IN-VITRO AND *IN-SILICO* ANALYSIS TO IDENTIFY NOVEL LEAD COMPOUND FROM *Berberis aristata* AGAINST *Paramoxyviridae* INFECTION

A PROJECT REPORT

Submitted by

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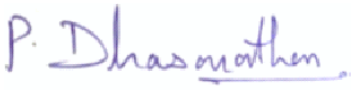
BONAFIDE CERTIFICATE

Certified that this project report on **"*IN-VITRO* AND *IN-SILICO* ANALYSIS TO IDENTIFY NOVEL LEAD COMPOUND FROM *Berberis aristata* AGAINST *Paramoxyviridae* INFECTION"** is the bonafide work of **"YUKTHA. S. SHREENIVASAN (111416214034) and KEERTHANA. S (111416214014)"** who carried out the project under my supervision.



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DECLARATION

We hereby declare that the project entitled **"*IN-VITRO* AND *IN-SILICO* ANALYSIS TO IDENTIFY NOVEL LEAD COMPOUND FROM *Berberis aristata* AGAINST *Paramoxyviridae* INFECTION"** submitted to the Department of Biotechnology, Prathyusha Engineering College,Thiruvallur-602025,Anna University, Chennai, in partial fulfillment of the award of the degree Bachelor of Technology in Biotechnology in the record of the original work carried by us under the guidance of **Dr. A. Praveena**, **SUPERVISOR** Associate Professor, Department of Biotechnology, Prathyusha Engineering College, Thiruvallur, during the period of December 2019 to March 2020. We further declare that the results of the work have not been previously submitted for the award of any degree or diploma.

Place: Chennai

Date:

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ABSTRACT

Berberis aristata is associated with the family *Berberidaceae* which is well renowned for its role in various medical applications including treatment for diabetes and cancer. In order to explore its antiviral activity, from the bark of *Berberis aristata*, the phytochemicals in it were extracted using acid dye method and it was validated based on the preliminary tests for alkaloids based on colour change. To determine the anti-viral activity, haemagglutination assay was performed which provided an antiviral activity of the extract at 1/16 dilution in 4 HA viral concentration. At this concentration of the extract, the viability on *Vero* cell lines was found to be 92.8% precisely. The GC-MS analysis enabled in identifying 6 molecules present in the extract. Amidst the 6 compounds present in the extract, 5 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. Upon performing docking studies with the predicted nontoxic moieties using iGEMDOCK, with the Sialic acid complexes host receptor, the highest binding energy was calculated to be -213 kcal/mol for Alpha.-d-mannofuranoside, 1-o-decyl- respectively, and providing potential new avenue for a novel lead compound.

KEYWORDS: Acid dye method, haemagglutination assay, Lipinski's drug filter, developmental non-toxicant.

I. LIST OF TABLES

II. LIST OF FIGURES

III LIST OF SYMBOLS

µl - Microliter

Ml - Millilitre

℃ - Celsius

Min - Minutes

g - Gram

V - Volume

Nm - Nanometre

Sec - Seconds

Hrs - Hours

IV LIST OF ABBREVIATION

DMEM	-	Dulbecco's Modified Eagle Media
DMSO	-	Dimethyl Sulfoxide
FBS	-	Foetal Bovine Serum
GC-MS	-	Gas Chromatography Mass
HA	-	Haemagglutination
HCL	-	Hydrochloric Acid
MTT	-	(3-[4,5- Dimethylthiazol-2-yl]-2,5 Diphenyltetrazolium bromide) assay
nt	-	Nucleotide
QSAR	-	Quantitative Structure Activity Relationship
RBC	-	Red Blood Cell
RdRP	-	RNA dependant RNA polymerase Relationship
SMILES	-	Simplified Molecular Input Line Entry System Spectrometry

V. LIST OF NOMENCLATURE

MDEN33	-	Molecular distance edge between all tertiary nitrogen
MW	-	Molecular weight
ic	-	Information content
MATS2p	-	Moran autocorrelation of lag 2 weighted by atomic polarizabilities
icycem	-	Mean information on the vertex cycle matrix equality
MATS5m	-	Moran autocorrelation of lag 5 weighted by mass
MATS6m	-	Moran autocorrelation of lag 6 weighted by atomic masses
MATS5p	-	Moran autocorrelation of lag 5 weighted by atomic polarizabilities
GATS3v	-	Geary autocorrelation of lag 3 weighted by atomic volume
SssOacnt	-	Single sign-on
BELm5	-	lowest eigenvalue n. 5 of Burden matrix / weighted by atomic masses
BEHp6	-	highest eigenvalue n. 6 of Burden matrix / weighted by atomic polarizabilities
MATS3v	-	Moran autocorrelation of lag 3 weighted by van der Waals volume
NH	-	Imidogen
CH3	-	Methyl group
CH	-	Methylidyne

NH2	-	Azanide
C14H24N2O7	-	Spectinomycin
C13H18O5	-	Methyl-3-o-Benzyl. Aloha.D-Xylopyranoside
C16H3206	-	Alpha-D-Mannofuranoside,1-o-Decyl
C7H12O6	-	2,7-Anhydro-1-galacto-heptulofuranose
C15H30O6	-	Alpha-D-Mannofuranoside,1-nonyl-

AIM AND OBJECTIVE

AIM:

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

OBJECTIVES:

- To extract and identify the phytochemical from the *Berberis aristata* through GC MS analysis.
- To perform the antiviral assay in order to determine the efficacy of the prospective lead compound.
- To carry out *in-vitro* cytotoxicity studies in order to determine the toxic range of the extract.
- To predict the drug likeliness of the compounds identified and molecular interaction with the target molecules using *in-silico* studies to acquire novel lead compound.

CHAPTER 1

INTRODUCTION

Berberidaceae Consisting of 14 genera and 701 species of perennial herbs and shrubs, *Berberidaceae*, the barberry family of the buttercup order (*Ranunculales*), occurs in most temperate regions across the globe. The most important and largest genus is *Berberis*, with about 500 species, including *B.aristata, B.vulgaris, B.darwinii, B.jaeschkeana* respectively (Watson and Dallwitz, 1992).

1.1 *Berberis aristata*

Noted as Indian barberry, "chutro" or tree turmeric, *Berberis aristata* (Fig 1-a) grows predominantly in the temperate and sub-tropical regions of Asia, Europe, and America respectively. Existing between 2 to 3 m (6.6 to 9.8 ft) in height, *B.aristata* is a woody plant, with bark that appears yellow to brown from the outside and deep yellow from the inside (Parmar and Kaushal, 1982) (Fig 1-b). In India, *B.aristata* is used in traditional herbal medicine where its stem, roots, and fruits are used extensively in Ayurveda (Kala *et.al.*, 2006).

(a) (b)

Fig 1: a) *B.aristata* plant b) Bark of *B.aristata*

1.2 *Paramoxyviridae* FAMILY OF VIRUSES

The family *Paramyxoviridae* consists of large enveloped RNA viruses infecting mammals and birds and in certain cases reptiles and fishes too. Many *Paramyxoviruses* are host-specific and many others such as *Measles* virus, *Mumps* virus, *Nipah* virus, *Hendra* virus and numerous *Parainfluenza* viruses are pathogenic for humans (Bert *et.al.*, 2019*)*.

1.2.1 GENOME STRUCTURE

Paramyxovirus genomes are non-segmented, negative-sense single-stranded RNA molecules. Total RNA sequences for known members of this family are approximately 15200-15900 nucleotides in length. The RNA consists of six to eleven tandemly linked genes, which namely encode for three nucleocapsid-associated proteins (N or NP; P and L), three membrane-associated proteins, a matrix protein (M) and two envelope proteins (F and G, or H, or HN). It is these envelope proteins found to be predominantly involved in fusion and entry in the host cells (David *et.al.*, 2007).

1.2.2REPLICATION

After attachment to cell receptors, virion entry is achieved by fusion of the virion envelope with the cell surface membrane. It is observed this reaction occurs at the neutral pH. Virus replication occurs in the cell cytoplasm and is thought to be independent of host nuclear functions. The genome is transcribed processively from the 3′-end by the virion-associated RdRP into 6–8 separate positive-sense mRNAs. Transcription is guided by short (10–13 nt) conserved gene start (GS) and gene end (GE) signals flanking the intergenic sequence. The mRNAs are capped by the guanylyl- and methyltransferase activities of the L

protein and possess 3′-poly(A) tracts synthesized by reiterative copying of U tracts in GE sequence. Intergenic regions are highly conserved in length (3 nt) and sequence in the *Orthoparamyxoviruses* and *Metaparamyxoviruses*. Neither, the length or sequence of the intergenic sequences is conserved in *Avulavirus* or *Rubulavirus* genomes. RNA replication occurs through an intermediate, the antigenome, which is an exact positive-sense copy of the genome (Bert *et al*., 2019*).*

1.3 SUB FAMILY OF *Paramoxyviridae* FAMILY

The family *Paramyxoviridae* consists of three genera: *Paramyxovirus*, which includes the parainfluenza viruses and mumps virus; *Pneumovirus*, which includes respiratory syncytial virus; and *Morbillivirus*, which includes the measles virus. Amidst those viruses the *Avulavrinae* family of viruses exists, around which this research work revolves around.

1.3.1 *Avian avulavirus*

Avian paramyxoviruses (APMVs) have been reported from a wide variety of avian species around the world. Avian *Paramyxoviruses* are economically significant because of the huge mortality and morbidity associated with it. Twelve different serotypes of APMV have been reported till date. Avian *Paramyxoviruses* belong to the family *Paramyxoviridae* under genus *Avulavirus*. *Newcastle disease virus* (APMV-1) is the most characterized members among the APMV serotypes. The most important and best characterized *Avulavirus* is *Newcastle disease virus*, a variant of *Avian paramyxovirus 1* (species *Avian avulavirus*. *Avulavirus* can be separated into distinct serotypes using haemagglutination assay and neuraminidase assay (Gogoi *et.al.*, 2015).

1.4 RECEPTOR OF ENTRY

Avian avulavirus infections are mediated by specific interactions between the viral hemagglutinin (HA) and cell oligosaccharides containing sialic acid (SA) residues. The majority of *Avian avulavirus* bind to receptors with sialic acids having an α2,3 linkage to the penultimate galactose (SAα2,3-gal), while human viruses prefer receptors that are present with an α2,6 linkage (SAα2,6-gal). *Avian avulavirus* replicate poorly in humans partially due to restrictions in receptor specificity (Beare and Webster, 1991).

1.5 VIRAL SURFACE PROTEINS

The entry of *Newcastle disease virus* (NDV), a prototype *Paramyxovirus,* is directed by two virion glycoproteins, the hemagglutinin-neuraminidase (HN) protein and the fusion (F) protein. HN protein, the virus attachment protein, binds to sialic acid-containing receptors, and F protein mediates membrane fusion. In contrast to many viral fusion proteins, *Paramyxovirus* F proteins do not require the acid pH of endosomes to activate fusion activity. As a consequence, infected cells expressing both attachment proteins and F proteins can fuse with adjacent cells to form multinuclear cells, or syncytia, a process that is assumed to mimic virus-cell fusion (Lamb *et.al.*, 2001)

1.5.1 FUSION PROTEIN

The NDV fusion protein is synthesized as a 553-amino-acid precursor, F_0 (Gorman *et.al.*, 1988*)*.The mature F protein is a homo-trimer (Russell *et.al.*, 1994). Fusion activity of the protein requires a proteolytic cleavage of F_0 at amino acid 117 to produce disulfide-linked F_2 and F_1 polypeptides derived from the amino-terminal and carboxyl-terminal domains, respectively (Lamb *et.al.*, 2001). The F_1 polypeptide has several domains important for fusion activity. The

protein has one and perhaps two fusion peptides (Peisajovich *et.al.,* 2000), one located at the amino terminus of the F_1 polypeptide and the other at a more internal location (Peisajovich *et.al.,* 2000). Upon activation of fusion, fusion peptides are thought to insert into target membranes docking the protein to these membranes (Eckert *et.al.,* 2001). *Paramyxovirus* F_1 proteins have two heptad repeat (HR) regions, one (HR1) located just carboxyl terminal to the more amino-terminal fusion peptide and the other adjacent to the transmembrane domain (HR2) (Lamb *et.al.*, 2001).

1.5.2 HEMAGGLUTININ-NEURAMINIDASE PROTEIN

The attachment protein is required for fusion in most *Paramyxovirus* systems, since most F proteins expressed alone do not mediate membrane fusion (Lamb, 1993). It is also clear that HN protein provides more than an attachment function, since mutations in the HN protein stalk domain can retain attachment activity but are defective in fusion promotion (Sergel *et.al.,* 1993). The HN protein and the F protein must be from the same virus, with a few exceptions, indicating that virus-specific interactions between HN and F proteins are required for fusion directed by the F protein (Hu *et.al.*, 1992). It has been proposed that the attachment of HN protein to its receptor serves to activate the F protein (Lamb, 1993).

1.6 *IN-SILICO* APPROACH FOR QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP PREDICTION

QSAR grew out of physical organic chemistry based on studies to show how differential reaction rates of chemical reactions depend on the differences in molecular structure. Characterization of these differences in structure, which are due to functional group substitutions into a fixed core structure, led to the development of substituent constants. The substituent constants may encode the

electronic, hydrophobic, and steric aspects of a series of compounds by which QSAR models can be generated. Substituent constants are basically physicochemical descriptors that are designed on the basis of factors, which govern the physicochemical properties of chemical entities. Due to changes in physicochemical properties, absorption, distribution, and transport of chemical entities may be changed (Kunal *et.al.,* 2015). When the three-dimensional structure of the target, even from experiments or computing, exists, a frequently used technique to design inhibitor molecules is structure-based drug design (SBDD). The most popular method in SBDD is molecular docking. Initially, docking – a term which was coined in the late 1970s – meant the refinement of a model of a complex structure by optimization of the separation between the partners, but with fixed relative orientations (Jerome *et.al.,* 2016).

CHAPTER 2

REVIEW OF LITERATURE

Herbal medicine is an important component of the health care system of many developing countries. The utilization of herbal medicine and its associated medicinal plants has been documented by many authors. The history of herbal medicine and innovative utilization of plants as a source of medicine in India has been passed down through generations as oral tradition.

2.1 BERBERINE

Berberine was first isolated from *Xanthoxylon cava* in 1826 by Chevallier and Pelletan (Perrins, 1862). This structure, which contains quaternary nitrogen, forms salts with mineral acids such as HCI and H_2SO_4 through elimination of one molecule of water; these salts are correctly designated as berberinium compounds. Berberinium nitrate is poorly soluble, and the addition of nitric acid to aqueous solutions of the alkaloid precipitates the nitrate. The research thirst began when understanding about the compound existing as an equilibrium mixture of three tautomeric forms (Gadamer, 1905) began, wherein the quaternary base possessing an extraordinary high pK_α of 15.23 in water was grasped. This compound happened to be berberine which caught the attention of the budding scientist as us. It is a yellow coloured alkaloid known to occur in numerous plants (Jeffs, 1967). The compound was of immense significance after gaining knowledge regarding the antimicrobial properties of the compound. This was known from the experiment performed (Odebiyi and Sofowora, 1979) wherein the powdered root extracts of *Fagarazanth oxyloides* known to have been containing chelerythrine chloride and berberine chloride were found to possess antimicrobial activity.

2.2 *Paramoxyviridae* INFECTION

According to their epidemiology all *Paramyxoviridae* are extremely infectious, and there is little chance for anybody living in a cosmopolitan community to get through a full life without being infected by wild or attenuated forms of all the *Paramyxoviruses* adapted to humans. Their common structure is labile and all viruses of this family are dependent on transmission by close association of hosts. They do not survive drying on a solid surface, nor are they sufficiently stable in water to be transmitted by this vehicle with regularity (Black, 1991).

Hendra and *Nipah* viruses are the only identified zoonotic member of the *Paramoxyviridae* family, and both are highly pathogenic in humans. *Nipah* virus emerged in Malaysia in 1999 causing an outbreak of viral encephalitis that led to 105 human fatalities out of 265 reported cases (Eaton *et.al.*, 2006).The β –propeller fold of HNV-G consists of 4 antiparallel β- strands per blade and is similar to the sialic acid pockets of attachment of the new castle disease virus respectively. This insight probed the research to shift using the NDV pathogen instead of the bsl-4 pathogen respectively. (Hashiguchi *et.al.*, 2007). To enter host cells, *Paramyxoviruses* must go through the key steps of viral attachment to the target cell, followed by the fusion of the viral membrane to a host cell membrane. Two major viral glycoproteins promote these events: the attachment protein facilitates primary receptor binding of the virus to the target cell, while the F protein promotes subsequent membrane fusion events (Smith *et.al.*, 2009). It is being observed according to fusion activity for the *Hendra* and the *Nipah* is inversely proportional to the strength of the F attachment protein interaction, in contrast to the results from other *Paramoxyvirus* system (Aguilar *et.al.*, 2009).

2.3 EXTRACTION OF BERBERINE

Berberis aristata is useful as anti-pyretic, anti-bacterial, anti-microbial, anti-hepatotoxic, anti-hyperglycaemic, anti-cancer, anti-oxidant and

anti-lipidemic agent *B.aristata* extracts and its formulations are also useful in the treatment of diarrhoea, haemorrhoids, gynaecological disorders, HIV-AIDS, osteoporosis, diabetes, eye and ear infections, wound healing, jaundice, skin diseases and malarial fever (Dipti *et.al.,* 2012). It is because of this purpose, the plant was chosen for extraction. By hot extraction protocol described, (Shingwan *et.al.,* 2013), the yield obtained was only 800 g. Further the time consumed was 3 hours and the solvent used was methanol. The extraction of the B*erberis aristata* phytochemicals involved the protocol of acid dye extraction (Mimansha, 2013) which involved use of less toxic ethanol and time was only 1.5 hours and the yield was greater.

Despite the presence of various plant sources which were known to contain medicinally important alkaloids, such as *Coscinium fenestratum* (Akowuah *et.al.,* 2013), *B.aristata* was selected due the part of the plant being chosen to the barks of the plant, and also the solvent used is ethanol due to which the hazards it could impact to the experiment while handling and testing also seemed to have reduced respectively unlike the difficulties that had to be met while making use of other plant species.

2.4 EPHRIN B2 RECEPTOR

When millions of receptors were known to exist in a human body, it might seem strange for one to see the limelight being awarded to this specific receptor. The motivation behind the scrutiny is the biological function this particular receptor is responsible in the human body. It is because, more recent studies have shown that EphB4, a member of the tyrosine kinase receptor Eph Family (Yongmei *et.al.,* 2014). Ephrin-B2, a cell-surface protein linked to the cell membrane by a single transmembrane segment, contributes to cancer cell survival, invasion, and migration (Chen *et.al.,* 2016).

The reason behind starting the research from berberine and also for selecting Ephrin B2 receptor is the binding affinity berberine is known to possess with the receptor of interest respectively. In addition to the binding affinity berberine possess with the Ephrin B2 receptor thereby inhibiting the proliferation and migration of breast cancer ZR-75-30 cells, it was suspected to be capable of inhibiting the entry and proliferation of a deadly virus belonging to the *Paramoxyviridae* family (Ma *et.al.*, 2017).

2.5 LABORATORY BASED TECHNIQUES FOR DECIPHERING THE ANTIVIRAL ACTIVITY

Understanding about the characterisation of viruses belonging to *Paramoxyviridae* family felt necessary before proceeding in finding antivirals. Hence upon studying the paper, prevalence and characterizing the CaPV, determining the CaPV-PPRV co-infection prevalence and regarding the phylogenetic relationship between the fusion protein of the two organisms enable in opening new possibilities in this work (Bwihangane *et.al.*, 2017) It helped in understanding how the cure for any one of the virus under this family could be opening an avenue of possibility to other viruses belonging to the same family as well.

Hemagglutination inhibition (HAI) assay and ELISA do not corroborate the presence of neutralizing antibodies (nAbs); they are used to measure protection and immune response against NDV (Ana *et.al.*, 2017). The novel work of *Oleaeuropaea* leaves extract down regulating Newcastle disease virus gene expression in cancer cells, was observed how after certain levels of passaging, the virus could adapt to *Vero* and also *Hela* cell lines (Raajaa *et.al.*, 2017).

2.6 *IN-SILICO* STUDIES

Bio-informaticians in drug discovery use high-throughput molecular data in comparisons between symptom-carriers (patients, animal disease models, cancer cell lines, *etc.*) and normal controls. The key objectives of such comparisons are to 1) connect disease symptoms to genetic mutations, epigenetic modifications, and other environmental factors modulating gene expression, 2) identify drug targets that can either restore cellular function or eliminate malfunctioning cells, 3) predict or refine drug candidates that can act upon the drug target to achieve the designed therapeutic result and minimize side effects, and 4) assess the impact on environmental health and the potential of drug resistance (Xuhua, 2017). For characterisation of the extracted moieties, GC-MS procedure was adopted to help in QSAR *in-silico* studies as in the work of (Jayashree *et.al.,* 2017). (Keruli *et.al.,* 2019) has orchestrated in his work regarding the inhibition of the aldose reductase receptor based on QSAR studies using V LIFE MDS 4.0 software. This helped in understanding the role of descriptors and the possible developmental toxicity it could produce be predicted by understanding the structure of the molecule.

In the work of (Rahman *et.al.,* 2019) 3C-like protease also called the main protease which is an essential enzyme for the completion of the life cycle of Middle East Respiratory Syndrome *Coronavirus* have been studied. In their research they have predicted compounds which are capable of inhibiting 3C-like protease, and thus inhibit the lifecycle of Middle East Respiratory Syndrome Coronavirus using iGEMDOCK. Hence in the present study molecular docking is preferred to do *in-silico* using the iGEMDOCK software.

CHAPTER 3

MATERIALS AND METHODS

The following methodologies have been performed to achieve the objectives of the present study.

3.1 COLLECTION AND SAMPLE PREPARATION OF *Berberis aristata* BARK

The bark of *Berberis aristata* was collected from Tambaram, Chennai, Tamilnadu, India. The collected sample was confirmed by Botanist Prof.P.T.Kalaichelvan of Department of Botany, Madras University. The collected sample was shade dried for 2 weeks and coarsely powdered (Fig 2a and b).

(a) **(b)**

Fig 2: a) Shade drying of *Berberis aristata* bark b) Coarsely powdered sample

3.2 STERILISATION OF GLASSWARES

Glass wares were cleaned with detergent solution and rinsed several times with tap water and finally in distilled water. To get rid of the sticking water

molecules, it was air dried. The glass wares were sterilized in an autoclave at 15 Psi for 20 minutes at 120˚C.

3.3 SOLVENT EXTRACTION USING SOXHLET EXTRACTOR

Precisely 10 grams of the powdered bark sample was added to the soxhlet apparatus with 80% ethanol as the solvent of extraction (Fig 3). Extraction was continued for 1.5 hours with 3 cycles per hour at 40-60˚C. After that the mantle was switched off and the water flow was stopped.

The syrup was dissolved in 25 ml of hot water and it was filtered in Whattman filter paper and 5 ml of hot water was added to the residual mass and filtered again. Following this, the extract was precipitated using 15 ml of 36.5% HCL respectively (Fig 3). This was cooled in ice bath for 30 minutes. The precipitate was filtered and was stored in the refrigerator (Mimansha, 2013)

Fig 3: Sohxlet extraction setup of the phytochemical and filtration using HCL.

3.4 PHYTOCHEMICAL SCREENING OF *Berberis aristata* EXTRACT

3.4.1 TEST FOR ALKALOIDS

The presence of alkaloids in the extract was confirmed using the following standard procedures (Rahman *et.al*, 2017).

3.4.1.1 MAYER'S TEST

A sterile test tube was taken to which 1 ml of the *B.aristata* bark extract was added. This was kept as a control. Another sterile test tube was taken to which 1 ml of the extract was added and a few drops of potassium mercuric iodide solution were added and the resultant colour change was observed.

3.4.1.2 WAGNER'S TEST

In a sterile test tube 1 ml of the *B.aristata* extract was added. This was kept as a control. Another sterile test tube was taken to which 1 ml of the extract was added and a few drops of iodine in potassium iodide solution were added and the resultant colour change was observed.

3.4.1.3 HAGER'S TEST

A sterile test tube was taken to which 1 ml of the *B.aristata* extract was added which was maintained as control and a few drops of picric acid was added. The resultant colour change was observed.

3.5 HAEMAGGLUTINATION ASSAY

Haemagglutination assay was performed based on the standard procedure explained by (Zacoure *et.al.,* 2016). Haemagglutination is a specific property executed by many different viruses with red blood cells of various species. In case, the virus and RBCs in suspension reacted, they would adhere to each other thus being bridged by the viral particles.

3.5.1 PREPARATION OF RED BLOOD CELLS

Chicken blood of volume 10 ml was collected and was 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 minutes. The supernatant was discarded. Following this twice the volume of the pellet the anticoagulant was added and the centrifugation was carried out in the above mentioned conditions. This step was repeated twice after which the RBCs was observed to have settled down completely. The supernatant was discarded without losing the RBCs. To the RBCs 10 times the volume, the anticoagulant was added and this 0.1% RBC were stored in a refrigerator.

3.5.2 STANDADISATION OF HAEMAGGLUTINATION UNITS

Normal saline of 50 µl was added to all the 12 wells of a sterile microtiter plate. To it, 50 µl of 1/5 dilution of virus was serially diluted up to 11^{th} well respectively. Washed 0.1% RBC was added to all the wells. The microtiter plate was covered with a lid and was incubated at 37°C for 30 minutes (Table 1). Following the incubation period the results were duly noted and used for the successive processes.

3.5.3 HAEMAGGLUTINATION INHIBITION TEST

Precisely 25 µl of normal saline was added to all the 12 wells of the sterile microtiter plate. To it 25 µl *Berberis aristata* extract was serial diluted up to 9^{th} well and 25 µl of the presently studied antiviral solution was added to the 11^{th} well. The 11^{th} well was used as the antiviral control. 25 µl of 4 HA virus was added up to 10^{th} well where 10^{th} well was seen as the virus control. 50 µl of 0.1% washed RBCs was added to all the 12 wells and the 12^{th} was considered as RBC control respectively. Following which, this was incubated at 37°C for 45

minutes precisely (Table 2). The agglutinations were observed keenly to predict the antiviral activity of the *Berberis aristata* extract respectively.

Table 1: Standardisation of HA units

NUMBER OF WELL	Normal saline (µl)	Serial diluted virus in 1/5 dilution (µl)	0.1% RBC washed (µl)		Derived virus dilution
1	50	50	50	Incubation at 37°C for 30 minutes	1/10
2	50	50	50		1/20
3	50	50	50		1/40
4	50	50	50		1/80
5	50	50	50		1/160
6	50	50	50		1/320
7	50	50	50		1/640
8	50	50	50		1/1280
9	50	50	50		1/2560
10	50	50	50		1/5280
11	50	50	50		1/10560
12	50	⊗	50		RBC control

⊗ -discarded serially diluted virus by 11th well

Table 2: HAI test protocol

NUMBER OF WELL	Normal saline (µl)	Serial diluted antiviral preparation (µl) in constant dilution	Specific HA virus (µl)	0.1% RBC washed (µl)		Antiviral dilution
1	25	25	25	50		1/2
2	25	25	25	50		1/4
3	25	25	25	50		1/8
4	25	25	25	50		1/16
5	25	25	25	50		1/32
6	25	25	25	50	**Incubation at 37°C for 45 minutes**	1/64
7	25	25	25	50		1/128
8	25	25	25	50		1/256
9	25	25	25	50		1/352
10	25	✖	25	50		Virus control
11	25	25	✖	50		Antiviral control
12	25	✖	✖	50		RBC control

✖ - Sample not to be added to the respective wells

3.6 GAS CHROMATOGRAPHY MASS SPECTROSCOPY ANALYSIS

3.61 GAS CHROMATOGRAPHY:

A Shimdzu GC-2010 Plus gas chromatograph (Briyan *et.al*, 1974) was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35°C, hold for 2minutes, then ramp at 20°C per minute to 450°C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode) respectively.

3.62 MASS SPECTRUM:

A Direct connection with capillary column metal quadruple mass filter period mass spectrometer operating in electron ionization (EI) mode with software GC-MS solution ver. 2.6 was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 1000 at 0.3 seconds per scan with a 0.2 second inter-scan delay. 26 on the High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 1000 at 1 second per scan.

3.6.3 MASS SPECTOMETRY LIBRARY SEARCH

Identification of the components of the compound was matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software. GC/MS metabolomics Database was used for the similarity search with retention index.

3.7 CYTOTOXICITY ASSAY

3.7.1 CELL LINE AND CULTURE

Cell line was obtained from NCCS, Pune. The cells were maintained in DMEM with 10%FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a humidified atmosphere of 50 μg/ml CO_2 at 37°C.

3.7.2 *IN-VITRO* ASSAY FOR TOXICITY DETERMINATION

Cells (1×10^5/well) were plated in 24-well plates and incubated in 37°C with 5% CO2 condition. After the cell reaches the confluence, the sample was added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. A volume of 100μl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation 1ml of DMSO was added in all the wells .The absorbance at 570nm was measured with UV-Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically (Mosmann *et.al.*, 1983) .The %cell viability was calculated using the following formula:

% cell viability = A570 of treated cells / A570 of control cells ×100

3.8 *IN-SILICO* STUDIES

3.8.1 LIPINSKI'S DRUG FILTER

The Lipinski's rule of 5 (Andrés *et.al.,* 2015) has been applied to verify if the moieties were to possess the attributes of a prospective lead compound respectively. The required properties such as Molecular mass (Less than 500 Daltons), Hydrogen bond donors (Less than 5), acceptors (Less than 10) and molar refractivity (Between 40 and 130) to validate the drug likeliness property of the compound. The PUBCHEM database was utilised to retrieve the canonical smiles notation of the moieties that were found to be present in the extract. Using the canonical smiles notation, SWISS-ADME online tool (Dainaet *et.al.,* 2017) was utilised to determine if the moieties obeyed the Lipinski's rule of 5 respectively.

3.8.2 QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP PREDICTION

In order to determine the quantitative structure activity relationship of those compounds possessing drug-likeliness property, the Toxicity Estimation Software Tool (TEST version 4.2.1) was used (Martin, 2016). The toxicity for a given query compound was estimated using the weighted average of the predictions from several different models. The different models were obtained by using Ward's method to divide the training set into a series of structurally similar clusters. A genetic algorithm-based technique was used to generate models for each cluster. The models were generated prior to runtime using hierarchical method respectively. To obtain the bio-developmental toxicity range of the compounds scrutinized, 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.

3.8.3 DOCKING STUDIES

iGEMDOCK (Yang and Chen, 2004) was the automated docking tool utilised for the present study. The two types of tags in iGEMDOCK include docking/screening tags and post analysing tag. The binding of chemical molecules were predicted by docking/screening tags. The proteins and ligands that were prior found can also be taken for post analysis under the post-analysis tag. This tool iGEMDOCK allows one to perform docking in either standard docking or quick docking. For the present work, standard docking was used. The ligand and the receptor were fed as input in PDB format respectively using Open babel online server (Boyle *et.al.,* 2011). After setting the protein ligand output path and parameters, the docking process was commenced. The status was displayed on the screen. The result of the docking process was examined by selecting the "view docked poses and the post analysis" icon. The list of energy of the poses and the amino acids interacted along with the different bonding energies involved were displayed for analysis respectively.

CHAPTER 4

RESULTS

From the various observations recorded during experimentation, the relevant results are being compiled and represented below.

4.1 SOLVENT EXTRACTION USING SOXHLET EXTRACTOR

The acid dye method of extraction enabled in procuring the extracts of *Berberis aristata*. The extracted solution was observed to be yellow coloured which appear to be containing alkaloids.

4.2 PRELIMINARY CONFIRMATORY TESTS OF ALKALOID

From the three preliminary tests for alkaloids, positive results indicating the presence of alkaloids based on the foreseeable colour changes obtained (Table 3 and Fig 4 a, b, c).

4.3 GAS CHROMATOGRAPHY MASS SPECTROSCOPY ANALYSIS

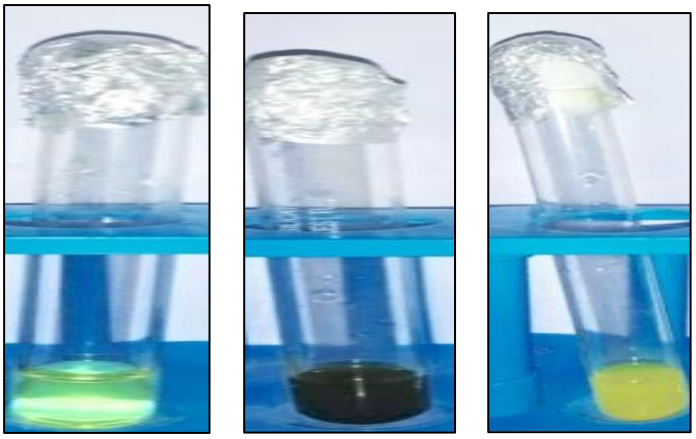
The gas chromatography mass spectroscopy process performed enabled in identifying the compounds present in the extract of *B.aristata*. Based on the retention volume and the highest peaks obtained in the chromatogram (Fig 5). The graph was plotted with the X axis as m/z values and the corresponding Y axis as percentage of retention volume respectively. Amongst them, 6 compounds were selected based on the highest peaks obtained for further studies. The compounds includes 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 includes 56.09 minutes, 73.02 minutes, 98.1 minutes, 116.022 minutes, 146.122 minutes, 267.128 minutes respectively have been represented (Table 4)

Table 3.0: Preliminary screening of Alkaloid in the extracts

Test performed	Observation	Positive (+)/Negative (-)
Mayer's Test	Cream coloured precipitate	+
Wagner's Test	Brown coloured precipitate	+
Hager's Test	Yellow coloured precipitate	+

Table 4: Compounds identified by GC-MS based on RT

HIT	RT (minutes)	COMPOUND NAME	FORMULA
1	56.09	spectinomycin	$C_{14}H_{24}O_7N_2$
2	73.02	Methyl-3-o-benzyl.α.d-xylopyranoside	$C_{13}H_{18}O_5$
3	98.1	α –D-mannofuranoside,1-o-decyl	$C_{16}H_{32}O_6$
4	116.022	2,7-anhydro-L-gallacto-heptulofuranose	$C_7H_{12}O_6$
5	146.122	α –D-mannofuranoside , 1-nonyl-	$C_{15}H_{30}O_6$
6	267.128	HeptanoicAcid,Heptyl Ester	$C_{14}H_{28}O_2$



(a) (b) (c)

Fig 4: a) Mayer's Test b) Wagner's Test c) Hager's Test

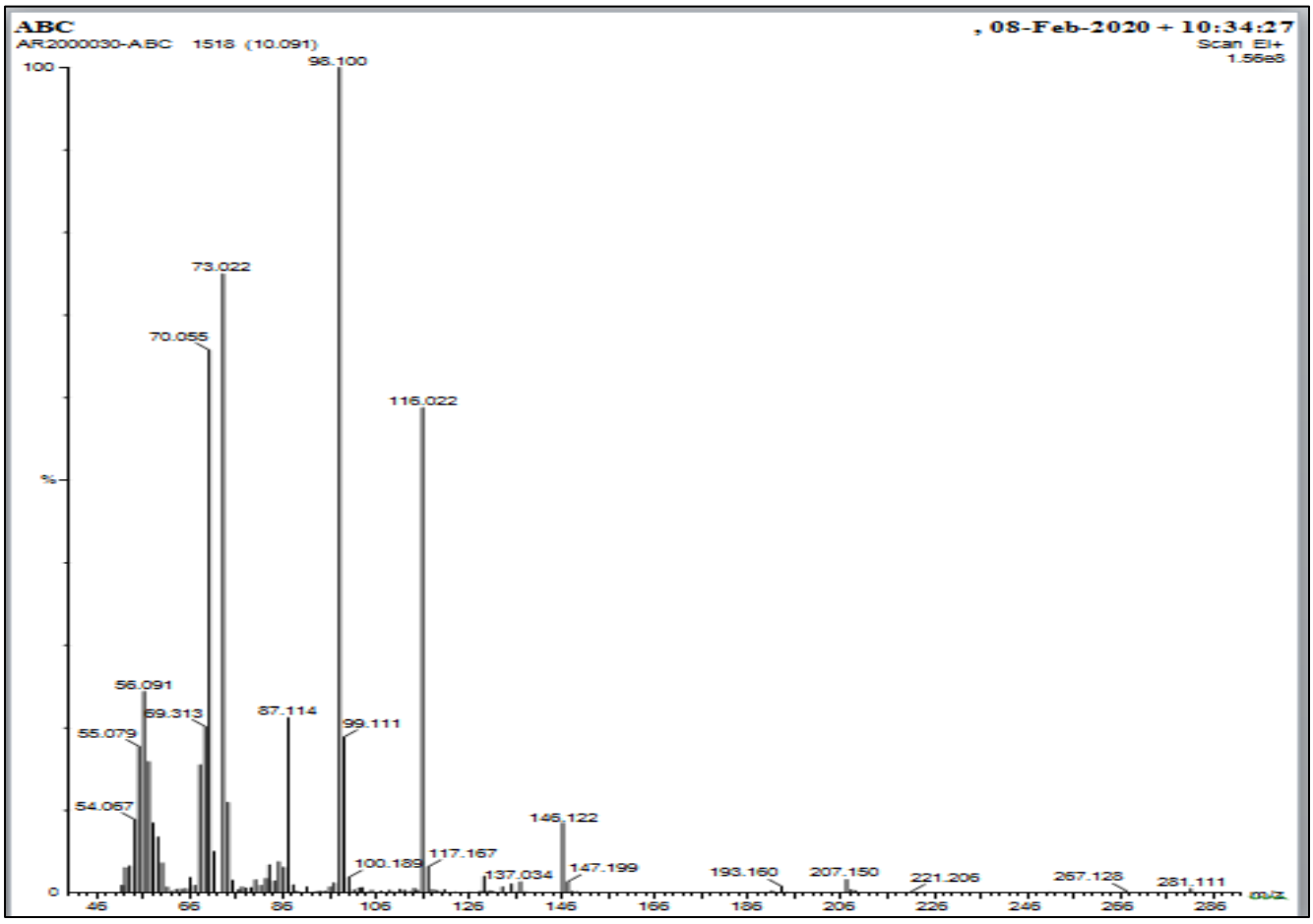


Fig 5: Chromatogram on m/z ratio peaks of various moieties present in the *Berberis aristata* extract

4.4 HAEMAGGLUTINATION INHIBITION TEST

The red blood cells of *Gallus gallus domesticus* was isolated using the standard protocol and the standardisation of the HA units produced the results (Fig 6). By observing the microtiter plate after the incubation period, formation of HA units was seen up to the 7^{th} well succeeding which the wells depicted a lack of HA units formation thereby marking the end point titre at the 7^{th} well. Using the results obtained from the preceding step 4 HA viral concentration was diluted proportioned with 1/640 diluted virus has 1 HA viral activity in 50µl volume respectively. To test the antiviral activity of the *Berberis aristata*, the HAI test was carried out and the results are depicted in (Fig 7). Due to the formation of HA up to $1/16^{th}$ dilution of the extracts of *Berberis aristata* extracts, its antiviral activity has been inferred to be 62.5µg/ml dilution of the extract for 4HA viral concentration. Hence this result (Table 5) implies that *in-vitro* the *Berberis aristata* extract up to the dilution of 62.5µg/ml could combat *the Paramoxyviridae* infection by interacting with the host cell receptors present in the *erythrocytes* of *Gallus gallus domesticus* provided in the microtiter plate. Due to the formation of HA up to the 4^{th} well we could conclude that replication of the viral progeny has been inhibited due to the presence of the phytochemicals present in the extract respectively.

4.5 CYTOTOXICITY ASSAY

In order to study the toxicity of the extract, *in-vitro* cytotoxicity assay was performed. It is observed from the cytotoxicity assay performed that, at 62.5µg/ml concentration of the *B.aristata* extract (which is detected to possess antiviral activity for 4HA viral concentration), the cell viability percentage is 92.8% respectively (Table 6). The higher cell viability percentage tends to lower the threat this extract could impose in terms of toxic effects respectively (Fig 8). From the illustration it is seen how more than 90% of the *vero* cellline

was not inhibited of its normal growth and survived the presence of the extract upto 62.5µg/ml concentration precisely (Fig 9). This is a positive result supporting the safety and efficacy of the extracts possesing the inhibitory effects for the *Paramoxyviridae* infection.

Table 5 : Antiviral activity observed in haemagglutination assay

S.no	Dilution	Final observation :HA /No HA
1	1/2	No HA
2	1/4	No HA
3	1/8	No HA
4	1/16	No HA
5	1/32	HA
6	1/64	HA
7	1/128	HA
8	1/256	HA
9	1/352	HA

HA- Haemagglutination

Table 6: Cytotoxicity effect of the extracts on *Vero* cell lines

S.No	Concentration µg/ml	Absorbance 540nm	% cell Viability
1	1000	0.23	18.2
2	500	0.32	25.3
3	250	0.67	53.1
4	125	0.95	75.3
5	62.5	1.17	92.8
6	31.2	1.23	97.6
7	DMSO	1.25	99.2
8	Control Cells	1.26	100

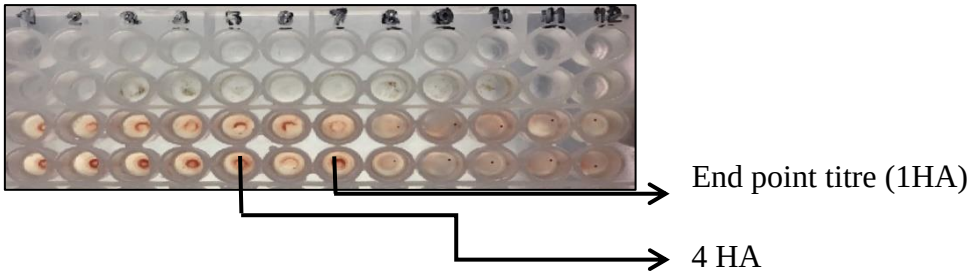


Fig 6: Microtiter plate depicting the end point titre for standardisation of HA units

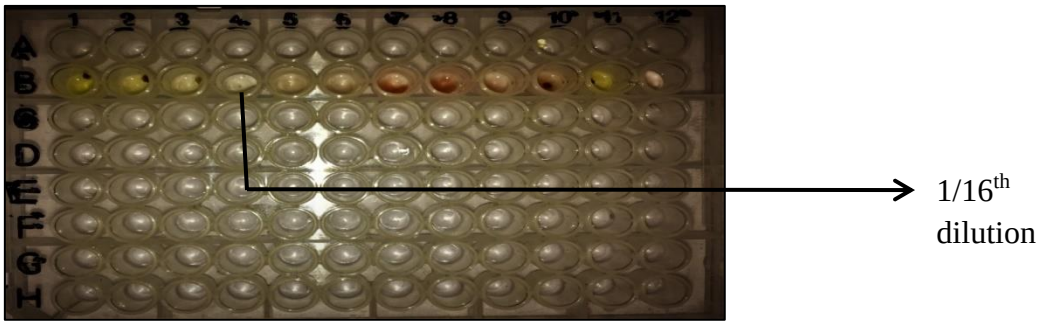


Fig 7: Antiviral titre of the *Berberis aristata* bark extract

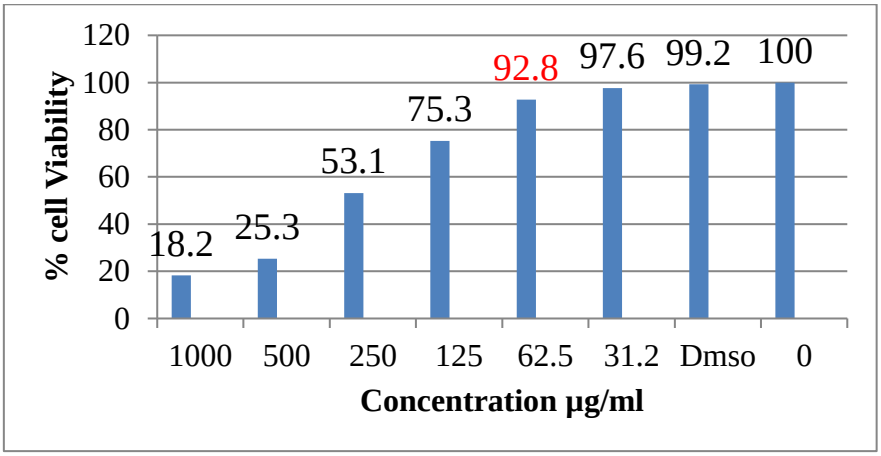
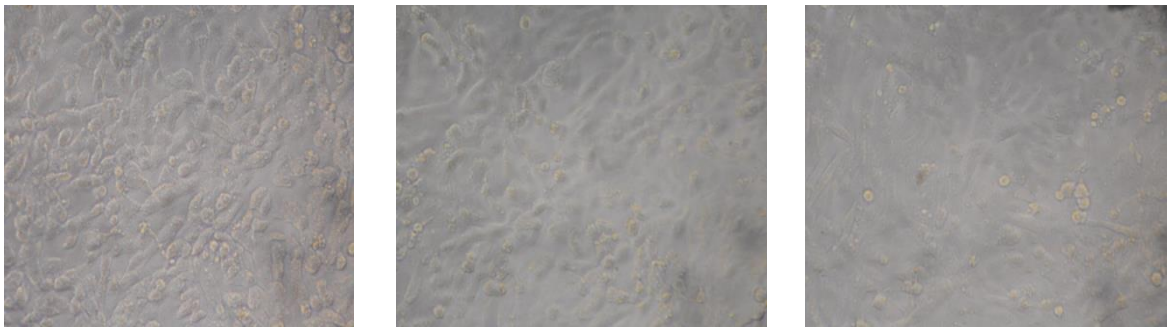
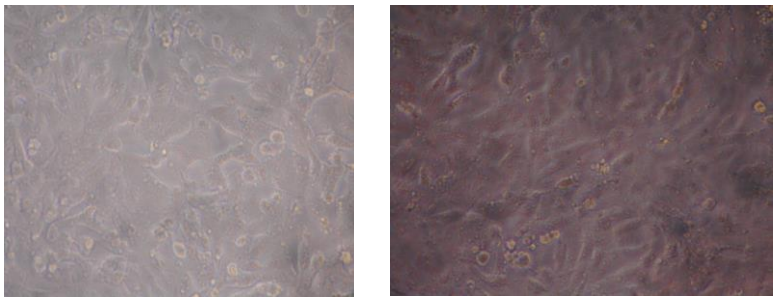


Fig 8: Graphical representation of Cell viability

1000µg 500 µg 250 µg



125 µg 62.5 µg 31.2 µg



DMSO VERO Control cells

Fig 9: Cytotoxic effect of the extracts in various dilutions

4.6 *IN-SILICO* STUDIES

4.6.1 LIPINSKI'S DRUG FILTER

Lipinski's rule of five is a rule of great significance in determining if a chemical compound possesing certain chemical and biological activities is capable of acting as a potent drug compound when administered orally. There are certain parameters based on which these are assesed.The compounds found

to be present in the extract were tested to posses drug likliness property by passing them through lipinski's drug filter using SWISS ADME (Table 7). Upon validation of the molecules, they seemed to not express any violation of the lipinski's rule. In case of spectinomycin, the molecular weight is 332.35 , H-bond acceptors are 9, Log_p value is 1.69. In methyl-3-o-benzyl.alpha.d-xylopyranosidethe molecular weight is 254.28, H-bond acceptors are 5, and Log_p value is 2.08. Upon analysing alpha.-d-mannofuranoside, 1-o-decyl-the molecular weight is 320.42, H-bond acceptors is 6, , Log_p value is 2.99 , for 2,7-Anhydro-l-galacto-heptulofuranose the molecular weight is 192.17 , H-bond acceptors is 6 , Log_p value is 0.46 and the alpha-D-Mannofuranoside, 1-nonyl-has molecular weight is 306.4 , H-bond acceptors is 6 ,Log_p value is 2.86. Overall there isn't any violation of the Lipinski's rule of 5 and hence they pass the drug filter.

4.6.2 QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP PREDICTIONS

Following this, for those compounds possessing drug likeliness property, QSAR study was performed. The results were obtained for the 5 compounds amidst which those which were not known to be considered as a developmental toxicant possessing drug likeliness property descriptors values along with the predicted value (Table 8). The predicted value of spectinomycin (Table 8.1) is observed to be 0.12 which is less than 48.78% respectively. Due to this it is considered to be developemetal non-toxicant according to the Caesar model for toxicity development employed in TEST version 4.2.1 respectively. In case of alpha-D-Mannofuranoside, (Table 8.2) 1-nonyl- the predicted value being 0.42, once again falls within the acceptable range and hence this is also considered to be a developmental non-toxicant. Similarly, the predicted value Alpha.-d-mannofuranoside, 1-o-decyl- (Table 8.3) is around 0.32 lesser than 0.4878 due to which it is also developmental non-toxicant respectively. On the other hand,

the methyl-3-o-benzyl.alpha.d-xylopyranoside and 2,7-Anhydro-l-galacto-heptulofuranose (Table 8.4 and Table 8.5) were predicted to possess developmental toxicity as they have the predicted value as 0.86 and 0.51 precisely. Due to this predicted value being greater than 48.78% according to Caesar model for toxicity development employed in TEST version 4.2.1 they are predicted to be capbable of interfering with the physiology of the organisms. Hence they were not considered for further studies.

4.6.3 MOLECULAR DOCKING PREDICTIONS

Cell binding by using glycosylation mutants reveals binding of the N-terminal domain of chicken galectin-8 (CG-8N) to α-2, 3-sialylated and galactose-terminated glycan chains. Co-crystals with lactose and its 3'-sialylated derivative disclose Arg58 as a key contact for the carboxylic acid and differences in loop lengths to the three homo-dimeric chicken galectins. The sialic acid receptor, facilitating the viral entry, was retrieved from PDB database with PDB id as 4WVW (Fig 10).

To predict the binding efficacy of the ligand with the receptor the docking study was performed and the following results were obtained (Table 9). Upon docking the ligands Spectinomycin, Alpha.-d-mannofuranoside, 1-o-decyl-, Alpha-d-mannofuranoside, 1-nonyl- with the host cell receptor, the docking energies were obtained as -197.74kcal/mol, -213kcal/mol, and -180.6kcal/mol respectively, This study enables in predicting that Alpha.-d-mannofuranoside, 1-o-decyl- with a binding energy of -213kcal/mol could 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 the viral entry and proliferation into the host cell (Fig 11 a, b, c). It is predicted that, Ser37, Ile38, Phe96, Gln95, Arg100, Pro101, and Phe102 residues of the receptor tend to bind by Vanderwaal's with the Alpha.-d-mannofuranoside, 1-o-decyl- to

provide with a binding energy of -198.97kcal/mol and hydrogen bonds are formed with the binding energy of -14kcal/mol. Due to the lowest overall binding energy exhibited by the interaction of Alpha.-d-mannofuranoside, 1-o-decyl- and Chicken Galectin-8 N-terminal domain complexes with 3'-sialyl-lactosereceptor, the resultant entropy is least resulting in more stable interaction. As this moiety (Alpha.-d-mannofuranoside, 1-o-decyl-) is observed to possess drug likeliness property by not violating the Lipinski's rule of 5 and also has the predicted QSAR value within the acceptable range for it to be a developmental non toxicant, in addition to its considerably high binding energy for a phytochemical, this is concluded to be a prospective lead compound to combat the *Paramoxyviridae* infection respectively.

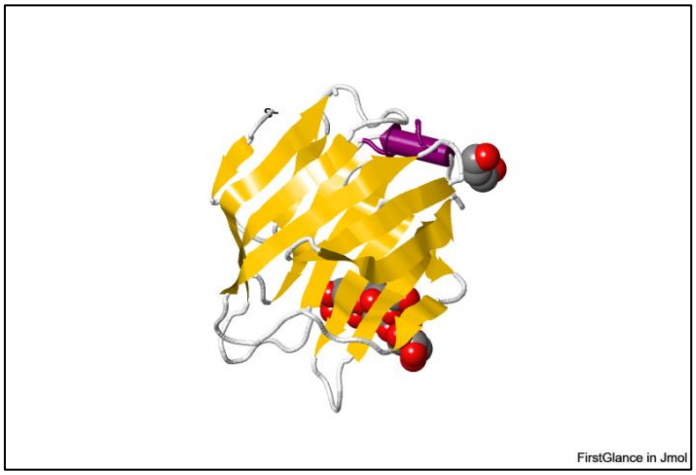


Fig 10: Chicken Galectin-8 N-terminal domain complexed with 3'-sialyl-lactose

Table 7 : Noted observations upon passing the compounds through Lipinski's drug filter

Molecule	MW	H-bond acceptors	H-bond donors	Log_p	Lipinski's violations
Spectinomycin	332.35	9	5	1.69	0
Methyl-3-O-Benzyl.Alpha.D-Xylopyranoside	254.28	5	2	2.08	0
Alpha.-d-mannofuranoside, 1-o-decyl-	320.42	6	4	2.99	0
2,7-anhydro-L-Galacto-Heptulofuranose	192.17	6	4	0.46	0
Alpha-d-mannofuranoside, 1-nonyl-	306.4	6	4	2.86	0

Table 8: Quantitative Structure Activity Relationship of identified compounds

Table 8.1 Descriptor Values of Spectinomycin

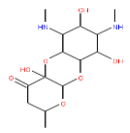
Descriptor	Value	Coefficient	Value × Coefficient	structure
MDEC33	13.3089	0.0249	0.33	
MATS5m	-0.0554	1.3442	-0.07	
MATS6m	-0.0202	0.4615	-0.01	
MATS5p	-0.0484	0.6944	-0.03	
GATS3v	0.9089	0.8598	0.78	
-NH- [aromatic attach]	0.0000	0.6840	0.00	
Model intercept	1.0000	-0.87	-0.87	
Predicted value			0.12	

Table 8.2: Descriptor Values Alpha.-d-mannofuranoside, 1-o-decyl-

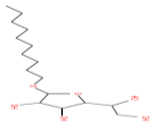
Descriptor	Value	Coefficient	Value × Coefficient	structure
SssO_acnt	2.0000	0.2305	0.46	
MATS3v	0.3750	-1.0092	-0.38	
Model intercept	1.0000	9.18	9.18	
Predicted value			0.32	

Table 8.3: Descriptors value of Methyl-3-o-benzyl.alpha.D-xylopyranoside

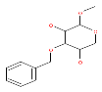
Descriptor	Value	Coefficient	Value × Coefficient	structure
Model intercept	1.0000	-7.16	-7.16	
Predicted value			0.86	

Table8.4: Descriptor Values Alpha-d-mannofuranoside, 1-nonyl-

Descriptor	Value	Coefficient	Value × Coefficient	structure
ic	0.0000	-0.0096	0.00	
icycem	0.7919	0.4907	0.39	
MDEN33	0.0000	0.4846	0.00	
MATS2p	-0.1654	0.4541	-0.08	
-CH3 [aromatic attach]	0.0000	-0.1317	0.00	
-CH= [aromatic attach]	0.0000	-0.4264	0.00	
>C= [aromatic attach]	0.0000	0.1666	0.00	
-NH2 [aliphatic attach]	0.0000	0.1766	0.00	
-S(=O)(=O)- [aliphatic attach]	0.0000	0.5328	0.00	
Model intercept	1.0000	0.48	0.48	
Predicted value			0.42	

Table 8.5: Descriptors of 2, 7-Anhydro-l-galacto-heptulofuranose

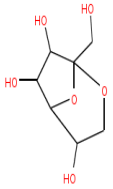
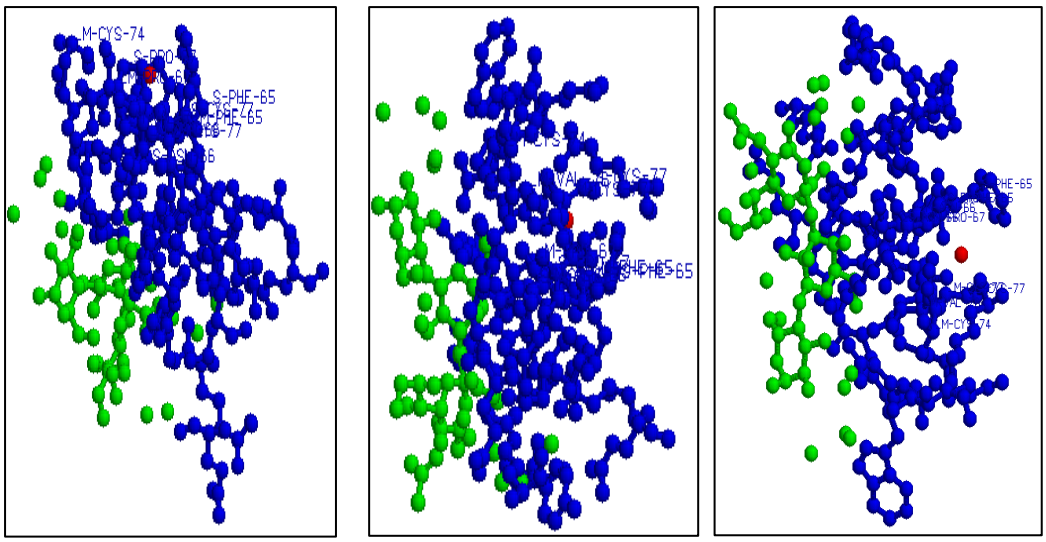
Descriptor	Value	Coefficient	Value × Coefficient	structure
-NH- [aromatic attach]	0.0000	-0.8014	0.00	
-C(=O)O- [nitrogen attach]	0.0000	0.4333	0.00	
Model intercept	1.0000	2.05	2.05	
Predicted value			0.51	

Table 9: Molecular interaction between the target and identified ligand using iGEMDOCK

Compound	Energy (kcal/mol)	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



(a) (b) (c)

Fig 11: Docked pose of ligand and Chicken Galectin-8 N-terminal domain complexed with 3'-sialyl-lactose

a) Alpha.-d-mannofuranoside, 1-o-decyl-
b) Spectinomycin
c) Alpha-d-mannofuranoside, 1-nonyl

CHAPTER 5

DISCUSSION

Considering the present times, there is no treatment for *New castle disease virus.* Although, for the past six decades, vaccines have been proven effective to immunise the susceptible chickens, in present day scenario this seems to exist as a challenge than being a solution. This is due to the questionable bio-safety security being difficult to manage especially during an endemic, adaptation of the virus to other species of birds such as pigeons due to mutation are a couple of factors menacing the vaccination strategy for NDV.

According to the World Organisation of Animal Health (OIE) has included this among the list of diseases that requires immediate notification upon recognition (Bello *et.al.,* 2018). In addition to this many viruses belonging to the same family pose similar threats to the world. The WHO states according to its 2018 annual review that there is no effective treatment available for people or animals. To overcome the existing approach of pathogen targeted drug discovery prone to succumbing to limitations of microbial drug resistance and consequent resurgence of once contained infection, the present study focussed on lead identification based on the host factors facilitating the emergence and proliferation of the pathogen. In the present study, to discover a novel lead compound from *Berberis aristata* extracts containing alkaloids was chosen after close observation of the work on the antiviral activity exhibited by isoquinoline such as berberine as effective anti-influenza agent respectively (Sidra *et.al.,* 2011). Since the entry mechanism is conserved for the *Paramoxyviridae* family, there exists a possibility for the lead compound predicted be potent in inhibiting other diseases caused by the virus belonging to this family such as *Nipah* and *Hendra* viruses as well (Rebecca *et.al,* 1999).

The extract which showed the promising antiviral activity was characterised using the haemagglutination inhibition assay which was similar to

the work performed using oil inactivated NDV vaccines by Saleh *et.al.,* (2018). In addition, *in-vitro* cytotoxicity was analysed by means of the cytotoxicity assay on *Vero* cell lines. The current study showed 92.5% of *Vero* cells viability in 62.5 µg concentration of extract. That shows the low risk toxicity of extract which has been supported by the study conducted by Sidique *et.al.*, 2017.

The *in-silico* portion of experimentation in the present study has given emphasis to the pre-clinical studies involving structure based drug design which was supported by many researchers using the moieties present in *B.aristata* identified by GC-MS analytical technique (Amuthavalli *et.al.,* 2019 and Subramanian *et.al.*, 2019).

Similar to the toxicity estimation approach to confirm efficacy and safety of pharmaceuticals adopted by Yi *et.al.,* (2018) the moieties were analysed by toxicity estimation software tool for *in-vitro* developmental toxicity presence. In the present study molecular dockings showed the lowest energy value of -213 kcal/mol for Alpha.-d-mannofuranoside, 1-o-decyl- with the selected ligand which supports antiviral activity of identified compounds from the extract through the stable molecular complex formation. The similar analysis with the research of using iGEMDOCK on evaluation of the antiviral efficacy of *Punicagrantum L* on *Herpes virus* was explained by Divyadharshini and Rajandran, 2019. Thus this research strongly supports the moieties present in the extract to contain the lead compound capable of possessing a therapeutic advantage.

CHAPTER 6

SUMMARY CONCLUSION

Though vaccination has existed for *New castle disease virus* for 6 decades, the need for a cure for the disease has arose due to the increased incidence of the disease and reduced bio-safety security in vaccination. The novel lead compound has been discovered from *in-vitro* and *in-silico* studies involving the extracts of medicinally meritorious *Berberis aristata*. The haemagglutination assay provided an antiviral activity of the extract at 1/16 dilution in 4 HA viral concentration and the cytotoxicity assay at 62.5 µg concentration supported 92.5% of *Vero* cells viability. The compounds identified using the GC-MS analysis was subjected to *in-silico* studies to predict the bio-developmental toxicity and binding efficacy of the compounds.

The *in-silico* approach provided evidences supporting the drug likeliness of the lead compound in foreseeable future. Alpha.-d-mannofuranoside, 1-o-decyl- obeyed the Lipinski's rule of 5 and further was calculated as development non-toxicant with the predicted value as 0.32 respectively. The molecular docking between Chicken Galectin-8 N-terminal domain complexed with 3'-sialyl-lactose (PDB id: 4wvw) and the compound presumed a binding energy of -213kca/mol with Vanderwaal's force involved constituting -198.97 kcal/mol and hydrogen bonding between the amino acid residues of the host receptor and the compound as -14kcal/mol respectively. Hence this compound is concluded as a prospective lead compound.

In future research, the work can be further developed and clinical trials can be done to test its effectiveness and for social benefit. Further we aim to test the efficacy of the extracts of *Berberis aristata* in combatting other deadly viruses belonging to the *Paramoxyviridae* family of viruses.

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