JEB®

Journal of Environmental Biology

p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

Journal website : www.jeb.co.in ★ **E-mail :** editor@jeb.co.in

Triveni Enterprises (Educational Services Set-up)

Original Research

DOI : http://doi.org/10.22438/jeb/43/4/MRN-2006

Revealing *Berberis aristata* potential as an anti-viral agent to combat *Paramyxoviridae* infection

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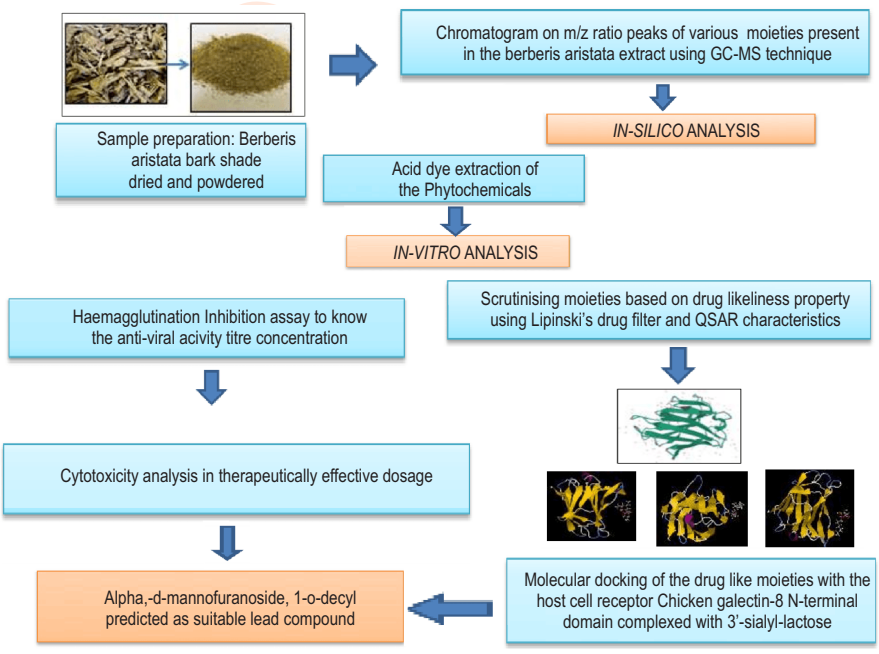
Received: 06.04.2021 *Revised*: 10.08.2021 *Accepted*: 03.01.2022

Abstract

Aim: To study the anti-viral activity of extracts of *Berberis aristata* and to scrutinize the novel lead compound present in it probably to combat *Paramoxyviridae* infection.

Methodology: The phytochemicals present in the barks of *Berberis aristata* were extracted and screened by GC-MS analysis. Haemagglutination inhibition and cytotoxicity assay was performed to determine the anti-viral property, and the novel lead compound was selected using *in-silico* methods.



Results: Haemagglutination assay provided anti-viral activity of the extract at 1/16 dilution in 4 HA viral concentration. At this concentration, the viability on Vero cell lines was precisely 92.8%. The GC-MS analysis enabled in identifying six molecules present in the extract. Among the six compounds present in the extract, five moieties exhibited drug likeliness property when passed through the Lipinski's drug filter. QSAR predictions using T.E.S.T projected 3 compounds to be developmental non-toxicant with the predicted values of 0.12, 0.32 and 0.42 respectively. On performing docking studies with the predicted nontoxic moieties using iGEMDOCK, with the Sialic acid complexes host receptor, the highest binding energy was -213 kcal mol^{-1} for alpha-d-mannofuranoside, 1-o-decyl-, respectively.

Interpretation: These findings enabled in understanding the anti-viral potency of bark extracts of *B. aristata* at 62.5mg ml^{-1} promoting high cellular viability of uninfected cells of host cell with low toxic effects. Probing molecularly, the *in-silico* analysis helped to predict alpha-d-mannofuranoside, 1-o-decyl as the possible lead molecule supporting its therapeutic efficacy as an anti-viral drug compound in future.

Key words: Acid dye method, Developmental non-toxicant, Haemagglutination assay, Lipinski's drug filter

How to cite : Shreenivasan, Y.S., S. Keerthana, A. Praveena and P. Dhasarathan: Revealing *Berberis aristata* potential as an anti-viral agent to combat *Paramyxoviridae* infection. *J. Environ. Biol.*, **43**, 520-526 (2022).

Introduction

Globally, there has been a steep surge in the incidence of infectious disease of zoonotic origin in particular (Dikid *et al.,* 2013; Bhatia *et al.,* 2012). Emerging Infectious Diseases (EIDs) upgraded with new clinical features extend their menace to open avenues for numerous pandemics in the future (Taylor *et al.,* 2001). Amidst them, *Paramoxyviridae* family of viruses are renowned as highly infectious, leaving little room for survival of susceptible life forms because of their high mutation rates (James MacLachlan, 2011). Scientific communities are focussed to pose detrimental effects of the EIDs through development of vaccines for economic expansion and national harmony (Fauci, 2001). Although vaccines are effective in immunising susceptible organisms, in present day scenario the cons seem to outweigh the pros. This is due to the questionable biosafety security being difficult to manage especially during an endemic (Dortmans *et al.,* 2011). Such adverse situations aid the birth of a drug discoverer. The phases of clinical trials enable in developing a drug molecule possessing high safety and efficacy when administered to the victim (Browne *et al.,* 2014). To scrutinize a lead molecule having potential of becoming a drug, a series of *in-vitro* analysis proposed as Phase 0 clinical trial has been established in the process of drug discovery and development (Kinders *et al.,* 2007).

The four phases have revolutionized since the advent of molecular biology and genomic sciences in particular (Drews, 2000). Pioneering computational biology for development of drugs is observed to streamline the process by rationalizing the time and overall expenditure of marketing the final product (Nayarisseri, 2020). Further, an increased inclination has been observed towards the development of lead molecules from natural sources such as plants, microbes or animals due to their low toxic effects (Harvey, 2008). In fact, utilization of herbal medicine and its associated medicinal plants has been an important component of health care system of many developing countries, particularly in the Indian subcontinent *Berberis aristata* is used in traditional herbal medicine where their stem, root, and fruits are extensively used in Ayurveda (Kala *et al.,* 2006). Noted as Indian barberry, "chutro" or tree turmeric, *Berberis aristata* grows predominantly in the temperate and sub-tropical regions of Asia, Europe and America (Parmar and Kaushal, 1982). The rich Ethno pharmacology history of *Berberis aristata* in ancient medication as an anti-diabetic, anti-cancerous and anti-inflammatory agent has raised curiosity to explore its anti-viral potential in this study.

For this research, *Newcastle disease virus* (APMV1) under *Avian Paramyxoviruses* belong to the family *Paramyxoviridae* under genus Avula virus was selected as it is the most characterized members using haemagglutination assay and neuraminidase assay among the APMV serotypes due to high mortality and morbidity associated with it. (Gogoi *et al.,* 2015). The entry of *Newcastle disease virus* (NDV), a prototype *Paramyxovirus*, is directed by its two glycoprotein subunits, the hemagglutinin-neuraminidase (HN) protein and the fusion (F) protein (Lamb *et al.,* 2001). Entry into the host cells is facilitated by two viral glycoproteins: the attachment protein facilitates primary receptor binding of the virus with the target cell, while F protein promotes subsequent membrane fusion events (Smith *et al.,* 2009). The viral protein domain is observed to be similar in the prone hosts due to the conserved viral replication mechanism. Similarity in the binding motifs encourage exploitation of sought after technique of Structure based drug design (SBDD) to predict suitable lead molecule by using three dimensional structure of the host receptor using the tools of computational biology for developing a lead molecule effective against a family of viruses (sialic acid receptor of present in the chickens) (Jerome *et al.,* 2016). With this compassion towards the prone hosts, the study aimed to identify a novel lead compound from the bark extracts of *Berberis aristata* to combat *Paramoxyviridae* infection using *in-vitro* and *in-silico* studies.

Materials and Methods

Collection and sample preparation of *Berberis aristata* bark: The bark of *Berberis aristata* was collected from Tambaram, Chennai, Tamilnadu, India. The collected sample was shade dried and powdered coarsely.

Solvent extraction and phytochemical screening of *Berberis aristate*: In 80% ethanol, precisely 10 g of powdered barks of *Berberis aristata* was dissolved to execute acid dye extraction of for 1.5 hrs (3 cycles per hour), at 40-60 °C. The syrup was dissolved in 25 ml of hot water and filtered. To the filtrate, 5 ml of hot water was added and was filtered again. Following this, the extract was precipitated using HCl and was cooled in ice bath for 30 min (Mimansha, 2013). The presence of alkaloids in the extract was confirmed by Mayer's test, Wagner's test and Hager's test (Harborne, 1998).

Determination of anti-viral activity: Haemagglutination assay was performed following the standard procedure (Allan *et al.,* 1974). Firstly, 10 ml of chicken blood was collected and added to sterilize graduating tube containing equal amount of anticoagulant solution. The blood was then mixed gently by rotating between the palms and was centrifuged at 1000 rpm for 2 min and the supernatant was discarded. To the pellet, anticoagulant was added twice its volume and centrifuged under the aforementioned conditions. Upon repeating this step twice, supernatant was discarded without losing the red blood cells and 1% RBC was obtained. Secondly, the standard Haemagglutination (HA) Units were obtained following the incubation period of 37°C for 30 min. Haemagglutination Inhibition (HAI) test was performed in a sterile microtiter plate wherein the extract was serially diluted to test the antiviral concentration against the standard viral HA units with an incubation period of 45 min at 37°C. The agglutinations were observed keenly to predict the antiviral activity of *Berberis aristata* extract.

Cytotoxicity assay: *Vero* cell line was obtained from NCCS, Pune. The cells were maintained in DMEM under standard

conditions. Cells (1 × 105 per well) were plated in 24-well plates and incubated at 37°C in 5% CO_2 condition until they reached confluence after which the *Berberis aristata* extract was added and incubated for 24 hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4). A volume of 100µl per well (5mg ml^{-1}) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hrs. After incubation, 1ml of DMSO was added in all the wells and the absorbance was read at 570nm using DMSO as blank (Mosmann, 1983), and percent cell viability was calculated.

Ligand preparation: Gas Chromatography Mass Spectroscopy was performed (Bryan *et al.,* 1974) in order to determine the presence of moiety in the extract. Following that, six molecules were identified based on highest peaks obtained from chromatogram. The ligand possessing drug likeliness property and existing as developmental non-toxicant were fed as input in PDB format using Open babel online server for molecular docking process.

Estimation of drug likeliness property: The Lipinski's rule of 5 (Lipinski *et al.,* 2001) was applied to verify if the moieties possessed the attributes of a prospective lead compound. For this purpose, the canonical smiles notation of the moieties present in the extract were retrieved from PUBCHEM database and were passed through Lipinski's drug filter using SWISS-ADME online tool (Daina *et al.,* 2017).

Quantitative Structure Activity Relationship Prediction (QSAR): Toxicity Estimation Software Tool (TEST version 4.2.1) was used to obtain the bio-developmental toxicity range of the compounds scrutinized. In this tool, their SMILES notation was given as input based on which its structure was retrieved from the internal database of TEST which was used to provide the toxicity range of the molecule.

Protein preparation and molecular docking: The 3D structure of Sialic acid receptor which facilitates the viral entry was retrieved from PDB database (PDB id 4WVW). In the present study, standard docking software iGEMGOCK (Yang and Chan, 2004) was used. After setting the protein ligand output path and parameters, the docking process was commenced against the prospective ligand moieties. The list of energy of the poses and the amino acids interacted along with different bonding energies involved were displayed for analysis.

Results and Discussion

The acid dye method of extraction enabled in procuring the extracts of *Berberis aristata*. The extracted solution was yellow colour which evidently supported the presence of alkaloids (Peter *et al.,* 1986). Alkaloids of microbial origin have also been observed to be present in yellow colour irrespective of the extraction procedure adopted (Omura *et al.,* 1977). Further, there exists a strong relation between the extraction method adopted and the chemicals and solvents used to yield the desired phytochemicals like alkaloids possessing medicinal advantage (Saxena *et al.,* 2020). Supportively positive results such as cream coloured precipitate, brown coloured precipitate and yellow coloured precipitate was obtained from the Mayer's, Wagner's and Hager's test respectively to confirm the presence of alkaloids as analysed previously to conclude the qualitative presence of a class of phytochemicals (Raghavendra *et al.,* 2010).

The gas chromatography mass spectroscopy process performed enabled in identifying the compounds present in the extract of *B. aristate*, while dealing with moieties of different polarities that needs to be identified, it is suggested to separate them to hexanoic and methanolic fractions (Lam-Gutiérrez *et al.,* 2019). Based on the retention time and the highest peaks obtained in the chromatogram, six compounds were identified based on retention time. The compounds included

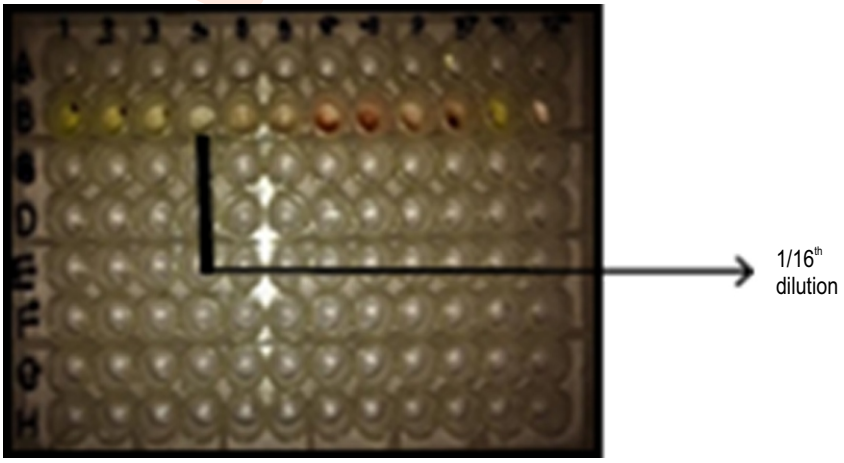


Fig 1: Antiviral titre of *Berberis aristata bark* extract.

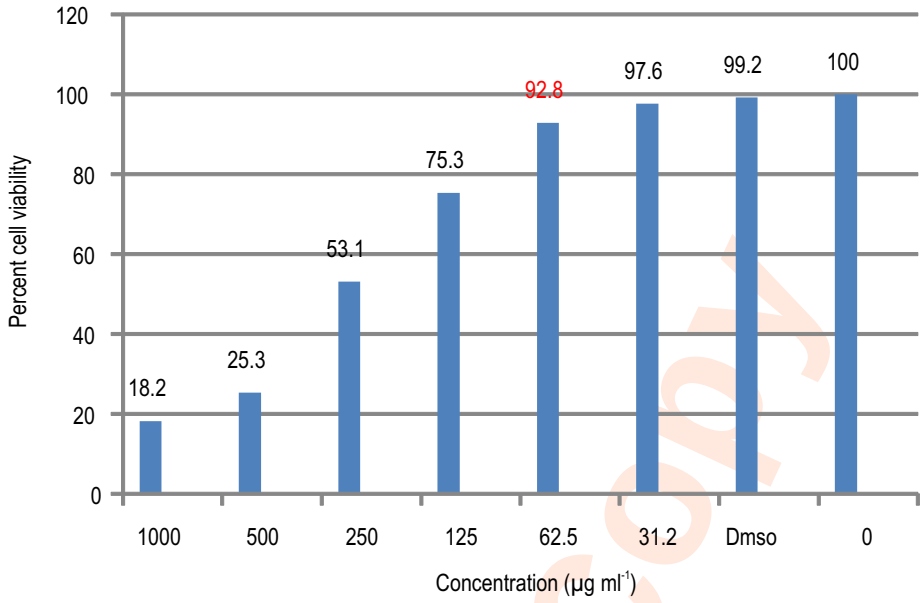


Fig 2: Graphical representation of cell viability.

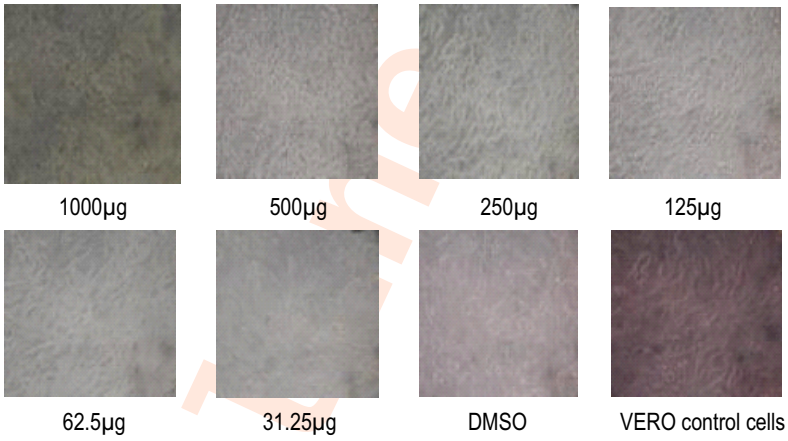


Fig 3: Cytotoxic effect of *B. aristata* extract in various dilutions

spectinomycin, Methyl-3-o-benzyl.α.d-xylopyranoside, α –D-mannofuranoside,1-O-decyl, 2,7-anhydro-L-gallacto-heptulofuranose, α –D-mannofuranoside , 1-nonyl-, Heptanoic Acid, Heptyl Ester and their retention time were 56.09, 73.02, 98.1, 116.022, 146.122, 267.128 min, respectively.

Medicinal herbs and shrubs have been utilized for ages to treat chronic infectious ailments such as those caused by highly mutating organisms belonging to genus Mycoplasma (Yassin *et al.,* 2021). To determine the antiviral activity of *B. aristata* extracts, standardization of HA units produced was 4 HA viral concentration. To test the antiviral activity of *Berberis aristata*, the HAI test was carried out and formation of HA up to 1/16th dilution of the extracts of *Berberis aristata* extracts was obtained, which implies that *in- vitro Berberis aristata* extract up to the dilution of 62.5µg ml^{-1} could combat the *Paramoxyviridae* infection by interacting with the host cell receptors present in the erythrocytes of *Gallus gallusdomesticus* provided in the microtiter plate (Fig. 1). Similarly, Haemagglutination assay was used to quantify the viral activity using oil inactivated NDV vaccines to develop a Vero cell-adapted thermostable NDV I-2 vaccine by Sidiqque *et al.,* (2017). The cytotoxicity assay performed on *Vero* cell line showed (which is detected to possess antiviral activity for 4HA viral concentration), the cell viability percentage was 92.8% at 62.5 µg ml^{-1} (Fig. 2) of *B. aristata* extract. The surfaces envelop glycoprotein of Human immunodeficiency virus in tissue and

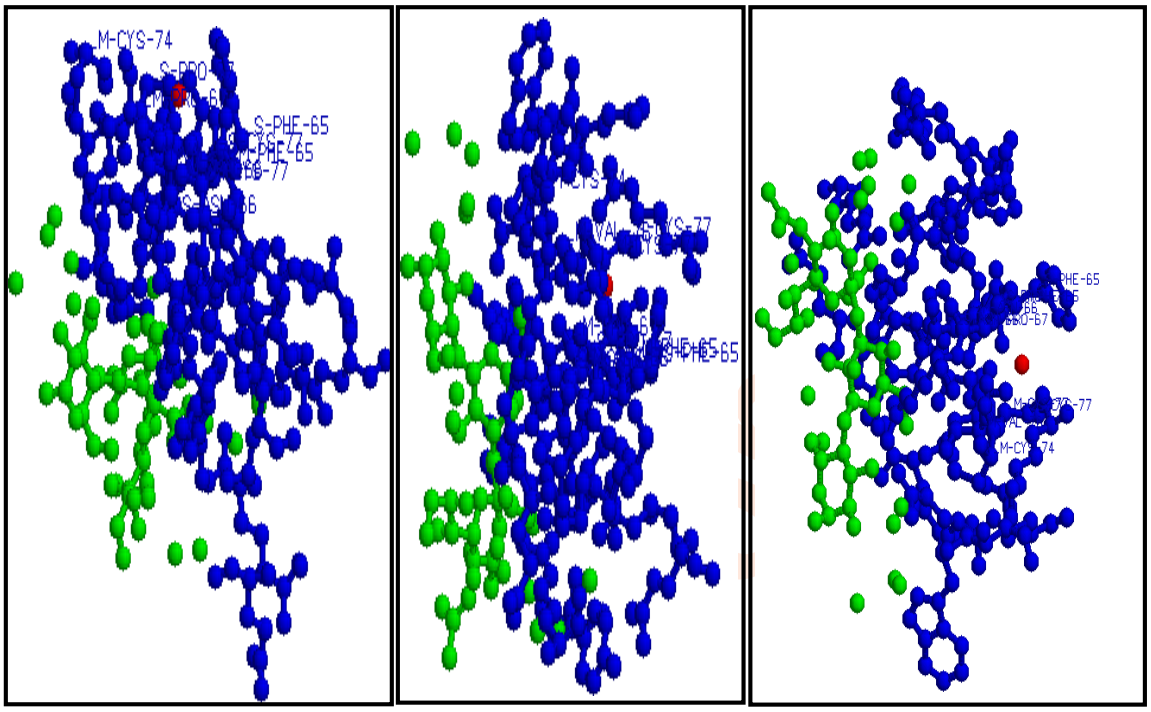


Fig. 4: Docked pose of Alpha -mannofuranoside 1_o-decyl, Spectinomycin and Alpha d mannofuranoside, 1-nonyl with Chicken Galectin 8 N terminal domain respectively.

Table 1: Molecular interaction between the target and identified ligand using iGEMDOCK

Compounds	Energy (k cal mol^{-1})	Binding site amino acids
Alpha.-d-mannofuranoside, 1-o- decyl-	-213	Ser37, Ile38, Pro95, Phe96, Gln95, Arg100, Pro101, Phe102, Gln97
Spectinomycin	-197.74	Phe65, Asn66, Pro67, Cys74,Val76, Cys77, Pro67,Val76
Alpha-d- mannofuranoside, 1- nonyl-	-180.6	Phe65, Asn66, Pro67, Cys74, Val76, Cys77

peripheral blood sample was studied formerly by the cytotoxicity assays (Zhang *et al.,* 2002). The higher cell viability percentage tends to lower the threat this extract could impose in terms of toxic effects (Fig 3). With a conserved entry mechanism, the low toxic effects of *B. aristata* extracts on *Vero* cell lines could be hypothesized suitable for other hosts of *Paramoviridae* family of viruses (Baker *et al.,* 1999). Cell proliferation assay enables in optimising the toxic dosages for clinical purposes (Junius *et al.,* 1966).

In addition to studying the cytotoxic effects, MTT assay enables to probe the anti-cancerous activity of various chemotherapeutic agents (Sreenivasa *et al.,* 2021). At molecular levels, the six compounds identified from the GC-MS study upon retrieval from PUBCHEM database were tested to possess drug likeliness property from which five compounds such as Spectinomycin, Methyl-3-O- Benzyl. Alpha. D-Xylopyranoside, Alpha.-d- mannofuranoside, 1-o-decyl-, 2,7-anhydro-L-Galacto-Heptulofuranose, Alpha-d-annofuranoside,1-nonyl-) were observed to possess drug like property (Xiaoxia *et al.,* 2020). The developmental toxicity was predicted by QSAR study as 0.12, 0.32, 0.86, 0.42, 0.51 for Spectinomycin, Alpha.-d-mannofuranoside, 1-o-decyl- Methyl-3-o-benzyl, alpha.D-xylopyranoside, Alpha-d-mannofuranoside, 1-nonyl- 2, 7-Anhydro-l-galacto-heptulofuranose, respectively. The non-toxicants were predicted according to the Caesar's model for toxicity development employed in TEST version 4.2.1. Similar studies enabled in understanding the importance of risk assessment wherein the moieties predicted as developmental toxicants were avoided to mitigate any metabolic hindrance the compound could possibly cause if included in drug designing in future (Larbi EIMchichi *et al.,* 2020). Knowledge on suitable lead moieties with least toxic effects helped to scrutinise compounds for further molecular docking studies.

Alpha-D-Mannofuranoside, 1-nonyl-, Spectinomycin and Alpha.-d-mannofuranoside, 1-o-decyl-showed a predicted value of 0.42, 0.12 and 0.32. These compounds were considered as

developmental non-toxicant since they fall under the acceptable range of 0.4878 according to Caesar's model for toxicity development employed in TEST version 4.2.1. On the other hand, the methyl-3-o-benzyl.alpha.d-xylopyranoside and 2,7-Anhydro-l-galacto-heptulofuranose were predicted to possess developmental toxicity as their predicted value was precisely 0.86 and 0.51. The predicted non-toxicants were scrutinized further for docking process to prove the binding efficacy of ligand with the receptor (Table 1). Molecular docking process is used to study the binding efficacy of biological compounds or its mimics from various sources for their therapeutic potential (Sivagamai *et al.,* 2021).

In present study, upon docking the ligands Spectinomycin, Alpha.-d-mannofuranoside, 1-o-decyl-, Alpha-D-Mannofuranoside, 1-nonyl- with the host cell receptor with PDB id 4WVW (Ruiz *et al.,* 2015), the docking energies were obtained as -197.74 kcal mol^{-1}, -213 kcal mol^{-1} and -180.6 kcal mol^{-1}, respectively. This study enables in predicting that Alpha.-d-mannofuranoside, 1-o-decyl- with a binding energy of -213kcal mol-1could possibly bind with the host cell receptor and thereby prevent the *New castle disease virus* from binding with the host cell receptor resulting in the inhibition of viral entry and proliferation into the host cell via molecular complex formation with lowest energy value (Fig 4). Studies using organic and inorganic moieties docked against biological receptors of diverse host using iGEMDOCK tool enabled the interpretation of binding efficacy of scrutinised moieties (Angamuthu Diviyadharshini and Rajarajan, 2019; Indu *et al.,* 2020). The present study strongly supports the moieties present in the extract containing lead compound possessed a therapeutic advantage. Hence, Alpha-d-mannofuranoside, 1-o-decyl compound is considered a prospective lead compound. This bioactive compound from the plant extract possibly supports to develop effective and affordable antiviral drugs in future.

Acknowledgments

We thank the Department of Biotechnology of Prathyusha Engineering College, Chennai for the support and facility to conduct the study.

Add-on Information

Authors' contribution: A. Praveena: Designed the study, concept, title of the paper, necessary corrections and improvement of paper; **Y.S. Shreenivasan:** Execution of *In-vitro* and *In-silico* research work; **S. Keerthana:** Performed laboratory experiments to observe antiviral titre, **P. Dhasarathan:** Facilitation and guidance for laboratory experiments.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that there is no conflict of interest.

Data from other sources: Not applicable.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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