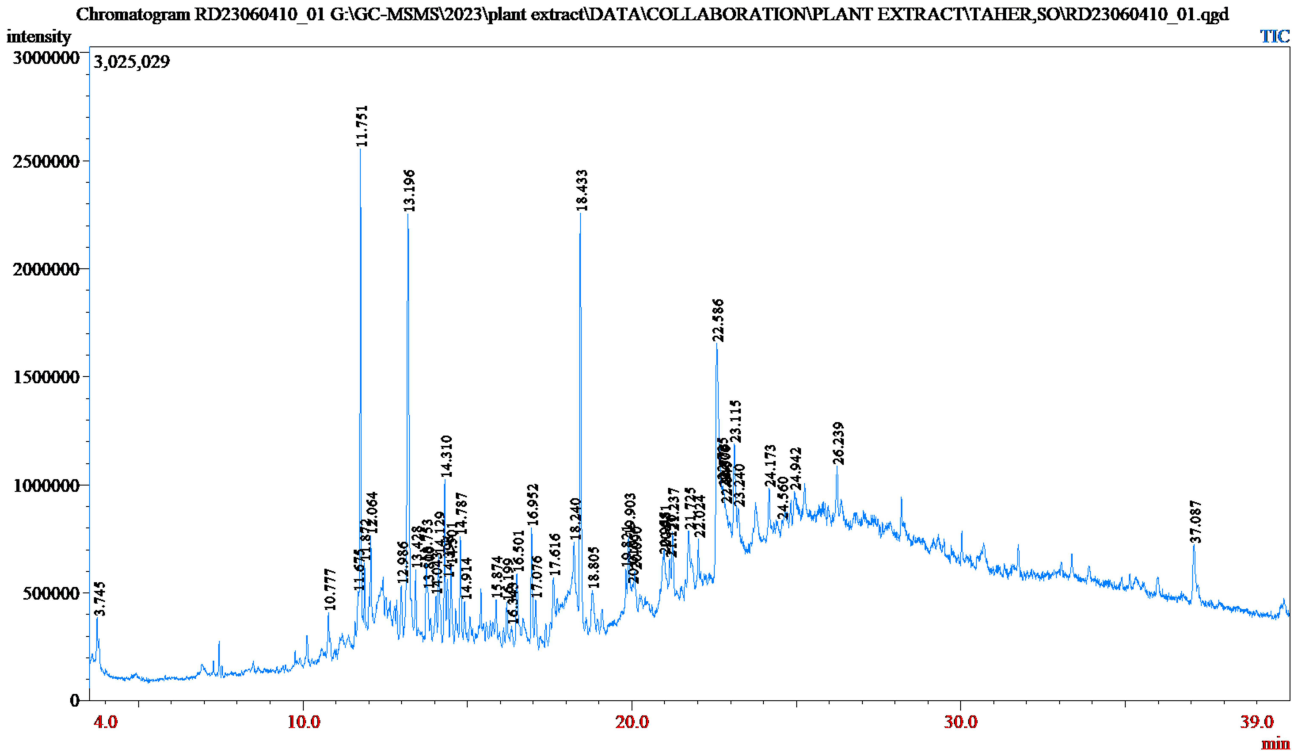
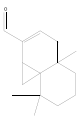
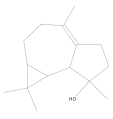


Figure 2 GC–MS/MS Chromatogram of Aqueous extract of *Canna indica*.

Antimicrobial Activity

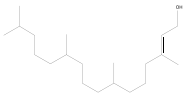
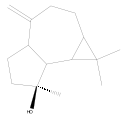
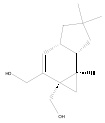
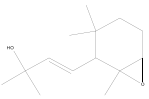
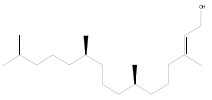
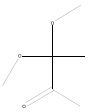
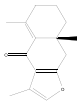
Antimicrobial activities of all partitions were evaluated against five gram-positive and eight gram-negative bacteria, with Azithromycin, Amoxicillin, Ciprofloxacin, and Fluconazole serving as reference standards for respective antimicrobial

Table 3 Phytochemicals Identified from GC–MS/MS Analysis of Aqueous (AQ) Extract of Canna Indica

S/N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
Aldehyde						
1	(1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydrocyclopropa[d]naphthalene-2-carbaldehyde	16.5	2.02	91	78	
Alcohol						
2	Isospathulenol	12.06	1.33	119	91	
3	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	13.75	1.59	119	–	

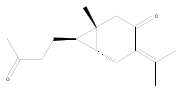
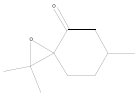
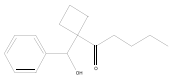
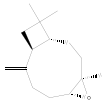
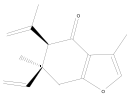
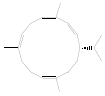
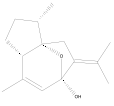
(*Continued*)

Table 3 (Continued).

S/N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	14.91	0.65	81	90	
5	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha).]-	15.87	0.89	119	76	
6	(1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethyldecahydro-1H-cyclopropa[a]naphthalen-7-ol	17.07	1.34	81	76	
7	Cycloprop[e]indene-1a,2(1H)-dimethanol, 3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-, (1a.alpha.,3a.beta.,6a.beta.,6b.alpha).-(-)	21.72	1.26	91	66	
8	3-Buten-2-ol, 2-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-	22.8	0.81	55	59	
9	1-Heptatriacotanol	23.11	3.06	81	74	
10	Phytol	18.43	8.66	71	97	
Ketone						
11	3,3-Dimethoxy-2-butanone	3.74	0.79	89	79	
12	(R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one	11.75	8.3	122	84	

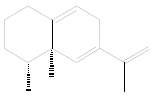
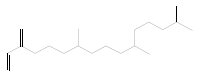
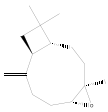
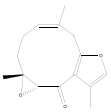
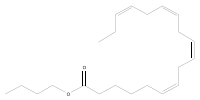
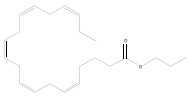
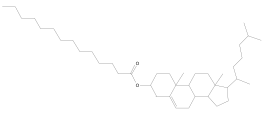
(*Continued*)

Table 3 (Continued).

S/N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
13	2-Pentadecanone, 6,10,14-trimethyl-	14.39	1.42	71	79	
14	Curcumenone	14.50	1.93	68	90	
15	1-Oxaspiro[2.5]octan-4-one, 2,2,6-trimethyl-, cis-	20.95	1.11	55	47	
16	1-Pentanone,1-(1-[hydroxy(phenyl)methyl]cyclobutyl])	20.98	1.09	55	52	
Terpene						
17	Caryophyllene oxide	11.67	1.16	79	75	
18	Epicurzerenone	11.87	1.07	122	83	
19	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	12.98	1.11	105	79	
20	Curcumenol A	13.19	12.98	105	90	

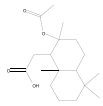
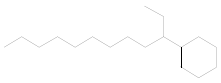
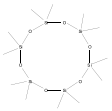
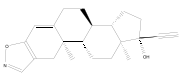
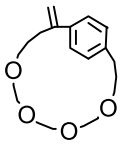
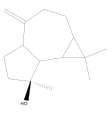
(*Continued*)

Table 3 (Continued).

S/N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
21	(4R,4aR)-4,4a-Dimethyl-6-(prop-1-en-2-yl)-1,2,3,4,4a,7-hexahydronaphthalene	14.04	0.82	91	–	
22	Neophytadiene	14.31	2.94	68	92	
23	Caryophyllene oxide	16.34	0.68	133	–	
24	Zederone	16.95	2.82	119	88	
25	Corymbolone	21.15	1.08	124	63	
Esters						
26	Butyl 6,9,12,15-octadecatetraenoate	10.77	0.8	191	74	
27	n-Propyl 5,8,11,14,17-eicosapentaenoate	14.12	1.85	107	72	
28	Cholest-5-en-3-ol (3.beta).-, tetradecanoate	37.08	1.69	73	74	

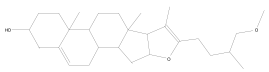
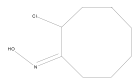
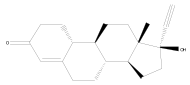
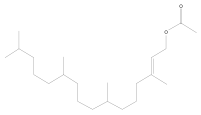
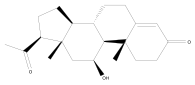
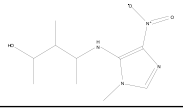
(*Continued*)

Table 3 (Continued).

S/N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
29	Acetic acid, 2-(2-acetoxy-2,5,5,8a-tetramethyldecalin-1-yl)-	24.94	1.1	124	59	
Hydrocarbon						
30	Decane, 1-bromo-2-methyl-	20.01	0.85	57	61	
31	Dodecane, 3-cyclohexyl-	22.77	1.42	69	56	
32	2-Methyltetracosane	23.24	0.74	57	79	
33	2,7-Octadiene, 1-butoxy-	24.56	0.7	71	53	
Others						
34	Cyclohexasiloxane, dodecamethyl-	13.42	1.08	73	–	
35	Danazol	13.8	0.97	109	–	
36	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha).-	14.78	2.06	107	–	
37	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha).]-	16.19	1.07	91	77	

(*Continued*)

Table 3 (Continued).

S/N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
38	Pseduosarsasapogenin-5,20-dien methyl ether	17.61	1.1	73	56	
39	alpha.-Chlorocyclooctanone oxime	18.24	1.09	53	62	
40	Norethindrone	19.90	2.22	53	–	
41	Phytol, acetate	20.09	0.88	57	–	
42	11.beta.-Hydroxyprogesterone	21.23	1.67	124	64	
43	1-Methyl-4-nitro-5-[(1,2-dimethyl-3-hydroxybutyl) amino]-(1H)-imidazole	22.84	0.7	55	54	

testing. The test samples exhibited a range of 6 mm to 18 mm in the zone of inhibition (ZOI), as outlined in Table 6. The aqueous fraction (AQSF) and dichloromethane fraction (DCMSF) displayed moderate antibacterial activity, particularly against *E. coli* (16.67 ± 0.47 mm for AQSF) and *P. aeruginosa* (17.67 ± 0.47 mm for AQSF). However, both ethyl acetate (EASF) and petroleum ether fractions (PESF) showed limited antibacterial efficacy, with mean inhibition zones generally below 12 mm across all bacterial strains. Notably, Gram-positive bacteria like *S. aureus* showed higher susceptibility to azithromycin (40.00 ± 0.82 mm) and ciprofloxacin (34.33 ± 0.94 mm) compared to the extracts, which demonstrated a maximum ZOI of 15 mm.

Cytotoxic Activity

The EASF and DCMSF fractions revealed the lowest LC_{50} values at 1.11 and 1.21 µg/mL, respectively, in comparison to the standard of 0.451 µg/mL, the AQSF and PESF fractions displayed moderate cytotoxic activity with LC_{50} values of 25.47 and 15.52 µg/mL, respectively (Figure 5).

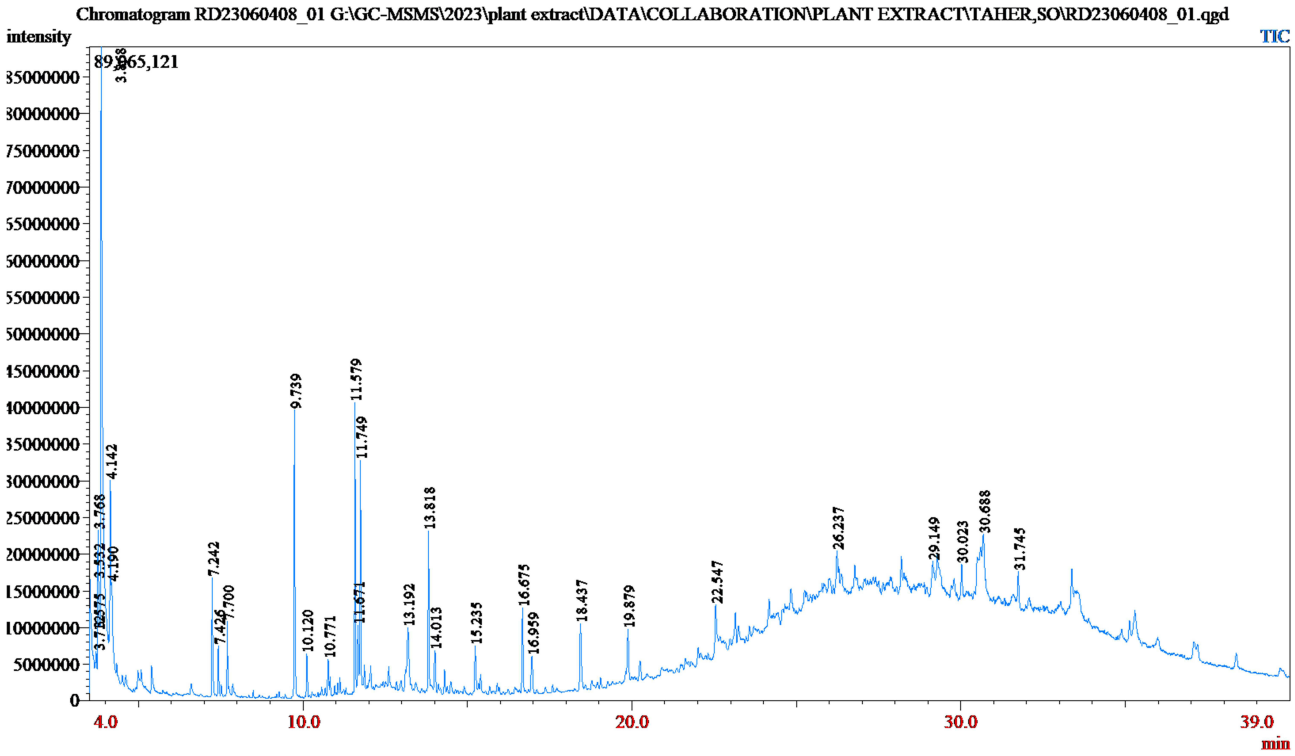
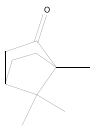


Figure 3 GC–MS/MS Chromatogram of Petroleum Ether extract of *Canna indica*.

Analgesic Activity

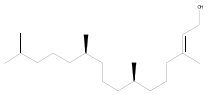
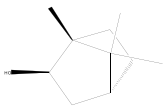
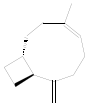
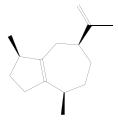
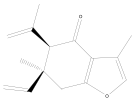
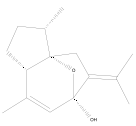
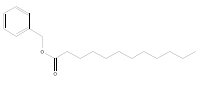
Figure 6 illustrated that highly significant ($p< 0.001$) analgesic activity was observed from most of the fractions of leaves of *C. indica*, while EASF showed very significant ($p< 0.01$). The highest reduction of writhing was observed for DCMSF, where 400 mg/kg dose reduced 53.57% writhing compared to the standard 81.34% reduction. Also, a dose-dependent effect was shown by all of these fractions.

Table 4 Phytochemicals Identified from GC–MS/MS Analysis of Petroleum Ether (PET) Extract of Canna Indica

S.N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
Ketone						
1	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	7.242	2.63	95	97	
Alcohol						
2	Z-10-Pentadecen-1-ol	11.671	1.68	55	85	

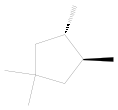
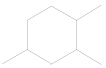
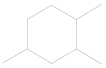
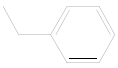
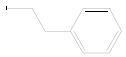
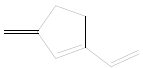
(*Continued*)

Table 4 (Continued).

S.N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
3	2,4-Pentadien-1-ol, 3-pentyl-, (2Z)-	16.959	1.41	83	79	
4	Phytol	18.437	2.35	71	97	
Terpene						
5	Isoborneol	7.426	1.1	95	95	
6	Caryophyllene	10.12	0.96	91	95	
7	alpha.-Guaiene	10.771	0.98	191	79	
8	Epicurzerenone	11.749	5.48	122	88	
9	Curcumenol	13.192	2.65	105	76	
Ester						
10	Dodecanoic acid, phenylmethyl ester	29.149	0.86	57	77	

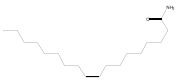
(*Continued*)

Table 4 (Continued).

S.N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
11	Benzenepropanoic acid, 3.5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	30.688	1.34	57	83	
Hydrocarbon						
12	Cyclopentane, 1,1,3,4-tetramethyl-, trans-	3.532	2.79	55	87	
13	Cyclohexane, 1,2,4-trimethyl-	3.575	1.58	69	85	
14	Cyclohexane, 1,2,4-trimethyl-	3.712	1.16	69	92	
15	Ethylbenzene	3.768	4.69	91	92	
16	Benzene, (2-iodoethyl)-	3.868	31.28	92	82	
17	Cyclopentene, 1-ethenyl-3-methylene-	4.142	6.05	91	94	
18	Nonane	4.19	3.42	57	96	
19	Dodecane	7.7	1.17	57	98	
20	Tetradecane	9.739	6.13	57	94	

(*Continued*)

Table 4 (Continued).

S.N	Compound Name	Ret. Time	Conc. %	m/z	MS similarity	Figures
21	Hexadecane	11.579	5.56	57	96	
22	Heneicosane	13.818	3.72	57	97	
23	7-Heptadecene, 1-chloro-	14.013	1.19	55	83	
24	Cyclopropane, 2-(1,1-dimethyl-2-propenyl)-1,1-dimethyl-	15.235	1.39	82	84	
25	Heneicosane	16.675	2.33	57	97	
26	Heneicosane	19.879	1.44	57	95	
Other						
27	9-Octadecenamide, (Z)-	22.547	1.56	59	93	

Antidiarrheal Activity

The various fractions of *C. indica* leaf extract illustrated a dose-dependent antidiarrheal effect. Notably, the DCMSF exhibited the most significant activity, with a 400 mg/kg dose resulting in a 45.21% reduction in diarrheal episodes compared to the standard loperamide, which achieved a 76.21% reduction. Additionally, the 400 mg/kg extracts of AQSF, PESF, and EASF displayed reductions of 40.5%, 26.21%, and 28.57%, respectively, in diarrhea incidence (Figure 6).

Molecular Docking

A comprehensive set of 103 compounds, originating from distinct fractions including DCM, AQ PET, and EA of the *C. indica* leaf extract, underwent meticulous computational docking studies. These investigations were conducted to assess the interactions and binding affinities of these compounds with five distinct receptors. The findings, including the detailed representation of binding affinities for each compound concerning the specified receptors, have been organized and presented in Table 7.

Compound C46 demonstrated the highest affinity towards EGFR, with a binding score of −9.9 kcal/mol, albeit slightly lower than the standard Lapatinib, with a score of −10.8 kcal/mol. Following closely were C92 and C99, both recording a binding score of −9.8 kcal/mol, trailed by C53 and C89 with scores of −9.2 kcal/mol and −9 kcal/mol, respectively. Notably, nine of these compounds exhibited binding scores lower than −8 kcal/mol, and an additional 30 compounds recorded scores lower than −7 kcal/mol.

For DHFR, the lowermost affinity was noted for C29, displaying a binding affinity value of −9.1 kcal/mol. Furthermore, C98, C95, C10, C29, and C92 showcased notable binding affinities toward DHFR, scoring −9, −8.7,

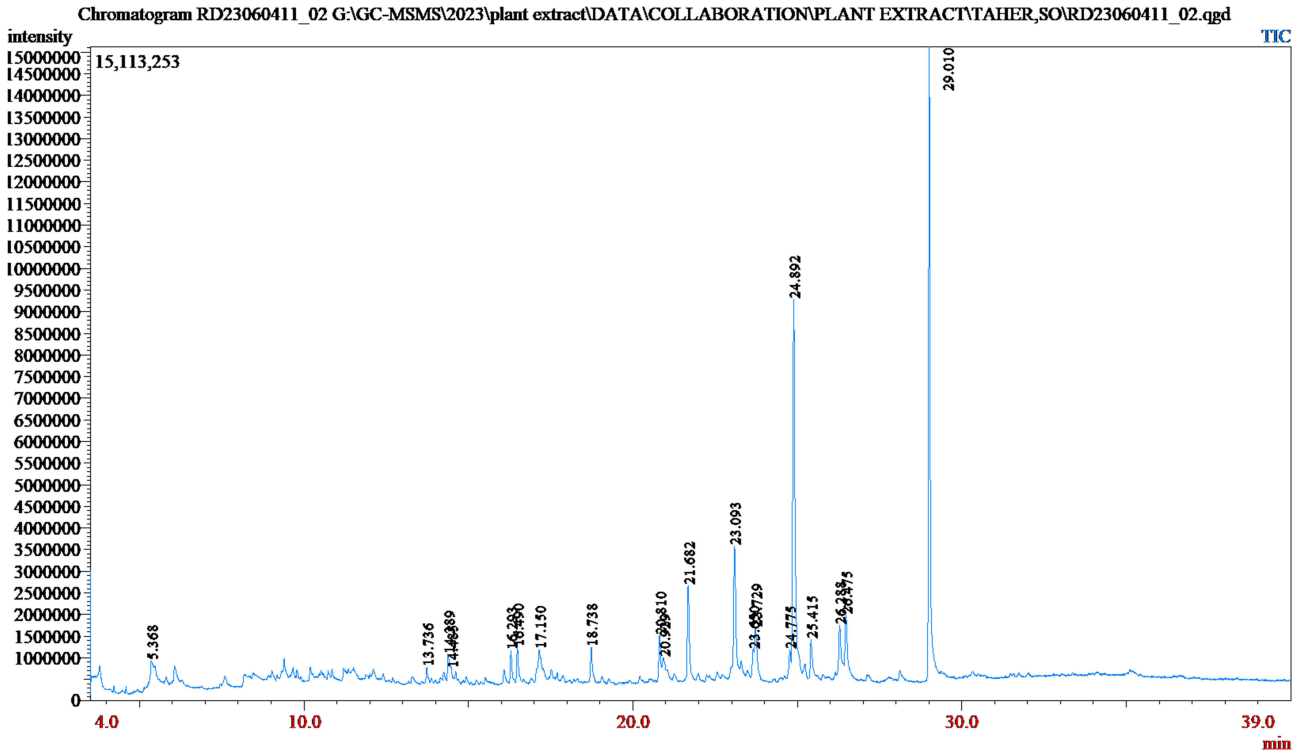
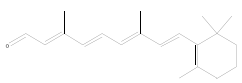


Figure 4 GC–MS/MS Chromatogram of Ethyl Acetate extract of *Canna indica*.

−8.6, −8.4, and −8.4 kcal/mol, respectively. Notably, these values surpassed the standard ciprofloxacin affinity of −8.2 kcal/mol.

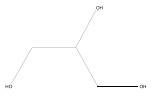
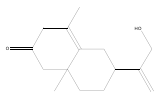
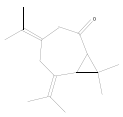
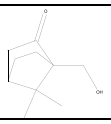
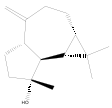
In the case of DOR, C95 exhibited the lowest affinity score of −10.1 kcal/mol, slightly trailing behind Loperamide with a score of −10.2 kcal/mol. Additionally, C96, C29, C99, C7, C46, C53, and C87 demonstrated binding scores of −9.8, −9.5, −9.5, −9.1, −9.1, −9, and −9 kcal/mol, respectively. Notably, 23 compounds displayed binding affinities lower than −8 kcal/mol.

Table 5 Phytochemicals Identified from GC–MS/MS Analysis of Ethyl Acetate (EA) Extract of Canna Indica

S/N	Compound Name	Ret. Time	Area %	m/z	MS similarity	Figures
Aldehyde						
1	Retinal	13.736	0.67	69	77	
2	D:A-Friedooleanan-28-al, 3-oxo-	16.293	1.65	137	75	

(*Continued*)

Table 5 (Continued).

S/N	Compound Name	Ret. Time	Area %	m/z	MS similarity	Figures
Alcohol						
3	Glycerin	5.368	2.23	61	88	
4	2,5,8,11,14,17-Hexaoxanonadecan-19-ol	14.485	0.69	59	66	
5	Sambucinol	24.892	27.37	124	66	
Ketones						
6	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	16.49	1.4	147	80	
7	Bicyclo[5.1.0]octan-2-one, 4.6-diisopropylidene-8,8-dimethyl-	18.738	1.95	163	69	
8	Androst-5-en-3-one, 4.4-dimethyl-	20.81	2.31	124	70	
9	1-Hydroxymethyl-7,7-dimethylbicyclo[2.2.1]heptan-2-one	26.475	4.57	153	76	
Terpene						
10	(-)-Spathulenol	23.093	6.92	81	69	
Hydrocarbon						
11	Nonadecanoic acid	17.15	1.31	55	77	

(*Continued*)

Table 5 (Continued).

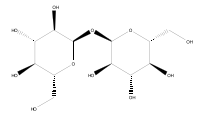
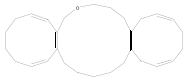
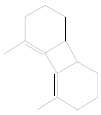
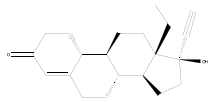
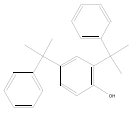
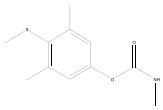
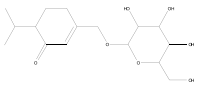
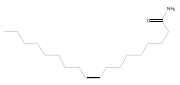
S/N	Compound Name	Ret. Time	Area %	m/z	MS similarity	Figures
Others						
12	Trehalose	14.389	1.58	73	87	
13	3-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22),7(16),9,13,23,29-hexaene	20.929	1.04	145	65	
14	Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl-	21.682	5.02	188	70	
15	Norgestrel	23.65	1.39	160	64	
16	Androst-5-en-3-one, 4.4-dimethyl-	23.729	2.95	124	69	
17	Phenol, 2.4-bis(1-methyl-1-phenylethyl)-	24.775	1.1	315	90	
18	Methiocarb	25.415	2	153	72	
19	(4-isopropyl-cyclohex-1-en-3-on-1-yl) methyl glucopyranoside	26.288	3.28	108	73	
20	9-Octadecenamide, (Z)-	29.01	30.57	59	91	

Table 6 The Antimicrobial Activity of Canna Indica Extracts and Standard Against Gram-Positive Bacteria and Gram-Negative Bacteria

Test Microorganisms	Zone of Inhibition						
	Azithromycin (30 µg/disc)	Amoxicillin (30 µg/disc)	Ciprofloxacin (30 µg/disc)	AQSF (100 µg/disc)	DCMSF (100 µg/disc)	EASF (100 µg/disc)	PESF (100 µg/disc)
Gram Positive							
Bacillus cereus	33.67 ± 1.25	33.00 ± 0.82	31.33 ± 0.47	15.00 ± 0.82	11.00 ± 0.82	9.33 ± 0.47	7.33 ± 0.47
Bacillus megaterium	32.33 ± 2.05	27.00 ± 0.82	27.67 ± 0.47	10.67 ± 0.94	9.67 ± 0.47	8.67 ± 0.47	–
Bacillus subtilis	31.33 ± 1.25	25.67 ± 1.70	31.00 ± 0.82	8.33 ± 0.47	8.67 ± 0.47	–	–
Staphylococcus aureus	40.00 ± 0.82	35.33 ± 0.47	34.33 ± 0.94	14.33 ± 0.47	13.00 ± 0.82	9.67 ± 0.47	7.67 ± 0.47
Sarcina lutea	37.67 ± 1.25	33.00 ± 0.82	28.00 ± 0.82	13.67 ± 0.47	10.00 ± 0.82	7.67 ± 0.47	6.33 ± 0.47
Gram Negative							
Escherichia coli	37.67 ± 1.70	35.67 ± 0.47	31.00 ± 0.82	16.67 ± 0.47	12.33 ± 0.94	11.33 ± 0.47	11.67 ± 0.47
Pseudomonas aeruginosa	41.33 ± 0.47	37.00 ± 0.82	37.00 ± 0.82	17.67 ± 0.47	15.00 ± 0.82	9.67 ± 0.47	8.67 ± 0.47
Salmonella paratyphi	33.67 ± 1.70	31.00 ± 0.82	25.67 ± 0.94	15.33 ± 0.47	10.67 ± 0.47	9.33 ± 0.47	–
Salmonella typhi	39.00 ± 0.82	30.33 ± 1.25	32.00 ± 0.82	18.00 ± 0.82	11.33 ± 0.47	–	6.33 ± 0.47
Shigella dysenteriae	35.67 ± 0.47	31.33 ± 0.47	34.00 ± 0.82	16.33 ± 0.47	12.33 ± 0.47	8.33 ± 0.47	7.67 ± 0.47
Vibrio mimicus	32.67 ± 1.25	27.00 ± 0.82	26.33 ± 0.47	12.33 ± 0.47	9.33 ± 0.47	–	–
Vibrio parahemolyticus	40.33 ± 0.47	34.00 ± 0.82	34.00 ± 0.82	14.33 ± 0.47	14.67 ± 0.47	6.33 ± 0.47	–

The most substantial binding affinity against TNF-α was documented for C96, boasting an affinity value of −9.8 kcal/mol, followed by C92 and C10 with scores of −9.5 and −9.1 kcal/mol, respectively. Several compounds surpassed the affinity score of the standard Diclofenac. Notably, C7 and C99 recorded −8.7 kcal/mol, while C20, C23, and C29 achieved −8.4 kcal/mol. Additionally, C60 and C95 exhibited affinity scores of −8.2 kcal/mol.

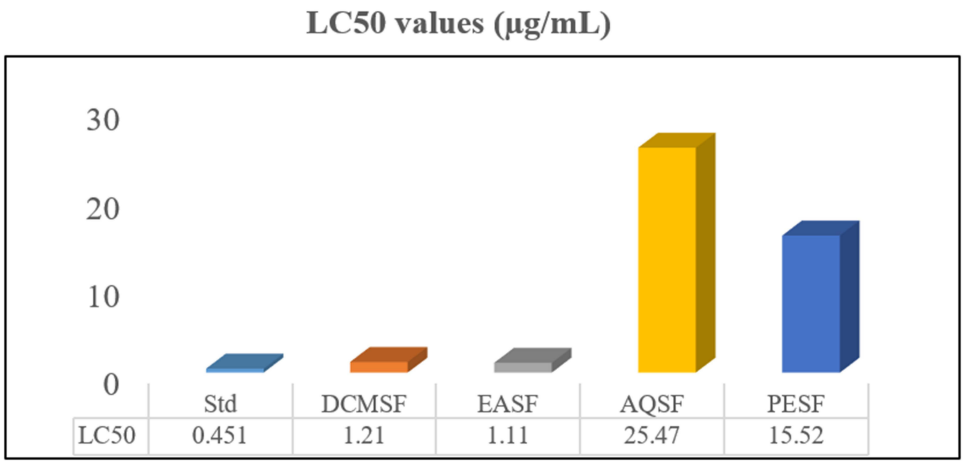


Figure 5 Cytotoxic effect of different fractions of leaves of *Canna indica*.

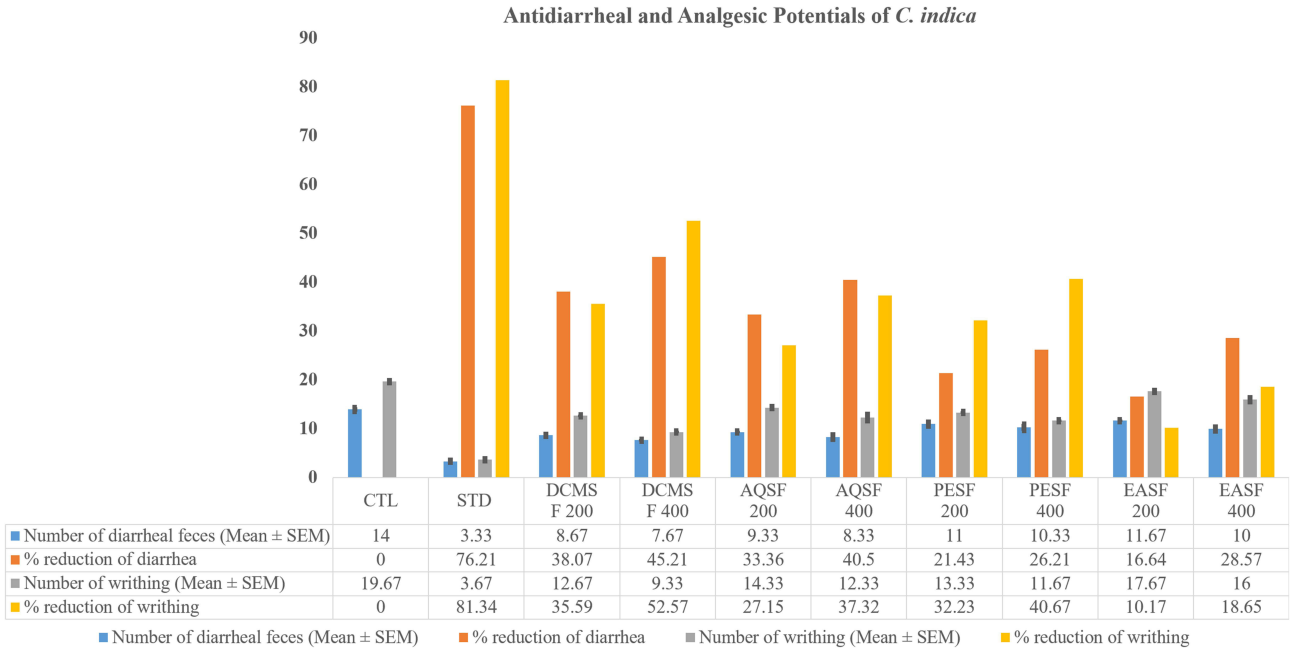


Figure 6 Antidihreal and Analgesic activities of different fractions of leaves of *Canna indica*.

Lastly, C99 demonstrated the most robust binding affinity against COX-2, registering a binding score of −9.2 kcal/mol. Notably, C87, C4, C11, C59, and C14 displayed substantial binding affinities of −8.7, −8.6, −8.1, −8.1, and −8 kcal/mol, respectively, surpassing the standard Diclofenac value of −7.9 kcal/mol.

Table 7 The Identified Compounds from Leaves of Canna Indica with Their PubChem CID and Binding Affinity Scores Against Different Receptors

Extract	Serial	Compounds	PubChem Cid	Targets				
				EGFR	DHFR	DOR	TNF-α	COX-2
Aqueous	1	3,3-Dimethoxy-2-butanone	140871	−4.7	−4.2	−4.7	−4.4	−4.5
	2	Butyl 6,9,12,15-octadecatetraenoate	91697552	−6.3	−5.7	−7.3	−5.9	−7.5
	3	Caryophyllene oxide	1742210	−7	−6.6	−7.5	−7.4	−6.4
	4	(R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one	91711266	−7.9	−7.4	−8.5	−7.7	−8.6
	5	Epicurzerenone	5317062	−6.5	−6.1	−7.4	−6.6	−7.6
	6	Isospathulenol	102303030	−7.7	−6.8	−8	−7	−6.7
	7	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	6,436,662	−7.6	−7	−9.1	−8.7	−6.4
	8	Curcumenol	167812	−8.3	−6.8	−8.1	−7.7	−6.4
	9	Cyclohexasiloxane, dodecamethyl-	10911	−1.7	−1.4	−1.6	−1.2	−1.5
	10	Danazol	28417	−8.4	−8.6	−8.1	−9.1	−7
	11	n-Propyl 5,8,11,14,17-eicosapentaenoate	91697570	−7.3	−6.3	−7.3	−6.4	−8.1

(*Continued*)

Table 7 (Continued).

Extract	Serial	Compounds	PubChem Cid	Targets				
				EGFR	DHFR	DOR	TNF-α	COX-2
	12	Neophytadiene	10446	−6.7	−5.7	−6.3	−5.8	−7.2
	13	2-Pentadecanone, 6,10,14-trimethyl-	10408	−6.8	−5.5	−7	−5.9	−7.2
	14	Curcumenone	153845	−7.5	−6.9	−7.7	−6.4	−8
	15	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha).-	10,544,720	−7	−6.1	−7.4	−6.7	−7.5
	16	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha).]-	6,432,640	−7.2	−7	−7.5	−7	−6.4
	17	(1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydrocyclopropa[d]naphthalene-2-carbaldehyde	12444331	−7.1	−6.6	−7.4	−6.9	−7.5
	18	Zederone	101286196	−7.2	−7.2	−7.9	−7.4	−6.2
	19	(1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethyldecahydro-1H-cyclopropa[a]naphthalen-7-ol	12309603	−6.6	−6.5	−8.2	−6.9	−5.8
	20	Pseduosarsasapogenin-5,20-dien methyl ether	552194	−8.8	−9.1	−8.8	−8.4	−7.2
	21	alpha.-Chlorocyclooctanone oxime	6505552	−5.3	−5.6	−6.2	−5.6	−6.1
	22	Phytol	5280435	−6.9	−5.5	−6.6	−5.8	−7.3
	23	Norethindrone	6230	−7.1	−7.9	−8.5	−8.4	−6.8
	24	Decane, 1-bromo-2-methyl-	545631	−5.1	−4.5	−5.4	−4.8	−5.4
	25	Phytol, acetate	6428538	−7	−5.9	−7.3	−6.3	−7.5
	26	1-Oxaspiro[2.5]octan-4-one, 2,2,6-trimethyl-, cis-	330109	−5.8	−5.8	−6.7	−5.8	−6.9
	27	1-Pentanone,1-(1-[hydroxy(phenyl)methyl]cyclobutyl])	557,977	−6.7	−6.1	−7.4	−6.9	−7.2
	28	Corymbolone	178931	−7.5	−6.9	−7.9	−6.9	−6.4
	29	11.beta.-Hydroxyprogesterone	101788	−8.9	−8.4	−9.5	−8.4	−6.9
	30	Cycloprop[e]indene-1a,2(1H)-dimethanol, 3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-, (1a.alpha.,3a.beta.,6a.beta.,6b.alpha).-(-)	565,048	−6.6	−6.3	−7.8	−6.9	−6.4
	31	Dodecane, 3-cyclohexyl-	524423	−6.9	−5.5	−6.8	−5.6	−7.3
	32	3-Buten-2-ol, 2-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-	5,363,622	−6.5	−6.1	−6.6	−6.5	−6.8
	33	1-Methyl-4-nitro-5-[(1,2-dimethyl-3-hydroxybutyl)amino]-(1H)-imidazole	566702	−5.8	−5.7	−6.9	−6	−6.9
	34	2-Methyltetracosane	527459	−6.3	−5.2	−6.5	−5.3	−7.1
	35	2,7-Octadiene, 1-butoxy-	534461	−5.5	−4.6	−5.4	−4.8	−5.6
	36	Acetic acid, 2-(2-acetoxy-2,5,5,8a-tetramethyldecalin-1-yl)-	536,559	−6.4	−6.6	−8.5	−7.6	−7.3
	37	Cholest-5-en-3-ol (3.beta).-, tetradecanoate	313252	−7	−7.1	−8.5	−7.2	−4.3

(*Continued*)

Table 7 (Continued).

Extract	Serial	Compounds	PubChem Cid	Targets				
				EGFR	DHFR	DOR	TNF-α	COX-2
Dichloromethane	38	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	2537	−5	−5	−6.6	−6	−4.8
	39	Isoborneol	6321405	−5	−5.3	−6.6	−5.8	−4.6
	40	endo-Borneol	6552009	−4.8	−5.1	−6.6	−6.1	−4.7
	41	Cyclohexene, 4-pentyl-1-(4-propylcyclohexyl)-	557,007	−8.2	−7	−8.2	−6.5	−7.5
	42	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	178322	−7	−6.7	−7	−7.3	−6.1
	43	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-enone	688492	−6.4	−6.4	−7	−7.2	−6.2
	44	(4R,4aR)-4,4a-Dimethyl-6-(prop-1-en-2-yl)-1,2,3,4,4a,7-hexahydronaphthalene	90470826	−7.6	−6.7	−8.3	−7	−7.2
	45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5366244	−7	−5.6	−6.6	−5.7	−7.3
	46	Ergostane-3,5,6,12,25-pentol, 25-acetate, (3.beta.,5.alpha.,6.beta.,12.beta).-	91,691,421	−9.9	−7.8	−9.1	−7.7	−6.9
	47	2(1H)-Azulenone, 4,5,6,7,8,8a-hexahydro-8a-methyl-, (S)-	22,216,158	−6.2	−6	−7.4	−6.2	−6.7
	48	Aromadendrene oxide-(2)	16,211,192	−7.6	−6.7	−8.2	−7	−7.2
	49	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	553039	−1.7	−1.4	−1.6	−1.2	−1.5
	50	Thunbergol	5363523	−5.7	−6	−7.6	−8.1	−5.2
	51	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	564373	−8	−6.7	−8.1	−6.9	−7.1
	52	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	5,369,997	−6.7	−5.9	−7.6	−6.6	−7.3
	53	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde	5363101	−9.2	−7.7	−9	−7.5	−6.7
	54	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	6329087	−1.7	−1.4	−1.6	−1.2	−1.5
	55	Acetic acid, tricyclo[3.3.1.1(3,7)]decylidene-, ethyl ester	600028	−6.2	−6.1	−7.7	−6.6	−6.1
	56	(Chroman-7-yl)methylamine	6424486	−6.2	−5.7	−6.3	−5.5	−6.6
	57	Cyclononasiloxane, octadecamethyl-	11172	−1.7	−1.4	−1.6	−1.2	−1.5
	58	1-(4-Hydroxy-3-isopropenyl-4,7,7-trimethyl-cyclohept-1-enyl)-ethanone	539314	−6.2	−6.5	−7.5	−7	−7.5
	59	Dihydroartemisinin, 6-deshydro-5-deshydroxy-3-desoxy-	541540	−8.1	−7.4	−8.7	−7.4	−8.1
	60	4,7-Methanofuro[3,2-c]oxacycloundecin-6(4H)-one, 7,8,9,12-tetrahydro-3,11-dimethyl-	5377116	−7.5	−7.2	−8.3	−8.2	−7
	61	Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl-	583087	−8	−6.7	−8	−7.4	−7.9
	62	9-Octadecenamide, (Z)-	5,283,387	−6.6	−5.4	−6.2	−5.3	−7.1
	63	1-Heptatriacotanol	537071	−5.8	−5.3	−6.7	−5.3	−6.3
	64	Eicosane	8222	−6	−4.8	−6.1	−5.2	−6.7

(*Continued*)

Table 7 (Continued).

Extract	Serial	Compounds	PubChem Cid	Targets				
				EGFR	DHFR	DOR	TNF-α	COX-2
	65	1S,3R,4S,5R,6S-1-Hydroxy-2,2,3,4,5,6-hexamethyl-8-oxo-7,9-dioxatricyclo[4.2.1.0(3,5)]nonane	539855	−6.6	−6.7	−7.5	−6.9	−6.5
	66	Triacontane, 1-bromo-	521082	−5.8	−5	−6.5	−5	−6.4
	67	2,3:5,6-Di-O-1-Cyclohexylieden-1,4-cyclohexandiallylether	15704917	−7	−7.4	−8.3	−8	−6.6
	68	11-Methyltricosane	530326	−6.2	−5.4	−6.5	−5.4	−7.1
	69	Eicosyl isopropyl ether	91691499	−6.2	−5.2	−6.4	−5.5	−7
Pet ether	70	Cyclopentane, 1,1,3,4-tetramethyl-, trans-	6432224	−5.5	−5	−5.8	−5.1	−5.4
	71	Cyclohexane, 1,2,4-trimethyl-	91517	−5.4	−4.9	−6	−5	−6.2
	72	Ethylbenzene	7500	−5.1	−4.7	−5.3	−4.9	−5.6
	73	Benzene, (2-iodoethyl)-	28,503	−5.2	−4.8	−5.4	−4.8	−5.8
	74	Cyclopentene, 1-ethenyl-3-methylene-	562142	−5.1	−4.6	−5.4	−5	−5.3
	75	Nonane	8141	−4.8	−4.3	−4.7	−4.5	−4.8
	76	Dodecane	8182	−5.2	−4	−5.3	−4.6	−5.5
	77	Tetradecane	12389	−5.6	−4.5	−5.4	−4.6	−5.9
	78	Caryophyllene	5281515	−7.1	−6.8	−7.5	−7	−6.7
	79	alpha.-Guaiene	6429358	−7.8	−6	−7.5	−7.3	−7.3
	80	Hexadecane	11006	−5.8	−4.5	−5.6	−4.7	−6.4
	81	Z-10-Pentadecen-1-ol	5364483	−6.1	−5.2	−6	−5	−6.2
	82	Heneicosane	12403	−6.1	−4.8	−6.4	−5	−6.7
	83	7-Heptadecene, 1-chloro-	5364485	−6	−5.5	−5.8	−4.9	−6.6
	84	Cyclopropane, 2-(1,1-dimethyl-2-propenyl)-1,1-dimethyl-	557547	−5	−4.4	−5.9	−4.9	−5.8
	85	2,4-Pentadien-1-ol, 3-pentyl-, (2Z)-	5,367,717	−5.2	−4.6	−5.7	−5.2	−5.3
	86	Dodecanoic acid, phenylmethyl ester	8791	−7	−5.7	−7.1	−6.1	−7.2
	87	Benzenepropanoic acid, 3.5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	16386	−7.3	−6.8	−9	−6.8	−8.7
Ethyl Acetate	88	Glycerin	753	−4.3	−3.3	−4.1	−3.5	−3.8
	89	Retinal	638015	−9	−7	−8.4	−7.1	−6.4
	90	Trehalose	7427	−5.9	−7.2	−7.4	−6.1	−5.7
	91	2,5,8,11,14,17-Hexaoxanonadecan-19-ol	90207	−4.9	−4.6	−5.2	−4.2	−5.4
	92	D:A-Friedooleanan-28-al, 3-oxo-	586214	−9.8	−8.4	−8.6	−9.5	−7.6
	93	Nonadecanoic acid	12591	−6.4	−5.2	−6.1	−5.5	−7
	94	Bicyclo[5.1.0]octan-2-one, 4.6-diisopropylidene-8,8-dimethyl-	534691	−7.9	−7.4	−7.8	−7.3	−7.5
	95	Androst-5-en-3-one, 4.4-dimethyl-	22216284	−8.5	−8.7	−10.1	−8.2	−7.3

(*Continued*)

Table 7 (Continued).

Extract	Serial	Compounds	PubChem Cid	Targets				
				EGFR	DHFR	DOR	TNF-α	COX-2
	96	3-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22),7(16),9,13,23,29-hexaene	5368332	−7.8	−7	−9.8	−9.8	−7.7
	97	(-)-Spathulenol	13854255	−7.8	−6.9	−8.4	−7.2	−7.3
	98	Norgestrel	13109	−7.9	−9	−8.7	−8.3	−6.5
	99	Phenol, 2.4-bis(1-methyl-1-phenylethyl)-	76,013	−9.8	−8	−9.5	−8.7	−9.2
	100	Sambucinol	5459101	−7.3	−6.6	−8.2	−7.4	−6
	101	Methiocarb	16248	−6.5	−6.4	−6.5	−5.7	−7
	102	(4-isopropyl-cyclohex-1-en-3-on-1-yl) methyl glucopyranoside	91704271	−7.6	−7.4	−8.8	−6.8	−7.2
	103	1-Hydroxymethyl-7,7-dimethylbicyclo[2.2.1]heptan-2-one	572679	−5.3	−5.1	−6.7	−6.2	−4.7
Standard		Lapatinib	208908	−10.8	–	–	–	–
		Ciprofloxacin	2764	–	−8.2	–	–	–
		Glibenclamide	3488	–	–	−10.2	–	–
		Diclofenac	3033	–	–	–	−7.1	− 7.9

Discussion

The quest for novel bioactive compounds applicable in emerging therapies highlights the prominence of medicinal plants. In developing nations, there is a growing focus on extensively utilizing plant-based remedies due to their multifaceted protective benefits and positive impact on human health, with traditional medicines being relied upon by approximately 80% of individuals, even in underdeveloped regions.[40,59] These medicinal plant extracts, intricate combinations of secondary metabolites from plants, animals, and microorganisms, typically contain 10 to 60 ingredients in varying concentrations, often relying on 2–4 key molecules for their biological features.[60,61] The exploration of the chemical composition and structure of these extracts unveils diverse biological potentials.

The DCM extract of *C. indica* yields 1-Heptatriacotanol, a compound exhibiting potent anti-hypercholesterolemic properties.[62] Oleamide, also known as 9-Octadecenamide, emerges as a versatile pharmacological candidate with capabilities such as sleep induction, hypomotility, and hypothermia.[63,64] Furthermore, Oleamide contributes to vasodilation and acts as a hypolipidemic agent, reducing serum triglycerides, total cholesterol, low-density lipoprotein cholesterol, and hepatic triglycerides.[65] Aromadendrene oxide 2 (AO-(2)) showcases anti-cancer potential against skin epidermoid cancer.[66] Curcumenol demonstrates efficacy against various cancers, particularly lung cancer, by inducing cell death and suppressing proliferation through the ferroptotic pathway.[67] It also inhibits the expression of the cervical cancer oncogene YWHAG.[68] In rats with chronic renal failure, Curcumenol improves renal function by suppressing inflammation and modulating the SIRT1 and NF-κB signaling pathways.[69] Additionally, Curcumenol exhibits a protective effect against intervertebral disc degeneration (IVDD) due to its anti-inflammatory activity.[70] Phytol, a medically significant compound, boasts diverse biological activities, including anti-anxiety, metabolism modulation, cytotoxicity, antioxidant properties, autophagy- and apoptosis-inducing effects, antinociception, anti-inflammatory action, immune modulation, and antimicrobial activity.[71] Neophytadiene is reported to have neurological effects, acting against anxiety and epilepsy.[72] Curcumenone, a sesquiterpene, demonstrates vasorelaxant and hepatoprotective activity and can prevent alcohol-induced intoxication.[73] Caryophyllene oxide exhibits notable analgesic and anti-inflammatory effects and possesses anticancer properties, making it a potential complementary medicine alongside conventional therapy.[74,75] Tetradecane shows antimicrobial and antifungal activity.[76] Isoborneol is reported to have cardioprotective effects and antiviral properties.[77,78] Heneicosane is a potent antimicrobial agent.[79] Caryophyllene, a bioactive

sesquiterpene, is effective in reducing inflammation and alleviating various neurodegenerative diseases, as well as demonstrating antimicrobial, anticonvulsant, analgesic, myorelaxant, sedative, and anti-depressive properties.[80] Spathulenol is reported to exhibit neuroprotective activity, being effective against 6-hydroxydopamine-induced neurotoxicity in SH-SY5Y neuroblastoma cells.[80] In another study, Spathulenol demonstrates significant antimycobacterial activity against *Mycobacterium tuberculosis* strains, including a resistant strain.[81]

Cytotoxicity assays, commonly utilizing brine shrimp as a zoological specimen, are frequently employed to evaluate the presence of cytotoxic compounds in plant material. The cytotoxic properties of terrestrial plants have been extensively studied through this method, as evidenced in various research studies.[82] This investigation found the potentiality of the plant extricates cytotoxic efficacy against brine shrimp. The results shown in Figure 5 indicate that the EASF and DCMSF fractions exhibited the highest cytotoxic activity, with LC_{50} values of 1.11 and 1.21 µg/mL, respectively. These values are significantly lower compared to the standard (Vincristine), which has an LC_{50} value of 0.451 µg/mL. In contrast, the AQSF and PESF fractions showed moderate cytotoxic activity. This suggests that while EASF and DCMSF are highly potent, AQSF and PESF have a less pronounced cytotoxic effect. Adding comparative data from the literature on *C. indica* highlights the strength of our findings, as dichloromethane and ethanol extracts of *C. indica* leaves exhibited significantly higher LC_{50} values of 273.9 µg/mL (167.8–447.0 µg/mL) and >1000 µg/mL, respectively, underscoring the exceptional efficacy of our EASF and DCMSF fractions.[83] These differences underscore the influence of solvent choice on the extraction of bioactive compounds, as certain solvents may selectively isolate compounds with heightened cytotoxic activity. However, it is essential to recognize that while the brine shrimp lethality assay provides a quick and cost-effective measure of toxicity, it serves as a preliminary indicator rather than a definitive predictor of anticancer potential in human cells. Therefore, further bioassay-guided fractionation and testing on human cell lines are recommended to isolate specific bioactive compounds and verify the therapeutic relevance of these extracts, allowing for a more comprehensive understanding of the plant's medicinal potential.

Bacteria possess an innate genetic inclination to build resistance, underscoring the imperative for continuously advancing novel antimicrobial medications to counteract a broad spectrum of microorganisms.[84] These antimicrobials selectively affect several mechanisms, including inhibiting microbial cell wall construction, altering crucial enzymatic pathways, and impeding the generation of genetic materials and proteins.[85,86] In this study, the antimicrobial activity of different fractions (AQSF, DCMSF, EASF, PESF) was evaluated against various Gram-positive and Gram-negative bacteria, and compared with standard antibiotics (Azithromycin, Amoxicillin, Ciprofloxacin) (Table 6). The standard antibiotics demonstrated significant inhibition zones across all tested microorganisms, ranging from 25 to 42 mm. Among the fractions, AQSF exhibited the highest activity, with inhibition zones between 8 to 18 mm, showing relatively better efficacy against Gram-negative bacteria. DCMSF, EASF, and PESF displayed lower antimicrobial activity, with inhibition zones ranging from 8 to 15 mm, 6 to 14 mm, and 6 to 12 mm, respectively. In another research illustrates the antibacterial potential of its methanolic and ethyl acetate extracts against three bacteria. Methanolic extracts of *C. indica* leaves and flowers demonstrated activity against *Bacillus subtilis*, and ethyl acetate extracts of flowers and stems/barks also inhibited *B. subtilis*. However, hexane and distilled water extracts from various parts showed no antibacterial effects, indicating that solvent choice significantly affects the extraction of active compounds. Additionally, oil extracts of *C. indica* displayed notable activity against *S. aureus* but limited action against *B. subtilis*, which aligns with our findings that certain fractions, like AQSF, exhibit more promising antimicrobial activity than others.[87] These comparisons underscore that while the tested plant fractions in our study display potential antimicrobial activity, particularly AQSF. However, their efficacy is lower than that of standard antibiotics. Furthermore, solvent selection appears to play a crucial role in extracting bioactive components with antibacterial properties. Given the relatively moderate antibacterial activity of these fractions, further isolation of bioactive compounds and testing against resistant strains is recommended. This approach could potentially identify novel compounds with more potent antimicrobial effects, addressing the urgent need for effective alternatives to conventional antibiotics in combating resistant bacteria.

The study also demonstrates the dose-dependent antidiarrheal effects of tested plant extracts, notably observed compared to loperamide, a standard antidiarrheal drug. Upon ingesting castor oil, ricinoleic acid is released into the intestinal lumen, causing irritation and inflammation in the mucosa. This process hinders the re-absorption of Na^+, K^+, and water, triggering the release of inflammatory mediators like prostaglandins, histamine, and nitric oxide.[8,88] In castor oil-induced diarrhea, the extract, particularly DCMSF and AQSF, exhibits significant inhibition of diarrheal faces, which could be by blicking release of Ricinoleic acid. Acetic acid exhibits a significant peripheral antinociceptive effect, with the writhing experiment indicating its

impact on both central and peripheral analgesic drug activity linked to arachidonic acid synthesis inhibition.[89] Pain may arise from the release of substances like serotonin and bradykinin, affecting damaged peripheral neurons sensitive to opioids and non-steroids.[90] Intraperitoneal acetic acid injection activates chemosensitive nociceptors, causing visceral inflammation with histamine, prostaglandins, serotonin, and bradykinin secretion.[91] In our experiment, different fractions of *C. indica* showed potential efficacy in pain management by reduction of writhing. Specially DCMSF and PESF exhibited the highest activities. Comparatively, these findings underscore the potential of these fractions for pain relief applications; however, they remain less effective than standard analgesics. Further comparative studies could help understand their exact mechanistic pathways in pain modulation, especially when targeting opioid and non-steroidal pathways.

Evidence reveals that elevated EGFR levels in gastric cancer, when co-expressed with ligands like EGF, are linked to poor overall survival. In breast cancer, increased EGFR expression is associated with reduced patient survival, advanced clinical stage, and resistance to endocrine therapy. The role of EGFR in colorectal cancer is less clear but is linked to tumor grade, stage, and survival, albeit to a lesser extent than in other cancers. Overall, the EGFR signaling pathway remains significant in these tumors, indicating its prognostic relevance.[92] The analysis of the compounds revealed that various types of bonding interactions play critical roles in determining bioactivity. For instance, alkyl bonds contribute to the lipophilicity of the compounds, enhancing their ability to penetrate cellular membranes and thereby increasing their bioavailability.[93] Additionally, pi-pi interactions are significant as they can stabilize the binding of ligands to their target receptors, facilitating effective molecular recognition and enhancing biological activity. These interactions not only improve the affinity of compounds for their targets but also influence the pharmacokinetic properties, which are essential for therapeutic efficacy.[94] Our investigation uncovers the capability of the identified phytochemicals to bind with EGFR, providing insights into their potential. Notably, C46 exhibits the lowest binding affinity to EGFR, forming three conventional hydrogen bonds, one unfavorable acceptor-acceptor bond, and nine alkyl bonds (Table 8, Figure 7). In

Table 8 Bond and Binding Site of Highly Active Compounds Against Different Targets Including EGFR, DGFR, DOR, TNF-α, and COX-2

Receptor	Compounds	Binding Affinities (kcal/mol)	Bond Type	Amino acids
EGFR	C46	−9.9	Conventional Hydrogen Bond	Ser 720, Thr 854, Asp 855
			Unfavorable Acceptor- Acceptor	Asn 842
			Alkyl	Leu 718, Val 726, Ala 743, Lys 745, Leu 777, Leu 788, Cys 797, Leu 844, Leu 858
	C53	−9.2	Conventional Hydrogen Bond	Tyr 998
			Carbon Hydrogen Bond	Phe 795
			Alkyl	Leu 718, Val 726, Ala 743, Leu 792, Cys 797, Leu 844, Leu 1001
	C89	−9	Conventional Hydrogen Bond	Met 766
			Alkyl	Leu 718, Val 726, Ala 743, Leu 777, Leu 788, Leu 792, Met 793, Cys 797, Leu 844.
	C92	−9.8	Conventional Hydrogen Bond	Cys 797, Thr 854
			Carbon Hydrogen Bond	Asp 800
			Alkyl	Val 726, Arg 841, Leu 844
	C99	−9.8	Pi- Carbon	Lys 745
			Pi- Sigma	Leu 718, Val 726, Leu 844
			Alkyl	Ala 743, Leu 788, Cys 797

(*Continued*)

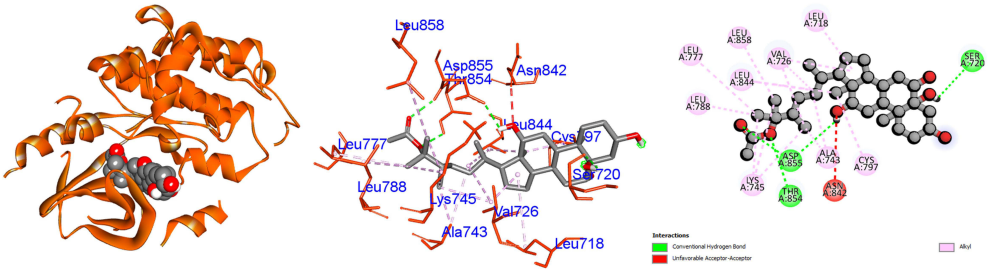
Table 8 (Continued).

Receptor	Compounds	Binding Affinities (kcal/mol)	Bond Type	Amino acids
	Lapatinib	−10.8	Conventional Hydrogen Bond	Lys 745, Thr 790
			Carbon Hydrogen Bond	Ser 720
			Alkyl	Leu 718, Val 726, Ala A: 743, Leu 844
			Pi- Sigma	Met 766
			Pi-Pi T-shaped	Phe 856
			Halogen(Fluorine)	Cys 775, Arg 776
DHFR	C10	−8.6	Carbon Hydrogen Bond	Tyr 121
			Alkyl	Leu 22, Lys 55
	C20	−9.1	Carbon Hydrogen Bond	Ala 9
			Alkyl	Leu 22,Phe 34, Lys 55
	C29	−8.4	Carbon Hydrogen Bond	Ser 118
			Alkyl	Ile 16, Leu 22
	C92	−8.4	Conventional Hydrogen Bond	Gly 20
			Alkyl	Lys 55
	C95	−8.7	Alkyl	Ile 16, Leu 22
	C98	−9	Conventional Hydrogen Bond	Ala 9
			Carbon Hydrogen Bond	Val 8
			Alkyl	Ile 16, Leu 22, Lys 55
	Ciprofloxacin	−8.2	Conventional Hydrogen Bond	Ala 9
			Carbon Hydrogen Bond	Val 8, Thr 56, Ser 118
			Alkyl	Ile 16, Leu 22, Lys 55
DOR	C29	−9.5	Alkyl	Val 316, Ala 319, Phe 329
	C46	−9.1	Unfavorable Donor-Donor	Arg 76
			Alkyl	Val 68, Leu 69
			Pi- Sigma	Phe 72
	C95	−10.1	Alkyl	Val 62, Leu 69, Val 70, Pro 315, Val 316, Ala 319, Phe 329
	C96	−9.8	Alkyl	Met 71, Val 75
	C99	−9.5	Pi- Sigma	Leu 69
			Pi- Pi Stacked	Phe 329
			Alkyl	Val 62, Val 70, Pro 315, Val 316,Ala 319, Leu 332
	Loperamide	−10.2	Conventional Hydrogen Bond	Leu 69
			Alkyl	Val 70, Ala 319, Phe 325, Cys 328, Leu 332
			Pi -Pi Stacked	Phe 72

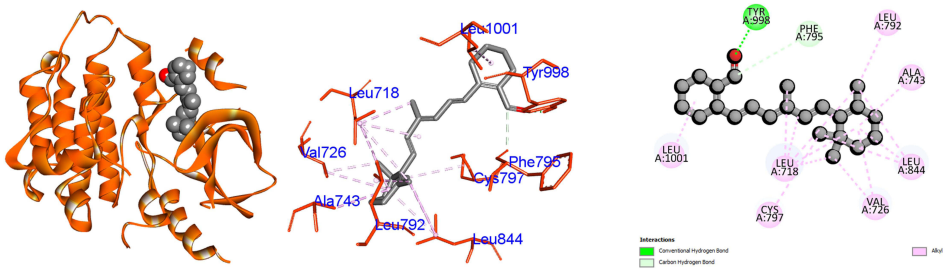
(*Continued*)

Table 8 (Continued).

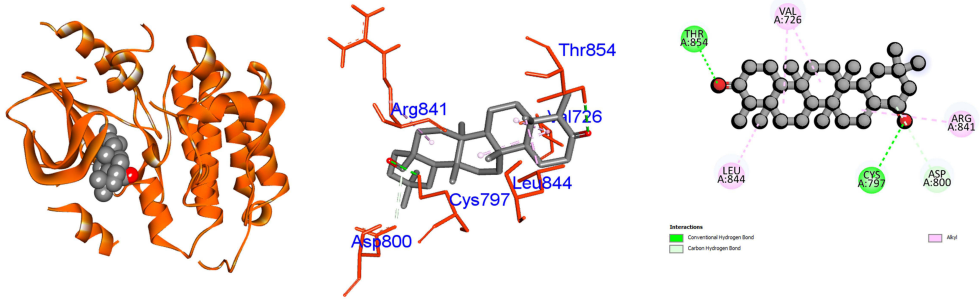
Receptor	Compounds	Binding Affinities (kcal/mol)	Bond Type	Amino acids
TNF-alpha	C7	−8.7	Pi- Sigma	Tyr 59
			Alkyl	Leu 57, Tyr 59, Tyr 151
	C10	−9.1	Conventional Hydrogen Bond	Ser 60
			Pi- Sigma	Tyr 59
			Alkyl	Leu 57, Tyr 59, Tyr 151
	C92	−9.5	Pi- Sigma	Tyr 59
			Alkyl	Tyr 59, Tyr 119, Tyr 151
	C96	−9.8	Carbon Hydrogen Bond	Gly 121
			Alkyl	Ala 96
	C99	−8.7	Pi- Pi Stacked	Tyr 59, Tyr 119, Tyr 119
			Alkyl	Leu 57
	Diclofenac	−7.1	Conventional Hydrogen Bond	Leu 120
			Pi- Pi Stacked	Tyr 59, Tyr 119
COX-2	C4	−8.6	Carbon Hydrogen Bond	Val 523,Ser 530
			Pi- Pi Stacked	Phe 518, Gly 526
			Alkyl	Val 349, Leu 352, Tyr 385, Trp 387, Ala 527, Leu 531
	C11	−8.1	Alkyl	His 90, Val 116, Val 349, Leu 352, Leu 359, Tyr 385, Trp 387,Ala 516, Met 522, Val 523, Ala 527, Leu 531
	C14	−8	Conventional Hydrogen Bond	Ser 530
			Alkyl	Val 349, Leu 352, Tyr 385, Trp 387, Phe 518, Met 522, Ala 527
	C59	−8.1	Alkyl	Tyr 348, Val 349, Leu 352, Leu 384, Tyr 385, Trp 387, Phe 518, Met 522, Val 523, Ala 527
	C87	−8.7	Van der Waals	Ala 516
			Pi- Sigma	Thr 94
			Amide -Pi Stacked	Asp 515
			Alkyl	Val 116, Val 349, Leu 352, Leu 359, Tyr 385, Trp 387, Pro 514, Phe 518, Val 523, Ala 527, Leu 531,
	C99	−9.2	Pi- Sigma	Val 349, Val 523, Ala 527
			Pi- Pi T -shaped	Trp 387
			Alkyl	Tyr 348, Leu 352, Tyr 355
			Pi- sulfur	Met 522
	Diclofenac	−7.9	Conventional Hydrogen Bond	Tyr 355
			Pi- Sigma	Val 349, Ala 527
			Amide -Pi Stacked	Gly 526
			Alkyl	Leu 352, Leu 531



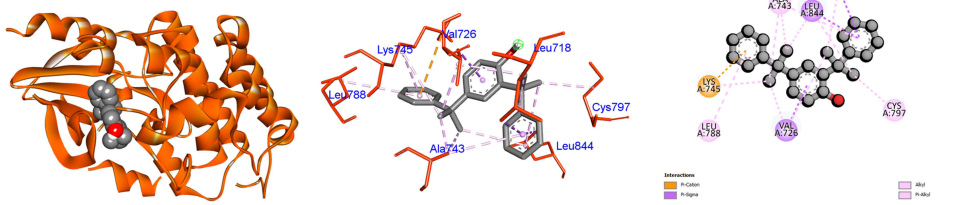
A: EGFR+C46



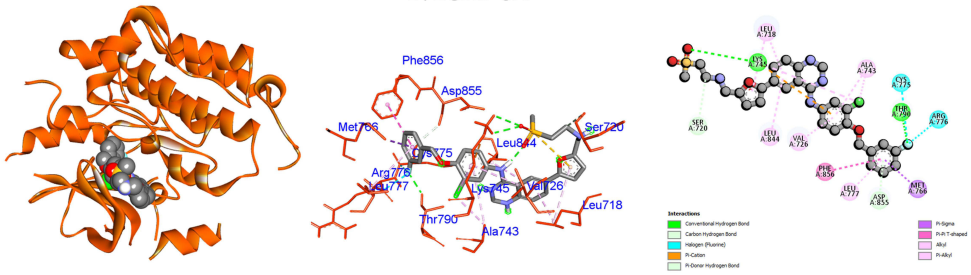
B: EGFR+C53



C: EGFR+C92



D: EGFR+C95



E: EGFR+Lapatinib

Figure 7 Molecular Interactions of Phytochemicals with EGFR enzyme with the most prominent phytocompounds, here (**A**) interactions of Compound 46 and EGFR enzyme, (**B**) interactions of Compound 53 and EGFR enzyme, (**C**) interactions of Compound 92 and EGFR enzyme, (**D**) interactions of Compound 95 and EGFR enzyme, (**E**) interactions of standard Lapatinib and EGFR.

contrast, C92 and C99 demonstrate significant binding affinity to the receptor. C92 establishes two conventional hydrogen bonds, one carbon-hydrogen bond, and three alkyl bonds, while C99 forms one pi-carbon bond, three pi-sigma bonds, and three alkyl bonds. Comparably, Lapatinib interacted with the receptor through six different bond types and showed a binding affinity of −10.9 kcal/mol.

Bacterial DHFR, crucial for thymidylate biosynthesis in the folic acid pathway, is a promising target for treating infections. Inhibitors targeting DHFR may induce bacterial death, offering a potential avenue for infection treatment.[95] Concerning DHFR, C20 demonstrated a robust interaction, forming a carbon-hydrogen bond and three alkyl bonds, resulting in an impressive binding score of −9.1 kcal/mol, surpassing the affinity of Ciprofloxacin, which scored −8.2 kcal/mol (Table 8, Figure 8). Additionally, C10 and C29 established bonds with the receptor through alkyl and carbon-hydrogen interactions, displaying binding scores of −8.6 and −9.1 kcal/mol, respectively.

The opioid receptors (μ, ƙ, and δ) in the human gastrointestinal (GI) tract significantly impact GI signaling. They work by inhibiting enteric nerve activity, blocking neurotransmitter release, and disrupting excitatory and inhibitory motor pathways. This causes a slowing down of intestinal transit, decreased excitability of enteric neurons, and changes in fluid transport and secretion mechanisms. These complex alterations ultimately lead to variations in GI motility and stool consistency.[96] Numerous compounds identified in the study demonstrated notable activity against DOR, with C95 and C96 standing out with the highest affinities, scoring −10.1 and −9.8 kcal/mol, respectively. They achieved this through the formation of different bonds like alkyl, pi-sigma, and unfavorable donor donors. In contrast, Loperamide established conventional hydrogen, alkyl, and pi-stacked bonds, resulting in a binding score of −10.2 kcal/mol (Table 8, Figure 9).

Inflammation is a physiological response to injury or infection, with acute inflammation involving cytokines and neutrophils. Chronic inflammation, involving additional immune cells, is associated with diseases, including cancer. TNF-α plays a crucial role in inflammation by activating NF-ƙB, leading to the expression of various inflammatory genes. TNF-α produced in the tumor microenvironment can promote tumor cell survival and contribute to multiple steps in tumorigenesis, including cellular transformation, promotion, proliferation, invasion, angiogenesis, and metastasis.[97] With a remarkable binding score of −9.8 kcal/mol, C96 displayed the highest affinity towards TNF-α, forming interactions through a single carbon-hydrogen bond and alkyl bonds (Table 8, Figure 10). Notably, numerous identified compounds showed significant suppression of the docking score compared to the standard diclofenac. This suggests the potential for a potent anti-inflammatory effect from the plant crude extract.

COX-2, or cyclooxygenase-2, is implicated in inflammation as it generates prostaglandins that play a pro-inflammatory role during the initial stages of inflammation. However, recent studies suggest that later-stage COX-2-generated prostaglandins may contribute to the resolution of inflammation, indicating a dual role in the inflammatory process. Inhibition of COX-2 has been shown to reduce inflammation in certain experimental models, highlighting its complex involvement in inflammatory responses.[98] Like TNF-α, various identified compounds demonstrated the suppression of the standard diclofenac binding score towards COX-2, indicating the potential for analgesic activities in the plant extract. Notably, C99 exhibited the highest affinity, interacting through four different bond types: pi-sigma, pi-pi, pi-sulfur, and alkyl (Table 8, Figure 11). Conversely, C87, with a score of −8.7 kcal/mol, displayed the second-highest activity toward the receptor, forming bonds such as van der Waals, pi-sigma, amide-pi, and alkyl bonds.

The ADMET study was conducted to determine the drug-likeness profile of the most active compounds, assessing their potential as drug candidates. This analysis included evaluating pharmacokinetic properties such as absorption, distribution, metabolism, excretion, and toxicity (ADMET), which are essential for understanding the pharmacological properties of substances and their suitability for drug development.[99] Table 9 demonstrate that C4, C14, C29, C89, and C98 exhibit high GI absorption with favorable drug-likeness profiles. These compounds have moderate lipophilicity, as indicated by their log P values (between 2.97 and 5.21), making them potentially suitable for oral administration. Notably, C29 and C98 meet all drug-likeness criteria, signifying their potential as orally bioavailable therapeutic agents. Compounds such as C7, C11, C53, and C96 have lower GI absorption, which may impact their oral bioavailability. In particular, C11, with a high lipophilicity (log P = 6.31) and more than seven rotatable bonds, may have reduced intestinal permeability and an unfavorable pharmacokinetic profile. Similarly, C53 and C96 also have high lipophilicity (log P values >5), which could limit their absorption and distribution. Concerning hepatotoxicity, most compounds were non-

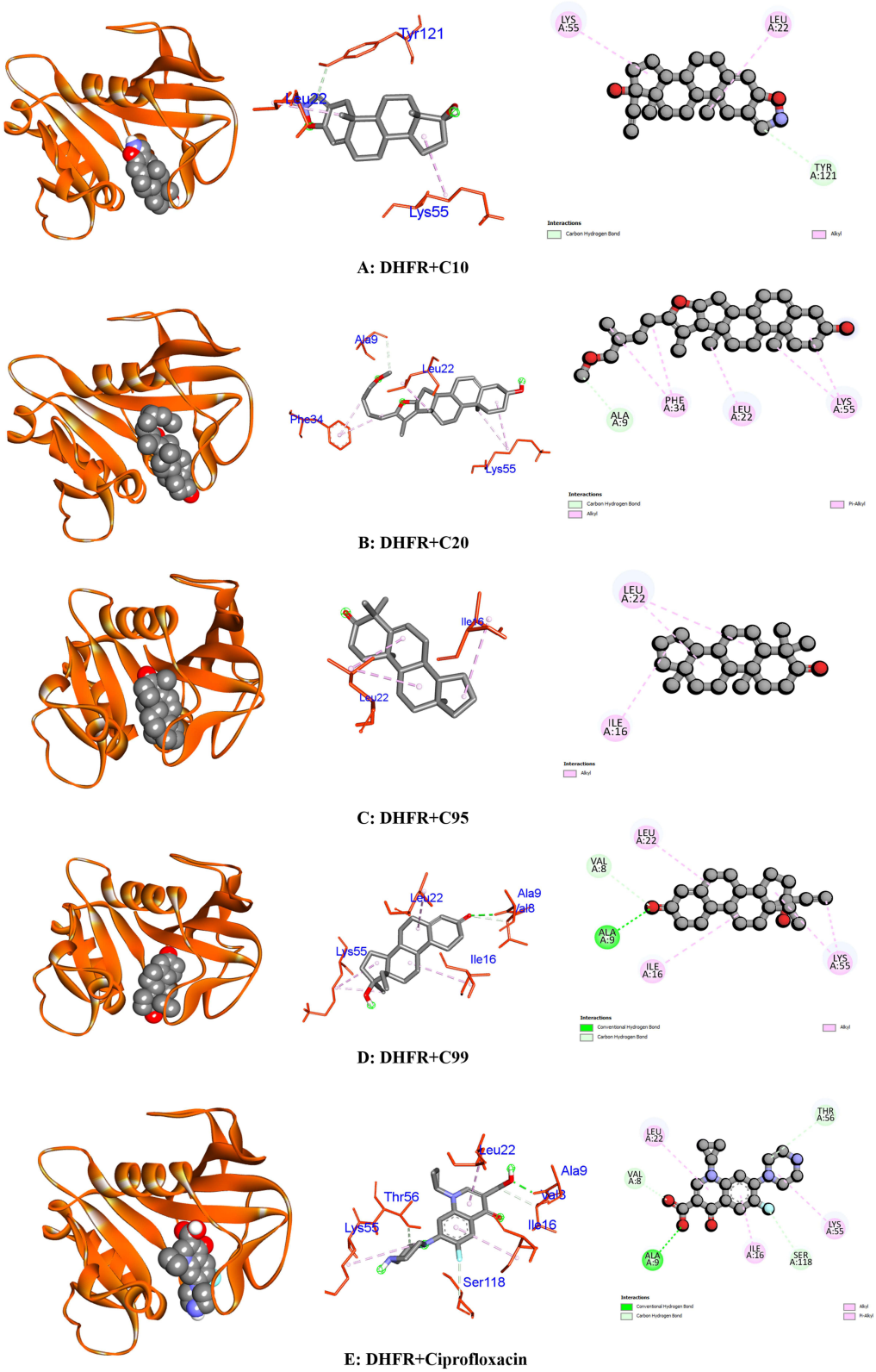
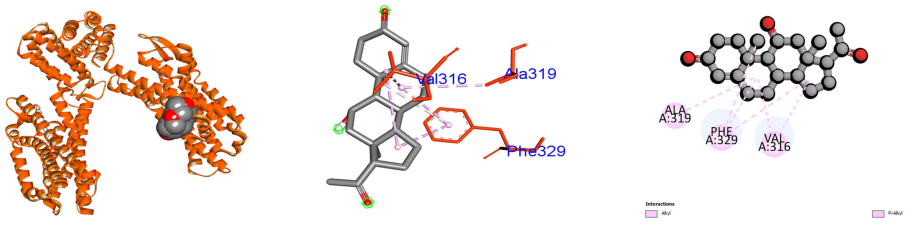
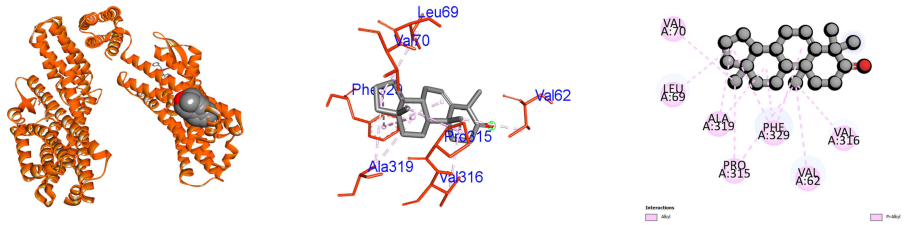


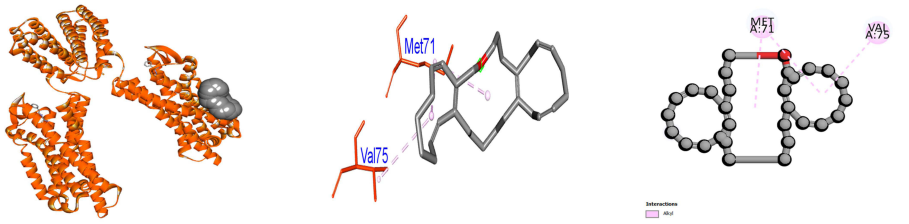
Figure 8 Molecular Interactions of Phytochemicals with DHFR enzyme with the most prominent phytocompounds, here (**A**) interactions of Compound 10 and DHFR enzyme, (**B**) interactions of Compound 20 and DHFR enzyme, (**C**) interactions of Compound 95 and DHFR enzyme, (**D**) interactions of Compound 99 and DHFR enzyme, (**E**) interactions of standard Ciprofloxacin and DHFR.



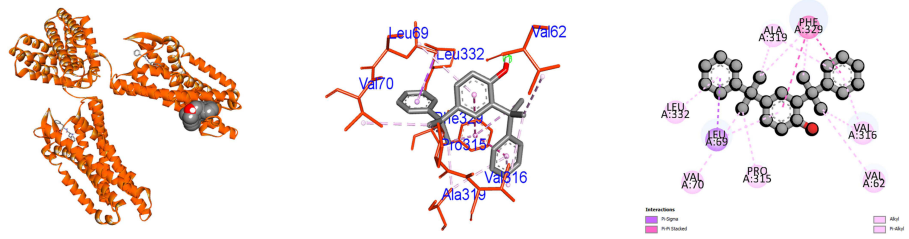
A: DOR+C29



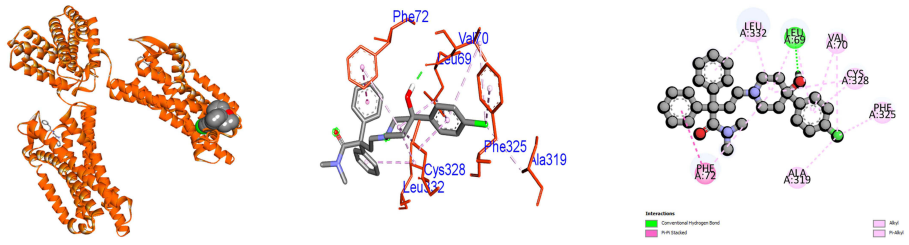
B: DOR+C95



C: DOR+C96



D: DOR+C99



E: DOR+ Lopeamide

Figure 9 Molecular Interactions of Phytochemicals with DOR enzyme with the most prominent phytocompounds, here (**A**) interactions of Compound 29 and DOR enzyme, (**B**) interactions of Compound 95 and DOR enzyme, (**C**) interactions of Compound 96 and DOR enzyme, (**D**) interactions of Compound 99 and DOR enzyme, (**E**) interactions of standard Loperamide and DOR.

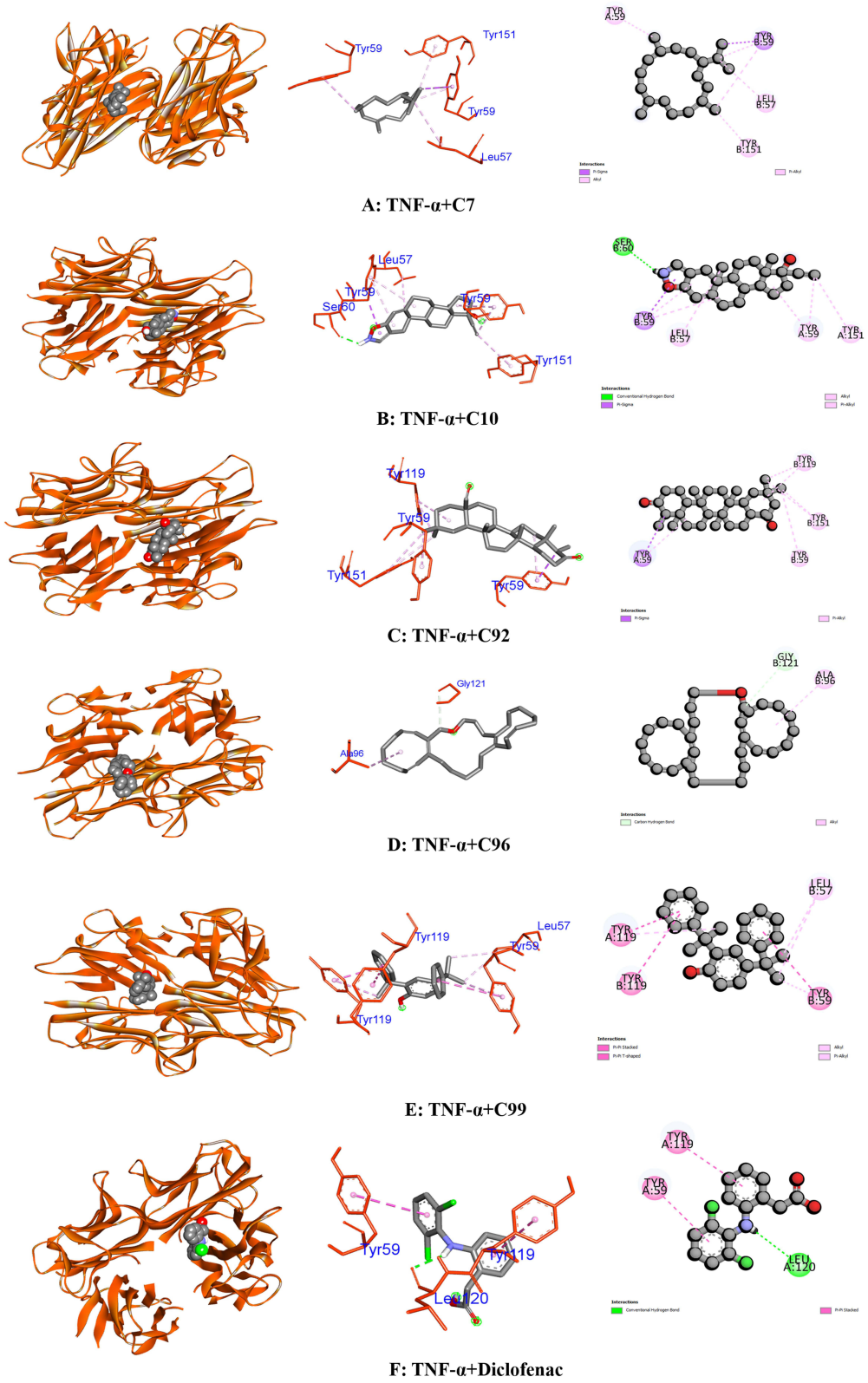


Figure 10 Molecular Interactions of Phytochemicals with TNF-α enzyme with the most prominent phytocompounds, here (**A**) interactions of Compound 7 and TNF-α enzyme, (**B**) interactions of Compound 10 and TNF-α enzyme, (**C**) interactions of Compound 92 and TNF-α enzyme, (**D**) interactions of Compound 96 and TNF-α enzyme, (**E**) interactions of Compound 99 and TNF-α enzyme, (**F**) interactions of standard Diclofenac and TNF-α enzyme.

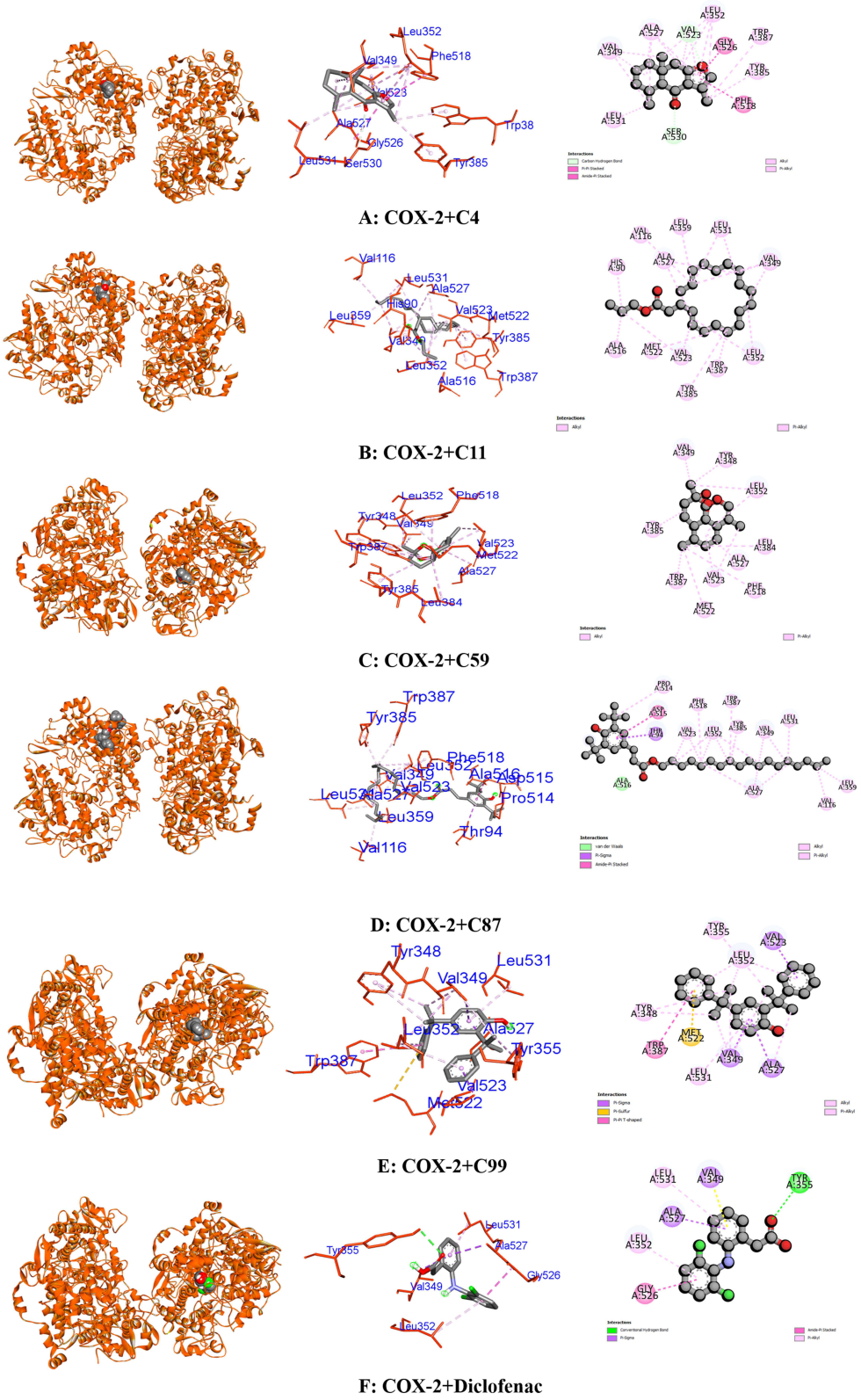


Figure 11 Molecular Interactions of Phytochemicals with COX-2 enzyme with the most prominent phytocompounds, here (**A**) interactions of Compound 4 and COX-2 enzyme, (**B**) interactions of Compound 11 and COX-2 enzyme, (**C**) interactions of Compound 59 and COX-2 enzyme, (**D**) interactions of Compound 87 and COX-2 enzyme, (**E**) interactions of Compound 99 and COX-2 enzyme, (**F**) interactions of standard Diclofenac and COX-2.

Table 9 ADMET Analysis Result of the Best-Bonded Compounds of Leaves of Canna Indica

Compound No	H-bond Donor	H-bond Acceptors	Lipophilicity - log P (o/w)	GI Absorption	AMES Toxicity	Hepatotoxicity	Drug likeliness	Bioavailability score
C4	0	2	3.37	High	No	No	0.55	No; 1 violation: MW<250
C7	0	0	5.45	Low	No	No	0.55	No; 1 violation: XLOGP3>3.5
C10	1	3	3.88	High	No	Yes	0.55	No; 1 violation: XLOGP3>3.5
C11	1	2	6.31	Low	No	No	0.55	No; 2 violations: Rotors>7, XLOGP3>3.5
C14	0	2	2.97	High	No	No	0.55	No; 1 violation: MW<250
C20	1	3	5.33	High	No	Yes	0.55	No; 2 violations: MW>350, XLOGP3>3.5
C29	1	3	3.09	High	No	No	0.55	Yes
C46	4	6	4.22	High	No	No	0.55	No; 2 violations: MW>350, XLOGP3>3.5
C53	0	1	5.85	Low	No	Yes	0.55	No; 1 violation: XLOGP3>3.5
C87	1	3	10.72	High	No	No	0.55	No; 2 violations: MW>350, XLOGP3>3.5
C89	0	1	5.21	High	No	Yes	0.55	No; 1 violation: XLOGP3>3.5
C92	0	2	6.57	Low	No	No	0.55	No; 2 violations: MW>350, XLOGP3>3.5
C95	0	1	4.96	High	No	Yes	0.55	No; 1 violation: XLOGP3>3.5
C96	0	1	6.7	Low	No	No	0.55	No; 2 violations: MW>350, XLOGP3>3.5
C98	1	2	3.71	High	No	Yes	0.55	Yes
C99	1	1	5.83	High	Yes	Yes	0.55	No; 1 violation: XLOGP3>3.5

hepatotoxic, suggesting a favorable safety profile for potential liver toxicity. However, compounds C10, C20, and C89 exhibit AMES toxicity, indicating possible mutagenic properties that could limit their therapeutic applications without further structural modifications or alternative usage routes. In terms of drug-likeness, violations of the Lipinski Rule of Five were observed in compounds with either high molecular weight (eg, C20, C46, C87, C92, C96) or elevated log P values (eg, C7, C10, C11, C20, C53, C87, C89, C95, C96, C99). These violations suggest potential challenges in achieving optimal bioavailability and metabolic stability, especially for highly lipophilic compounds. Overall, C4, C14, C29, and C98 emerge as favorable candidates, demonstrating high GI absorption, minimal toxicity, and compliance with drug-likeness criteria. Future studies may focus on optimizing these compounds to enhance their bioavailability while mitigating any potential toxic effects indicated by their ADME/T profiles.

These interpretations are significant for the discovery of novel medicinal products, clinical trials, and the biological analysis of isolated compounds. The study emphasizes that compounds adhering to pharmacokinetic and toxicological criteria can be potent candidates for drug discovery, particularly for oral use, and safer for therapeutic applications. The findings suggest that the investigated compounds possess attributes rendering them promising contenders for drug development targeting a range of health concerns, including cancer, diarrhea, microbial infections, inflammation, and

pain management. While these initial outcomes are positive, further preclinical investigations involving animal experiments and clinical trials with human subjects are essential to thoroughly evaluate the efficacy and safety of these potential treatments. These supplementary studies are crucial to substantiate the potential therapeutic applications of these substances and pave the way for developing potent drugs addressing various illnesses.

In summary, this study highlights several promising phytochemicals from *C. indica* leaf extracts that exhibit significant biological activities, underscoring their potential applications in drug development. Among the compounds analyzed, C99, C96, and C20 demonstrated notable pharmacological properties. Specifically, C99 showed strong affinity for both COX-2 and EGFR, indicating potential roles in anti-inflammatory and anticancer applications. C96 exhibited the highest binding affinity toward TNF-α, which suggests an effective anti-inflammatory action, while C20 showed promising binding interactions with DHFR, which is crucial in targeting bacterial infections. Furthermore, ADMET analysis indicated that C29 and C98 meet Lipinski's Rule of Five, suggesting high oral bioavailability, although some compounds, such as C99, showed hepatotoxicity, which necessitates further investigation for safety.

These findings collectively illustrate the therapeutic potential of *C. indica* extracts, with key compounds emerging as candidates for anti-inflammatory, antimicrobial, and anticancer drug development. However, additional studies, including clinical trials and in-depth toxicological assessments, are essential to validate these effects and ensure safety in human applications. By expanding research on these bioactive compounds, this study lays a foundation for the future exploration of *C. indica* as a valuable source for natural drug development.

Limitations

While this research demonstrates promising findings for *C. indica* leaf extracts, several limitations warrant careful consideration. Although animal models, particularly mice, were used to investigate the antidiarrheal and analgesic effects, translating these results to human applications requires caution. Further examination of potential synergistic effects within the extract could deepen our understanding of its pharmacological profile. Conducting human clinical trials will be essential to accurately assess the extract's efficacy, safety, and optimal dosing in humans. Additionally, the complex processes involved in drug discovery and development underscore the importance of understanding the ADME properties, as these are fundamental to predicting drug behavior in the body. Drug metabolism studies, in particular, are vital to identifying active metabolites, addressing safety concerns, and refining dosage guidelines. Incorporating in silico data may also provide valuable leads for further research, supporting these efforts. In summary, while this study offers significant insights, future research, including human clinical trials, will be crucial for substantiating and expanding upon these findings, advancing the potential of *C. indica* in natural medicine.

Conclusion

This comprehensive exploration highlights the impressive pharmacological versatility of *Canna indica* L. leaves, emphasizing their substantial therapeutic potential through significant bioactivities, including antidiarrheal, antimicrobial, analgesic, and cytotoxic properties. In our study, EASF and DCMSF fractions of *C. indica* demonstrated strong cytotoxic effects against brine shrimp, with LC_{50} values suggesting anti-cancer potential. Additionally, the AQSF fraction showed antimicrobial efficacy, albeit lower than standard antibiotics, supporting the need for bioassay-guided isolation of potent antimicrobial agents. Antidiarrheal activity was observed in DCMSF and AQSF, likely through inhibition of intestinal inflammation. On the other hand, Compounds C99, C96, and C20 exhibited notable pharmacological properties, with C99 showing strong affinity for COX-2 and EGFR, suggesting anti-inflammatory and anticancer potential. C96 demonstrated the highest binding affinity for TNF-α, indicating effective anti-inflammatory action, while C20 showed promising interactions with DHFR, a critical target in combating bacterial infections. ADMET analysis further revealed that compounds C29 and C98 meet Lipinski's Rule of Five, suggesting good oral bioavailability, though C99 requires additional safety evaluation due to indications of hepatotoxicity. The multi-target activities of these compounds not only enhance the overall pharmacological effects of the leaf extract but also pave the way for exciting future research and pharmaceutical applications. To build on these findings, future studies should focus on in vivo efficacy, mechanisms of action, and formulation development to further elucidate the therapeutic potential of *C. indica* extracts. These efforts could ultimately yield promising leads for clinical trials. Given the compelling evidence, *C. indica* leaves present a significant opportunity for the development of new medicinal products, advancing the pursuit of innovative and effective treatments.

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Disclosure

The authors have declared no competing interests.

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