International Journal on Science and Technology (IJSAT)
E-ISSN: 2229-7677 ● Website: www.ijsat.org ● Email: editor@ijsat.org

Investigating The Phytochemical Activity and Anti-Ebola Virus Properties of Corn Silk: In Silico

Aniket Kamble[1], Rajat Patil[2], Dikshant Kamble[3], Mansi Katwate[4], Pooja Dhale[5]

[1,3,4,5]Final Year B Pharmacy, [2]Assistant Professor
[1,2,3,4,5]Mahadevrao Wandre Institute of Technology, Turkewadi, Kolhapur.

ABSTRACT

Corn silk, a byproduct of maize cultivation (Zea Mays), is made up of thread-like stigma structures observed in the female flowers. It is often underutilized, serving as manure, agricultural waste, or animal feed. India produces 30 million tons annually. Rich in nutrients like fibers, proteins, vitamins, and bioactive compounds, corn silk also contains flavonoids (0.1% to 3%), exhibiting antioxidant, antibacterial, antidiabetic, and antifatigue properties. These components enhance its clinical and nutritional potential for diverse applications.

Ebola virus (EBOV) remains a global health threat due to its high mortality rate and lack of widely available antiviral treatments. Natural products, particularly flavonoids from medicinal plants, have shown promising antiviral properties. This study investigates the potential of bioactive compounds found in corn silk (Zea mays) as inhibitors of Ebola virus proteins, using in-silico methods such as molecular docking. Investigating quercetin's antiviral properties through in-silico research shows potential for creating innovative therapeutic approaches because of the life-threatening Ebola virus, which remains a serious risk to public health.

The Ebola virus remains a critical global health treat due to its high mortality rates and limited therapeutic options. In this study, an in silico approach was employed to evaluate the binding potential of Quercetin, a naturally occurring flavonoids which is found in Corn Silk, against a key Ebola virus protein using Schrödinger molecular modelling tools. The ligand was created and optimized using LigPrep, and the protein structure was refined using the Protein Preparation Wizard. Molecular docking was performed using Glide, revealing that Quercetin forms stable interactions with active site residues, including ARG247, THR249, SER246, supported by hydrophobic contacts with PHE248, ALA288, & TRP288. The docking results suggest a strong binding affinity and favourable positioning of Quercetin within the protein's active site. These results indicate quercetin's potential as an effective antiviral treatment for the Ebola virus.

KEY WORDS: Corn Silk, Ebola Virus, Flavonoids, Phytochemical, in-silico study, Quercetin

ABBREVIATIONS AND ACRONYMS

Abbreviations and Acronyms	Full form
EBOV	Ebola virus
PABA	Para amino benzoic acid
EVD	Ebola virus disease
EHF	Ebola hemorrhagic fever
UV-VIS spectroscopy	Ultra violet visible spectroscopy
Vit.	Vitamin
HCl	Hydrochloric acid
TFC	Total flavonoid content
PDB	Protein data bank
Kg/ha	Kilogram/hectare
cm	Centimeter
°C	Degree Celsius
mL	Milliliter
g	Gram
mgQE/100g	Milligram quercetin per 100 grams
nm	Nanometer

1. INTRODUCTION

1.1 OVERVIEW OF CORN SILK

Corn Silk is a byproduct of corn cultivation known as Maydis Stigma. Corn silk is made up of stigmas, which are yellowish, thread-like structures that are part of the female flower of maize (Zea mays).[1,2]
Due to its inefficient usage, corn silk is the major by-product of processing corn is often collected at an average rate of 123-283 kg/ha and utilized as manure, agricultural waste, or animal feed. With an annual production of 30 million tons and a global production of 1148 million tons, maize is the third most harvested crop in India. Corn makes about 10% of the nation's total food grain supply and is used in many industrial processes to produce paper, gum, textiles, extruded goods, protein, sweeteners, and medications.[3]
Corn silk contains abundant flavonoids. The total amount of flavonoid concentration ranges from less than 0.1% to 3%, with significant variation according to the variety. Flavonoid show a variety of biological activities, such as antioxidant, antibacterial, antidiabetic, and antifatigue, and also have some clinical applications. [1,4]

However, corn silk is a good source of essential nutrients, such as fibers, mucilage, resins, proteins, carbs, and vitamins and minerals. Furthermore, it contains a variety of bioactive substances, including steroids, volatile oil, and other naturally occurring antioxidants like flavonoids and polyphenols.[4]

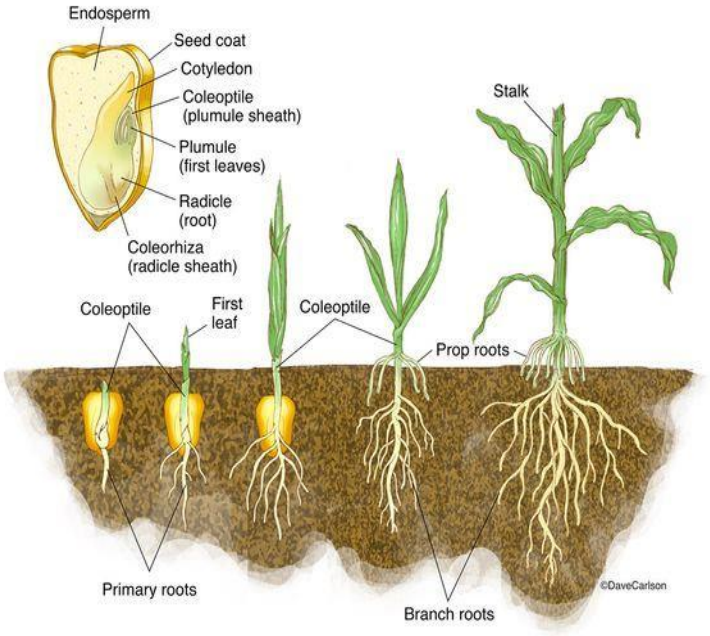


Fig. 1. Germination of Corn (Zea Mays)

1.2 HISTORICAL USE IN TRADITIONAL MEDICINE:

Since ancient times, corn silk has been used extensively in traditional medicine, especially in European, Chinese, and Native American herbal medicine. Corn silk's diuretic qualities have been used by many cultures to treat conditions involving the urinary system, inflammation and even diabetes.[5]

Corn silk, known for its cooling and detoxifying properties, has been part of Ayurvedic treatments for centuries.

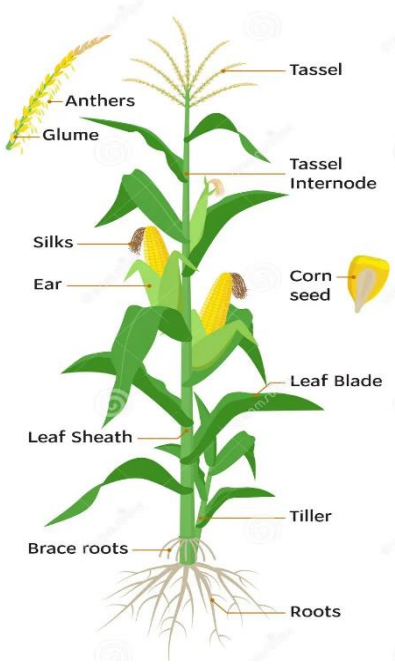


Fig. 2. Structures of Flavonoids derivatives

Urinary tract support: used to treat burning urination and bladder infections. Kidney stone prevention: Believed to help break kidney stones.

Detoxification: Thought to cleanse the liver and blood
Indigenous tribes in North America valued corn silk for its ability to treat urinary and kidney problems. During the 17th -19th centuries, European herbalists adopted corn silk for treating kidney related issues. In traditional Chinese medicine, corn silk has been used for centuries to clear dampness, reduce heat, and promote urination.

1.3 CHEMICAL CONSTITUENT:

Corn silk contains a variety of bioactive substances that have a major impact on human health, including alkaloids, saponins, organic acid, polysaccharides, flavonoids, cross fiber, and other compounds.[11]
It contains polyphenols such as tannins, saponins, flavonoids, alkaloids, steroids, cardiac glycosides, allantoins, anthocyanins, hesperidin and resins which boost digestion and brain health as well as protect against heart disease, type 2 diabetes and certain cancers. [12]
It contains phenolic acids like para-aminobenzoic acid (PABA), vanillic acid, p-coumaric acid, chlorogenic acid, protocatechuic acid, caffeic acid, ferulic acid, maizenic acid, hydroxycinnamic acid ester and 3-O-caffeoylquinic acid. Useful for prevent cellular damage due to free-radical oxidation reactions, promote anti-inflammatory conditions. [13]
It also contains carotenoids, steroids, tannins, volatile compounds, sugars, vitamins (C, K, E) which are useful in various healthcare conditions.
It contains also flavonoids, such as quercetin, luteolin, catechin, protocatechin, rutin, 3-hydroxy-1, 4-hydroxy, 5-hydroxy, 7-hydroxy flavones, isoflavones, C-3-deoxyglucosyle-3-methoxy luteolin and 6,4-dihydroxy-3-methoxyflavone-7-O-glucoside. It is important for human health because of their antioxidant, antiviral, and anti-inflammatory nature.[14]

Table 1 Phytochemical composition of Corn silk

Class of Phytochemical	Component of Phytochemical	Health benefits	Reference
Polyphenols	tannins, saponins, flavonoids, alkaloids, steroids, cardiac glycosides, allantoins, anthocyanins, hesperidin and resins	Boost digestion and brain health as well as protect against heart disease, type 2 diabetes and certain cancers	[12]
Phenolic acids	para-aminobenzoic acid (PABA), vanillic acid, p-coumaric acid, chlorogenic acid, protocatechuic acid, caffeic acid, ferulic acid, maizenic acid, hydroxycinnamic acid ester and 3-O-caffeoylquinic acid	Prevent cellular damage due to free-radical oxidation reactions, promote anti-inflammatory conditions	[13]
Flavonoids	quercetin, luteolin, catechin, protocatechin, rutin, 3-hydroxy-1, 4-hydroxy, 5-hydroxy, 7-hydroxy flavones, isoflavones, C-3-deoxyglucosyle-3-	Important for human health because of their antioxidant, antiviral, and anti-inflammatory nature	[14]

	methoxy luteolin and 6,4-dihydroxy-3-methoxyflavone-7-O-glucoside		
Carotenoids	Bata-carotene, zeaxanthin	Beneficial antioxidants that can protect from disease & enhance your immune system	[17]
Steroids	Phytosterols like stigmasterol, beta-sitosterol	Help to lower cholesterol, treat cancer & prevent heart attack	[15]
Tannins	Gallotannins, phlobatannins	To accelerate blood clotting, reduce BP, decrease serum lipid level, produce liver necrosis & modulate immune responses	[18]
Volatile compounds	Menthol, carvacrol, thymol, neo-iso-3-thujanol, cis-sabinene hydrate, cis-R-terpinol & neo-iso-3-thujanol	Helps to improve human health beyond flavoring properties & reduce the usage of chemical additives	[12]
Sugars	Dextrose, xylose	Effectives treatments for low blood sugar	[16]
Vitamins	Vitamin C, Vitamin K, Vitamin E,	Helps in shore up ones, heal wounds & boost your immune system	[19]

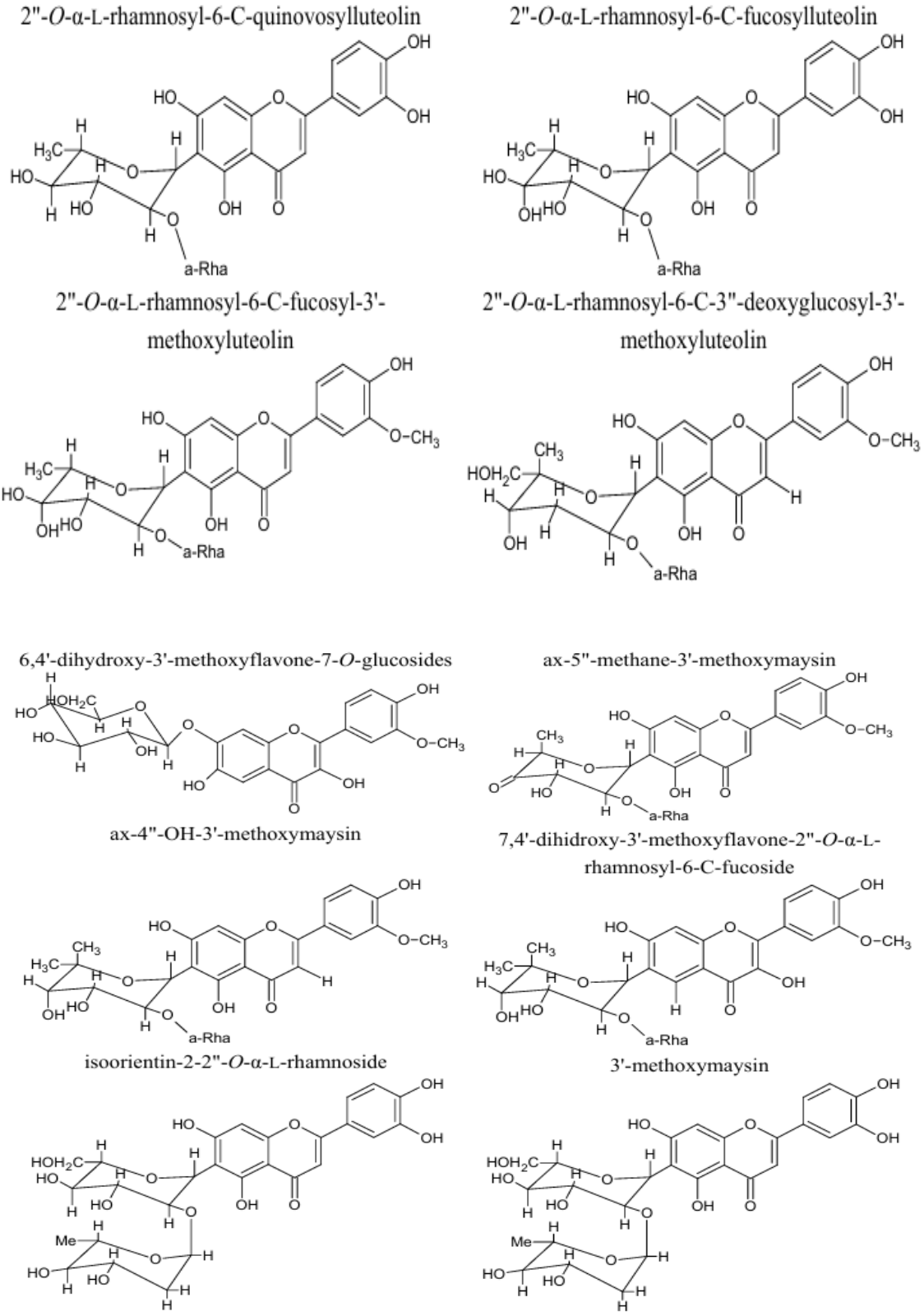


Fig 3. Structure of terpenoid compounds

1.4 NUTRITIONAL COMPOSITION OF CORN SILK:

Corn silk, particularly in its various forms, is a nutritionally rich agricultural byproduct with significant potential for functional food and medicinal applications. Variations in nutrient composition among different corn silk types indicate their suitability for different health benefits, including digestive health, cardiovascular support, and metabolic balance.

Key parameters such as moisture, lipid content, protein content, dietary/crude fiber, carbohydrate content and mineral composition were examined.

Table 2 Nutritional Composition of Corn Silk (puneet kaur, december 2022)

Variety	Moisture (%)	Lipid content	Protein content	Dietary/Crude fiber	Carbohydrate content	Mineral content
Corn silk	9.65-17.4%	0.29-4.74%	9.42-17.6%	7.34%	65.5-74.3%	Rich in Na, K, Ca, Mg, Fe, Zn, Mn & Cu
Processed corn silk	-	-	Crude Protein-13%	Crude fiber-13%	69%	-
Immature corn silk	89.13%	1.27%	12.96%	48.5%	27.8%	Rich source of Ca, Mg, Cu and Zn
Mature corn silk	84.4%	0.66%	8.95%	41.25%	29.4%	Rich source of Na, K, Fe and Mn
Corn silk powder	7.89%	0.55%	15.29%	-	56.16g	Rich source of Na, Mg, K, Ca, Cu, Fe, and Mn
Sweet corn silk	72.2%	3.5%	13%	67.9% hemicellulose, 31.4% cellulose, 0.7% lignin	41%	Rich in Mg, P and K, Vitamin A, D, E & K
Purple corn silk	89.14-89.57%	0.89-0.97%	3.74-4.01%	52.08-53.49%	0.06-0.68%	

1.5 EBOLA VIRUS: AN OVERVIEW

Ebola virus disease (EVD) is a serious and frequently lethal sickness caused by the Ebola virus. There are multiple species of the virus, some of which are more lethal than others, and it is a member of the *Filoviridae* family.[20]

Ebola is a viral hemorrhagic fever that affects humans and other primates and is caused by the Ebola viruses. It is also referred to as Ebola virus disease and Ebola hemorrhagic fever (EHF). After infection, symptoms usually appear two days to three weeks later.[20]

Usually, fever, headaches, sore throats, & muscles pain are the initial symptoms. Some people start bleeding internally and externally after these, which are typically followed by vomiting, diarrhoea, rash, and reduced liver and kidney function. Although it can kill up to 90% of infected individuals, it typically kills 50% of them. Direct contact with bodily fluids, such as blood from infected humans or other animals, or interaction with interns who have just come into touch with contaminated bodily fluids are two ways that the virus is disseminated.[20]

1.6 HISTORY OF EBOLA VIRUS:

Ebola was first discovered in 1976 during two concurrent outbreaks in the South Sudanese town of Nzara and the Democratic Republic of the Congo village of Yambuku, which is close to the Ebola River and is where the illness got its name. In sub-Saharan Africa's tropical regions, Ebola epidemics happen sporadically. The World Health Organization reports that there were 24 Ebola outbreaks between 1976 and 2012, resulting in 2,387 cases and 1,590 deaths overall. [20,21] West Africa experienced the greatest Ebola outbreak to date, with 28,646 cases and 11,323 deaths between December 2013 to January 2016. [22,23,24] It was declared no longer an emergency on the 29th March 2016.[25] In May 2017 and 2018, other outbreaks in Africa started in the Democratic Republic of the Congo. [26,27,28] The Congo Ebola epidemic was declared a global health emergency by World Health Organization in July 2019.[29]

1.7 TRANSMISSION OF EBOLA VIRUS:

Ebola disease spreads through direct contact with blood or body fluids, including saliva, mucus, vomit, feces, sweat, tears, breast milk, urine, and semen. The virus enters through the nose, mouth, eyes, open wounds, cuts, and abrasions. Large droplets may occur when a person is very sick, and contact with contaminated surfaces or objects, particularly needles and syringes, may also transmit the infection.[30]

Ebola Virus can persist in semen for over three months after recovery, potentially leading to sexual intercourse infections. It may also be found in breast milk and the eye, making it uncertain when to breastfeed again. Recovered individuals are not infectious.

Ebola infections pose a law risk in countries with proper medical systems, but unprotected contact with infected corpses during burial rituals can lead to widespread infections. Symptoms often require assistance, and 69% of cases in Guinea occurred during this period.

Healthcare workers treating Ebola patients face the highest risk of infection due to inadequate protective clothing, improper handling, and poor health systems. Transmission has occurred in African countries with reused needles, and some centers lack running water. Inadequate training and procedures have been criticized.[32]

EBOV transmission during EVD outbreaks is not reported, with airborne transmission only observed in strict laboratory conditions. No spread by water, food, or insects has been observed.

Ebola Virus transmission among humans is theoretically possible due to saliva particles, but the actual risk is low. Studies suggest that pigs with Ebola Virus disease (EVD) can spread the virus through droplets in the air or on the ground when they sneeze or cough. Primates accumulate the virus in their blood but not in their lungs, leading to pig-to-primate transmission without physical contact.

Fig.4. Transmission of Ebola Virus

Ebola spread from animals to humans through direct contact with infected wild animals of bats. Animals may become infected by eating partially eaten fruit by bats. Domestic dogs and pigs can also be infected, but their role in spreading the disease to humans is unclear. Factor such as fruit production and animal behavior may trigger outbreaks.[31]

1.8 PATHOLOGY:

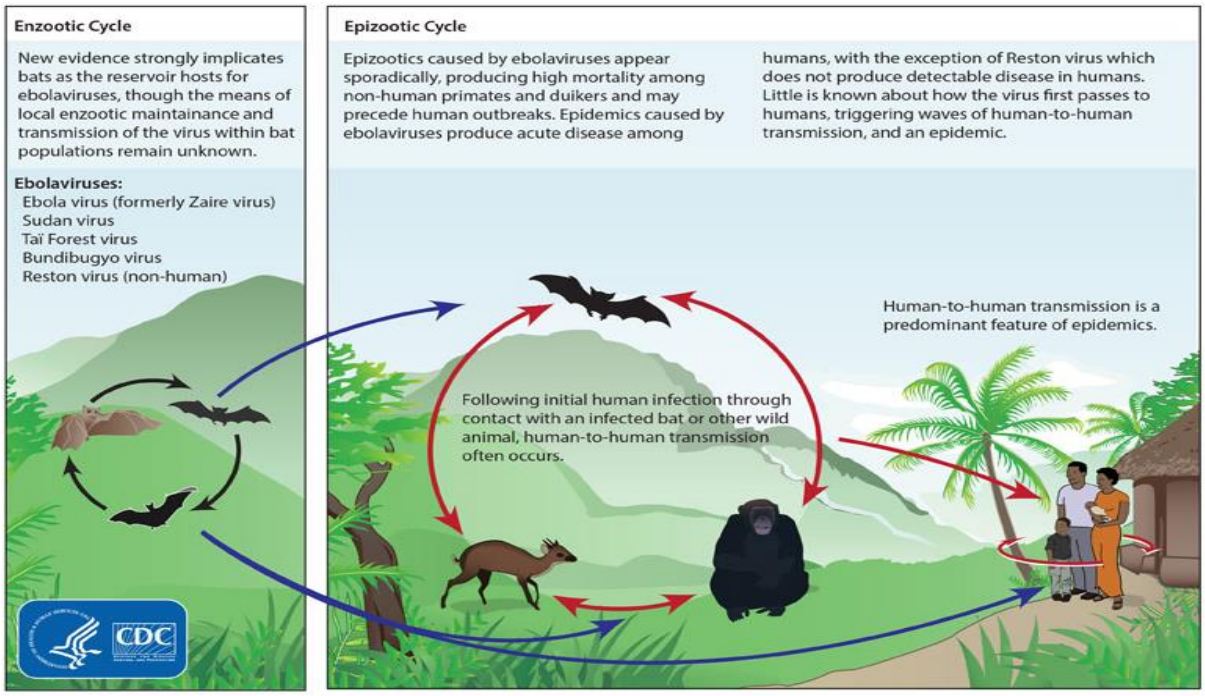


Fig 4.

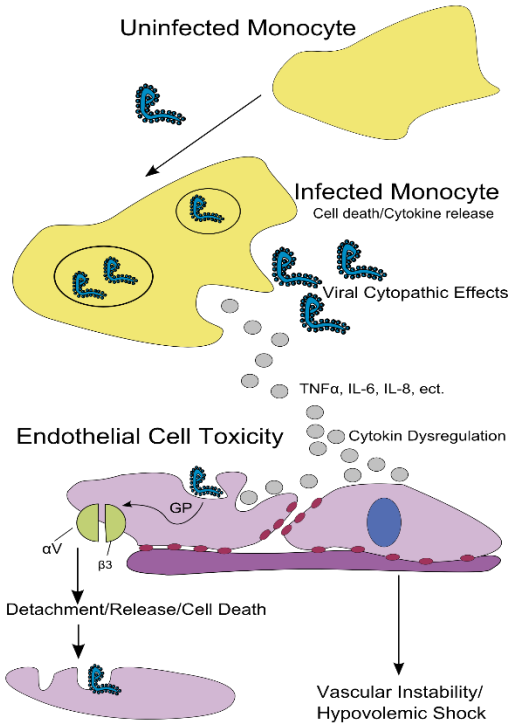


Fig.5. Pathology of Ebola Virus

Ebola Virus (EBOV) is a highly efficient virus that replicates in various cells, including monocytes, macrophages, dendritic cells, liver cells, fibroblasts, and adrenal gland cells. It infects humans through contact with mucous membranes or skin breaks, targeting endothelial cells, liver cells, and immune cells like macrophages, monocytes, and dendritic cells. The virus then enters the bloodstream and lymphatic system, spreading throughout the body. The virus's glycoproteins, such as Ebola virus glycoprotein (GP), cause damage to endothelial cells, liver damage, and improper clotting. The virus also disrupts cellular metabolism, allowing it to evade the immune system. EBOV proteins also interfere with the human immune system's response to viral infections by interfering with the production and response of interferon proteins. The VP24 and VP35 structural proteins of EBOV to play a crucial role in this interference, blocking the production of antiviral proteins of EBOV to spread quickly throughout the body. [33,34,35]

2. OBJECTIVES

AIM:

Aim of the study is **"INVESTIGATING OF PHYTOCHEMICAL PROFILE AND ANTI-EBOLA VIRUS ACTIVITY OF CORN SILK: *IN SILICO* STUDY"**

RESEARCH OBJECTIVES

2.2.1 Preparation of herb extract

- To collect fresh corn silk of Zea mays and authenticate the same.
- To prepare fine powder of the procured sample
- To obtain ethanolic herb extract by Soxhlet extraction method

2.2.2 Pre-formulation studies

- Phytochemical screening of extract (flavonoids)
- Quantification of herb extract by UV-VIS spectroscopy

2.2.3 Post formulation studies

- To investigate the Anti-Ebola activity of corn silk by the in-silico studies
- Determine which compounds may have antiviral properties that could be effective against the Ebola virus.

2.2.4 Evaluation & Result

In-silico anti-viral studies

3. METHODOLOGY

MATERIALS

The following materials used were of either analytical grade, food grade, or the best possible pharma grade available as supplied by the manufacturer.

Table 3 List of chemicals use with the manufacturer

Sr. No.	Materials	Manufacturer
1.	Corn Silk extract	Local field of Chandgad, Maharashtra
2.	Ethanol	Labline Laboratories

EQUIPMENTS USED

Table 4 List of equipments with their manufacturer

Sr. No.	Instruments	Manufacturer
1.	Electronic Weighing Balance	Labline Laboratories

2.	Soxhlet Apparatus	Universal Scientific, Bangalore, Karnataka.
3.	Hot Air Oven	Biotech India Ltd. PSI030 hot air oven
4.	UV Spectrophotometer	Shimadzu, Japan
5.	High Performance Liquid Chromatography (HPLC)	Shimadzu, Japan

3.3 METHODOLOGY

1. Sample collection and sample preparation
2. Extraction of sample (Corn Silk)
3. Phytochemical Investigation
4. Evaluation
5. Result

DRUG PROFILE OF CORN SILK (STIGMA MAYDIS)

Biological Source:

Fig.6. CORN SILK

The biological source of Corn Silk (*Stigma Maydis*) is the stigma and female flowers of maize (*Zea Mays Linn*), which belongs to the family *Poaceae*. (puneet kaur, december 2022)

Morphological Features:

Fresh corn silk is greenish-yellow to light green, but as it matures or dries, it turns yellowish-brown to dark brown. It is long thread-like structures and smooth, soft and silky to touch. When it dried, it becomes coarse, fibrous, and bittle.

Each thread (style) can be about 10-30 cm long. It has a slight, characteristic odor; when it dried, it has a faint, hay-like odor. It has bland or slightly sweet taste when chewed fresh. Corn silk emerges in clusters from the tip of the ear and extends outside the husk. (puneet kaur, december 2022)

Synonyms:

Hindi (Makka ke reshe), Sanskrit (Yavakanthakah, Madhurakesharam), English (Corn silk, Maize silk, Stigma & style of maize) Telugu (Makkajonna tutha), Bengali (Bhutter reshmi, Makai reshmi), Cambodian (Sorsay poat), Tamil (Cholam mudi), Gujarati (Makaaina resha), Kannada (Jola reshe), Thai (Mai khao phot)

Ecology:

Corn silk comes from maize (Zea mays), which is a tropical and subtropical plant but also grow in temperature regions. Native to Central America and Mexico, but now extensively cultivated worldwide including India, USA, China, Brazil, Africa, and many other regions. It requires a warm climate with a temperature range of 18ºC to 27ºC. Grow best in fertile loamy soils that are well-drained and high in organic matter. (puneet kaur, december 2022)

Taxonomy:

- Kingdom: Plantae
- Sub-kingdom: Tracheobionta (vascular plants)
- Division: Magnoliophyte (Angiosperms)
- Class: Liliopsida (Monocotyledons)
- Order: Poales
- Family: Poaceae (Gramineae)
- Genus: Zea
- Species: Zea mays Linn

Chemical Constituents:

Corn Silk	Maysin, Luteolin, Apigenin, Quercetin, Rutin, Steroidal saponins, Alkaloids, Vit. C, Vit. K, Potassium, Calcium, Magnesium, Phosphorus, Polysaccharides, Sitosterol, Stigmasterol, Campesterol, Tannins, Volatile oil

Uses:

- **Diuretic:**
  Used to increase urine output and treat conditions like urinary tract infections, cystitis, and kidney stones.
- **Anti-inflammatory:**
  Helps in reducing inflammation in urinary tract and other tissues.
- **Antioxidant:**
  Rich in flavonoids and vitamin C, it helps neutralize free radicals, protecting cells from oxidative damage.

- **Hypoglycemic agent:**
  Used to help lower blood sugar level on case of diabetes mellitus
- **Blood pressure regulation:**
  The potassium content helps in managing hypertension by acting as a mild vasodilator and diuretic.
- **Liver tonic:**
  Used to detoxify the liver and improve liver function in traditional medicine.

- **Supports prostate health:**
  Traditionally used to treat men's prostate enlargement problems.

3.3.1 Sample collection and Sample preparation:

Selection and collection of Corn:

Samples of Zea mays corn silk are obtained from an agricultural farm. Choose healthy corn plant are free from disease, pests, and chemical contamination. Choose organically farmed corn for medical or research applications. Collect samples during the silking stage, when the corn silk is fresh and yellowish in color. The best time to collect corn silk is early morning or late afternoon to avoid moisture loss. Collection is done when the silk is fresh (yellowish-green to golden color), before it turns brown and dries. (labomir lapcik, May 2023)

Method of collection:

Open the corn husk gently so that the kernels are not damaged. Gently pull out the silk strands from the cob using clean hands. Avoid collecting silk that is already dried or brown as its potency may be reduced. Collect a sufficient quantity based on the study or purpose. Store in clean, labelled paper bags or perforated plastic bags for transportation. Do not mix silk from different sources if testing for purity. Ensure hands, tools, and collection containers are clean and free from chemicals. (labomir lapcik, May 2023)

3.3.2 Authentication of the plant

The fresh corn silk (Stigma maydis) originated from *Zea mays* Linn (family: Poaceae) authenticated by concerning authority.

A. Sample preparation of Corn Silk

After collection, the corn silk must be thoroughly prepared for analysis, storage, or usage. The preparation process includes cleaning, drying, grinding, and storage.

Cleaning of Corn Silk:

Remove any contaminants, such dust, insects, or pieces of husk. Fresh silk was cleaned with tap water and drenched with distilled water. Pat dry with a clean cotton cloth or allow it to air dry under shade. (labomir lapcik, May 2023)

B. Drying process:

Drying is essential to preserve the bioactive compounds in corn silk. Spread the corn silk thinly across a clean dying tray or mesh. Place in a well-ventilated, shaded area with no direct sunlight to prevent degradation of active compounds. Allow to dry for 5-7 days, turning it occasionally for uniform drying. After drying, the ideal moisture content is less than 10% to avoid microbial growth and ensure a long shelf life. (labomir lapcik, May 2023)

C. Grinding:

a) b) c)

Fig.7. Powdered corn silk processing: a) Corn silk during the silking stage, b) corn silk after harvest c) corn silk powder

Once completely dried, corn silk can be ground into powder using a laboratory grinder or mortar and pestle. For extraction or analysis, the powder needs to be homogeneous and fine. To achieve a consistent particle size, use a mesh sieve. (labomir lapcik, May 2023)

D. Storage of Corn Silk samples:

Whole dried silk: Store in airtight glass containers, paper bags, or vacuum-sealed bags
Powdered form: Store in moisture-proof, light-resistant containers to maintain potency.
Keep in a cool, dark place (temperature: 10-25ºC; relative humidity: <50%).
Properly labelled with collection date, location, drying method, and storage conditions. (labomir lapcik, May 2023)

3.4 EXTRACTION OF CORN SILK USING SOXHLET APPARATUS:

The Soxhlet extraction method is widely used for extracting bioactive compounds from plant material, including corn silk (Zea Mays). The solvent is continually passed through the sample in this procedure, which guarantees optimal efficiency.

Principle of Soxhlet extraction:

The principle of Soxhlet extraction is continuous solvent reflux and percolation. The solvent repeatedly dissolves the soluble components from the corn silk and collects in a separate chamber, allowing for high-yield extraction of bioactive compounds. Soxhlet extraction is an efficient method for obtaining high purity corn silk extract.

Material and equipment required:

A. Material:

- Dried and powdered corn silk (15g)
- Solvent (Ethanol- 300mL)
- Filter paper or cotton wool

B. Equipment

- Soxhlet apparatus
- Water bath or Heating mantle

- Rotary evaporator (For solvent recovery)
- Analytical balance (for weighing the sample)
- Beaker and glassware for handling extract

Extraction Procedure: (labomir lapcik, May 2023)

Step 1: Preparation of corn silk sample

Ensure the corn silk is dried properly (moisture content below 10%) to prevent microbial growth. Use a grinder or mortar and pestle to obtain a fine powder (sieve through 80 mesh for uniformity). Accurately weigh 10-50g of powdered corn silk using an analytical balance.

Step 2: Setting up the Soxhlet apparatus

Place the weighed corn silk powder (15g) inside a thimble or wrapped in filter paper. Insert it into the Soxhlet extractor chamber. Add pure ethanol as a solvent (300ml) into the round bottom flask. Attach the round-bottom flask to the extraction chamber. Attach the condenser on top to cool the evaporated solvent.

Step 3: Extraction process

Place the round-bottom flask on the heating mantle or in a water bath. Maintain a temperature just below the boiling point the of the solvent (ethanol ~78ºC). The solvent vaporizes, travels up into the condenser into the extraction chamber. It percolates through the corn silk, dissolving bioactive compounds. The liquid syphons back into the flask after the extraction chamber is filled. Allow 4-6 hours of the continuous reflux for efficient extraction. Ensure the solvent remains in constant circulation. (labomir lapcik, May 2023)

Step 4: Solvent recovery and concentration

When the cycle is complete, turn off the heat and allow the setup to cool. Filter the extracted solution using Whatman filter paper. Use rotary evaporator at reduced pressure to remove the solvent and concentrate the extract. As an alternative, evaporate the solvent in a low-temperature water bath. Store the extract in an airtight, amber-colored bottle at 4ºC to prevent degradation.

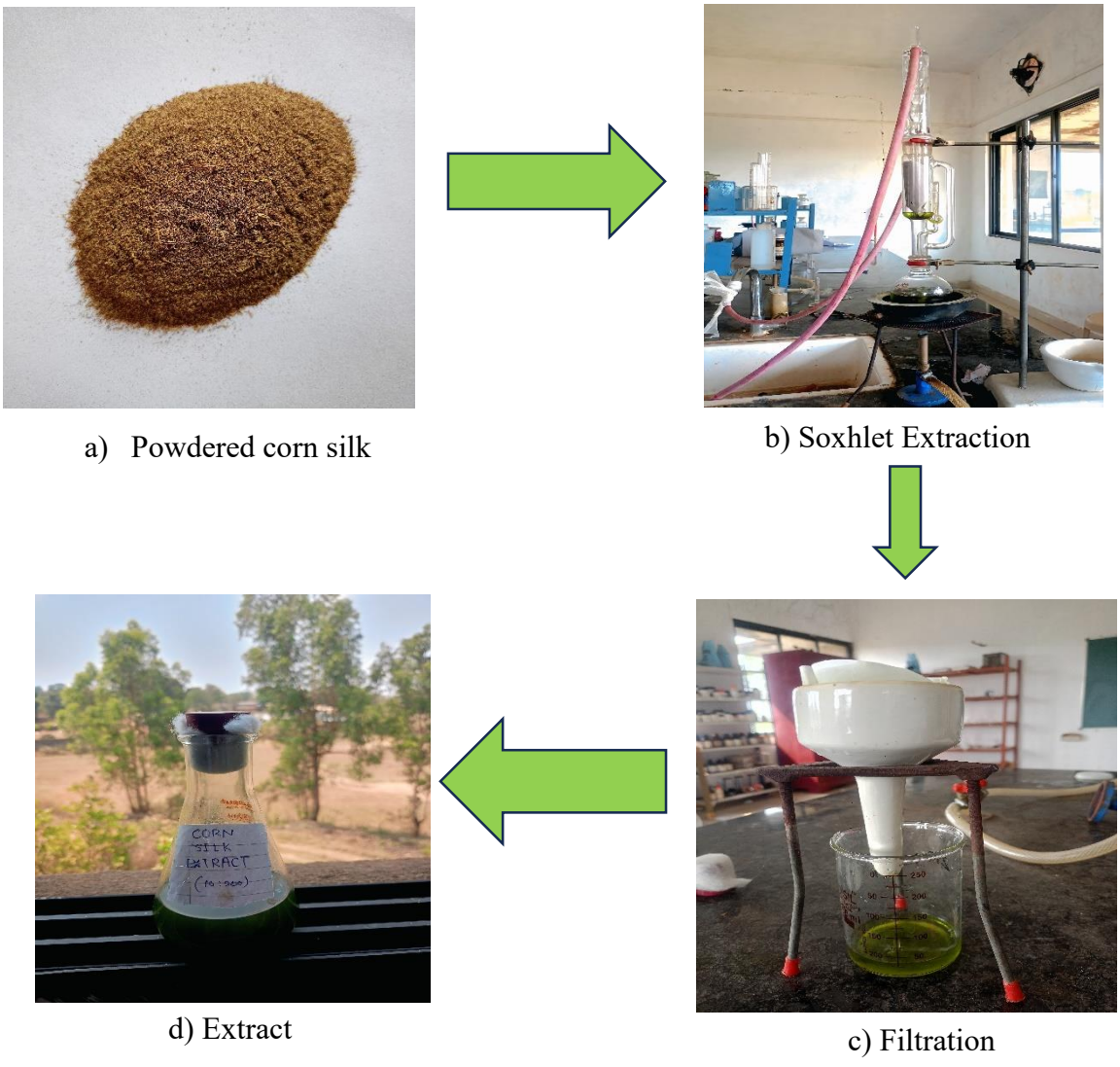


Fig.8. Process of Extraction

3.5 PHYTOCHEMICAL SCREENING OF CORN SILK OF *Zea mays Linn*

Phytochemical investigation was performed on corn silk extract in order to identify its chemical components through both qualitative and quantitative examination.

Qualitative and quantitative analysis are used to find out the chemical composition, bioactive components, and concentration of important phytochemicals.

Table 5 Phytochemical Tests

Test	Observation	Inference
Test for Flavonoids		
SHINODA TEST- 1 ml of Extract + few magnesium ribbons + conc. HCl	Red or pink coloration	Flavonoid Present

FERRIC CHLORIDE TEST- Extract + Few drops of 10% w/v ferric chloride solution	Blackish green coloration	Flavonoids Present
Test for Alkaloids		
DRAGENDORFF'S TEST- Extract + few drops of Dragendorff's reagent	Reddish brown precipitate	Alkaloids Present
MAYER'S TEST- Extract + Mayer's reagent	Creamy white precipitate	Alkaloids Present
Test for Tannins		
FERRIC CHLORIDE TEST- Extract + few drops of ferric chloride	Blue-black or greenish coloration	Tannins Present
Test for Saponins		
FOAM TEST- Extract is shake with water	Persistent froth formation	Saponins Present
Test for Terpenoids		
SALKOWSKI TEST- Extract + chloroform + conc. Sulfuric acid	Reddish brown coloration at the interface	Terpenoids Present

3.6 QUANTIFICATION OF PLANT EXTRACT BY UV SPECTROSCOPY

a) Total Flavonoid Content (TFC):

Two milliliters of corn silk extract solution are mixed with two milliliters of 5% sodium nitrite. 0.2 mL of aluminium chloride was then added and stirred well. A 0.1M of Sodium hydroxide (2mL) added & the sample is kept aside for 6 minutes. In the solution 0.275 mL of distilled water is added and check absorbance at 510 nm by UV-Vis spectrophotometer. Flavonoid content is calculated in milligrams of quercetin equivalent (QE), using quercetin as the standard curve. per extract gram (mgQE/100g).

4. IN-SILICO STUDY OF QUERCETIN (FLAVONOID) AGAINST EBOLA VIRUS:

Quercetin, a natural flavonoid found in numerous fruits and vegetables, has attracted significant attention for its potential medicinal benefits against viral infections. Investigating quercetin's antiviral properties through in-silico research shows possibility for creating innovative therapeutic approaches because of the life-threatening Ebola virus, which remains a serious risk to public health. This article explores quercetin's in-silico research against the Ebola virus, looking at its mechanisms of action, molecular docking studies, possible uses in treating the disease, as well as its limits and future directions. This study intends to provide important insights into the fight against this fatal infectious disease by computationally clarifying the interactions between quercetin and Ebola virus proteins.

METHODOLOGY:

The study employed molecular docking simulations using Schrodinger software to analyze the binding interactions between Quercetin and target Ebola virus proteins, such as VP35 and glycoproteins. The protein structural data were retrieved from protein data bank (PDB), and ligand preparation was performed using PubChem and Schrodinger software.

Protein and Ligand preparation:

The 3D structure of Ebola virus glycoprotein (PDB ID – 5JQ3) were retrieved from the Protein Data Bank (https://www.rcsb.org) essential for viral replication.

The protein preparation wizard can be used to reduce the protein structure, replace missing side chains or loops, optimize the H-bond network, remove water molecules, add missing hydrogen atoms, and assign the correct charges (using Gasteiger charges). Define the active site. The file should be saved in PDB format.

Get 2D structure of Quercetin from PubChem (https://pubchem.ncbi.nlm.nih.gov) (PubChem CID-5280343) Use LigPrep (Schrodinger) to generate 3D conformations, optimize geometry, assign protonation states, and minimizes energy.

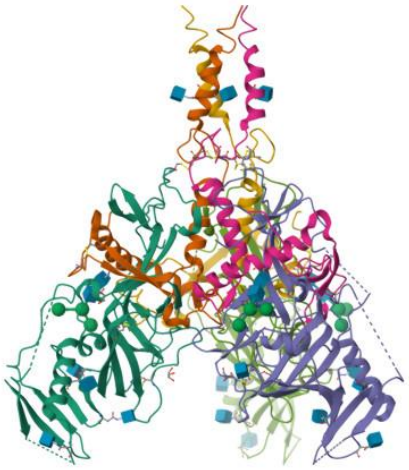


Fig. 9. Ebola Virus Glycoprotein

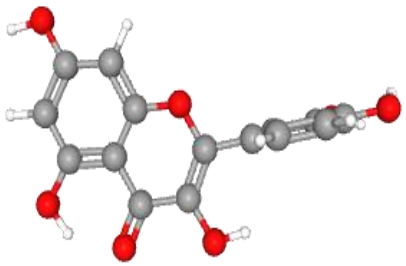


Fig.10. 3D structure of Quercetin

Molecular docking

Define the active binding site using the co-crystallized ligand or active site residues. Generate a receptor grid using Glide. Perform Glide Docking (standard precision [SP] or extra precision [XP]) of Quercetin into the prepared receptor grid. Examine or analyse binding positions, docking scores, and important molecular interactions.

Post-Docking Analysis: Visualize docking poses. Identify H-bonds, hydrophobic interactions, and π-π stacking between Quercetin and the protein.

5. RESULT & DISCUSSION

PHYTOCHEMICAL SCREENING:

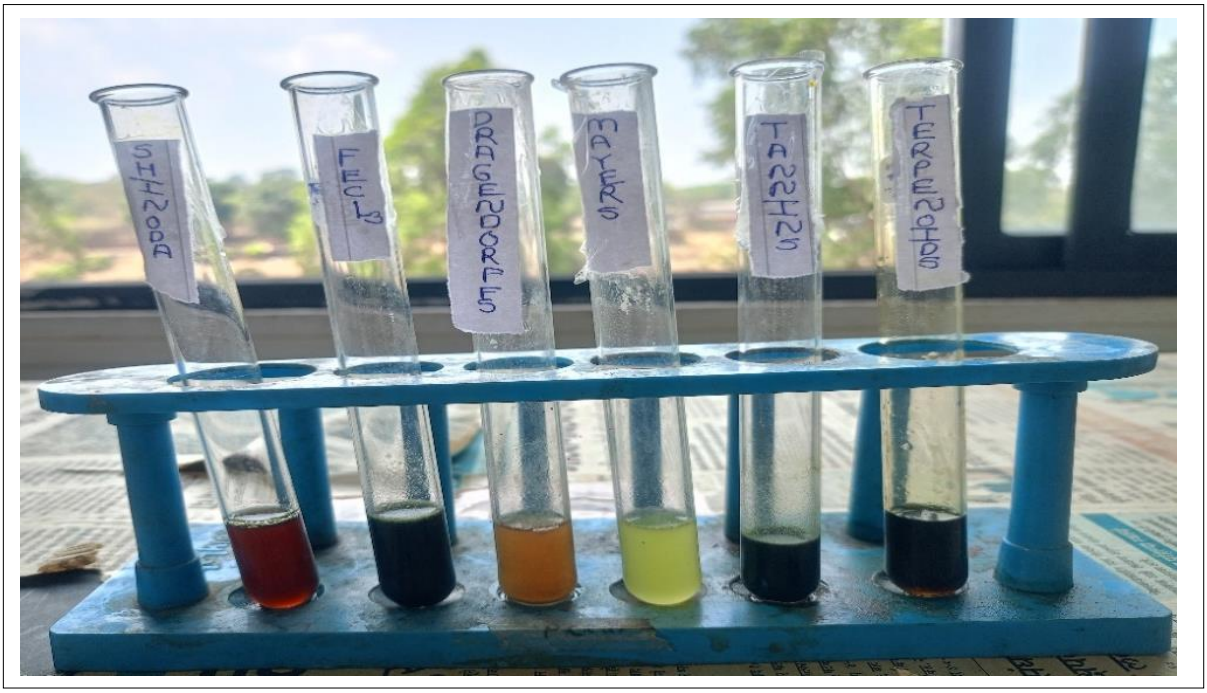


Fig.9. Phytochemical Screening of plant extract

Table 6 Result of phytochemical screening of plant extract

Sr. No.	Test	Observation	Report
1.	Test for Flavonoid		
	Shinoda test	Red color appears	Presence of flavonoids
	Ferric chloride test	Blackish green coloration	Presence of flavonoids
2.	Test for alkaloids		
	Dragendorff's test	Reddish brown color	Presence of alkaloids
	Mayer's test	Creamy white precipitate	Presence of alkaloids
3.	Test for Tannins	Greenish color	Presence of tannins
4.	Test for Saponins	No froth formation	Absence of Saponins
5.	Tast for Terpenoids	Reddish brown color at the interface	Presence of terpenoids

The phytochemical screening reveals that the sample contains flavonoids, alkaloids, tannins, & terpenoids, while saponins are absent. The rich presence of bioactive compounds suggests that the sample could possess significant pharmacological activities, making promising candidate for further biological evaluation and medicinal use.

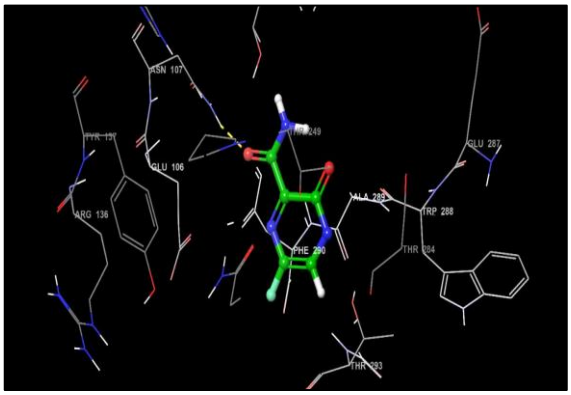
QUANTIFICATION OF HERBAL EXTRACT BY UV SPECTROSCOPY:

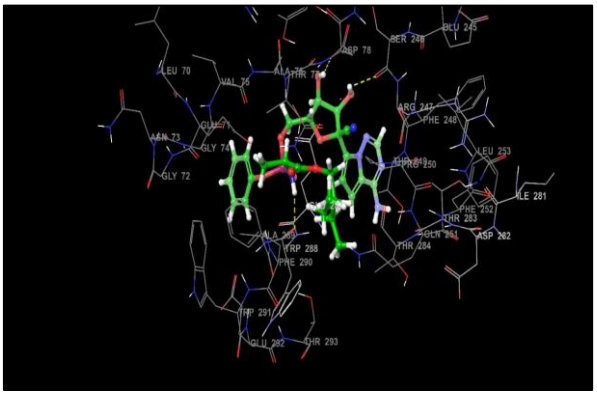
Total flavonoid content (TFC):

Corn silk is frequently considered a good source of antioxidant content in which include polyphenolic composites, flavonoids, and ascorbates. These factors give quality and nutritive value, as well as anti-

inflammatory, anti-diabetic, antiviral, and antioxidant properties, which are important in human fitness. Medicinal plant secondary metabolites act as low molecular weight antioxidants, although their mechanisms of action vary depending on the environment and structure. According to the current findings, corn silk has a high flavonoid concentration 163.93 ± 0.83 mg QE/100g.

IN-SILICO STUDY:





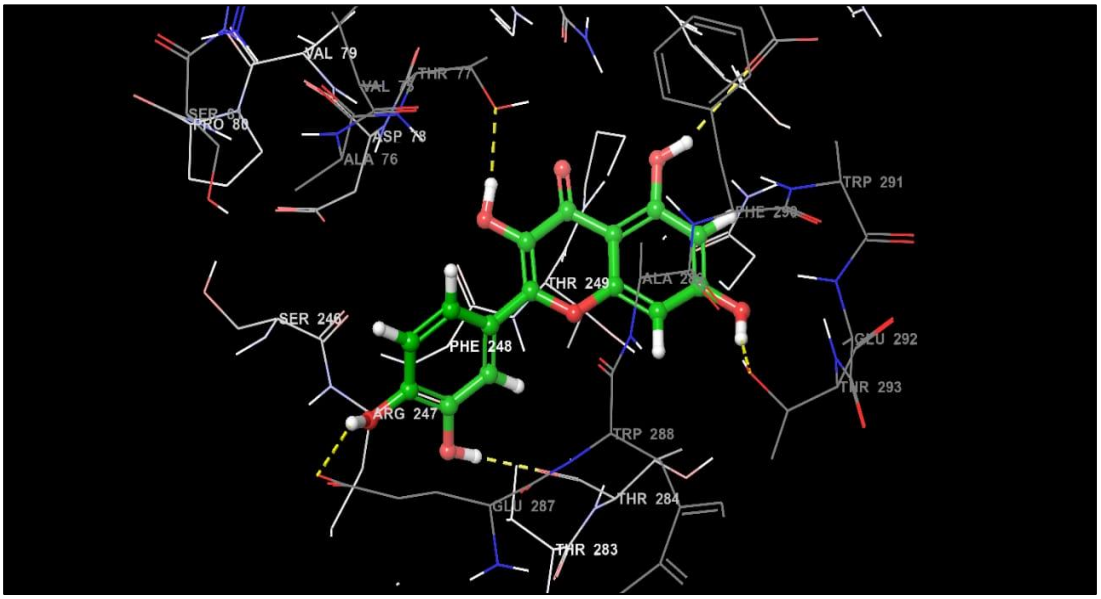
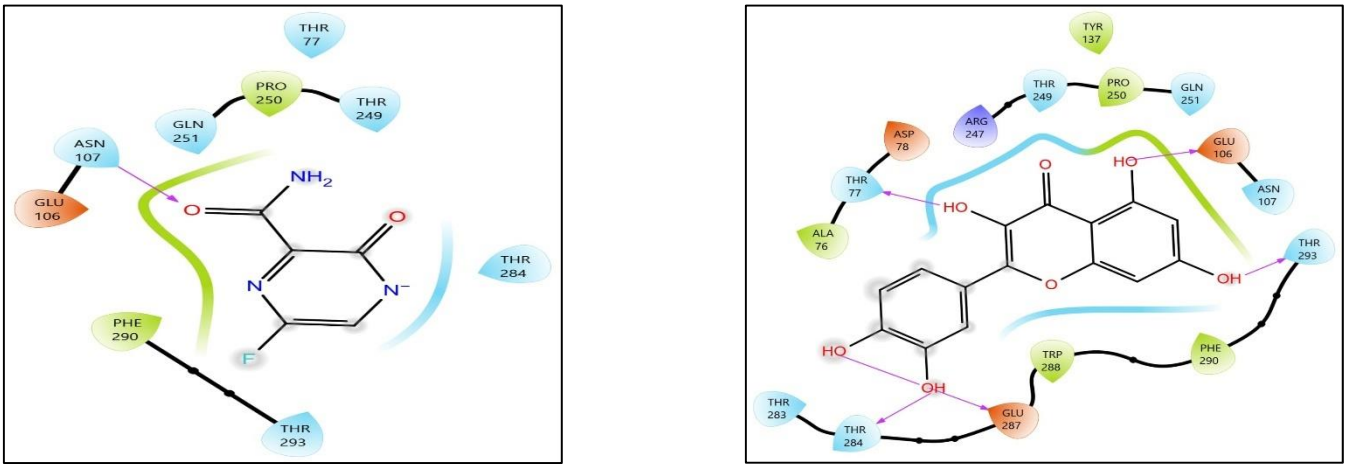


Fig.10. 3D binding orientation of Quercetin

The molecular docking study demonstrated that Quercetin binds effectively within the active site of the Ebola virus target protein. The 3D docking visualising revealed multiple hydrogen bonds formed between Quercetin and key residues, including ARG247, THR249, and SER246, contributing significantly to the stability of the ligand-protein complex. Additionally, hydrophobic interactions with residues such as PHE248, ALA288, and TRP288 further enhanced binding affinity.

The ligand is well-accommodated within the binding pocket, exhibiting an optimal orientation and a network of stabilizing interactions. These results indicate that quercetin may work as a possible inhibitor by efficiently occupying and interacting with the Ebola virus protein's active site, thereby impairing its biological activity.



(a) (b)

Fig.11. 2D binding orientation of a) Binding interaction of Quercetin, b) Ebola virus glycoprotein pocket (PDB ID: 5JQ3)

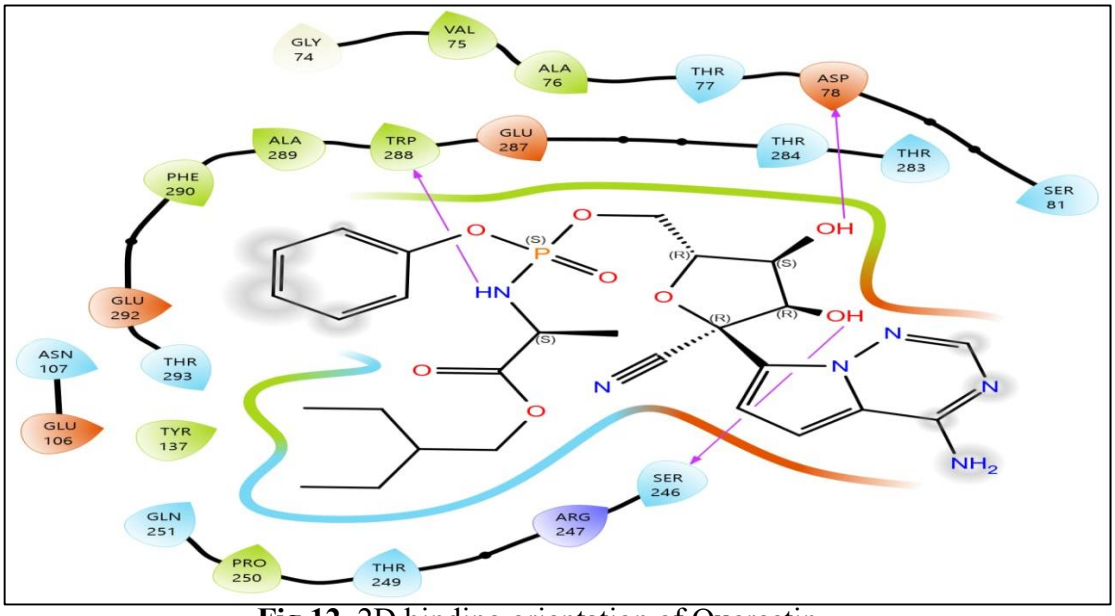


Fig.12. 2D binding orientation of Quercetin

In this in silico study, Quercetin extracted was docked against the Ebola virus glycoprotein. The 2D interaction analysis revealed that Quercetin forms strong hydrogen bonds with key active site residues, including ASP78, TRP288, SER246, & THR249. Hydrophobic interactions with residues such as GLU287, PHE290, & ARG247 further contributed to the stability of the complex. The binding orientation of Quercetin showed a deep fitting into the glycoprotein's active pocket, supported by a favourable network of polar and non-polar interactions. These results suggest that Quercetin has significant potential as an Ebola virus glycoprotein inhibitor, and could be considered a promising compound for further antiviral drug development.

Table 7 Key residues obtained after the interaction analysis

Interaction type	Residues Involved
Hydrogen bonds	ARG247, THR249, SER246
Hydrophobic contacts	PHE248, ALA288, TRP288, GLU287, PHE290

DISCUSSION

The results in this in silico study highlight Quercetin as a promising inhibitor of the Ebola virus glycoprotein, based on its strong and specific binding interactions within the glycoprotein's active site. Quercetin forms several hydrogen bonds with important residues ASP78, TRP288, SER246, and THR249, according to molecular docking. These interactions suggest a high degree of affinity and specificity, which are essential for effective inhibition of viral entry mechanisms mediated by the glycoprotein.

In addition to polar interactions, hydrophobic contacts involving residues such as GLU287, PHE290, and ARG247 further stabilizes the ligand-protein complex. This combination of hydrogen bonding and hydrophobic interactions suggest that Quercetin can effectively engage with the polar and nonpolar regions of the active site, increasing its overall binding efficiency.

The binding exposure of Quercetin also indicates a deep insertion into the glycoprotein's active pocket. Such a conformation is advantageous, as it implies that Quercetin can occupy and potentially block critical functional regions of the glycoprotein necessary for host cell attachment and viral fusion. The fitting of the molecule is supported by a favourable interaction network, reinforcing the hypothesis that Quercetin can interfere with glycoprotein function.

Overall, these findings suggest that Quercetin possesses favourable structural and chemical properties to serve as a lead compound for further antiviral drug development targeting Ebola virus. Still, it's important to admit the limitations of computational studies. While in silico docking provides valuable insights into binding potential and interaction profile; experimental validation through in vitro and in vivo assays is essential to confirm biological efficacy and pharmacokinetic behaviour.

Future research should focus on the experimental evaluation of Quercetin's antiviral activity against the Ebola virus, as well as possible chemical modifications to enhance its potency, bioavailability, and selectivity.

CONCLUSION

The in silico docking study revealed that Quercetin, a flavonoid extracted from corn silk, exhibits a strong binding affinity towards the Ebola virus glycoprotein. According to the 2D and 3D interaction investigations, quercetin exhibits strong hydrophobic interactions with TRP288, PHE248, and GLU287 as well as stable hydrogen bonds with significant amino acid residues such ASP78, SER246, THR249, and ARG247. The binding conformation indicated that Quercetin fits snugly into the active pocket of the complex through a combination of polar and nonpolar interactions.

These result suggest that Quercetin could be a promising natural inhibitor of the Ebola virus glycoprotein, supporting its potential development as a lead compound for anti-Ebola therapeutics. However, additional experimental validation, including in vitro antiviral assays and in vivo efficacy studies, will be necessary to confirm its therapeutic potential and safety.

BIBLOGRAPHY

1. Bai, H., Hai, C., Xi, M., Liang, X., Liu, R.: Protective effect of maize silks (Maydis stigma) ethanol extract on radiation-induced oxidative stress in mice. Plant Foods Hum. Nutr. 65(3), 271–276 (2010). https://doi.org/10.1007/s11130-010-0172-6
2. Alam, E.A.: Evaluation of antioxidant and antibacterial activities of Egyptian Maydis stigma (Zea mays hairs) rich in some bioactive constituents. J. Am. Sci. 7(4), 726–729 (2011)
3. Singh, J., Rasane, P., Nanda, V., Kaur, S.: Bioactive com pounds of corn silk and their role in management of glycaemic response. J. Food Sci. Technol. (2020). https:// doi. org/ 10. 1007/ s13197-022-05442-z
4. Hasanudin, K., Hashim, P., Mustafa, S.: Corn silk (Stigma maydis) in healthcare: a phytochemical and pharmacological review. Molecules 17(8), 9697–9715 (2012). https:// doi. org/ 3390/ molecules17089697
5. Subedi, K.D., Ma, B.L.: Corn crop production: growth, fertilization, and yield. Agric. Issues Policies (2009). https:// doi. org/ 10. 13140/2.1.3515.9040
6. Nawaz, H., Muzaffar, S., Aslam, M., Ahmad, S.: Phytochemical composition: antioxidant potential and biological activities of corn. In: Corn Production and Human Health in Changing Cli mate, vol. 10. pp. 49–68. Intech Open, London (2018)
7. Singh, J., Inbaraj, B.S., Kaur, S., Rasane, P., Nanda, V.: Phyto chemical analysis and characterization of corn silk (Zea mays, G5417). Agronomy 12(4), 777 (2022). https:// doi. org/ 10. 3390/ agronomy12040777
8. Rahman, N.A., Rosli, W.I.: Nutritional compositions and antioxidative capacity of the silk obtained from immature and mature corn. J. King Saud. Univ. Sci. 26(2), 119–127 (2014). https:// doi. org/10.1016/j.jksus.2013.11.002
9. Swapna, G., Jadesha, G., Mahadevu, P.: Sweet corn–a future healthy human nutrition food. Int. J. Curr. Microbiol. Appl. Sci. 9(7), 3859–3865 (2020). https:// doi. org/ 10. 20546/ ijcmas. 2020. 907.452
10. Sarepoua, E., Tangwongchai, R., Suriharn, B., Lertrat, K.: Influence of variety and harvest maturity on phytochemical content in corn silk. Food Chem. 169, 424–429 (2015). https:// doi. org/ 10. 1016/j.foodchem.2014.07.136
11. Chen S., Chen H., Tian J., Wang Y., Xing L., & Wang J. (2013). Chemical modification, antioxidant and α-amylase inhibitory activities of corn silk polysaccharides. Carbohydrate Polymers, 98, 428–437
12. Câmara, J.S., Albuquerque, B.R., Aguiar, J., Corrêa, R.C., Gonçalves, J.L., Granato, D., Pereira, J.A., Barros, L., Ferreira, I.C.: Food bioactive compounds and emerging techniques for their extraction: polyphenols as a case study. Foods 10(1), 37 (2020). https://doi.org/10.3390/foods10010037
13. Wang, Z., Ding, Y.N., Zhao, S., Prakash, W.L., Liu, J., Han, J., Wang, Z.: Effects of lutein particle size in embedding emulsions on encapsulation efficiency, storage stability, and dissolution rate of microcapsules through spray drying. LWT-Food Sci. Technol. 146, 111430 (2021). https://doi.org/10.1016/j.lwt.2021.111430
14. Guo, Q., Ma, Q., Xue, Z., Gao, X., Chen, H.: Studies on the binding characteristics of three polysaccharides with different molecular weight and flavonoids from corn silk (Maydis stigma). Carbohydr. Polym. 198, 581–588 (2018). https:// doi.org/10.1016/j.carbpol.2018.06.120

15. Tian, S., Sun, Y., Chen, Z.: Extraction of flavonoids from corn silk and biological activities in vitro. J. Food Qual. (2021). https:// doi.org/10.1155/2021/7390425
16. Li, C.C., Lee, Y.C., Lo, H.Y., Huang, Y.W., Hsiang, C.Y., Ho, T.Y.: Antihypertensive effects of corn silk extract and its novel bioactive constituent in spontaneously hypertensive rats: the involvement of angiotensin-converting enzyme inhibition. Molecules 24(10), 1886 (2019). https:// doi. org/ 10. 3390/ molecules2 4101886
17. Moongngarm, A., Homduang, A., Hochin, W.: Changes of phytochemical contents in sweet and waxy corn (Zea mays L.) as affected by cultivars and growth stages. Nutr. Food Sci. 16(2), 162–169 (2020). https:// doi. org/ 10. 2174/ 15734 01315 66618 1218125559
18. Limmatvapirat, C., Nateesathittarn, C., Dechasathian, K., Moohummad, T., Chinajitphan, P., Limmatvapirat, S.: Phytochemical analysis of baby corn silk extracts. J. Ayurveda Integr. Med. 11(3), 344–351 (2020). https:// doi. org/ 10. 1016/j. jaim. 2019. 10. 005
19. Kirrella, A.A., Abdo, S.E., El-Naggar, K., Soliman, M.M., Aboelenin, S.M., Dawood, M.A., Saleh, A.A.: Use of corn silk meal in broiler diet: effect on growth performance, blood bio chemistry, immunological responses, and growth-related gene expression. Animals 11(4), 1170 (2021). https:// doi. org/ 10. 3390/ ani11041170
20. Modrow S, Falke D, Truyen U, Schätzl H (2013). "Viruses: Definition, Structure, Classification". In Modrow S, Falke D, Truyen U, Schätzl H (eds.). Molecular Virology. Berlin, Heidelberg: Springer. pp. 17–30. doi:10.1007/978-3-642-20718-1_2. ISBN 978-3-642-20718-1. S2CID 83235976
21. Dixon MG, Schafer IJ (June 2014). “Ebola Viral Disease outbreak – West Africa, 2014”. MMWR Morb. Mortal. Wkly. Rep. 63 (25): 548-551. PMC 5779383. PMID 24964881. Archieved from the original on 23 October 2020. Retrieved 7 April 2020.
22. Ebola Virus disease (Report). World Health Organization. Retrieved 6 June 2019.
23. CDC urges all US residents to avoid nonessential travel to Liberia, Guinea and Sierra Leone because of an unprecedented outbreak of Ebola". Centers for Disease Control and Prevention (CDC). 31 July 2014. Archived from the original on 9 August 2014. Retrieved 2 August 2014
24. "2014 Ebola Outbreak in West Africa". Centers for Disease Control and Prevention (CDC). 4 August 2014. Archived from the original on 2 October 2014. Retrieved 5 August 2014
25. "Statement on the 9th meeting of the IHR Emergency Committee regarding the Ebola outbreak in West Africa". World Health Organization (WHO). 29 March 2016. Archived from the original on 31 March 2016. Retrieved 30 March 2016.
26. "Statement on Ebola in the Democratic Republic of the Congo". World Health Organization (WHO). Archived from the original on 12 May 2017. Retrieved 12 May 2017.
27. Hodal K (12 May 2017). "Ebola outbreak declared in Democratic Republic of the Congo after three die". The Guardian. United Kingdom. ISSN 1756-3224. OCLC 60623878. Archived from the original on 12 May 2017. Retrieved 12 May 2017.
28. Scutti S, Goldschmidt D. "Ebola outbreak declared in Democratic Republic of Congo". CNN. Archived from the original on 8 May 2018. Retrieved 9 May 2018.
29. Grady D (17 July 2019). "Congo's Ebola Outbreak Is Declared a Global Health Emergency". The New York Times. ISSN 1553-8095. OCLC 1645522. Archived from the original on 17 July 2019. Retrieved 17 July 2019.

30. Funk DJ, Kumar A (November 2014). "Ebola virus disease: an update for anesthesiologists and intensivists". Can J Anaesth. **62** (1): 80–91. doi:10.1007/s12630-014-0257-z. PMC 4286619. PMID 25373801.
31. "Transmission of Ebola virus". virology.ws. 27 September 2014. Archived from the original on 29 January 2016. Retrieved 22 January 2016
32. Osterholm MT, Moore KA, Kelley NS, Brosseau LM, Wong G, Murphy FA, et al. (19 February 2015). "Transmission of Ebola viruses: what we know and what we do not know". mBio. **6** (2):e00137. doi:10.1128/mBio.0013715. PMC 4358015. PMID 25698835.
33. Funk DJ, Kumar A (November 2014). "Ebola virus disease: an update for anesthesiologists and intensivists". Can J Anaesth. **62** (1): 80–91. doi:10.1007/s12630-014-0257-z. PMC 4286619. PMID 25373801.
34. Smith T (2005). Ebola (Deadly Diseases and Epidemics). Chelsea House Publications. ISBN 978-0791085059.
35. Tang H, Abouleila Y, Saris A, Shimizu Y, Ottenhoff TH, Mashaghi A (May 2023). "Ebola virus-like particles reprogram cellular metabolism". Journal of Molecular Medicine. **101** (5): 557–568. doi:10.1007/s00109-023-02309-4. PMC 10036248. PMID 36959259