ORIGINAL ARTICLE

Phytochemical Analysis and Anti-cryptosporidial Effects of *Zingiber officinale* Extracts: In-vivo and In-Silco Studies

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Key words:
Zingiber officinale; Cryptosporidium; antioxidant; phenolic content; anti-protozoal

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ABSTRACT

Background: *The intracellular protozoan* Cryptosporidium *species is a major cause of waterborne disease worldwide, highlighting the urgent need for new treatments.* ***Objective:*** *This study evaluates the therapeutic potential of Zingiber officinale (Z. officinale/ginger) against* Cryptosporidium parvum (C. parvum), *analyzing its antioxidant properties, phenolic content, and anti-protozoal effects.* ***Methodology:*** *Methanol extraction of air-dried* Z. officinale *rhizomes was followed by organic solvent fractionation. Total phenolic content was assessed with Folin-Ciocalteu's assay, and antioxidant activity was measured by the phosphomolybdenum method. An* in vivo *study on dexamethasone-immunosuppressed mice included seven groups: uninfected control, infected model, and five infected groups treated with different extracts (methanol, ethyl acetate, aqueous, petroleum ether) or nitazoxanide as a reference drug.* ***Results:*** *The most effective extract regarding oocyst reduction was methanol (89%). Molecular docking revealed robust binding to a target protein in C. parvum, and gas chromatography-mass spectrometry allowed for the identification of important active chemicals. The highest levels of antioxidant activity and phenolic content were found in the ethyl acetate fraction.* ***Conclusion:*** *The ethyl acetate fraction and methanol extract of* Z. officinale *exhibited strong antioxidant properties and high phenolic content. Methanol extract demonstrated significant anti-cryptosporidial effects, reducing oocyst shedding, suggesting it as a promising natural therapeutic option. By linking ginger's chemical makeup to its biological activity using molecular docking, the study shed light on how ginger works against C. parvum opening the door for further therapeutic advancement.*

INTRODUCTION

Medicinal plants play an essential role in modern medicine, as they contain bioactive compounds that have been shown to have various therapeutic effects[1]. These herbs are widely employed in medicines, herbal supplements, and complementary therapies to treat a wide range of medical conditions[2]. Scientific research both confirms ancient uses and investigates novel applications for medicinal plants in modern medicine[3]. By harnessing the healing properties of medicinal plants, healthcare professionals can offer patients natural and effective treatment options that complement conventional medical practices[4]. The incorporation of medicinal plants into modern medicine emphasizes the significance of combining traditional knowledge with evidence-based research to improve patient care and increase general well-being[5]. *Cryptosporidium* is a class of protozoan parasites that can cause gastrointestinal disease in both humans and animals[6]. These microscopic organisms are spread by the consumption of polluted water or food, resulting in complaints such as diarrhoea, stomach cramps, and nausea[7]. *Cryptosporidium* is a serious health hazard, especially in places with inadequate sanitation and water quality. Understanding the biology and transmission of *Cryptosporidium* is crucial for preventing and managing outbreaks of this parasite[6]. Research on Cryptosporidium has focused on various aspects, including its life cycle, genetic diversity, and drug

resistance[8]. According to several previous studies, *Cryptosporidium* has a complicated life cycle that includes both sexual and asexual stages, which adds to its capacity to survive and spread in a variety of situations. Genetic analysis has revealed the presence of multiple species and genotypes of *Cryptosporidium*, each with unique characteristics and potential for causing disease[9]. Concerns regarding the efficacy of the available treatment methods have also been raised by the advent of drug-resistant forms of *Cryptosporidium*[10]. Researchers are exploring alternative therapies and preventive measures to combat this parasite and reduce its impact on public health[8]. In addition, measures are being taken to enhance sanitation standards and water quality to reduce the possibility of environmental *Cryptosporidium* contamination[9].

Ginger, or *Zingiber officinale* (*Z. officinale*), is a flowering plant that is frequently utilized as a seasoning and has medicinal properties. It is a member of the Zingiberaceae family[11]. Originating from Southeast Asia, it has been grown for thousands of years for both medicinal and culinary uses. Ginger is known for its distinct flavor and aroma, which comes from its bioactive compounds such as gingerol and shogaol[12]. In traditional medicine, ginger has been used to aid digestion, reduce inflammation, and alleviate nausea[13]. It is a versatile plant with a long history of use in various cultures for its culinary and medicinal benefits. Recent research has revealed that ginger may have health benefits, such as its antioxidant and anti-inflammatory qualities[11].

Debilitating action is required to curb the spread of the parasite infection known as *Cryptosporidium*. Exploring the potential of ginger in combating *Cryptosporidium* spp. infections can provide valuable insights into natural and alternative treatments for this parasitic disease. The purpose of this study was to evaluate the potential effect of *Z. officinale*, the antioxidant effect, phenolic content, and its anti-protozoal effect in the treatment of *Cryptosporidium parvam.*

To reduce lab effort and assist in determining the most likely molecular targets and/or signalling pathways, molecular modelling has become crucial in biomedical research. The molecular docking technology has gained popularity recently since it has the potential to greatly boost productivity and reduce research expenditures. Furthermore, in computer-assisted drug design, nowadays, it is essential to use approaches like predicting affinity for binding and studying interaction mode[14].

METHODOLOGY

Preparation and Extraction of Plant Material

The dried rhizomes of *Z. officinale* were used in this study. Plant was bought from Al-Azhar Street - Egypt, in November 2023, the plant was identified by an expert taxonomist in the herbarium of the Botany Department, Faculty of Science, Cairo University. A voucher specimen was preserved in the herbarium of the Medicinal Chemistry Department, Theodor Bilharz Research Institute. The sample was allowed to dry at room temperature in the shade before being processed with a grinding machine into a fine powder. The powder (800g) was extracted with methanol (MEOH) (1 liter × 3 times), petroleum ether (Pet. ether) (1.150 liters × 3 times), ethyl acetate (EtOAc) (1 liter × 3 times), and aqueous (water) (1.850 liters × 3 times) by soaking at room temperature. The solvents were evaporated using a rotary evaporator (BUCHI R-300, Switzerland), under vacuum at 40°C, and then the extract was heated in a water bath at 60°C. The proportion of the yield of each extract was calculated using the following formula:

(% yield = (weight of obtained extract/weight of plant sample) × 100).

Total phenolic content estimation:

The total phenolic content was determined using Folin-Ciocalteu's assay[15], the basic idea of preparation is to use a solvent to extract the phenols from the sample, which makes them available for reaction. The principle of reagent addition states that phenolic compounds undergo a redox reaction with the Folin-Ciocalteu reagent, reducing the reagent and resulting in the creation of a blue complex. The incubation period ensures that all of the phenolics have reacted and permits the full development of the blue hue. Absorbance Measurement Principle: The intensity of the blue color at 760 nm is correlated with the absorbance, which is proportional to the sample's phenolic concentration. The quantification principle involves converting the absorbance values into a concentration of total phenolics, which is then expressed in gallic acid equivalents (GAE), using a standard curve (such as citric acid).

Phosphomolybdenum assay the total antioxidant capacity (TAC):

TAC was calculated using the established protocols for the phosphomolybdenum test[16]. According to the phosphomolybdenum technique, a green phosphate/Mo (V) combination forms at an acidic pH when Mo(VI) is reduced to Mo(V) in the presence of antioxidant chemicals. Usually detected at 695 nm, this color shift can be evaluated spectrophotometrically and is related to the sample's antioxidant capacity. Mix sodium phosphate, sulfuric acid, and ammonium molybdate to create the reagent solution. In a test tube, combine the reagent solution with a predetermined volume of the sample. The mixture should be incubated for 90 minutes at 95°C.Use a spectrophotometer to measure the green complex's absorbance at 695 nm once it has cooled. Determine the TAC using a standard curve that was created using a recognized antioxidant (such as trolox or ascorbic acid) for comparison. The antioxidant capacity of the sample is quantitatively measured by this process

and expressed in terms of equivalent antioxidant concentration.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

A Thermo Scientific Trace Gas Chromatograph-Tandem Quadrupole Mass Spectrometer (Austin, TX, USA) was used to analyze the chemical composition of the *Zingiber officinale* methanolic extract. A TG-5MS direct capillary column (diameter 0.25 mm, length 30 m, film thickness 0.25 µm) was used for the analysis. Starting at 50°C, the temperature in the column oven was increased by 5°C per minute until 250°C, where it remained for two minutes. Following that, it was elevated to 300°C for two minutes at a rate of 30°C per minute. The temperature of the injector and the mass spectrometry (MS) transfer line was kept at 270°C and 260°C, respectively. The carrier gas used in the experiment was helium, which moved at a constant rate of 1 milliliter per minute. Diluted samples of 1 µl were automatically injected by an auto-sampler AS1300 linked to a gas chromatograph (GC) in split mode following a 4-minute solvent delay. The ion source was set at a temperature of 200°C. Electron ionization (EI) mass spectra were gathered in full scan mode, covering mass-to-charge ratios (m/z) from 50 to 650, at 70 electron volts (eV) ionization voltages. It was possible to ascertain the chemical contents of the methanolic extract by comparing the peak retention time with standards. Following that, the generated mass spectra were contrasted with those in the mass spectral databases of National Institute of Standards and Technology (NIST) 14 and WILEY 9

Molecular docking simulation analysis:

To assess the potential affinity of the investigated chemicals isolated from ginger against Calcium-Dependent Protein Kinase 1 (CpCDPK1) (pdb code: 3ncg), which was acquired from the protein data bank, molecular docking analysis was performed.

First, the target protein's water molecules were eliminated. Preparation alternatives were then employed to address empty valence atoms and crystallographic abnormalities. CHARMM force fields were used to reduce the energy of the protein structure. Chem-Bio Draw Ultra16.0 was used to create the 2D structures of the compounds under study. The SDF files were then saved and opened, and the 3D structures were protonated while the energy was reduced using the MMFF94 force field. The ligand preparation methods were then used to obtain the reduced structures appropriate for docking[17]. The Autodock Vina 1.5.7 program was used to complete the docking procedure via the docking option[18]. All the ligands were left Then, using the Discovery Studio 2016 visualizer, 3D figures were created based on the docking scores (affinity energy) of the best-fitted poses with the active sites[19].

Animals:

In this study, 42 male Swiss albino mice of the CD1 strain, 20 to 25 grams in weight were procured from Theodor Bilharz Research Institute (TBRI), situated in Giza, Egypt, specifically from the *Schistosoma* Biological Supply unit. To ensure optimal hygiene, mice were kept in air-conditioned rooms (24 ± 2°C) in well-ventilated plastic cages with unrestricted access to food and water. They were also shielded from the sun. The study was carried out at TBRI's Parasitology Department[20].

Animal groups:

Mice were grouped into seven groups, each group contain six mice. All of them are Immunocompromised.

Group I: Immunocompromised, non-infected (control negative);

Group II: Immunocompromised, infected (control positive);

Group III: Immunocompromised, infected receiving methanolic extract of *Z. officinale*;

Group IV: Immunocompromised, infected receiving ethyl acetate extract of *Z. officinale*;

Group V: Immunocompromised, infected receiving aqueous extract of *Z. officinale*;

Group VI: Immunocompromised, infected receiving petroleum ether extract of *Z. officinale*;

Group VII: Immunocompromised, infected receiving Nitazoxanide (NZD) drug.

Immunosuppression:

Before receiving *Cryptosporidium* oocysts, the animals were administered oral dexamethasone (0.5 mg) at a rate of 0.25 µg/g/day for 14 days to suppress their immune systems. Kahira Pharmaceuticals and Chemical Industries Company, Shoubra, Cairo, Egypt, supplied the medication. During the entire trial, the mice were given the same amount of dexamethasone[21].

Infection:

Oocysts of *Cryptosporidium* were collected from spontaneously infected diarrheal calves at the Animal Reproduction Research Institute in Giza, Egypt. To ensure that the stool samples from the ill calves were free of pee or water, they were taken in sterile, clean stool cups. Following stool sample collection, oocysts underwent purification as per[22]. When needed, purified oocysts were maintained at 4 °C in a 2.5% potassium dichromate solution. An infectious inoculum was made[23]. To find out how much fluid volume each mouse received from the inoculum, the concentrated stock inoculum's oocyst count was determined. Oral-gastric gavage was used to infect mice orally with *Cryptosporidium* oocysts. About 3×10^3 oocysts of *Cryptosporidium* oocysts were used to infect each mouse[24]. To ensure infection establishment, fecal pellets were collected and studied after one week of mice infection (7th day post-infection (PI)).

Drugs administration:

At the 7th day PI, drugs were administered via oral gavage:

-The dose of *Zingiber officinale* extracts was 100 mg/kg BW[25].

NTZ (Nanazoxid, 100 mg/5 mL suspension, Medizen Pharmaceutical industries for Utopia Pharmaceuticals) at 100 mg/kg every day for 5 successive days[26]. The doses were derived by extrapolating therapeutic human doses to animal doses[27].

Ethics approval and consent to participate:

All procedures followed were in accordance with the ethical standards of the ethics committee of Theodor Bilharz Research Institute (under Federal Wide Assurance No. FWA00010609) and with the Helsinki Declaration of 1975, as revised in 2008.

Statistical analysis

Data were analyzed using Microsoft Excel 2016 and a statistical package for social science (IBM SPSS Statistics for Windows, version 26, IBM Corp., Armonk, NY, USA). Quantitative data were expressed as mean _ SD. ANOVA and Duncan's multiple range test as a post hoc test were used to detect statistically significant differences among all the study groups. A p-value < 0.05 was significant and a p-value < 0.001 was highly significant.

RESULTS

Antioxidant activity and total phenolic content

According to the results presented in Table 1, the examined extracts of *Z. officinale* exhibited varying levels of total phenolic content and antioxidant activity. In the phosphomolybdenum assay, the total antioxidant capacity (TAC) results were as follows: Ethyl Acetate (EtOAc**)** extract demonstrated the highest antioxidant capacity (575.3 mg Ascorbic Acid Equivalent per gram dry extract), followed by Methanol (MeOH) extract (428 mg Ascorbic Acid Equivalent per gram dry extract), Aqueous extract (214.7 mg Ascorbic Acid Equivalent per gram dry extract), and Petroleum Ether (Pet. ether) extract (94 mg Ascorbic Acid Equivalent per gram dry extract).

Additionally, the total phenolic contents of the extracts were in the following order: Ethyl Acetate (EtOAc) extract (238.5 mg Gallic Acid Equivalent per gram dry extract) > Methanol (MeOH) extract (209.4 mg Gallic Acid Equivalent per gram dry extract) > Aqueous extract (117.7 mg Gallic Acid Equivalent per gram dry extract) > Petroleum Ether (Pet. ether) extract (79.2 mg Gallic Acid Equivalent per gram dry extract).

Table 1: Tab Total antioxidant capacity and total phenolic content of Methanol, Ethyl acetate, Aqueous & Pet-Ether of *Z. officinal*

Sample	Total antioxidant capacity (mg AAE/g dry extract)[1,2]	Total phenolic content (mg GAE/g dry extract)[3]
MeOH	428 ± 40	209.4 ± 6.2
EtOAc	575.3 ± 50	238.5 ± 4.6
Aqueus	214.7 ± 50	117.7 ± 6.5
Pet-Ether	94.0 ± 3.50	79.2 ± 7.9

GC-MS analyses of *Z. officinale* MeOH extract.

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *Z. officinale* methanol (MeOH) extract revealed the presence of 27 compounds, accounting for 98.25% of the total number of compounds. Table 2 contains a list of the detected chemicals and the proportion of each compound's region. Additionally, Figure 1 shows the GC-MS total ion chromatogram of the *Z. officinale* methanol (MeOH) extract. Figure 2 illustrates that the most predominant components of the methanol (MeOH) extract were 1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one (32.80%), followed by gingerol (17.42%), 1-(4-Hydroxy-3-methoxyphenyl)tetradec-4-en-3-one (11.61%), 1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one (8.42%), and (E)-1-(4-Hydroxy-3-methoxyphenyl) dec-3-en-5-one (3.02%). Other significant compounds included 8-Isopropyl-1-methyl-3-methylenetricyclo [4.4.0.02, 7] decan-4-ol (2.85%), Diepicedrene-1-oxide (2.53%), 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl) (2.45%), and 1-(4-Hydroxy-3-methoxyphenyl) decane-3, 5-diyl diacetate (2.21%). It was noted that oxygenated and hydrocarbon sesquiterpenes were the main components in this extract, followed by diterpenes, monoterpenes, and fatty acids, respectively.

Table 2: Chemical constituents of *Zingiber officinale* MeOH extract.

No.	Name	t_R	Area %	MW	MF

1	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl (α-curcumene)	16.87	1.00	202	$C_{15}H_{22}$
2	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl (Zingiberene)	17.26	0.89	204	$C_{15}H_{24}$
3	Cyclohexene 3-(1,5-dimethyl-4-hexenyl)-6-methylene (α-Sesquiphellandrene)	17.90	0.76	204	$C_{15}H_{24}$
4	1-methyl-4-(6-Methylhept-5-en-2-yl) cyclohex-2-enol	19.90	0.35	222	$C_{15}H_{26}O$
5	2-Butanone, 4-(4-hydroxy-3 methoxyphenyl)	20.11	2.45	194	$C_{11}H_{14}O_3$
6	2-Naphthalenemethanol,decahydro- α, α,4 α -trimethyl-8-methyle	20.58	0.62	222	$C_{15}H_{26}O$
7	2-Naphthalenemethanol, decahydro- α, α,4 α -trimethyl-8-methylene-	20.71	0.53	222	$C_{15}H_{26}O$
8	α –Bisabolol	21.52	0.38	222	$C_{15}H_{26}O$
9	8-Isopropyl-1- methyl-3-methylenetricyclo [4.4.0.02,7] decan-4-ol	21.62	2.85	220	$C_{15}H_{24}O$
10	Diepicedrene-1-oxide	23.93	2.53	220	$C_{15}H_{24}O$
11	*n*-Hexadecanoic acid	27.51	1.32	256	$C_{16}H_{32}O_2$
12	Phenol, 5-[2-(3-hydroxy-5 methoxyphenyl) ethyl]-2-methoxy-	29.51	0.86	274	$C_{16}H_{18}O_4$
13	9,12-Octadecadienoic acid (Z,Z)	30.52	1.45	280	$C_{18}H_{32}O_2$
14	9-Octadecenoic acid (Z)	30.66	0.71	282	$C_{18}H_{34}O_2$
15	1-(4-Hydroxy-3-methoxyphenyl) dec-3-en-5-one	31.41	3.02	276	$C_{17}H_{24}O_3$
16	3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)	31.60	0.60	278	$C_{17}H_{26}O_3$
17	1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one	32.81	32.80	276	$C_{17}H_{24}O_3$
18	1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-dione	33.21	0.49	292	$C_{17}H_{24}O_4$
19	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one	33.46	1.45	294	$C_{17}H_{26}O_4$
20	Gingerol	34.36	17.42	294	$C_{17}H_{26}O_4$
21	4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	35.78	0.93	180	$C_{10}H_{12}O_3$
22	1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one	36.05	8.42	304	$C_{19}H_{28}O_3$
23	1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-diyl diacetate	36.39	2.21	380	$C_{21}H_{32}O_6$
24	5-Hydroxy-1-(4-hydroxy-3-methoxy phenyl)dodecan-3-one	37.54	0.88	322	$C_{19}H_{30}O_4$
25	1-(4-Hydroxy-3-methoxyphenyl)tetradec -3-en-5-one	38.04	1.03	332	$C_{21}H_{32}O_3$
26	1-(4-Hydroxy-3-methoxyphenyl) tetra dec-4-en-3-one	39.31	11.61	332	$C_{21}H_{32}O_3$
27	1-(4-Hydroxy-3-methoxyphenyl) tetra decane-3,5-dione	39.73	0.69	348	$C_{21}H_{32}O_4$
	Total area % of identified compounds		98.25		
	Total area % of identified compounds		98.25		

t_R; retention time, MF; Molecular formula, MW; Molecular weight.

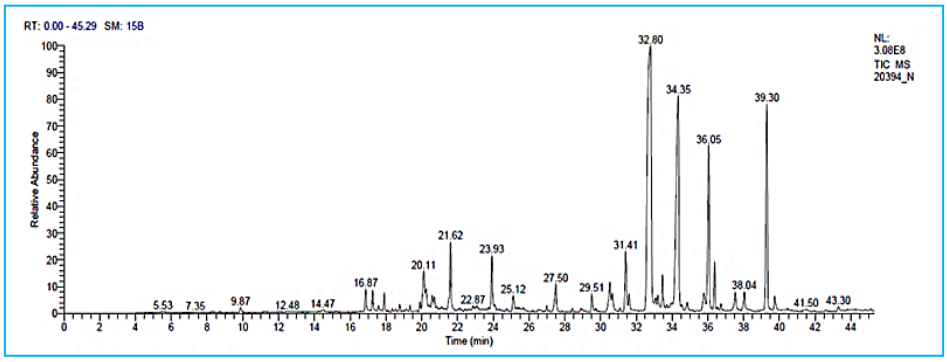

Fig. 1: GC-MS total ion chromatogram of *Zingiber oficinale* extract

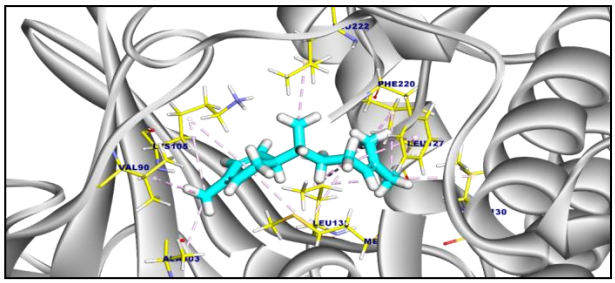

Fig. 2: 3D figure of compound 2 against (CpCDPK1)

Molecular docking simulation Results (CpCDPK1 inhibition):

Compound 2's binding mode against Cryptosporidium parvum Calcium-Dependent Protein Kinase 1 (CpCDPK1) showed a binding energy of -6.90 kcal/mol. Compound 2 interacted through fifteen hydrophobic π-interactions with Leucine (Leu) 222, Phenylalanine (Phe) 220, Leucine (Leu) 27, Leucine (Leu) 138, Leucine (Leu) 130, Methionine (Met) 136, Valine (Val) 90, Alanine (Ala) 103, and Lysine (Lys) 105 (Fig. 2).

Meanwhile, the binding mode of compound 4 against CpCDPK1 exhibited an affinity score of -6.87 kcal/mol. It formed eighteen hydrophobic π-interactions with Phenylalanine (Phe) 220, Leucine (Leu) 127, Leucine (Leu) 138, Methionine (Met) 136, Valine (Val) 90, Alanine (Ala) 103, Isoleucine (Ile) 150, Lysine (Lys) 105, and Isoleucine (Ile) 218. Furthermore, compound 4 established two hydrogen bonds at distances of 3.03 and 1.70 Å with Aspartic Acid (Asp) 219 and Phenylalanine (Phe) 220 (Fig. 3).

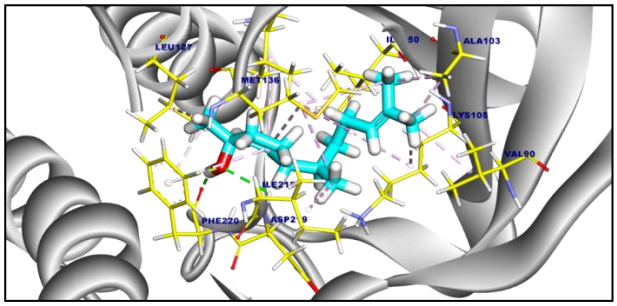

Figure 3: 3D figure of compound 4 against (CpCDPK1)

Compound 16 had a binding energy of -7.53 kcal/mol when it produced five hydrophobic π-interactions with Leucine (Leu) 82, Leucine (Leu) 205, Isoleucine (Ile) 218, Valine (Val) 90, and Methionine (Met) 136 in its binding mode against Cryptosporidium parvum Calcium-Dependent Protein Kinase 1 (CpCDPK1). Furthermore, it participated in three hydrogen bond exchanges at distances of 2.43, 2.93, and 2.04 Å with Tyrosine (Tyr) 155, Aspartic Acid (Asp) 219, and Lysine (Lys) 105 (Fig. 4).

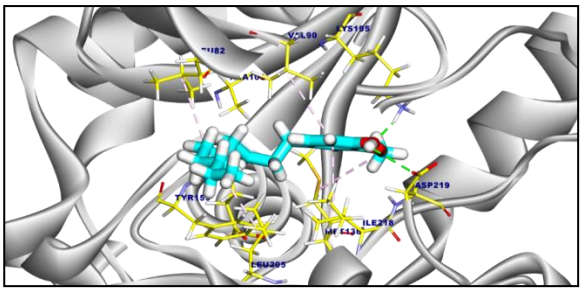

Fig. 4: 3D figure of compound 16 against (CpCDPK1)

Additionally, compound 18 had an affinity score of -6.95 kcal/mol for the proposed binding mechanism against CpCDPK1. Tyrosine (Tyr) 155, Valine (Val) 90, Isoleucine (Ile) 218, Alanine (Ala) 103, Leucine (Leu) 154, and Leucine (Leu) 205 were found to have six hydrophobic π-interactions. At separations of 2.50 and 2.08 Å, compound 18 connected with Lysine (Lys) 105 and Serine (Ser) 86 by two hydrogen bonds (Fig. 5)

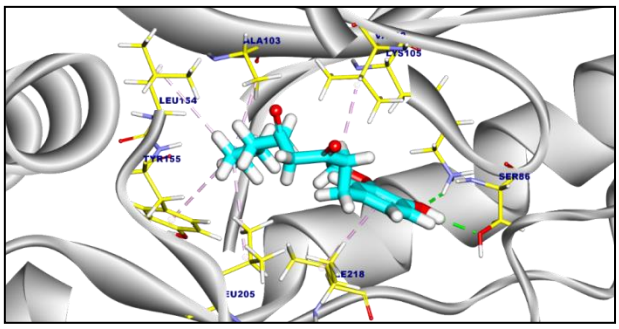

Fig. 5: 3D figure of compound 18 against (CpCDPK1)

In their binding mode against Cryptosporidium parvum Calcium-Dependent Protein Kinase 1 (CpCDPK1), compounds 20, 23, and 25 showed binding energies of -7.22, -7.67, and -8.36 kcal/mol, respectively. Compound 20 exhibited eleven hydrophobic π-interactions with Leucine (Leu) 222, Phenylalanine (Phe) 220, Leucine (Leu) 138, Isoleucine (Ile) 135, Methionine (Met) 136, Alanine (Ala) 103, Lysine (Lys) 105, Isoleucine (Ile) 218, and Valine (Val) 90, whereas compound 23 produced nine such interactions. Furthermore, compound 20 displayed two hydrogen bonds at distances of 2.95 and 2.29 Å with Lysine (Lys) 105 and Phenylalanine (Phe) 220 (Fig. 6).

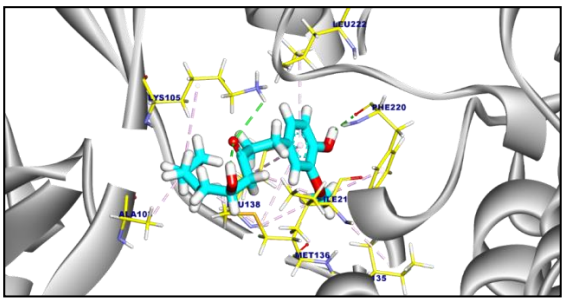

Fig. 6: 3D figure of compound 20 against (CPCDPK1)

In contrast, compound 23 created three hydrogen bonds with Aspartic Acid (Asp) 219, Glutamic Acid (Glu) 159, and Lysine (Lys) 105 at distances of 2.02,

2.63, and 2.82 Å, respectively (Fig. 7). Additionally, compound 25 formed two hydrogen bonds at distances of 2.29 and 2.53 Å with Lysine (Lys) 105 and Serine (Ser) 86, respectively, to reinforce its interactions with Leucine (Leu) 205, Isoleucine (Ile) 218, Methionine (Met) 136, Valine (Val) 90, Alanine (Ala) 103, Isoleucine (Ile) 150, Leucine (Leu) 222, Leucine (Leu) 138, and Glutamic Acid (Glu) 159 (Fig. 8).

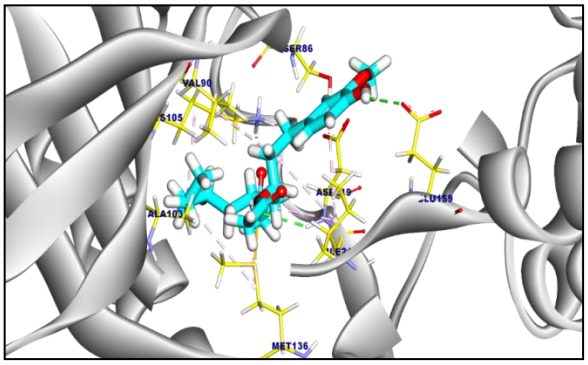

Fig. 7: 3D figure of compound 23 against (CPCDPK1)

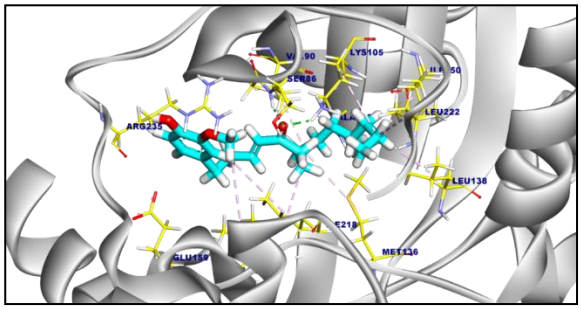

Fig. 8: 3D figure of compound 25 against (CPCDPK1)

With an affinity score of -7.03 kcal/mol, the co-crystallized ligand complexed with CpCDPK1 (Protein Data Bank (PDB) code: 3NCG) to establish fifteen hydrophobic π-interactions with Lysine (Lys) 105, Methionine (Met) 136, Valine (Val) 90, Isoleucine (Ile) 218, Leucine (Leu) 205, and Alanine (Ala) 103. Furthermore, two hydrogen bonds were observed with Tyrosine (Tyr) 155 and Glutamic Acid (Glu) 153 at distances of 2.05 and 2.12 Å, respectively (Fig. 9).

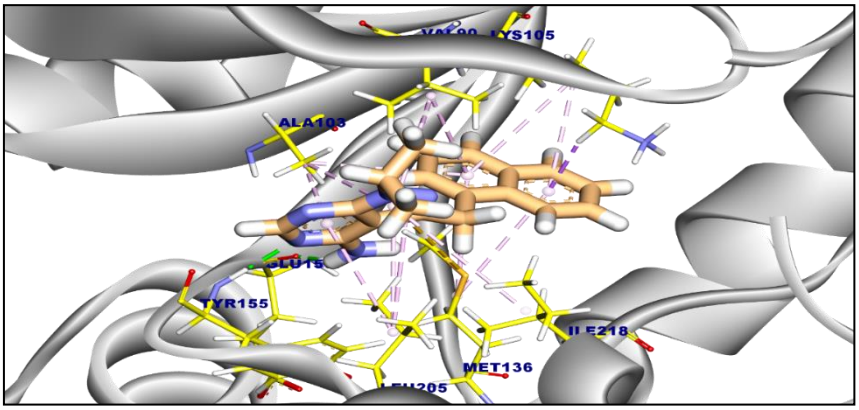

Fig 9: 3D figure of the co-crystalized ligand complexed with (CPCDPK1).

Table 3: shows the molecular docking results of the tested Compounds identified from ginger extract against (CPCDPK1).

Target	Tested compounds	RMSD value (Å)	Docking (Affinity) score (kcal/mol)
(CPCDPK1)	Compound 1	0.81	-6.39
	Compound 2	1.95	-6.90
	Compound 3	1.89	-6.67
	Compound 4	1.81	-6.87
	Compound 5	1.61	-6.35
	Compound 6	1.84	-1.77
	Compound 7	1.83	-3.63
	Compound 8	1.90	-4.96
	Compound 9	2.16	-3.65
	Compound 10	3.12	-1.90
	Compound 11	3.04	-6.01
	Compound 12	2.11	-6.09
	Compound 13	4.12	-6.20
	Compound 14	3.12	-6.12
	Compound 15	1.87	-6.89
	Compound 16	1.65	-7.53
	Compound 17	2.95	-6.78
	Compound 18	1.79	-6.95
	Compound 19	1.92	-6.87
	Compound 20	1.82	-7.22
	Compound 21	2.78	-5.23
	Compound 22	2.90	-6.22
	Compound 23	1.88	-7.67
	Compound 24	3.03	-6.90
	Compound 25	2.01	-8.30
	Compound 26	3.70	-6.62
	Compound 27	3.04	-6.05
	Co-crystalized ligand	0.87	-7.03

The values are presented as mean ±SD. The Mann–Whitney U test was used to compare two independent groups. A p-value equal to or less than 0.05 was considered significant.

Oocyst shedding:

The mice in the negative control group (GI) that were not infected did not have *C. parvum*. On the other hand, every infected mouse began to expel *C. parvum* oocysts, which, seven days following the infection, were confirmed (PI). At the end of the experiment (12 days PI), the GII, infected, untreated model had the highest oocyst intensity, with a mean score of 157.5. In comparison, the oocyst intensities in all treatment groups were lower than those in model GII, with GIII mean score of 17.9 demonstrating the highest efficacy at 89% suppression followed by GIV with 86% suppression and a mean score of 22.3 (Table 4).

The number of *Cryptosporidium* oocysts per gram of stool was expressed as (mean ± SD) x 10^3. The following formula was used to depict the reduction as a percentage: percent of reduction = [(mean count in the control infected group - mean count in the study group)/mean count in the control infected group] x 100

Table 4: *Cryptosporidium* oocysts count/ gram x 10^3 in different groups

Groups	*Cryptosporidium* oocysts count / gram x 10^3 in different groups		
	Mean ± SD	% inhibition	ANOVA P. value
GII	157.5 ± 12.8	-	0.000
GIII	17.9 ± 3.5*^#	89%	
GIV	22.3 ± 5*^#	86%	
GV	25.6 ± 7.7*^	84%	
GVI	35.8 ± 4*^	77%	
GVII	63.6 ± 4.1*	60%	

Key: Values expressed as Mean ± SD, * Significant VS +VE CON, ^ Significant VS NTZ, o Significant VS ginger MeOH, + Significant VS ginger EtOAc, @ Significant VS ginger Aqueous, # Significant VS ginger Pet-Ether. Different superscripts (*, ^, o, +, @, #) indicate significant differences at *p*-value ≤ 0.05.

DISCUSSION

Recently, the incidence of parasitic diseases that infect humans through contaminated food or water has increased. On the other hand, the phenomenon of oxidative stress resulting from the accumulation of free radicals within the body causes the body to suffer from several pathological phenomena, including cancer,

inflammation, and heart disease. Therefore, we are in urgent need of discovering some safe therapeutics from natural sources such as medicinal plants. In the same context, several medicinal plants have shown strong effectiveness as anti-parasitic and antioxidant agents due to the presence of some bioactive secondary metabolites like phenolic compounds[28].

Ginger is known for its health benefits, as it contains different nutrients, such as ginger essential oils, zingiberene, gingerols, sugars proteins, and amino acids[29]. In the present research, the main and the high constituents of *Z. officinale* MeOH extract were sesquiterpenes, diterpenes, monoterpene and fatty acids, respectively as shown in Table 1 and Fig.1. Sesquiterpenes were the highest content in this extract, in which 1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one, followed by gingerol, and 1-(4-Hydroxy-3-methoxyphenyl) tetradec-4-en-3-one were most abundant compounds. In addition, some compounds such as α-curcumene, Zingiberene, α-Sesquiphellandrene, 2-Butanone, 4-(4-hydroxy-3 methoxyphenyl), and 9-Octadecenoic acid (Z) have been identified in the essential oil and chloroform extract of Z. officinale[30,31]. Sesquiterpene concentrations were discovered to be rather high in Z. officinale oils from south Indian origin, while monoterpene concentrations were found to be low. Additionally, the volatile oil from Cuba contained a low concentration of monoterpenes and a high concentration of sesquiterpenes, which was consistent with our findings.

Additionally, the volatile oil from Cuba contained a low concentration of monoterpenes and a high concentration of sesquiterpenes, which was consistent with our findings[32]. The identified compounds in this investigation have a variety of biological actions, including anticancer, antibacterial, anti-inflammatory, and antioxidant properties, according to the literature. Antiparasitic, hypoglycemic, analgesic, antiplatelet, antiemetic, antithrombotic, antitumorigenic, radioprotective, and antifungal effects are among *Z. officinale's* additional pharmacological properties[33, 34]. The chemical makeup and biological effects of Z. officinale extracts and essential oils actions have been shown to be influenced by a number of intrinsic characteristics, including species or variant, climate, type of soil, maturity, and harvest time[35].

The present study's findings were corroborated by Abouelsoued[36] who found that ginger therapy significantly reduced the number of fecal oocysts in a dose-dependent manner. Both high and low dosages of ginger extract dramatically reduced the number of cryptosporidiosis oocysts in experimentally infected mice. The findings of present studies also indicate that using different ginger extracts reduced the number of oocytes, with the ginger methanol extract having the strongest anti-parasitic and anti-protozoal effects. Another study supported our results shows comparing *Cryptosporidium* oocyst excretion among the studied groups, the group treated with ginger CSNPs showed the highest reduction of the mean oocyst count, followed by the group treated with NTZ/ginger CSNPs combination, the group treated with CSNPs, the group treated with ginger then group treated with NTZ, and lastly positive control group. In comparison to the positive control group, all groups exhibited statistically significant decreases in oocyst count[37]. According to this study's latest findings, the rhizome of *Z. officinale* extracts like (methanol, pet-ether, aqueous, and acetyl acetate) especially methanol shows a high reduction of oocytes that's why it can be used as a safe and promising natural source of anti-parasitic and ginger acetyl acetate extract shows that it has a high antioxidant effect that's why it can also use as anti-oxidant drugs.

Table 5: A brief summary of in vitro and in vivo studies on the antiparasitic properties of extracts from ginger (Zingiber officinale).

Name of Parasite	Type of Study	Extract	Reduction Rate	Reference
Cryptosporidium parvum	In vitro/ In vivo	Aqueous ginger extract	65-85% reduction in oocysts Significant reduction in oocyst shedding in in-vivo	Elemi[38]
Trichomonas vaginalis	In vitro	Aqueous ginger extract	50-65% reduction in viability	Hassan[39]
Plasmodium falciparum	In vitro	Ethanolic ginger extract	45-70% inhibition	Massi[40]
Cryptosporidium parvum	In vitro	Ethanolic ginger extract	Reduced infectivity in cell cultures.	Abdou[41]
Giardia lamblia	In vivo	Ginger powder	~70% reduction in cyst shedding	Al-Attar[42]
Schistosoma mansoni	In vivo	Ethanolic ginger extract	Reduction in worm burden and liver pathology	Rizk[43]
Leishmania major	In vivo	Ginger ethanolic extract	Decreased lesion size and parasitic load	Omar[44]
Entamoeba histolytica	In vitro	Gingerol (active compound)	60-80% trophozoite inhibition	Khadeer[45]
Trichinella spiralis	In vivo	Gingerol (active compound)	Reduced larval burden in muscle tissues	Fayed[46]

CONCLUSION

The current work investigated the antioxidant and anti-protozoan properties of several solvent extracts from the rhizome of *Z. officinale* against *Cryptosporidium* spp. The extracts showed remarkable efficacy as antioxidant and anti-protozoan. The finding emphasizes the great ability of the ethyl acetate extract to scavenge free reactive species which correlated with their total phenolic content. In summary, ginger is thought to be a possible source of naturally occurring chemicals having medicinal uses. Future research is planned to isolate the main chemical constituents using the available chromatographic tools and to assess the isolated compounds in vitro and/ or in vivo.

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