JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/33868

RESEARCH ARTICLE

A Study on Biologically Active Components in *Morinda citrifolia* Leaf Extract and its Anti-cancer Effect by *In Vitro* Analysis and *In Silico* Molecular Docking Method

K. P. Greeshma[1*], R. Thamizselvi[2], S. Muthulingam[3], Sam John[4] and Dhanya B. Sen[5]

[1]Department of Chemistry, Sri Ramakrishna College of Arts and Science (SRCAS), Coimbatore - 641006, Tamil Nadu, India; kpmgreeshma@gmail.com
[2]Department of Chemistry, Government Arts College, Coimbatore - 641018, Tamil Nadu, India
[3]Department of Chemistry, Annapoorna Engineering College, Salem - 636308, Tamil Nadu, India
[4]Department of Chemistry, St. Berchamans College, Kottayam - 686101, Kerala, India
[5]Deparmtent of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara - 391760, Gujarat, India

Abstract

Nowadays, most antiviral drugs are plant-based due to their low toxicity and high resistance. *Morinda citrifolia* (Noni) is such a perennial shrub a popular plant-based medicine due to its wide therapeutic applications. The phytochemicals present in its fruit as well as its leaf have many antiviral properties and can enhance the human immune system. Herein, we report the identification of bioactive components present in the *Morinda citrifolia* (Noni) ethanolic leaf extract by Gas Chromatography Mass Spectrometry (GC-MS) analysis and their biological interaction with 6KN4 DNA of MCF-7 cell lines using Biovia Discovery Studio software. *In vitro* analysis was done by MTT assay of the same extract reported living cells of 59.74% at 100 µg/ml. The GC-MS analysis of prepared *Morinda citrifolia* ethanolic leaf extract showed the presence of 27 bioactive components, and out of these, five major components interacted with cancer DNA. The molecular docking interaction identified the most active bio component Phytol, with a binding energy of -27.0796 Kcal/mol. this value is much better than the commercially available doxorubicin. This novel study will provide insight into the development of bioactive components from *Morinda citrifolia* leaf extract against human breast cancer with minimal side effects.

Keywords: Anticancer Studies, Docking Interaction, GC-MS Analysis, *Morinda citrifolia*

1. Introduction

Plants are known to be the richest source of bioactive components like alkaloids, phenol, carotenoids, coumarins, terpenoids, and flavonoids[1]. Among the plant species available in our surroundings, nearly 20% exhibit activity against cancer and other life-threatening diseases. Plants are the sources of a diverse number of bioactive components and can protect against diabetics, skin infections, arthritis, and heart diseases. They are also effective as an anticancer agent[2]. Traditional medicines are based on various plants, which are mainly found in the Indian subcontinent. The major advantages of these medicines are low side effects and high activity. Plant extracts can provide high nutritional value as well as act as effective antioxidants[3].

Morinda citrifolia commonly known as Noni is a plant with versatile medicinal properties and is mostly cultivated in Southeast Asia and Australia[4]. Nearly 10–20 feet of mature noni trees have elongated and glossy leaves, which are similar to citrus fruit. The fruit, leaf, and other parts depend on plant variety and locality. This plant has many applications in various conditions like diabetes, skin infections, arthritis, and

*Author for correspondence

Article Received on: 25.05.2023 Revised on: 25.11.2023 Accepted on: 13.12.2023

heart diseases. Recently, many reports suggested its anticancer activities[5]. The ripe fruit and roots of this plant are effective in treating tuberculosis, respiratory infections, and dyes, respectively[6]. The extract from different parts of this noni plant has been reported as food and medicine in many places, like the Pacific Islands, Fiji, and India[7]. These extracts, through GCMS analysis and *in vitro* biological assays, show wide applications in antibacterial, antioxidant, and anticancer studies[8]. Sahabjada Siddiqui reported the interaction of phytocomponents present in *M. oleifera* against the COVID-19 protein by molecular docking[9].

Among the various phytochemicals present in noni leaves, polyphenols exhibit effectiveness in high-risk diseases like cancer and cardiac arrest. Recently, this plant was utilized in dietary supplements, and it was reported that its ethanolic extract was effective in suppressing immunological responses[10]. Assi *et al.,* reported important antibacterial and antifungal activities of the *M. citrifolia* plant[11]. Ratnoglik *et al.*, identified the activity of fruit and leaves for HIV and hepatitis C by *in silico* methods[12]. *M. citrifolia* has unique properties for preventing cancer and other immunological diseases[13]. This study aims to identify the major bioactive components from the *M. citrifolia* leaf extract and biological *in vitro* studies in antibacterial and anticancer effects. It also highlights the activity of phytocomponents with 6KN4 (Human parallel-stranded 7-mer-g-quadruplex) cancer protein DNA. These novel results will be an important tool in the development of natural medicines against breast cancer.

2. Experimental Methods

Fresh leaves of *M. citrifolia* leaves were collected from Coimbatore, Tamil Nadu, India, and chemicals used for extraction, phytochemical analysis, and biological studies were purchased from Sun Chemicals, Coimbatore. All chemicals were analytical grade and of high purity. MCF-7 cell lines of human breast cancer cells were initially collected from NCCS, Pune, India.

12g of fresh *M. citrifolia* (Noni) leaves, initially washed with double distilled water to remove dust and other contaminants were used to prepare the ethanolic plant solution. The leaves were then finely chopped and shadow dried and subjected to extraction in a soxhlet apparatus at 60°C for 3 hours and stored at 4°C for analysis. The stored ethanolic plant solution was used for phytochemical screening, GC-MS analysis, and antibacterial and anticancer studies[14].

2.1 Identification of Bioactive Components

Ethanolic leaf extract of *M. citrifolia* was subjected to Clarus SQ 8C Gas Chromatography - Mass Spectrometer (Perkin Elmer). Using the National Institute of Standards and Technology (NIST14) database, the mass spectrum of the GC-MS was interpreted. The known component's spectrum was compared to the library's internal database of known components.

2.2 Anti-bacterial Activity

The antibacterial activity of the prepared ethanol extract on *Escherichia coli* and *Bacillus subtilis* by disc diffusion method using standard procedure[15]. 10–50 µl of the plant extract was poured into the pre-prepared sterile Petri plates, and 20–25 µl of it was swabbed uniformly and incubated at 37°C (24 hours). Control plates were also maintained in the same manner and the inhibited zone was measured in mm and noted for comparative studies.

2.3 Anticancer Activity

The MTT (3-(4,5-dimethyl thiazole-2yl)-2,5-diphenyl tetrazolium bromide) assay was done to identify the cancer-inhibiting effect of the plant extract. The MCF-7 cell lines were maintained in Dulbecco's modified Eagle's medium and allowed to grow for about 24 hours. The cultured cell lines were suspended in a growth medium (10%) scattered in tissue culture plates and incubated at 37°C. The stock solution of the sample was prepared using 1mg of dry plan leaf powder in 0.1% DMSO using a cyclomixer. In order to ensure sterility, a 0.22µm millipore syringe filter was used to filter the sample solution again. The growth medium was removed after 24 hours, and MTT reagent was added to different sample concentrations and again incubated at the same previously mentioned temperature in a humidified 5% CO_2 incubator. The same procedure was repeated in the case of control cells, which were non-treated. The microscopic image was also recorded using an inverted phase contrast tissue culture microscope and finally,

the cell viability in percentage was calculated using the formula[16,17].

Cell viability(%) = (Mean OD of samples ÷ Mean OD of control) × 100

*OD-Optical density

2.4 *In Silico* Study using Molecular Docking and Ligand Preparation

The 3D structures of the selected phytocomponents were selected from the Pubchem compound database[18], and standard drugs used for docking interactions were saved in. mol format from the Drug Bank database[18,19]. The DNA utilized for this docking interaction was studied using the in Biovia Discovery Studio (C-Docker module). The structure of the selected DNA was downloaded from the PDB (protein data bank).

3. Results and Discussion

3.1 Identification of Bioactive Components

Table 1 shows the spectral analysis of the ethanolic *M. citrifolia* leaf and 27 compounds were identified from the leaf sample out of which nine compounds (highlighted in black) were selected for interaction

Table 1. GC MS analysis of ethanolic extract of *Morinda citrifolia* leaf extract

S. No.	Retention time	Compound name	Probability	Area %
1	3.083	Butane, 1-(1-ethoxyethoxy)-	94.4	0.618
2	3.143	1-Butanol, 3-methyl-, acetate	78.3	0.775
3	3.319	dl-Glyceraldehyde dimer	65.6	0.382
4	3.424	Pentanoic acid, ethyl ester	14.6	0.283
5	3.644	2-Propenoic acid, 3-ethoxy-, ethyl ester, (E)-	22.2	1.237
6	4.109	Butane, 1,1-diethoxy-3-methyl-	80.8	0.379
7	4.144	Pentane, 1,1-diethoxy-	17.4	0.341
8	4.759	Hexanoic acid, ethyl ester	78.3	0.444
9	5.915	Propane, 1,1,3-triethoxy-	93.8	0.679
10	6.32	3,3-Diethoxy-1-propanol, propyl ether	47.8	0.519
11	6.9	DL-Arabinose	25.2	0.253
12	6.985	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	90.1	0.407
13	8.381	Methyl 3-hydroxytetradecanoate	9.1	0.33
14	11.487	Sucrose	46.7	1.234
15	14.728	Diethyl Phthalate	76.8	22.777
16	15.223	Ethyl à-d-glucopyranoside	34.2	0.939
17	16.104	Triethyl citrate	96.4	30.523
18	21.056	Lidocaine	54.8	0.488
19	22.006	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	34.5	0.288
20	22.096	n-Hexadecanoic acid	20.6	0.665
21	22.196	Phthalic acid, butyl hept-3-yl ester	8.3	0.836
22	24.957	Phytol	74.3	12.506
23	25.383	17a-Methyl-3á-methoxy-17a-aza-D-homoandrost-5-ene-17-one	12	0.492
24	25.813	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a, 5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9atriacetate, [1aR-(1aà,1bá,4aá,5á,7aà,7bà,8à,9á,9aà)]-	14.4	0.448
25	26.433	Cholestan-26-oic acid, 3,7,12,24-tetrakis(acetyloxy)-, methyl ester, (3à,5á,7à,12à)-	41.2	0.329
26	28.929	á-Sitosterol	14.6	0.289
27	29.659	Triphenyl phosphate	39.6	0.437

with cancer DNA on the basis of their novelty in this particular study. Extraction using the Soxhlet apparatus has a higher efficiency than the other extraction processes, and the preferred solvents are usually methanol and ethanol because they can dissolve both polar and non-polar compounds[20,21].

3.2 Antibacterial Analysis

Antibacterial activity *M. citrifolia* leaf extract exhibits activity towards both bacteria and higher activity against exhibited in *E. coli* with a zone of inhibition of 11mm and the values are shown in Table 2. The experiment was repeated with the standard drug and showed a zone of inhibition of 12mm. This value is in good agreement with leaf extract[22,23]. Zone of inhibition and graphical representation are shown in Figures 1 and 2.

3.3 Anticancer Assay by MTT

The anticancer activity of ethanolic leaf extract was identified by MTT assay and exhibited significant activity towards breast cancer cell lines in a dose-dependent manner. The percentage viability was measured in different concentrations, including control, and found an effective value of 59.74 viability at 100 µg/ml. The experimental results and statistical analysis are shown in Table 3. The LC_{50} value calculated using ED50 plus V1.0 software was 125 µg/mL. A graphic representation depicting the anticancer effect of MC by MTT assay is shown in Figure 3. All experiments were done in triplicate, and results were represented as mean +/- SE. One-way ANOVA and Dunnett's test were performed to analyze the data. ***$p < 0.001$ compared to the control group. In addition to this, microscopic images were also recorded and shown in Figure 4. The detectable changes were recorded as images, and morphological changes like cell shrinkage, rounding, vacuolization, and granulation in the cytoplasm indicated cytotoxicity. Such a type of morphological effect was more observed at 100 µg/ml than at other concentrations. Phenolic compounds and flavonoids show a major role in cancer cell deactivation. These can inhibit the free radical chain reaction thereby preventing the conversion of hydroperoxide into oxygen free radicals. In addition to this flavonoids can able to form complexes with metal ions and prevent free radicals.

Table 2. Zone of inhibition of *E. coli* and *B. subtilis*

Concentration *Morinda citrifolia* ethanolic leaf extract (µl)	Zone of Inhibition (mm)	
	E. coli	*Bacillus subtilis*
2	3	2
5	5	4
10	6	5
15	7	9
20	11	10
Standard (Chloramphenicol)	12	12

3.4 *In Silico* Docking with 6KN4 Receptor

Among the 27 components identified by GC-MS analysis, 5 compounds were selected to dock with human parallel-stranded 7-mer g-quadruplexes. The components selected for interaction and their binding energies are shown in Table 4. The listed components interactions with cancer DNA have not yet been reported anywhere.

The C-docker binding energy of the phytol is -27.0796 Kcal/mol; it has a more binding affinity with

Table 3. The percentage of growth inhibition values at different concentrations

Sample Concentration (µg/ml)	OD I	OD II	OD III	Average Absorbance @ 540nm	Percentage Viability
CONTROL	0.7886	0.7982	0.7906	0.7925	100.00
SAMPLE CODE- MC					
6.25	0.7484	0.7434	0.7483	0.7467	94.42
12.5	0.6966	0.6941	0.6958	0.6955	87.80
25	0.634	0.6388	0.6334	0.6354	79.92
50	0.5929	0.5916	0.5953	0.5933	75.12

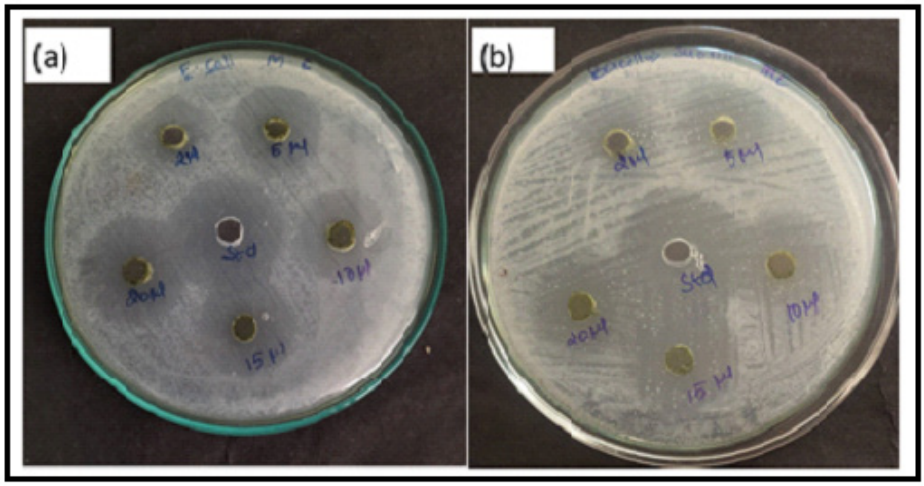

Figure 1. Zone of inhibition against **(a)**. *Escherichia coli,* **(b)**. *Bacillus subtilis.*

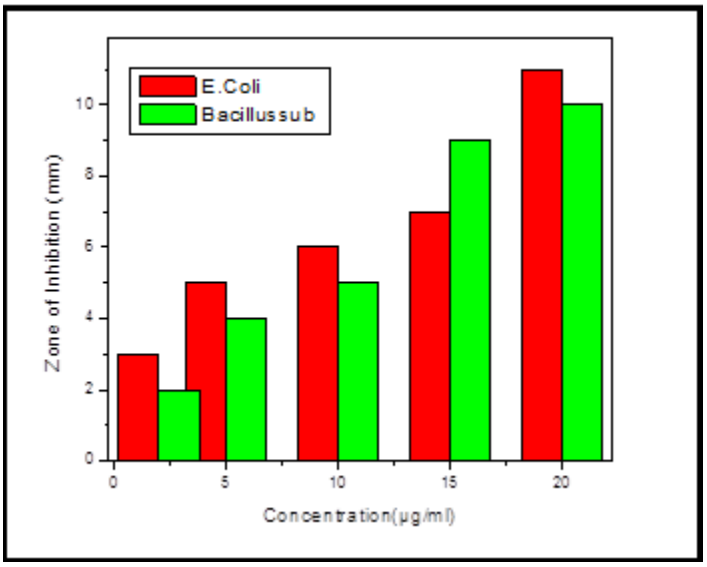

Figure 2. Antibacterial activity against *Escherichia coli* and *Bacillus subtilis.*

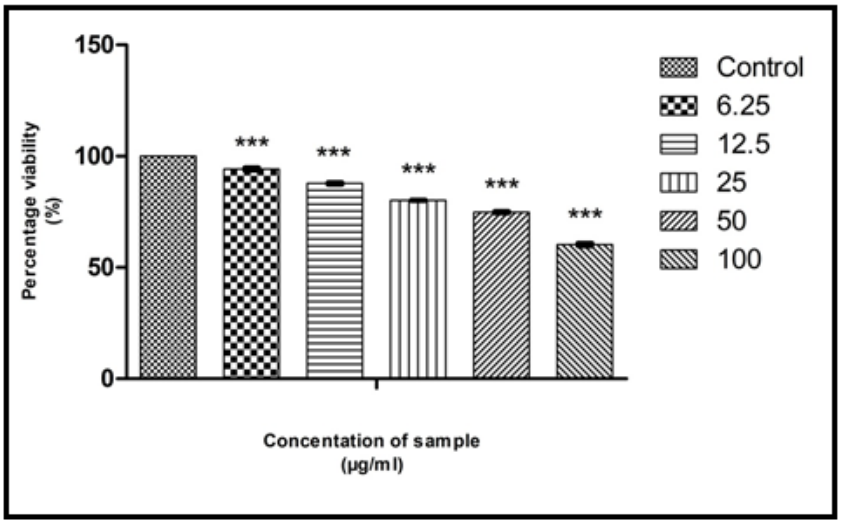

Figure 3. Graphical representation depicting the anticancer effect of *Morinda citrifolia* by MTT assay.

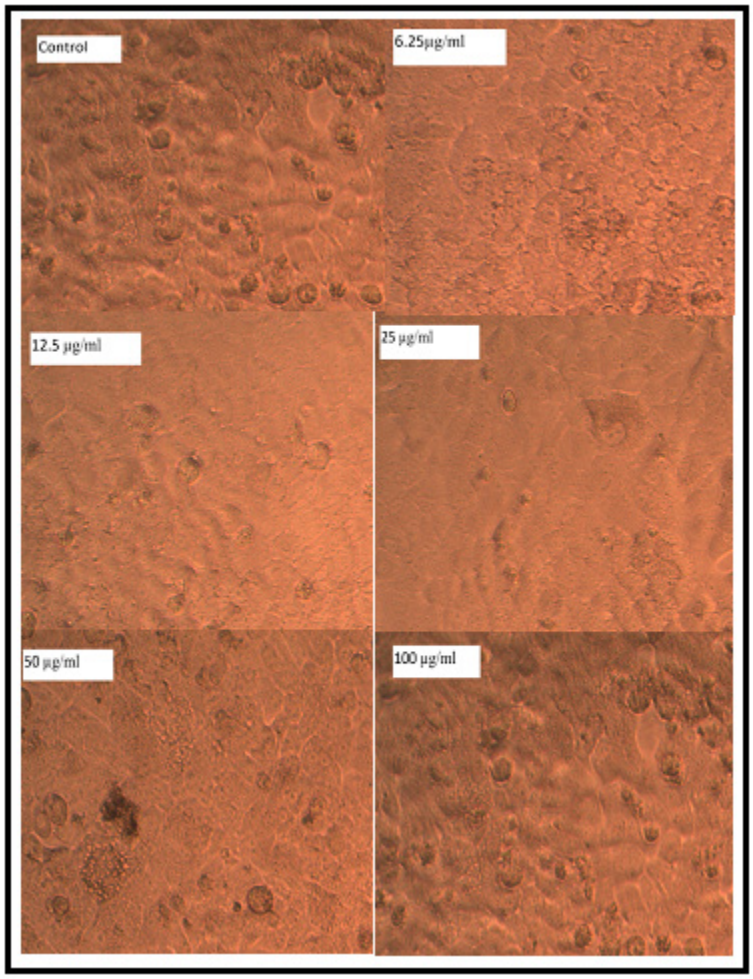

Figure 4. Images of cytotoxic observation by direct microscopic method.

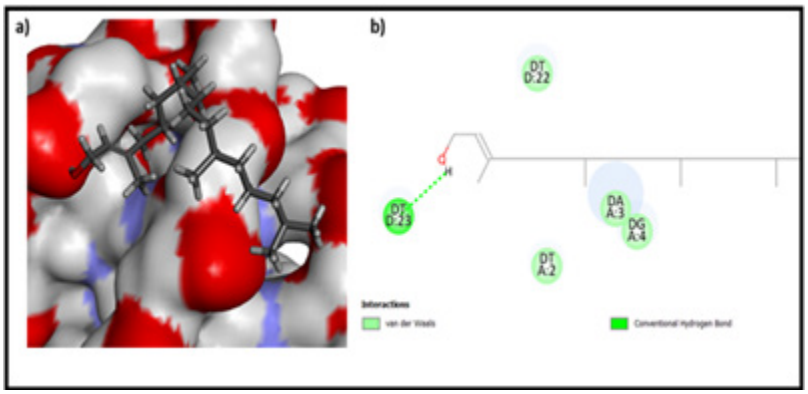

Figure 5. **(a).** 3D, **(b).** 2D binding interaction of the phytol with the Human parallel stranded 7-mer g-quadruplex.

an *in vitro* anticancer study on human breast cancer cells was also performed by MTT assay and showed an LC_{50} value of 125 g/mL and a cell viability value of 59.74% at 100 µg/ml. The selected phytocomponents also interacted with cancer DNA to confirm the anti-cancer effect of leaf extract theoretically. Based on the binding energies obtained from the *in silico* docking method, phytol exhibits a highly efficient result with the 6KN4 receptor with a binding energy of -27.0796 Kcal/mol. This value is better than the standard

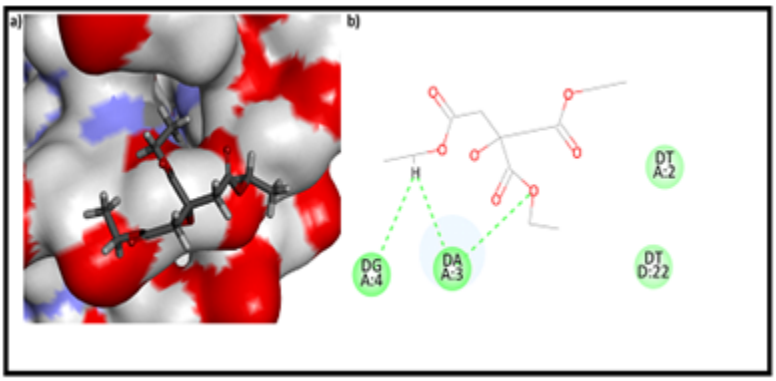

Figure 6. **(a).** 3D, **(b)**. 2D binding interaction of the Triethyl citrate with the Human parallel stranded 7-mer g-quadruplex.

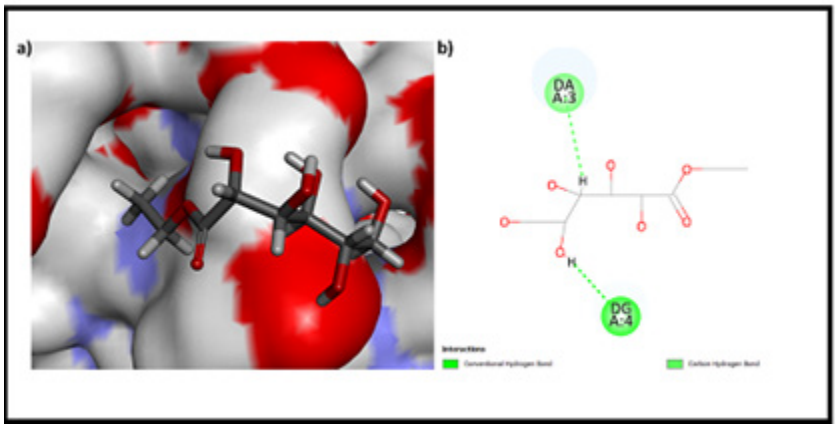

Figure 7. **(a).** 3D, **(b).** 2D binding interaction of the Ethyl hexonate with the Human parallel stranded 7-mer g-quadruplex.

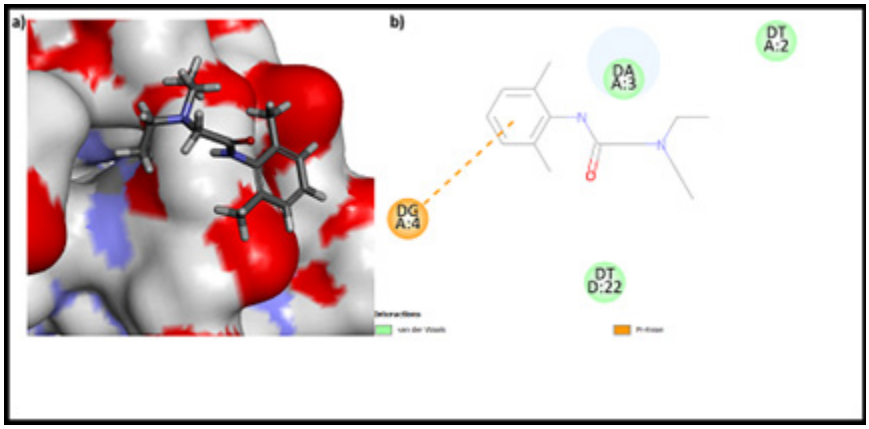

Figure 8. **(a).** 3D, **(b).** 2D binding interaction of the Lidocaine with the Human parallel stranded 7-mer g-quadruplex.

anti-cancer drug doxorubicin. These findings may provide insight into the experimental *in vivo* studies of isolated phyto components against MCF-7 cell lines. Bioinformatics and phytocomponents interaction play a major role in developing plant-based drugs for cancer therapy. These are safe eco-friendly and easily available. The molecular docking method is the best method to identify the efficient phytocomponents in plant extract and studies may lead to the development of plant-based drugs. These results may be an insight

Table 4. Molecular docking results of 6KN4 receptor

Name of the component	Binding Score (kcal/mol)
phytol	27.0796
Triethyl citrate	20.4431
Ethyl hexonate(hexanoic acid ethyl ester)	17.4985
Lidocaine	15.3188
1-(1-Ethoxyethoxy)butane	12.1538
Standard drugDoxorubicin	10.1435

into the development of eco-friendly drugs for cancer treatment.

5. Acknowledgements

We thank the Management, Principal, and Secretary of Sri Ramakrishna College of Arts and Science, Coimbatore for the successful completion of the work and also acknowledge lab facilities provided by the Department of Chemistry, Government Arts College, Coimbatore and Tamil Nadu Agricultural University, Coimbatore.

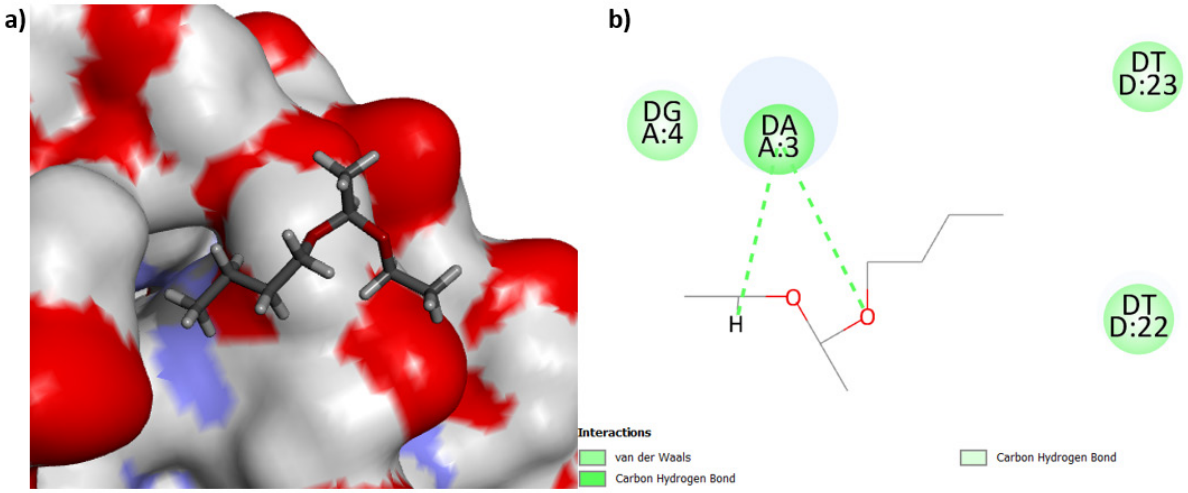

Figure 9. (a) 3D (b) 2D binding interaction of the 1-(1-Ethoxyethoxy)butane with the Human parallel stranded 7-mer g-quadruplex.

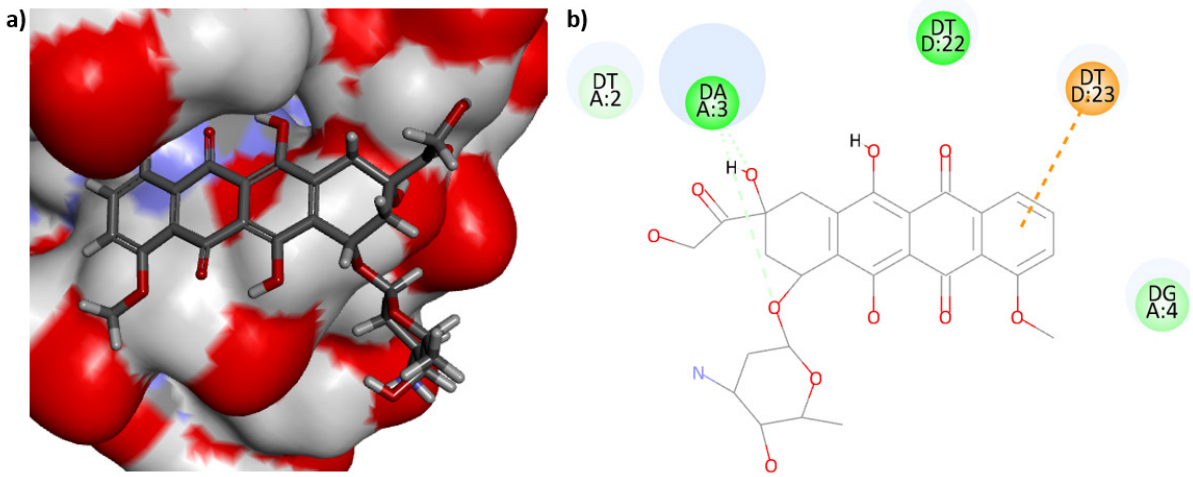

Figure 10. **(a).** 3D, **(b).** 2D binding interaction of the Doxorubicin with the human parallel stranded 7-mer g-quadruplex.

6. References

1. Chandraa S, Gahlot M, Choudharya AN, Palai S, de Almeida RS, de Vasconcelos JEL, dos Santos FAV, de Farias PAM, Coutinho HDM. Scientific evidence of the anticancer potential of medicinal plants. Food Chemistry Advances. 2023; 2:100239. https://doi.org/10.1016/j.focha.2023.100239
2. Patil MA, Sarkate AP, Nirmal NP, Sakhale BK. Alkaloids as potential anticancer agents. Recent Frontiers of Phytochemicals. 2023; 13:203-24. https://doi.org/10.1016/B978-0-443-19143-5.00034-7
3. Morton JF. The ocean-going noni, or Indian mulberry (*Morinda citrifolia*, Rubiaceae) and some of its "colourful" relatives. Economic Botany. 1992; 46(3):241-56. https://doi.org/10.1007/BF02866623
4. Almeida E, Oliveira D, Hotza D. Comprehensive Reviews in Food Science and Food Safety. 2019. p. 18. https://doi.org/10.1111/1541-4337.12456
5. Nagalingam M, Rajeshkumar S, Balu SK, Tharani M, Arunachalam K. Anticancer and antioxidant activity of *Morinda citrifolia* leaf mediated selenium nanoparticles. Journal of Nanomaterials. 2022; 2022:2155772. https://doi.org/10.1155/2022/2155772
6. Vuanghao L, Laghari M. *Morinda citrifolia* (noni): A comprehensive review on its industrial uses, pharmacological activities, and clinical trials. Arabian Journal of Chemistry. 2017; 10:691-707. https://doi.org/10.1016/j.arabjc.2015.06.018
7. Pawlus AD, Kinghorn AD. Review of the ethnobotany, chemistry, biological activity, and safety of the botanical dietary supplement *Morinda citrifolia* (noni). Journal of Pharmacy and Pharmacology. 2007; 59:1587–609. https://doi.org/10.1211/jpp.59.12.0001
8. Pino J, Márquez E, Quijano C, Castro D. Volatile compounds in noni (*Morinda citrifolia* L.) at two ripening stages. Ciencia E Tecnologia De Alimentos - CIENCIA TECNOL ALIMENT. 2010. p. 30. https://doi.org/10.1590/S0101-20612010000100028
9. Siddiqui S, Upadhyay S, Ahmad R, Barkat M, Jamal A, Alothaim A, Hassan M, Rahman M, Arshad M, Ahamad T, Khan M, Shankar H, Ali M, Kaleem S, Ahmad J. Interaction of bioactive compounds of *Moringa oleifera* leaves with SARS-CoV-2 proteins to combat COVID-19 pathogenesis: A phytochemical and *in silico* analysis. Applied Biochemistry and Biotechnology. 2022. p. 194. https://doi.org/10.1007/s12010-022-04040-1
10. Pongnaravane B, Goto M, Sasaki M, Anekpankul T, Pavasant P, Shotipruk A. Extraction of anthraquinones from roots of *Morinda citrifolia* by pressurized hot water: Antioxidant activity of extracts. The Journal of Supercritical Fluids. 2006; 37:390–6. https://doi.org/10.1016/j.supflu.2005.12.013
11. Abou Assi R, Darwis Y, Abdulbaqi IM, Vuanghao L, Laghari M. *Morinda citrifolia* (Noni): A comprehensive review on its industrial uses, pharmacological activities, and clinical trials. Arabian Journal of Chemistry. 2017; 10:691–707. https://doi.org/10.1016/j.arabjc.2015.06.018
12. Ratnoglik SL, Aoki C, Sudarmono P, Komoto M, Deng L, Shoji I, Fuchino H, Kawahara N, Hotta H. Antiviral activity of extracts from *Morinda citrifolia* leaves and chlorophyll catabolites, pheophorbide a and pyropheophorbide a, against hepatitis C virus. Microbiology and Immunology. 2014; 58:188–94. https://doi.org/10.1111/1348-0421.12133
13. Rastogi T, Ghorpade DS, Deokate U, Khadabadi S. Studies on antimicrobial activity of *Boswellia serrata*, *Moringa oleifera* and *Vitex negundo*: A comparison. Research Journal of Pharmacognosy and Phytochemistry. 2009; 1:75–7.
14. Greeshma KP, Thamizselvi R. Experimental and theoretical approach on green synthesized zinc oxide nanoparticles from combined leaf extracts of *Catharanthus roseus* and *Morinda citrifolia* for *in vitro* anti-cancer studies. Journal of Molecular Liquids. 2022; 351:118636. https://doi.org/10.1016/j.molliq.2022.118636
15. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1996; 45:493–6. https://doi.org/10.1093/ajcp/45.4_ts.493
16. Ciapetti G, Cenni E, Pratelli L, Pizzoferrato A. *In vitro* evaluation of cell/biomaterial interaction by MTT assay. Biomaterials. 1993; 14:359–64. https://doi.org/10.1016/0142-9612(93)90055-7
17. Merlin J-L, Azzi S, Lignon D, Ramacci C, Zeghari N, Guillemin F. MTT assays allow quick and reliable measurement of the response of human tumour cells to photodynamic therapy. European Journal of Cancer. 1992; 28:1452–8. https://doi.org/10.1016/0959-8049(92)90542-A
18. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH. PubChem substance and compound databases. Nucleic Acids Research. 2016; 44:1202–13. https://doi.org/10.1093/nar/gkv951
19. Wishart DS, Knox C, Guo AC. DrugBank: A comprehensive resource for *in silico* drug discovery and exploration. Nucleic Acids Res. 2006; 34. https://doi.org/10.1093/nar/gkj067
20. Thomsen R, Christensen MH. MolDock: A new technique for high-accuracy molecular docking. J Med Chem. 2006; 49:3315–21. https://doi.org/10.1021/jm051197e
21. Sriariyanun M, Muenmuang C, Narasingha M, Phusantisampan T. Chemical profiling of *Morinda citrifolia*

extract from solvent and soxhlet extraction method. 2017. p. 199-23. https://doi.org/10.1145/3121138.3121194

22. Shajahan E, Sekar C, Amutharaj P, Rahman Mohamed Esa SA, Khan KF. Evaluation of the antibacterial activity of *Morinda citrifolia*, *Vitex trifolia,* and *Chromolaena odorata*. African Journal of Pharmacy and Pharmacology. 2012; 6:783-8. https://doi.org/10.5897/AJPP11.435
23. Narayankar C, Charapale S, Samant I, Satpute, Gaikwad S, Gaikwad D. cytotoxic effects of ethanol extract of *Morinda citrifolia* fruit in human prostate cancer cell line. 2021; 53–61. https://doi.org/10.46243/jst.2021.v6.i3.pp53-61