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The potential of Brachionus plicatilis extract against bacterial infection and cancer : In Vitro and In Silico Studies

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Abstract

Drug discovery may open new horizons and new avenues to bring new neutral compounds into the drug trade. Furthermore, about 50% of modern synthetic drugs are originated directly or indirectly from natural products. The goal of the study is to investigate the effect of crude B. plicatilis extracts versus the three human cancer cell lines. Also, it was tested as an antimicrobial for5 strain Gram-positive and 2 strain Gram-negative bacteria. By using GC-MS, its chemical composition was also examined. A GC-MS analysis of the B. plicatilis extract revealed 31 components. The major identified compounds are Benzene propanoic acid,3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester (23.76%), and 1,4-benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl) (15.68%). B. plicatilis extract exhibited a moderate cytotoxicity activity of (IC50 = 83.78, 82.89, and 69.78 ug/ ml) against the cancer cell lines HepG2, A549, and Caco2, respectively. While, the cytotoxicity effect of staurosporine on the three cell lines was IC50 = 67.27, 64.97, and 63.75 ug/ ml, respectively. In the current study, the crude extract of B. plicatilis exhibited antimicrobial ability against three Gram-negative and four Gram-positive bacteria. The greater inhibition activities were achieved against Sarcina lutea and against Salmonella typhi. Additionally, investigations of compounds 1 and 2 using molecular docking in the DNA gyrase binding site was carried out, and the results matched the in vitro inhibitory findings. B. plicatilis extract provides opportunity for further investigation in the search for novel anticancer substances. Additionally, they are promising as an origin of bioactive compounds that can replace antibiotics in clinical settings

Keywords: *Brachionus plicatilis; Anticancer; Antimicrobial; GC-MS; Docking, Chemical constituents*

1. Introduction

Medicine is one of the biggest challenges that people constantly face in light of the spread of infections and diseases, especially those chronic diseases. Therefore, many researchers in many fields are striving to discover atypical drugs to treat large numbers of these diseases. In this regard, biologists, including aquatic biologists, have been interested in discovering new compounds from living organisms that can be used in the pharmaceutical industry [1, 2]. Drug discovery may open new horizons and new avenues to bring new neutral compounds into the drug trade. Furthermore, about 50% of modern synthetic drugs are originated directly or indirectly from natural products [3]. Natural products provide unique features compared to conventional synthetic compounds that provide the drug discovery procedure with benefits as well as challenges [4]. Natural products have a higher

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molecular mass, and more carbon and oxygen atoms, but fewer halogen and nitrogen atoms. Also, the molecular hardness of natural products is greater compared to synthetic ones [4-6]. These differences can be more useful in the pharmaceutical industry. For example, the high hardness of natural products can be valuable in drug discovery that manipulates protein-protein interactions [7]. Actually, natural products are a key source of oral medicines, beyond Lipinski's rule of five [8]. The increasing importance of medicines that do not meet Lipinski's rule is demonstrated by the increase in molecular mass of orally approved drugs over the past 20 years [9]. Consequently, significant formulations and promising compounds with the potential to be exploited as novel therapeutic agents for a range of diseases have been produced using natural products [10, 11].

Aquatic invertebrates are considered promising in order to produce natural compounds that can be used in medical fields. Because aquatic organisms face extreme ecological obstacles include variations in pressure, temperature, nutrition, and solar radiation intensity. Consequently, these aquatic invertebrates produce a variety of natural compounds that help them defend themselves and adjust to their environment. [12]. Additionally, many invertebrates secrete powerful chemicals to defend themselves against predation [13]. These chemical substances have biological effects against a wide range of diseases such as viral, cancer, cardiovascular, neurological, fungal, and microbial diseases [12, 14]. Accordingly, over the past few decades, numerous novel natural compounds have been identified from a variety of aquatic invertebrates, and these have increased rapidly to now exceed hundreds of newly discovered compounds [14]. On the other hand, studies on small invertebrates (zooplankton) as potential sources of medically active compounds are still rare; Because of these organisms' great nutritional value, the majority of applied studies have been on their significance as fish food. However, promising opportunities exist to isolate medically important compounds from these organisms [15-18].

Brachionus plicatilis is the most widely cultured zooplankton species, due to its tolerance of environmental changes, short life span, high fecundity, and high nutritional value, as well as feeding on many types of food [19]. Thus, the utilization of *B. plicatilis* as a food source for numerous aquatic larvae in aquarium cultivation has been the subject of multiple researches. [20]. Nonetheless, only one study tested an extract of B. plicatilis as an antibacterial and an anti-breast cancer drug [18]. Furthermore, this study was very preliminary, but it was a catalyst for more in-depth and detailed studies. Therefore, this study aims to further in-depth tests of the use of *B. plicatilis* extract as an antimicrobial and anti-colon cancer.

2. Experimental

2.1. The culturing of Brachionus plicatilis

Brachionus plicatilis was cultured under environmental conditions (28 ± 1 °C, salinity: 20 ‰, 12 h/day photoperiods, and continuous aeration) and feeding protocol (30% yeast, 70% sugar, and Cyclotella sp.) according to Hegab *et al*. [19]. *B. plicatilis* was cultivated in ceramic ponds (capacity 6000 L) and then half of the pond was filtered through a100 μm plankton net after 12 days to collect a *B. plicatilis* mass. The harvested mass was repeatedly cleaned with distilled water, before being dried for 24 hours at 50 °C to produce a powder that could be used for further examinations and applications.

2.2. Preparation of the crude extract

Using a checker at room temperature, 10 mL of 100% methanol (HPLC grade) was used for three days to thoroughly extract about one gram of dried material of *B. plicatilis*. To ensure complete extraction, the extraction procedure was done multiple times until no color was obtained. Filter paper Whatman no.1 was used for filtering the extracts. The obtained filtrates were then concentrated by a rotary evaporator (IKA Rotary Evaporator, IKA rv10) under vacuum at 40 °C and stored at -20 °C until being analyzed by GC/MS, cytotoxicity tests, and antimicrobial procedures were completed[21].

2.3. GC-mass analysis of the B. plicatilis extract

At the National Research Center in Dokki, Egypt, the bioactive component of the *B. plicatilis* extract was examined using an Agilent 8860 gas chromatograph and an Agilent 5977B GC/MSD system. The extract's bioactive components were determined to be those that were most likely to match the compounds listed in Agilent's Retention Time Locked (RTL) database and the NIST MS Spectrum Library (agilent.com/en/product/gas chromatography -mass-spectrumcluster-gc-ms/gc-ms-application-solutions/gc-ms-libraries).

2.4. Antitumor potential of the B. plicatilis extract

The Viability test of crude *B. plicatilis* extracts versus the following human cancer cell linesA549 (lung), HepG2 (liver), and Caco2 (colon), was examined. Employing the (MTT) procedure "3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide" as a functional assay according to Mosmann [22] . Staurosporine, a natural product with potent anticancer effects due to inhibition of multiple protein kinases was used as a positive control and produced comparable growth inhibition [23, 24].

2.5. Antimicrobial potential of the B. plicatilis extract

Using the agar well diffusion technique, the antibacterial activity was evaluated [25]. against both Gram negative and Gram positive bacteria, such as *Salmonella typhi*, *E. Coli*, and *Klebsiella pneumonia*, as well as Staphylococcus aureus, *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus fecalis*, and *Sarcina lutea*. Briefly, 100µl of 24hr bacterial cultures with 0.5 McFarland standard density (108cells/ ml-1) on trypticsoy broth medium (TSB, Difco Laboratories, Detroit,USA) were dispersed throughout the whole surface of Mueller–Hinton agar (Oxoid) plates, Using a sterile cork borer, 6 mm diameter wells were created in agar, and 50 µl of *Brachionus plicatilis* extract diluted in DMSO (15 mg/ml) were added to the wells. The widths of the inhibition zones were then determined after the inoculation plates were incubated for 24 hours at 37°C. Amikacin 30 mcg and Amoxicillin25 mcg were employed as positive control and DMSO as negative control.

2.6. Docking study

The MOE-Dock 2014.09 program was used to carry out the docking analysis [26]. Using the builder key, the structures of Comp. 1, Comp. 2, and Ligand were sketched. Then, these structures were subjected to energy reduction by the MMFF94x force field that was set as the default in the MOE software. The conformer search was used to obtain the compounds' three-dimensional conformers. After downloading the enzyme from the Protein Data Bank (PDB ID: 6F86) [27], it was opened in the MOE, where the missing hydrogens were added and water molecules were eliminated to give the protein structure the suitable ionization states. To find the active site, the MOE Alpha Site Finder was used with the standard settings. The dummy atoms, which make up the active site, were constructed using the alpha spheres that were obtained. To implement docking, MOE's "Docking" component was used. The usual docking procedure was then put into practice. MMFF94x was used to minimize and save the best thirty postures, as determined by London dG, inside the enzyme. Then, the GBVI/WSA dG ranking algorithm was employed to grade the created postures. Then the compounds' poses that have the highest scores are selected.

2.7. Statistical analysis

A one-way statistical ANOVA test was applied to compare the variance of the cytotoxic crude extract activity on tumor cells and staurosporine (control) by XLSTAT 2016.

3. Results and Discussion

3.1. Biochemical compounds of Brachionus plicatilis extract: GC-Mass analysis

GC-MS investigation of *B. plicatilis* extract includes 31 compounds (Table 1, Figure 1). The total peak area of the characterized compounds represents 66.46%. The major identified compounds are Benzenepropanoic acid,3,5-bis (1,1-dimethylethyl)-4-hydroxy-, octadecyl ester (23.76%), and 1,4-benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl) (15.68%). The description was accomplished utilizing computer search user-generated reference libraries, incorporating mass spectra [28-32].

Table 1: Chemical constitution of *B. plicatilis* extract

No.	Rt	Area %	M.W.	M. F.	Compound Name	Chemical Class
1	6.06	0.55	428	$C_{27}H_{40}O_4$	Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R)-	Spirostene derivatives
2	13.06	0.40	168	$C_{12}H_{24}$	1-Dodecene	Long chain alkene
3	14.91	1.13	220	$C_{14}H_{20}O_2$	2,5-Cyclohexadiene-1,4-dione,2,6Bis(1,1dimethylethyl)-	Benzoquinone derivatives
4	15.94	15.68	206	$C_{13}H_{18}O_2$	1,4-benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl)	Hydroquinone derivative
5	17.77	1.57	224	$C_{16}H_{32}$	Cetene	Long chain alkene
6	20.70	0.73	296	$C_{19}H_{36}O_2$	Cyclopentanetridecanoic acid, methyl ester	Long-chain fatty ester
7	22.10	1.85	242	$C_{16}H_{34}O$	1-Hexadecanol	Fatty alcohol
8	22.60	0.19	250	$C_{18}H_{34}$	Cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis-	Cycloalkane derivatives
9	24.35	1.18	268	$C_{17}H_{32}O_2$	(Z)-Methyl hexadec-11-enoate	Long-chain fatty ester
10	24.66	1.53	276	$C_{17}H_{24}O_3$	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	Lactone derivatives
11	24.77	2.52	270	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	Long-chain fatty ester
12	24.88	0.51	300	$C_{19}H_{40}O_2$	1,2-Nonadecanediol	Long-chain fatty alcohol
13	25.12	0.18	292	$C_{18}H_{28}O_3$	Benzenepropanoic acid,3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	Hydrocinnamic acid derivatives
14	25.48	0.63	306	$C_{18}H_{26}O_4$	Phthalic acid, butyl hex-3-yl ester	Phthalic acid derivatives
15	25.97	0.30	344	$C_{18}H_{16}O_7$	4H-1-Benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3, 5-dihydroxy-7-methoxy-	Benzopyran derivatives
16	26.05	1.47	252	$C_{18}H_{36}$	5-Octadecene, (E)-	Long chain alkene
17	26.79	0.42	230	$C_{13}H_{10}O_4$	Visnagin	Furanochromones
18	27.70	2.46	266	$C_{19}H_{38}$	1- Nonadecene	Long chain alkene
19	27.92	0.77	314	$C_{18}H_{34}O_4$	9,10-Dihydroxy-12Z-octadecenoic acid	Long-chain fatty acid
20	28.04	1.52	296	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)- methyl ester	Long-chain fatty ester
21	28.14	0.49	296	$C_{19}H_{36}O_2$	14-Octadecenoic acid, methyl ester	Long-chain fatty ester
22	28.51	0.85	298	$C_{19}H_{38}O_2$	Octadecenoic acid, methyl ester	Long-chain fatty ester
23	29.67	0.76	308	$C_{22}H_{44}$	1-Docosene	Long chain alkene
24	30.30	0.85	260	$C_{20}H_{20}$	1,5-Dimethoxy-3-propionyl-4-naphthol	Naphthol derivatives
25	30.75	0.47	306	$C_{20}H_{34}O_2$	1-Naphthalenepentanol,decahydro-5-(hydroxymethyl)-5,8a-dimethyl-ç,2-bis(methylene)-,(1à,4aá,5à,8aà)-	Naphthalene derivatives
26	31.75	1.00	364	$C_{26}H_{52}$	Eicosane, 2-cyclohexyl-	Alkane derivatives
27	33.05	1.04	344	$C_{18}H_{16}O_7$	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	Benzopyran derivatives
26	33.50	0.77	276	$C_{19}H_{32}O$	Androstan-16-ol,(5à,13à,16á)-	Steroid derivatives
27	38.98	0.55	390	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate	Phthalate esters
28	40.39	0.57	396	$C_{25}H_{48}O_3$	Tetracosanoic acid, 3-oxo-, methyl ester	Long chain alkene
29	45.45	0.47	280	$C_{20}H_{40}$	Eicosene <1->	
30	52.77	23.76	530	$C_{42}H_{26}$	Benzenepropanoic acid,3,5-bis(1,1-dimethylethyl)-4-hydroxy-,octadecyl ester	Hydrocinnamic acid derivatives
31	53.77	0.27	284	$C_{20}H_{28}O$	Dehydro abietal	Aldehyde derivatives
		66.46%				

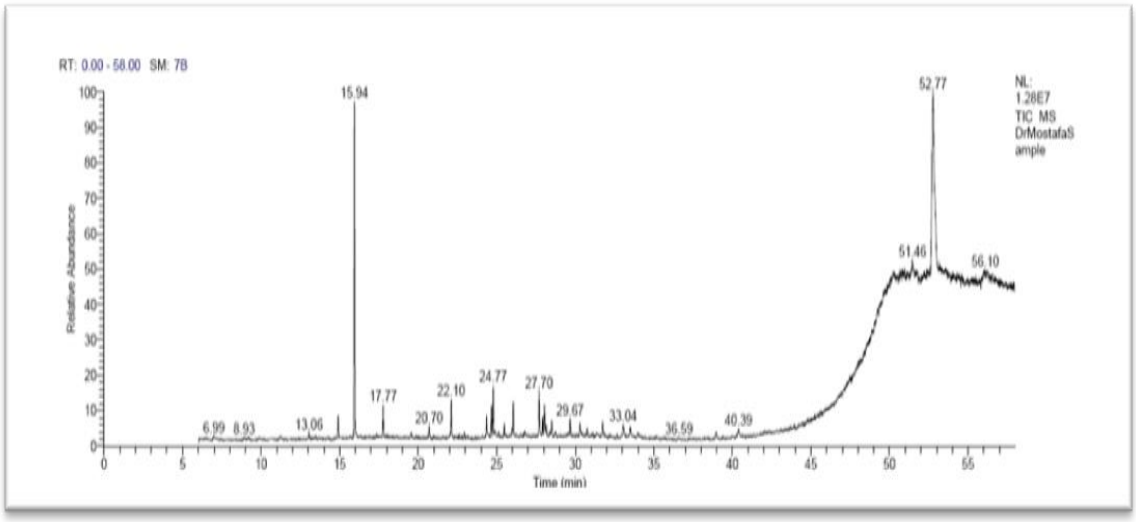

Fig. 1. GC/MS chromatogram of *B. plicatilis* extract.

The GC-MS results of *B. plicatilis* exhibited the existence of several components that possess various bioactivities. One of the active compounds is 2,5-Cyclohexadiene-1,4-Dione,2,6-Bis (1,1-Dimethylethyl) -it has been reported as antifungal compound [33]. On the same way, 2,4-Ditert-Butylphenol is another important natural compound that was detected in the *B.plicatilis* extract and has been reported to has antimicrobial [34-35] and anticancer activities [36, 37]. Furthermore, 1-Nonadecenee has the ability to activate the immune system and aid in the expression of inflammation-related cytokines [38]. Also, 9-Hexadecenoic acid, methyl ester is mono unsaturated fatty acid; it has anti-inflammatory activity and reduces risk of certain cardiac disease [39, 40]. As well as Hexadecanoic acid, methyl ester(Palmitic acid, methyl ester),9-Octadecenamide, 12-hydroxy-,[R-(Z)]- and 7,9-Di-Tert-Butyl-1-Oxaspiro[4.5]Deca-6,9-Diene-2,8-Dionealso were determined to have antioxidant and anti-inflammatory activities [41-43]. In addition the compounds1-Hexadecanol, an alcoholic compound, and 9-Octadecenoic acid (Z)-, methyl ester, Linoleic acid methyl ester, have been documented as having antimicrobial activity [44-45]. Whereas Khellin is a bioactive compound known with its anti-inflammatory, lipid-altering and anti-atherosclerotic activities, and it is the key active component of many drugs [46-47]. Furthermore, 11H-Indeno (1,2-B) Quinoline is one of quinoline derivatives which are characterized by their anticancer, anti-inflammatory, antioxidant, anti-microbial, antiplasmodial activities [48-50]. In the same way, the most abundant component in the *B. plicatilis* extract ("Area about 23.76%") is 1,2,5,8-tetra hydroxy anthraquinone, which has been shown to have anticancer action because these components' structural counterparts are closely related to the Hydroxy-9,10-anthraquinones, which form the basis of anthracycline anticancer drugs [51]. The outcomes of the *B. plicatilis* GC-MS examination have been verified by the antibacterial and anticancer activities, since it has confirmed the presence of numerous compounds with antibacterial and anticancer activities and showed that the *B. plicatilis* extract could be good precursor for medicinal drugs.

3.2. Bioactivity of crude extract of B. plicatilis on different tumor cell lines

B. plicatilis crude extract was tested for cytotoxic potentials in comparison with the activity of staurosporine (control) against three human cancers (liver, colon, and lung) of cancer cell lines (HepG2, Caco2, and A549) (Table 2). *B.plicatilis* extract exhibited a moderate cytotoxicity activity of (IC_{50} = 83.78, 69.78, and 82.89ug/ml) against the cancer cell linesHepG2, Caco2, and A549, respectively. While, the cytotoxicity effect of staurosporine on the three cell lines was IC_{50} = 67.27, 63.75, and 64.97ug/ml, respectively. The P values of ANOVA analysis between the cytotoxicity of *B. plicatilis* extract and staurosporine (control) against the three human

cancers were significant values. The anti-cancer ability of the *B. plicatilis* extract is corroborated by numerous further earlier investigations; it documented the source of aquatic invertebrates' pharmacologically powerful chemicals against cancer cells. [16-18, 52-55]. Additionally, a number of novel anticancer chemicals have been identified from a variety of aquatic invertebrates and are being tested for potential human applications [12].

Table 2: The cytotoxic activity of staurosporine and *B. plicatilis* on malignant cell lines (HepG2, Caco2, and A549) and P values (ANOVA test). The data is shown as mean ± SD for n = 3.

	Organ cell line		
	Liver	**Colon**	**Lung**
	HepG2	**Caco2**	**A549**
IC_{50} (ug ml^{-1}) of *B. plicatilis* extract	83.78±2.09	69.78±0.7	82.89±4.41
IC_{50} (ug ml^{-1}) of staurosporine (control)	67.27±0.55	63.75±0.28	64.97±0.38
P-value	0.012563	0.010874	0.004996
Significant	Yes	Yes	Yes

In comparison with the scarce previous studies, that tested zooplankton as an antitumor, we find that the current results of our crude extract were more robust than Abdelhameed *et al*. (2020) [18] who studied how the crude extract of *B. plicatilis* affected MCF-7 breast cancer cell lines and got an IC_{50} value of 967.85 µg/ml. However, Abdelhameed *et al*., (2020) used water as a solvent in the extraction process of *B. plicatilis*. On the other hand, our results were weaker than Hegab *et al*. [16], who studied the effect of copepod *Acanthocyclopstrojani* extract versus HCT, A549, HepG2 and MCF7 cancer cell lines and got IC_{50} values of 46.905, 18.377, 63.064, and 21.736 ug/ml, respectively. Additionally, the current results were weaker than Hegab *et al*. [17], who examined the cytotoxicity effect of *ostracod Heterocypris salina* extract against HCT, A549, HepG2 and MCF7 cancer cell lines and exhibited IC_{50} = 13.8, 17.6, 23.2 and 12.8 µg/ml, respectively . Conversely, however, in comparison with the previous studies, that tested large invertebrates as an antitumor, we find that the current results of *B. plicatilis* extract were weaker than the results obtained by Rady and Bashar (2020) who studied crude extracts of sponges, *Callyspongiasiphonella*, and Negombatamagnifica against breast cancer cell line (MCF-7) [56].Also, the *B. plicatilis* extract effect was less than that of some extracts that were produced from organisms from the molluscan, *Dolabella auricularia*, which affected on colon cancer cell model of HT-29, after 24 and 48 hrs (IC_{50} 5 and 0.10 µgml-1, respectively) [55]. The *B. plicatilis* extract's ability to inhibit cancer cell lines may be related to its major contents of aliphatic hydrocarbon compounds that have activity against cancer cells [57, 58]. Figueiredo *et al* [59] found that an extract of *Pyrostegiavenusta* heptane containing aliphatic hydrocarbons induces apoptosis in melanoma cells by induction of species of reactive oxygen, alterations in the membrane of mitochondria, fragmented DNA on the protective cell surface, and delayed apoptosis, which is manifested at the plasma membrane, chromatin condensation, inducing cancer cells' cell cycle halt in the G2/M stages. Also, *B. plicatilis* has a high content of Palmitic acid (24.2%), Palmitoleic acid (23.9%), and other important fatty acids [19]. Many studies have shown that these fatty acids have strong anti-cancer activity [60-62]. For example, Mericli *et al*. [61] found that almond oil, which contains a large proportion of Palmitic acid, has substantial impact on molecular signaling routes in colon cancer cells, suggesting that it may be a promising new therapeutic treatment. Also, Ito *et al*. [60] noted that palmitoleic acid significantly prolonged the survival of rats bearing Ehrlich's ascites. The total lipid and phospholipid content of Palmitoleic acid-treated cancer cells was reduced. Therefore,

according to the study's findings, this extract may have considerable potential as an anti-cancer, like many marine invertebrates. Where, the extensive research on marine natural compounds isolated from invertebrates has shown an opportunity to develop several strong anti-cancer drugs, as these molecules have been reported to have different modes of action, targeting cell receptors for the genetic material of cancer cells [63].

3.3. Antimicrobial activity

The increase in resistance of bacteria to common antibiotics is a critical global problem, it makes the needs to develop an alternative antibacterial compound be urgent [64]. In present study *B. plicatilis'*s crude extract exhibited antibacterial effects against four of five Gram positive bacteria and three Gram negative bacteria. *Sarcina lutea* and *Salmonella typhi* were found to exhibit the highest levels of inhibitory activity **(**Table 3**)**. The results of the current article showed moderate antibacterial efficacy in comparison to conventional antibiotics that have been used as positive control. The study's findings on antibacterial activity were in line with those of Hegab *et al*. [16] in their study of *copepod Acanthocyclops trajanin* extract , explain how it works against different types of bacteria, both Gram positive and Gram negative. Abdelhameed *et al*., [18] had studied *B. plicatilis* extract and its antimicrobial activity and revealed that the extract had antibacterial action against Gram positive bacteria "*Staphylococcus aureus* ATCC25923, *Streptococcus mutants* RCMB017 ATCC25175 and *Methicillin-Resistant Staphylococcus aureus* and Gram-negative bacteria *Salmonella typhimurium* RCMB006 ATCC14028.Several studies have shown that the invertebrate extracts contain active compounds that have antimicrobial activity [16,54, 65].

Table 3: Antimicrobial potential of methanolic extract of *B. plicatilis* against several Gram-positive and Gram-negative microorganisms

Extract	Clear Inhibition zone (Θmm)							
	Staphylococcus aureus	***Bacillus cereus***	***Bacillus subtilis***	***Enterococcus fecalis***	***Sarcina lutea***	***Escherichia coli***	***Klebsiella pneumonia***	***Salmonella typhi***
B. plicatilis	$12.0^{\pm1.63}$	$9.7^{\pm0.47}$	$14.0^{\pm0.82}$	0.0	$22.7^{\pm1.70}$	$11.7^{\pm0.94}$	$11.3^{\pm0.47}$	$13.7^{\pm1.25}$
AX	$9.0^{\pm0.82}$	$42.0^{\pm2.16}$	0.0	0.0	0.0	$26.3^{\pm1.25}$	$33.7^{\pm1.89}$	$23.3^{\pm1.70}$
AK	$19.3^{\pm0.94}$	$22.7^{\pm2.05}$	$18.3^{\pm1.25}$	$11.7^{\pm1.25}$	$20.0^{\pm0.82}$	$17.3^{\pm1.70}$	$14.3^{\pm0.47}$	$12.3^{\pm0.94}$

Results expressed as average of triplicate ± SD, AK: Amikacin 30 mcg and AX: Amoxicillin 25 mcg.

3.4. Docking into DNA Gyrase active site (PDB: 6F86)

It is well-known that the biggest challenge to treating microbial infections that lead to the recurrence of many infectious diseases is resistance to a broad spectrum of antimicrobial drugs [66-67]. Consequently, target selection is crucial in order to reduce the likelihood of resistance [68]. Using the bacterial enzyme DNA Gyrase B and the MOE software, the mechanisms of the recently identified compounds were examined; DNA Gyrase B is essential for DNA replication and repair and is present in the majority of bacteria [69]. The significance of this bacterial enzyme in modulating the topological state of DNA during replication was investigated by comparing it to its native ligand. Compounds 1 and 2 were docked inside DNA gyrase B's active site in order to study their binding modes and interactions. For validation re-docking of the co-crystallized ligand was done showing docking score value = -10.897 kcal mol-1 with (RMSD); a root-mean-square deviation = 0.79Å. The two NH of ureido group formed two hydrogen bonds with Asp73, while the carbonyl oxygen atom was bonded to Asn46. Also, the NH of the nicotinamide group displayed H-bond with Gly77. Compound 1 was able to fit inside the binding site employing the phenyl ring via C-H bond with Asp73 residue, besides two arene-H interactions were observed with Asn46, Thr165. Interestingly the phenyl ring in compound 2 interacted with Asn46 showing the same binding mode as compound 1, in addition to C-H interaction with Asp73. Findings showed that both compounds displayed high binding scores -9.34 and -11.02 kcal mol-1, respectively as compared to the ligand (Figure 2, Table 4).

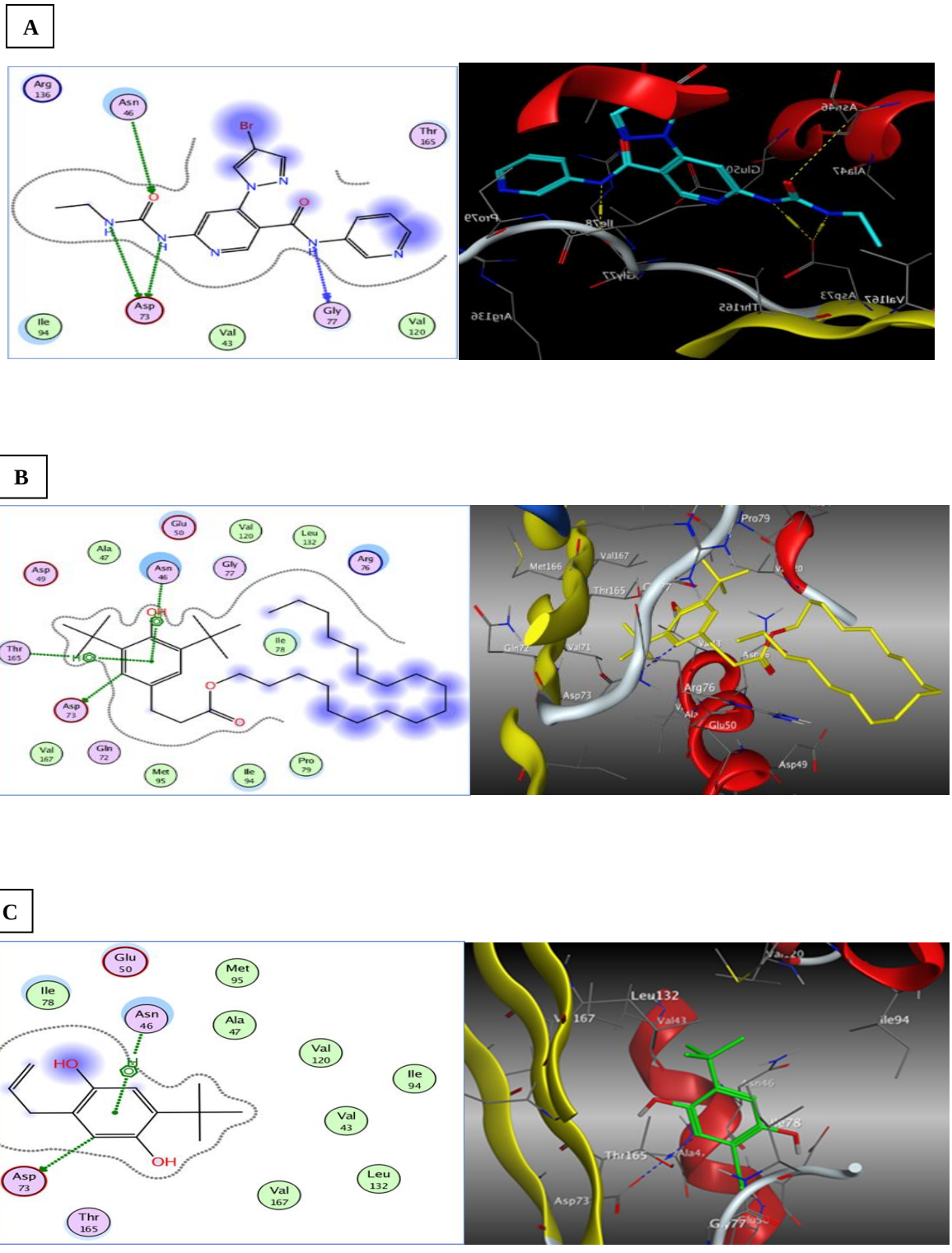

Fig. 2. Two dimensions (left) and three dimensions (right) proposed interactions of Ligand (A), compound 1 Benzenepropanoicacid,3,5-bis(1,1 dimethylethyl)-4-hydroxy-, octadecyl ester;(B), and compound 2 ; 1,4-benzenediol,2-(1,1-dimethylethyl)-5-(2-propenyl) (C) inside the DNA gyrase active binding site.

Table 4. The most significant compounds' docking scores and their binding interactions with the ligand within the DNA gyrase active binding site

Compound	Docking score (Kcal/mol)	Interacting amino acids	Type of interactions
Ligand	-10.89	Asn46	H- bond
		Asp73	H- bond
		Gly77	H- bond
Benzenepropanoicacid,3,5-bis(1,1 dimethylethyl)-4-hydroxy-, octadecyl ester	-9.34	Asp73	H- bond
		Asn46	Arene-H
		Thr165	Arene-H
1,4-benzenediol,2-(1,1-dimethylethyl)-5-(2-propenyl)	-11.02	Asn46	Arene-H
		Asp73	H- bond

4. Conclusion

B. plicatilis extract exhibited a moderate cytotoxic activity of (IC50 = 83.78, 82.89, and 69.78 ug/ ml) against the cancer cell lines HepG2, A549, and Caco2, respectively, so B. plicatilis has the potential to be further utilized in the search for novel anticancer agents. Additionally, they show promise as a source of bioactive compounds that can be substituted for antibiotics, especially against Gram-negative bacteria. The greater inhibition activities were achieved against *Sarcina lutea* and against *Salmonella typhi*. Our work paves the way for future investigations aimed at purifying bioactive substances from *B. plicatilis* and evaluating their activities against pathogenic bacteria and cancer cells. Finally, The DNA gyrase active site's molecular docking revealed appropriate binding with important residues in the active binding site with relatively high binding scores.

5. List of abbreviations

°C: Degree in Celsius.
Å: Angstrom
A549: Lung Cancer Cell Line
AK: Amikacin 30 mcg and
AX: Amoxicillin 25 mcg.
Caco2: Colon Cancer Cell Line
cm: Centimeter.
cm^{-1}: $Centimeter^{-1}$.
Conc.: Concentration.
DMSO- d6: Dimethyl Sulfoxide-deutrated6.
DNA: Deoxyribonucleic acid.
DPPH: 1,1'- Diphenyl-2-PicrylHydrazyl.
GC/MS: Gas Chromatography/Mass Spectrometry.
HepG-2: Liver Cancer Cell Line.
HPLC: High Performance Liquid Chromatography.
hr: hour.
HT-29: Colon Cancer Cell Model
IC50: Median Inhibitory concentration.
Kcal mol^{-1}: Killo Callori per Mole
l: Liter.
M.F.: Molecular Formula
M.W.: Molecular Weight
MCF-7: Breast cancer cell line
mg: milligram.
ml: Milli Liter.
mm: Milli meter.
MTT: "3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide" assay
No.: Number.
RMSD: Root-mean-square deviation
SD: Standard Deviation.
SW-480: Colon Cancer Cell Model
µg/ml: Microgram / Milliliter.
µl: Microlitre.
µm: Micro Meter.

6. Conflicts of interest

There are no conflicts of interest

7. References

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