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PHARMACOLOGICAL SCREENING AND DOCKING STUDY OF *BRASSICA OLERACEA* L. VAR. *ITALICA* PLENCK FOR ANTI-HIV ACTIVITY.

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ABSTRACT:-

The present study was aimed to investigate Anti-HIV activity of *Brassica Oleracea* L. var. *italica* Plenck. Different extracts from the plant was obtained by using different extraction techniques such as soxhlet and microwave assisted extraction. The assessment of Anti-HIV activity was carried out by using Enzyme pepsin inbition assay (In-Vitro Method) for ethanolic and aqueous extract of plant. Also docking study was carried out using VLife MDS software. In conclusion, ethanolic & aqueous extract of *Brassica Oleracea* L. var. *italica* Plenck exhibited good anti-HIV activity. But ethanolic extract showed maximum Anti-HIV activity as compared to aqueous extract.

KEYWORDS: Anti-HIV activity, *Brassica Oleracea* L. var. *italica* Plenck, Enzyme pepsin inhibition assay, Docking study.

1. INTRODUCTION:-

Natural products have played, and will continue to play, a key role in drug discovery and are therefore traditionally claimed as the cornerstones of drug discovery and development.

Drug discovery is leading to be a challenging scientific task to find robust and viable lead candidates, which is nothing but the process flow from a screening of natural product to a new isolate that requires expertise and experience. However, in addition to their chemical structure diversity and their biodiversity, the development of new technologies has revolutionized the screening of natural products in discovering new drugs.

Many drugs that are available in market today were discovered from natural sources. An important example is the analgesic activity of aspirin, which is so far the world's best known and most universally used medicinal agent. Its origin is from the plant genera Salix spp. and Populus spp. and it is related to salicin. A good example is serendipitous discovery of the antibiotic penicillin in the laboratory from the fungus Penicillium notatum.

Many other examples show the value and importance of natural products from plants and microorganisms in modern days. Paclitaxel (Taxol), which was first isolated from the bark of the Pacific yew tree *Taxus brevifolia* (Taxaceae), is the most recent example of an important natural product that has made an impact in medicine. Activity against a variety of retroviruses, including HIV, two compounds isolated from *Hypericum perforatum* (Guttiferae) are hypericin and pseudohypericin. They are of paramount importance due to inhibition of release of reverse transcriptase by stabilizing the structure of the HIV capsid and thus preventing the uncoating process.[1]

❖ HUMAN IMMUNO-DEFICIENCY VIRUS (HIV):-

Since its first discovery in 1981, Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) has killed more than 25 million people worldwide and is today the major threat to human health.[2]

HIV stands for human immunodeficiency virus. AIDS stands for acquired immuno deficiency syndrome.

HIV

H-It infects only human beings and also transmitted between humans not from animals. It is not transmitted from bites of mosquitoes, bats or any other species.

I-The body has immune system whose function is to protect our body from germs, infections etc. But a person suffering from HIV has inability to fight against diseases. However, immune system becomes deficient.

V-Virus is a small, simplest thing which is in inactive form outside the body and becomes active when it goes inside human body.

HIV/AIDS has always been one of the most thoroughly global of diseases. The human immunodeficiency virus (HIV) is a lent virus that causes HIV infection and AIDS. AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening infections and cancers to thrive. Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.[3]

a. Structure of HIV Virus:[3]

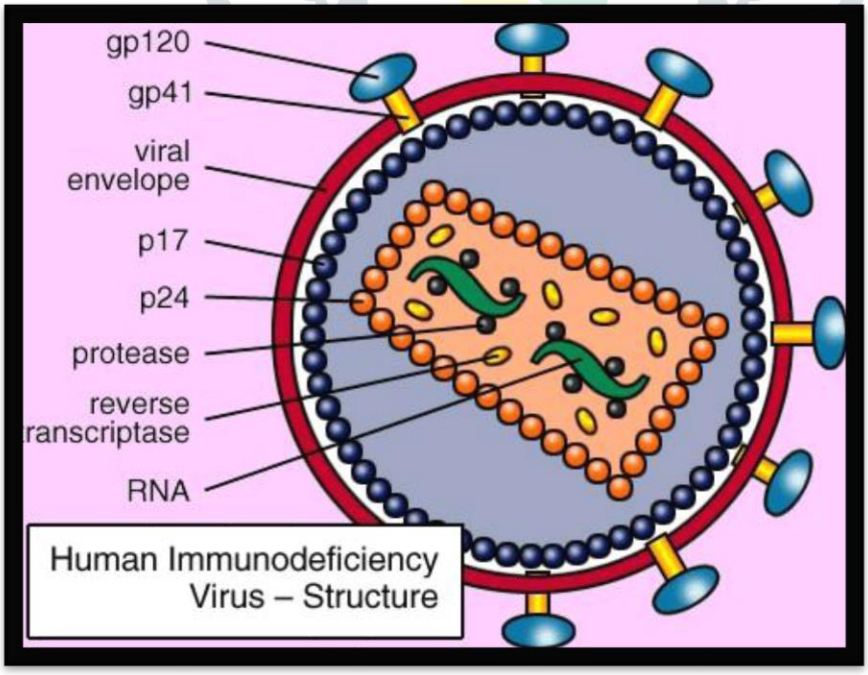

Fig.no.1- Structure of HIV Virus

Gp120:- The 120 in its name comes from its molecular weight. It is essential for virus entry into the cells as it plays vital role in attachment to specific cell surface receptors.

GP41:- It is a subunit of the envelope protein complex of retroviruses including human immuno deficiencies virus. It is family of enveloped viruses that replicate in host cell through process of reverse transcriptase. It targets a host cell.

Viral envelope:- It is envelope through which virus binds.

P17:- Viral core is made from protein. It is bullet shaped. Three enzymes required for HIV replication are reverse transcription, integrase and protease.

P24:- P24 is component of HIV capsid.

Protease:- It is a retroviral aspartyl protease that is essential for life cycle of HIV, the retrovirus that caused AIDS. This enzyme cleaves newly synthesized polyproteins at appropriate place to create nature protein components of infectious HIV virion.

Integrase:- Enzyme produce by retrovirus that enables its genetic material to be integrated into the DNA of infected cell.

RNA:- All organisms including most viruses store their genetic material on long strands of DNA. Retrovirus is exception because their genes are composed of RNA.

b. Life Cycle of HIV- AIDS:-[4]

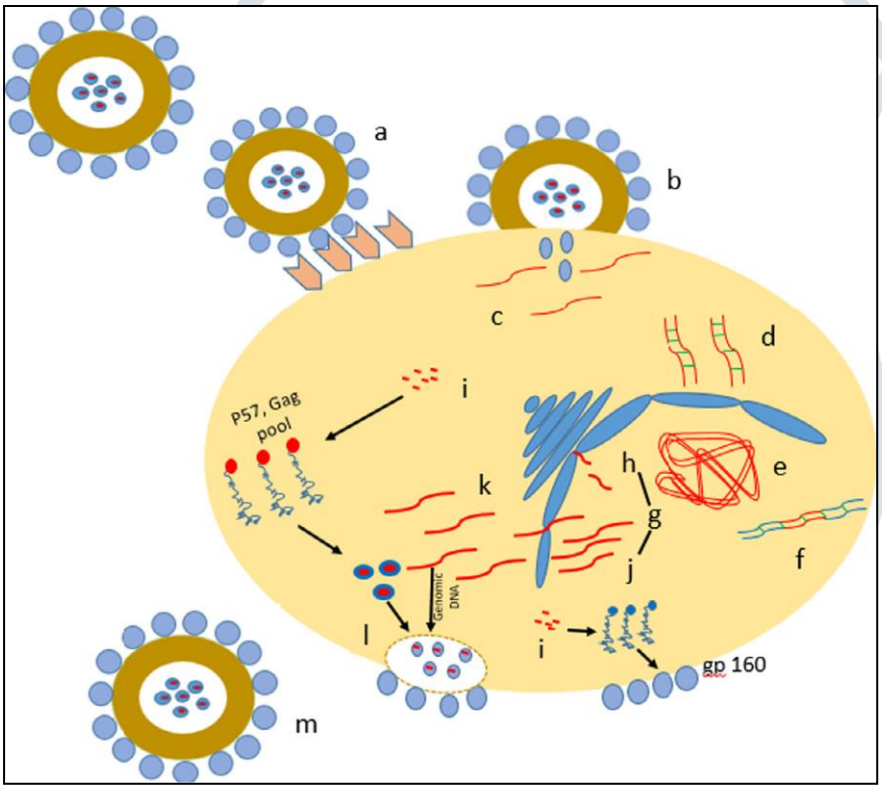

Fig.no.2- Life cycle of HIV- AIDS

(a) HIV attachment to CD4 antigen and a specific chemokine receptor.
(b) Virus fusion with the cell membrane and entry of the virion core into the cell.
(c) Release of viral RNA and core proteins and their transport into the nucleus.
(d) Formation of double-stranded DNA by reverse transcriptase.
(e) Transport of double-stranded viral DNA into the cell nucleus.
(f) Integration of viral DNA into cellular DNA.
(g) Synthesis of viral RNA by RNA polymerase II and production of RNA transcripts with shorter spliced RNA (h) and full length genomic RNA (j).
(h) Transport of shorter spliced RNAs to the cytoplasm and the production of several viral proteins that are then modified in the Golgi apparatus of the cell (i).
(j) Transport of full-length genomic RNAs into the cytoplasm (k).
(l) Assembly, budding and maturation of new virions.
(m) Release of mature virus.

c. Symptoms:[3]

- Large lymph nodes or "swollen glands" that may be enlarged, for more than three months.
- Frequent fevers and sweats skin rashes or flaky skin that does not go away.
- Short-term memory loss.
- Slow growth or frequent illness in children.
- Cough and shortness of breath.
- Seizures and lack of coordination.
- Difficult or painful swallowing.
- Confusion and forgetfulness nausea, cramps diarrhea or vomiting that do not go away.
- Vision loss.
- Unexplained weight loss.

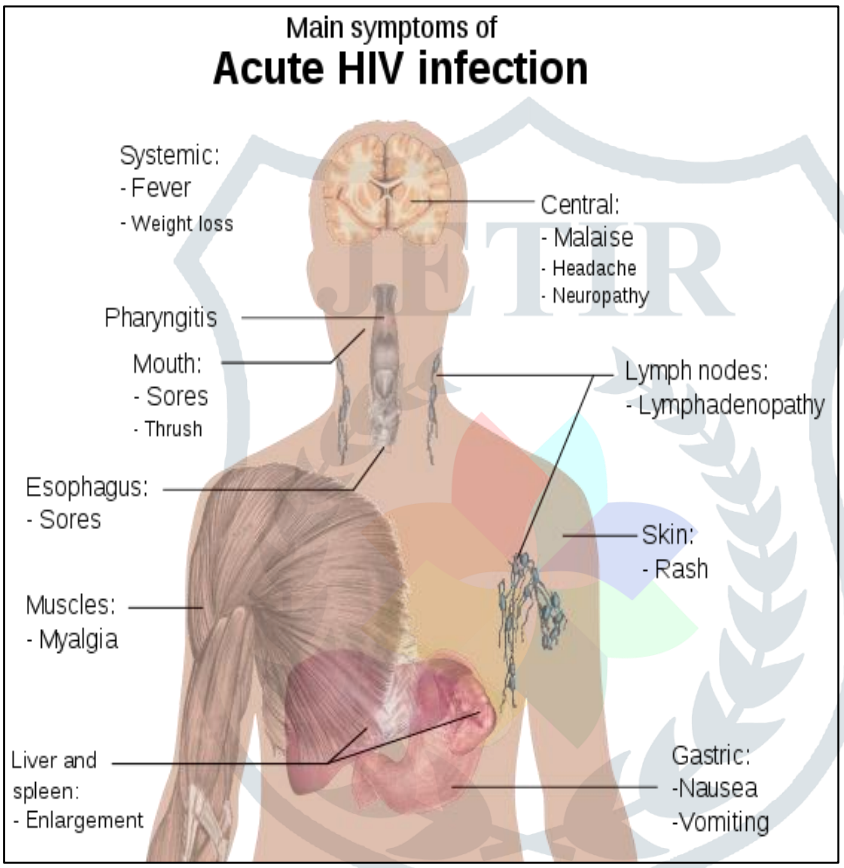

Fig.no.3- Symptoms of HIV infection.

d. Tramission:-[3]

HIV is transmitted principally in three ways: By sexual contact, by blood through transfusion, blood products or contaminated needles or by passage from mother to child. Although homosexual contact remains a major source of HIV within the United States, “hetero sexual transmission is the most important means of HIV spread worldwide today.” Treatment of blood products and donor screening has essentially eliminated the risk of HIV from contaminated blood products in developed countries, but its spread continues among intravenous drug users who share needles. In developing countries, contaminated blood and contaminated needles remain important means of infection. Thirteen to thirty-five percent of pregnant women infected with HIV will pass the infection on to their babies; transmission occurs before as well as during birth. Breast milk from infected mothers has been shown to contain high levels of the virus also.

HIV is not spread by the fecal-oral route; aerosols; insects; or casual contact, such as sharing household items or hugging. The risk to health care workers is primarily from direct inoculation by needle sticks. Although saliva can contain small quantities of the virus, the virus cannot be spread by kissing.

HIV can be transmitted from an infected person to another through:

• Blood (including menstrual blood)

• Semen
• Vaginal secretions
• Breast milk

e. Diagnosis:-[5]

Detection of the HIV virus in the blood is usually measured as viral RNA load and infection is associated with an acute symptomatic period that includes fever, general malaise, lymphadenopathy, rash, myalgias, however serious consequences such as meningitis have also been reported.

During the period of acute infection, the plasma levels of HIV RNA are at their highest and the severity of symptoms is associated with the level of viral load. It has been suggested that viral characteristics and viral load determine both the replication and pathogenesis. Thus, the clinical outcomes and disease progression are dependent not only on the host, but also on the viral genotype.

HIV is difficult to completely eradicate as it establishes a quiescent or latent infection within the memory CD4+ T cells, which have a stem-cell-like capacity for self-renewal. Once the HIV DNA is integrated into the host chromatin, the virus can repeatedly initiate replication as long as that cell exists. While ART can prevent new cells from becoming infected, it cannot eliminate infection once the DNA has successfully integrated into the target cell. The lymph nodes harbor the virus because of limited antiretroviral drug penetration, and limited host clearance mechanisms, and serves as a source of virus recrudescence in individuals who stop or interrupt their therapy. It has been suggested that ART therapy may be needed for several decades before the viral reservoir might decay to negligible levels.

f. Current Treatments for HIV/AIDS:[5]

Although HIV was recognized early in the 1980s, there is still no cure or an effective vaccine for HIV infection, but there have been some significant advances in treatment, control, and prevention. The introduction of anti-retroviral agents and highly active antiretroviral therapy (HAART) in 1996 significantly reduced the morbidity and mortality of HIV/AIDS.

Antiretroviral therapy is currently recommended for all adults with HIV. Recommendations for initial regimens include two nucleoside reverse transcriptase inhibitors (NRTIs; abacavir with lamivudine or tenofovir disoproxil fumarate with emtricitabine) and an integrase strand transfer inhibitor, such as dolutegravir, elvitegravir, or raltegravir; a nonnucleoside reverse transcriptase inhibitor (efavirenz or rilpivirine) or a boosted protease inhibitor (darunavir or atazanavir).

It is currently recommended that all HIV-infected patients with detectable virus, regardless of their CD4 cell count, should be treated with anti-retroviral therapy (ART) soon after diagnosis to prevent disease progression, improve clinical outcomes including reducing AIDS-associated events, non-AIDS-related events, and all-cause mortality, as well as to decrease transmission.

❖ **Future Scope:**[2][5]

The natural products calanolides (coumarins), ursolic and betulinic acids (triterpenes), baicalin (flavonoid), polycitone A (alkaloid), lithospermic acid (phenolic compound) have been proposed as promising candidates for anti-HIV agents.

Although, antiretroviral drugs can bring about the repression of the serum load of the virus to undetectable levels, economical, commercial, and political barriers have limited their accessibility to a good part of the population suffering from the diseases. Natural products, particularly those in traditional medicine have supplied a basis of new drug candidates for many diseases including HIV. Accordingly, there is need to evaluate traditional medicine, particularly medicinal plants and other natural products that may yield effective and affordable therapeutic agents.

Several plant species have shown remarkable anti-HIV activity. These plant species are worthy of further study for the development of new anti-HIV chemotherapeutic options. In particular, in vivo testing and, ultimately, human clinical trials need to be carried out on key lead plants and phytochemical isolates. In addition, continuous evaluation of medicinal plants for anti-HIV activity should be pursued.

2. ***Brassica oleracea* L. var. *italica* Plenck [Broccoli]*:-*** *Brassica oleracea* L. var. *italica* Plenck belongs to the family **Brassicaceae**. Commonly it is known as broccoli flower, sprouting broccoli, cape broccoli. Broccoli is an Italian word from the Latin brachium, meaning an arm or branch.[6]

Fig no.4. *Brassica oleracea* L. var. *italica* Plenck

➢ **Taxonomical classification of plant:[7]**

Table no.1-Taxonomical classification of plant

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Sub class	Dilleniidae
Order	Capparales
Family	Brassicaceae
Genus	Brassica
Species	Brassica L var italic Plenck

- **Vernacular Names in India:-**

Table.no.2- Vernacular Name

English	Broccoli
Marathi	Hiravi Kobi
Kannada	Kosugedde
Gujarati	Hari Phool Gobi
Hindi	Hari Phool Gobi

- **Occurrence and Description of Plant**

- **Geographical Distribution**

Brassicaceae family is southwestern and central Asia & the Mediterranean region whereas the arctic, western North America & the mountains of South America are secondary centers of variegation/ diversity.[8]
Broccoli is native to Mediterranean region. Broccoli is a cultivar of wild cabbage. Wild cabbages originate along the northern and western coasts of the Mediterranean, where it was apparently domesticated 1000 years ago. The family contains species of great economic importance, providing much of the world's winter vegetables.[9]

- **Plant Description**

The leaves are alternate (rarely opposite), sometimes arranged in basal rosettes; in the rare shrubby cruciferous of the Mediterranean, its leaves are found mainly in terminal rosettes and can be leathery and evergreen. Very often they are incised with pinnacles and don't have stipules.[8]

- **Chemical composition**

Broccoli is high in vitamins C, K, and A, as well as dietary fiber; it also contains multiple nutrients with potent anti-cancer properties, such as diindolylmethane and small amounts of selenium.[10]

3. Materials and Methods

A. Drugs, chemicals & solvents: (Analytical grade drugs & chemicals are used)

All the drugs chemicals were analytical grade. Drugs & chemicals used in this experimentation as follows chloroform**,** ethanol
Enzyme Pepsin (Himedia), Hemoglobin (Himedia), Valacyclovir, Silver nanoparticle of valacyclovir, Sodium Acetate tri hydrates (Qualigens), Sodium Chloride (Himedia), Tri Chloro Acetic acid (TCA) (Sdfine). Acetate buffer was prepared by 50 Mm Na Acetate tri hydrate and 0.1 M NaCl with pH- 3.5.

B. Collection of plant material:

The fresh flowering stalks of *Brassica oleracea* L. var. *italica* Plenck was collected in the month of September 2021 from the vegetable market (Frufiz Store) in Sangli region, Maharashtra.

C. Authentification of plant:

Authentication of the plant material was done at Department of Botany, Kasturbai Walchand College, Sangli by Mr. M. D. Wadmare, H. O. D of Botany Department.

D. Drying and size reduction of plant materials:

The collected plant flowering stalks were washed thoroughly to remove dirt and debris. The plant was cutting and spread out in thin layer on drying trays, kept in shade for 30 days. The drying trays were placed at a sufficient height above the ground to ensure air circulation and consistent drying of plant material and avoid mould formation. Once the plant material is dried, it can be stored for long period of time. After complete drying, the flowering stalks were powdered by mixer grinder to obtain coarse powder.

4. Extraction of plant material:

- **Soxhlet Extraction:**

Drug extract are preparation obtained by extracting herbal drug at certain particle size with suitable extraction medium. Extraction was carried out using Soxhlet apparatus. Successive extraction method was used for extraction. Generally, for successive solvent extraction, solvents from lower polarity to higher polarity are used.

In present study, petroleum ether was used former for successive extraction and ethanol was used later. Dried powder was used for extraction to avoid chemical changes taking place in drug.

Extraction was performed with organic solvents chloroform and ethanol. About 60 gm of powder was subjected to successive hot continuous extraction with chloroform. The temperature of heating mantle kept 50^0C below the melting point of solvent. The extraction was continued until the solvent in the thimble became clear. The chloroform extract was filtered and powder in the extraction apparatus is removed from extractor, dried and then it was used for extraction with ethanol. Then few drops were collected in a test tube during the completion of cycle (during siphoning) and chemical test of the solvent was performed. If the test for solvent is negative, then extraction is complete.

Fig no.5:- Soxhlet apparatus (Organic solvent extraction)

After the effective extraction, solvent was distilled off using rotary evaporator and the extracts were concentrated at low temperatures. The dried concentrated extracts were used for phytochemical investigation, and pharmacological activity.

- **Microwave Assisted Extraction:**

Procedure:

Table.no.3- Extraction procedure

Sr.no.	Plant powder (gm)	Solvent	Conc. (ml)	Power (watts)	Time (min)
1	150	Ethanol	400	340	15 min
2	150	Water	400	425	15 min

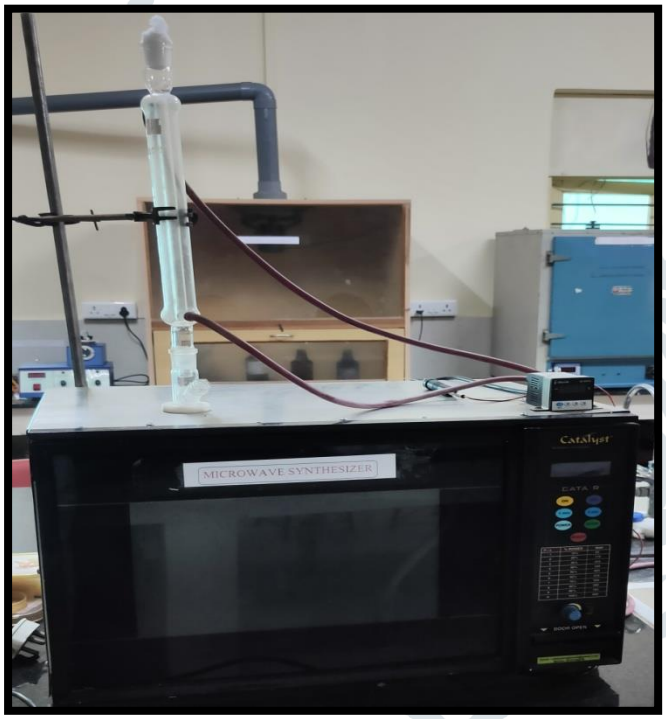

Fig no.6:- Microwave assisted Extraction

3 to 4 procelain pieces were added into the flask to each solvent extraction.
After heating each solution was filtered using muslin cloth to separate out the extract and residue. The obtained filtrate (extract) yields proportionally to the solubility and availability of phyto-chemicals in the plant. Extracts were then dried at the atmospheric temperature in open area.

5. Determination of extractive value:

Extractive values are useful for the evaluation of a crude drug. It gives an idea about the nature of the chemical constituents present in the crude drug. It is useful for the estimation of constituents extracted with the solvent used for extraction. Extractive values were calculated using following formula:

$$\% \text{ Extractive values} = \frac{\text{Weight of dried extract plant}}{\text{Weight of dried plant material}} \times 100$$

Table no.4- Extractives values of extract of *Brassica Oleracea* L. var. italica Plenck

Extract	Colour	Consistency	Extractive Value
Aqueous extract	Brown	Viscous	5.256%
Ethanolic extract	Green	Solid	6.02%

6. Anti-HIV activity screening:-

- **Introduction**

AIDS is a pandemic immunosuppressive disease which results in life-threatening opportunistic infections and malignancies. Since a retrovirus, designated human immunodeficiency virus (HIV) has been clearly identified as the primary cause of this disease[11][12]. The replicative cycle of HIV comprises ten steps that could be considered suitable targets for chemotherapeutic intervention[13]. A number of laboratories are actively involved in the development of antiviral agents that interfere with HIV at different stages of viral replication[14][15]. The high mutation rate of HIV frequently results in the rapid development of resistance towards the drugs used, and an attempt has been made to circumvent this problem by using a combination of drugs[16].

Based on the presence of the characteristic signature amino acid sequence, Asp-Thr-Gly, was suggested by Toh et al. in 1985 that the protease of HIV might belong to the family of aspartic proteases. The aspartic proteases are well characterized group of enzymes that can be found in vertebrates, plants, in addition to in fungi. Examples of proteases from the aspartic protease class are pepsin, cathepsin D, renin, chymosin, penicillopepsin, and Rhizopus pepsin, which are two-domain enzymes with more that 300 residues in length and contain the Asp-Thr-Gly sequence in each domain which forms the active site, and effectuates the cleavage reaction.

This enzyme was earlier perceived as a promising therapeutic target and its inhibition has been successfully used in the treatment of AIDS. Despite this achievement, the emergence of strains resistant to the currently available commercial inhibitors, the high cost of these drugs and its associated toxicity has driven a continuous interest in the development of new inhibitors. The search for new inhibitors often includes high throughput screening programs for detecting

HIV-PR inhibitor candidates. In order to identify and evaluate potential inhibitors either from natural sources or from rational designs, some groups have developed HIVPR activity assays. An indirect approach was used for screening HIV-Protease inhibitors through pepsin activity inhibition assay from crude plant extract, as many paper support the evidence in the resemblance in the activity of HIV Protease and Enzyme Pepsin[17][18][19].

- **Materials and Methods**

➢ **Chemical required:**

Enzyme Pepsin (Himedia), Hemoglobin (Himedia), Valacyclovir, Silver nanoparticle of valacyclovir, Sodium Acetate tri hydrates (Qualigens), Sodium Chloride (Himedia), Tri Chloro Acetic acid (TCA) (Sdfine). Acetate buffer was prepared by 50 Mm Na Acetate tri hydrate and 0.1 M NaCl with pH- 3.5.

➢ **Instruments required**

Spectrophotometer - Karry 100, Rami for 8000 rpm, Micropipette 5-40 µl, 40-200 µl, 200-1000 µl, Eppendorff (1.75ml), electronic balance.

- **Enzyme Pepsin activity inhibition Assay: -**

Pepsin has a quite close resemblance in proteolytic activity with HIV-1 protease one of key enzyme of HIV-1 life cycle as both of them belong to same Aspartate enzyme family. This enzyme was used as a substitute of

HIV-1 protease to check out anti HIV activity of valacyclovir and silver nanoparticle of valacyclovir in this experiment.

Protease has a quite resemblance in proteolytic activity with HIV-protease one key enzyme of HIV-1 life cycle as both of them belongs to same aspartate enzyme family18. This enzyme was used as a substitute of HIV-1 protease to check out anti- HIV activity of silver nanoparticle of valacyclovir in the present investigation. To measure the inhibition of the protease enzyme (Sigma Aldrich) used with their corresponding substrate (Sigma Aldrich).[20]

- **Procedure**

1. For this assay, 50µg pepsin, 800µg hemoglobin and different crude plant extracts were taken in 500µl of reaction mixture.
2. The mixture was allowed to incubate at 37^0C, after 20 min 700µl of 5% TCA was added to stop the reaction.
3. It was then centrifuged at 14000 g for 5 min and the supernatant was collected.
4. Optical Density (OD) was recorded spectrophotometrically at 280 nm.
5. Separate blanks were used or both positive and negative controls as well as for sample.
6. For negative control, enzyme and substrate were taken and followed the above procedure and for positive control protease was taken as a well-known inhibitor of HIV-protease, lopinavir was taken.
7. Each sample was taken in triplicate, so this assay gives reproducible results.
8. Percentage of inhibition was calculated by using a formula.

- Inhibition (%) = [(OD of negative control - OD of sample) /OD of negative control] ×100

Table.no.5- Procedure for anti-HIV activity

Parameter	**Optimum range**
pH	2- 4
Incubation period	30 min.
Reaction volume	1000 µL
Incubation temperature	37 ^{0} C
Centrifugation	14000 rpm.

7. Docking Study

Introduction:

Molecular docking is a process that involves the placement of molecules in appropriate configurations to interact with a receptor. It is a natural process that occurs within seconds in a cell.

A. **Receptor:** Receptor are the receiving molecules. Most commonly it involves a protein or other biopolymer.

B. **Ligands:** Ligands are the complementary partner molecules that bind to the receptors. Ligands are mostly tiny molecules but could also be another biopolymer.

C. **Docking:** A process that involves computational simulation of a candidate ligand binding to the receptor.

D. **Binding mode:** The position of the ligand to the receptor and the conformation of the ligand and receptor when bound to each other.

Study: Docking study was performed to check the anti-HIV activity of flavonoid Quercetin present in *Brassica oleracea* L. var. *italica* Plenck.

A docking study was performed at Appasaheb Birnale College of Pharmacy, Sangli.

Software used: VLife MDS

Drug molecule: Quercetin

Targeted receptor :- HIV-1 RT (PDB ID- 1FK9)

Preparation and modification of receptors:

1. Receptors were downloaded from RCSB PDB.
2. Addition of hydrogen
3. Incomplete residue treatment
4. Missing residue treatment (Loop insertion)
5. Deleting unwanted chains
6. Extraction of co-crystal ligand.

Drug Treatment:

The structure of quercetin was converted into Mol 2 format.

Fig.no.7- Structure of Quercetin (Mol 2 format)

Genetic Algorithm:

Ideas based on the language of natural genetics and biological evolution are used in the genetic algorithms. In molecular docking, the particular arrangement of the ligand and the protein describes the set of values that is translation, orientation, and conformation of the ligand concerning the protein. Translation, orientation and conformation are the ligand's state variables, and in the GA each state variable corresponds to the gene. Ligand's state corresponds to the genotype, whereas its atomic coordinates correspond to the phenotype. In the case of molecular docking, the total interaction energy of the ligand with the protein is said to be fitness. And the fitness is evaluated using the energy function.[3]

We were performed GA docking of the receptor.

Note down of dock score was carried out.

8. RESULTS AND DISCUSSION

A. Pharmacological Screening

RESULT FOR ANTI-HIV ACTIVITY:

The main aim of studying the anti-HIV activity of the ethanolic and aqueous extract of *Brassica Oleracea* L. var. *italica* Plenck is to either isolate bioactive agents for direct use as anti-HIV drugs or to find bioactive compounds that can be used as a lead material in the preparation of semi-synthetic anti-HIV drugs. The current study makes use of an ethanolic as well as an aqueous extract of *Brassica Oleracea* L. var. *italica* Plenck. In this study, we performed putative anti-HIV activity i.e Enzyme pepsin inhibition assay and observed that an ethanolic and aqueous extract of *Brassica Oleracea* L. var. *italica* Plenck possesses anti-HIV property. Among the various concentrations of the ethanolic and aqueous extract of *Brassica Oleracea* L. var. *italica* Plenck, the percentage inhibition of both the extracts was found to be 82.52 and 81.55 % at concentration 100 µg/ml respectively.

Table.no.6- Anti-HIV activity of Ethanolic extract of *Brassica Oleracea* L. var. *italica* Plenck by using Enzyme pepsin inhibition assay.

Sr. no.	Compound	Concentration	Reading	% inhibition
1	**Control**		**0.309**	
2	Pepstatin (std)	100 µg/ml	0.070	77.34
3	Ethanol extract	20 µg/ml	0.078	74.75
4	Ethanol extract	40 µl/ml	0.071	77.02
5	Ethanol extract	60 µl/ml	0.065	78.96
6	Ethanol extract	80 µg/ml	0.059	80.90
7	Ethanol extract	100 µg/ml	0.054	82.52

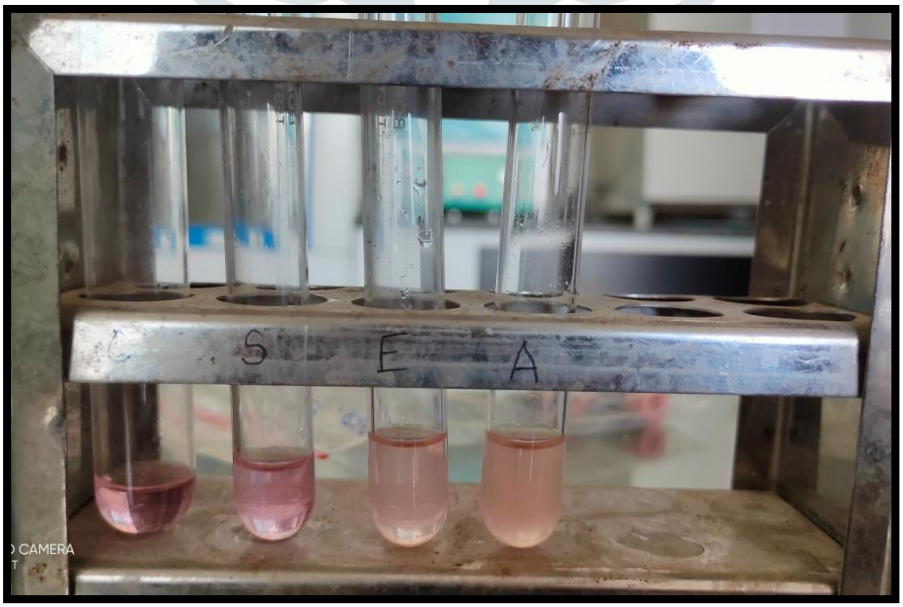

Fig.no.8- Enzyme pepsin inhibition assay

Table.no.7- Anti-HIV activity of Aqueous extract of *Brassica Oleracea* L. var. *italica* Plenck by using Enzyme pepsin inhibition assay.

Sr. no.	Compound	Concentration	Reading	% inhibition
1	**Control**		**0.309**	
2	Pepstatin (std)	100 µg/ml	0.070	77.34
3	Aqueous extract	20 µg/ml	0.076	75.40
4	Aqueous extract	40 µl/ml	0.072	76.69
5	Aqueous extract	60 µl/ml	0.068	77.99
6	Aqueous extract	80 µg/ml	0.062	79.93
7	Aqueous extract	100 µg/ml	0.057	81.55

Discussion:-

From the above anti-HIV activity i.e of Enzyme pepsin inhibition assay results obtained from table no. 6 and 7, the ethanolic and aqueous extract of *Brassica Oleracea* L. var. *italica* Plenck shows percentage inhibition at 82.52 and 81.55 % at concentration 100 µg/ml respectively. And when compared with the standard drug showed possible anti-HIV activity.

B. Docking Study :-

Table.no.8- Amino acid with type of interaction for anti-HIV activity

Sr.no.	Amino Acid	Atom of Ligand	Type of Interaction
1	**LYS101A**	**19O**	**HYDROGENBOND_INTERACTION**
2	**HIS235A**	**18O**	**HYDROGENBOND_INTERACTION**
3	**TYR188A**	**11C**	**AROMATIC_INTERACTION**
4	**TYR318A**	**1C**	**AROMATIC_INTERACTION**

Table.no.9- Result of GA Docking for 1FK9

Receptor	Drug/Ligand	Docking score
1FK9	Quercetin	-6.383849

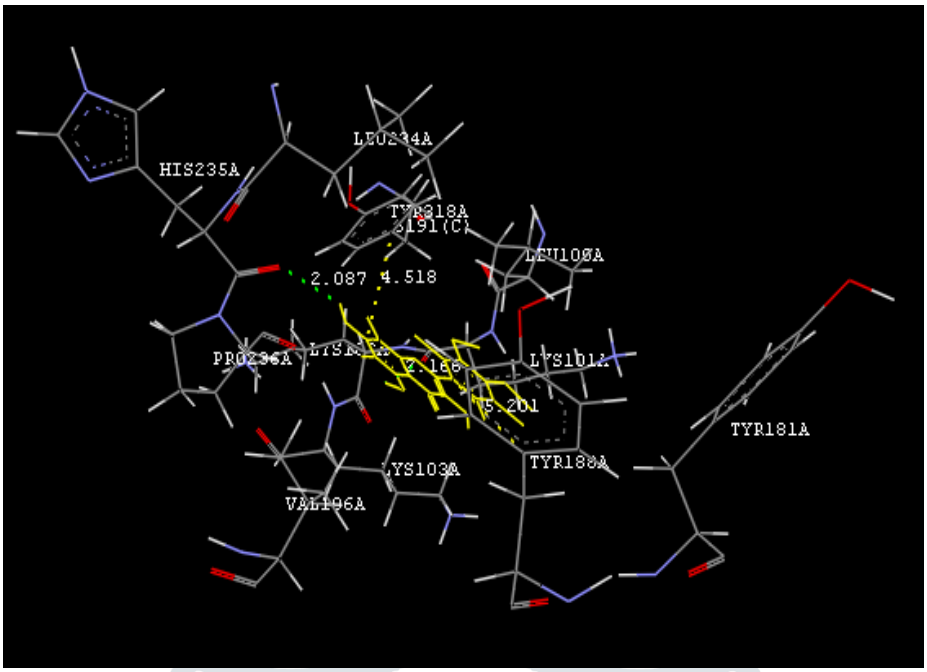

Fig.no.9- GA docking of 1FK9 and Quercetin

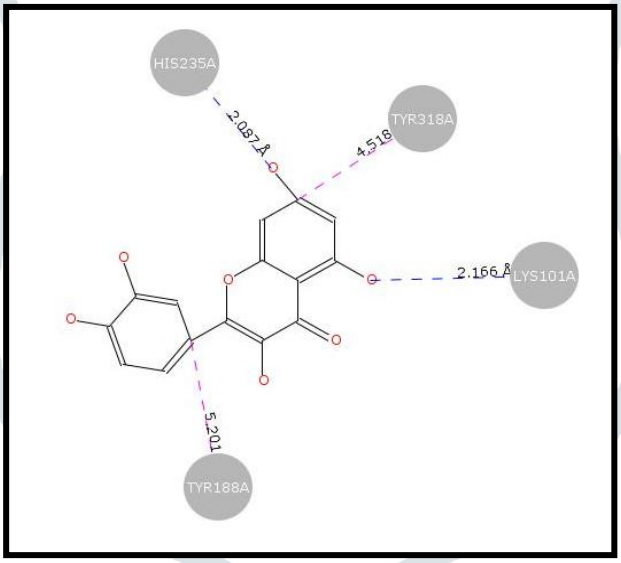

Fig.no.10- 2D representation of docking for Anti-HIV activity

9. CONCLUSION

Herbal extracts of broccoli was prepared by soxhlet apparatus and microwave assisted extraction using different solvents such as water and ethanol. Both Ethanolic & Aqueous extract of *Brassica Oleracea* L. var. *italica* Plenck exhibited good Anti-HIV activity. But ethanolic extract showed maximum Anti-HIV activity than aqueous extract by In-vitro method.

From GA docking study, quercetin exhibited -6.383849 dock score along with two hydrogen bond interactions & two aromatic interactions.

10. REFERENCES

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