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MOLECULAR DOCKING OF PHYTOCHEMICALS FROM *Terminalia chebula* FRUIT EXTRACT AGAINST SELECTED PROTEINS OF *Xanthomonas campestris* pv *vesicatoria*

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ABSTRACT

Background: In tomatoes, bacterial diseases directly damage the fruit and severe foliar infection leads to defoliation. Thereby, creating a huge loss in both organic and traditional farming systems. A biological approach is essential to gain pathogen-free tomato plants. Natural compounds obtained from plants have gained attraction because of their high specific nature and reduced toxicity. The structure based modelling and rapid screening method offer significant potential for identifying and developing antibacterial constituents.

Methods: The present study aimed to assess biologically active compounds present in methanolic extract of *Terminalia chebula* (*T. chebula*) fruit, using HR-LCMS (High Resolution- Liquid chromatography Mass Spectrometry) and Molecular docking methods. HR-LCMS was performed at IIT Bombay's Sophisticated Analytical Instrument Facility (SAIF), Mumbai. The Docking study was performed by PyRx –Virtual Screening Tool (https://sourceforge.net/projects/pyrx/). The three bioactive compounds gluconic acid, ellagic acid and 4-methoxycinnamic acid of *T chebula* fruit methanolic extract were selected. All the compounds were subjected to molecular docking studies against the selected protein receptors PDB ID-201X and 4FC9 from *Xanthomonas campestris* pv *vesicatoria*

Results: Among the three bioactive compounds, ellagic acid exhibited better docking score against both proteins. Ellagic acid forms three hydrogen bonds with amino acids in the active site of the target protein, with the least binding affinity -6.5 and -7.9 against the protein 201X and 4FC9 respectively and hence Ellagic acid is considered to exhibit the best docking conformation and good interaction. The present study supported the traditional claim of *T.chebula* methanolic fruit extract as potential treatment in control of *Xanthomonas campestris* pv *vesicatoria* that causes Bacterial spot in tomatoes.

Keywords: *T. chebula; HR-LCMS; Molecular docking; Ellagic acid; Xanthomonas campestris pv vesicatoria.*

INTRODUCTION

Agriculture is expected to meet a variety of customer needs while also creating value for the entire society. India has a favourable climate for cultivating a wide variety of fruits, vegetables, spices, and nuts, all of which provide significant nutritional benefits to mankind [1]. There are many distinct vegetable families, each containing hundreds of distinct plant components that are helpful to human health. Most of the vegetables have all essential properties and tomato is considered one among them. Tomato is known for its nutrient value rich in Vitamin C, pro-vitamin A, folate, β carotene, vitamin E, potassium, and secondary metabolites viz flavonoids, polyphenols, polysterols, and lycopene. Along with the nutritional value, bioactive compounds contribute towards the texture, aroma, and sensory quality of the tomato. Due to its nutrient value and health-promoting compounds, tomatoes have gained attraction to be a part of a balanced diet [2]. Tomato is the second most important vegetable crop after potato. Because of its widespread and health-promoting compounds, it is the model organism for research [3].

One of the limiting factors towards the decreased productivity of tomato yield is due to microbial diseases caused by bacteria, fungi, viruses, nematodes, insect pests, and viroids [4]. Bacterial diseases are common in plant disease outbreaks around the country, and they affect a wide range of vegetables. Tomatoes are one of the vegetable that are prone to numerous bacterial diseases [4]. *X.campestris* pv *vesicatoria* causative agent of the Bacterial spot in tomato was reported and isolated in many countries, this was supported by the cultural, morphological, and biochemical tests. This pathogen is a major concern in the production of tomatoes [5]. The disease causing bacteria double their population under favourable environmental conditions and colonize in the internal spaces of plants; Bacterial diseases are difficult to control [6].

The majority of currently accessible antimicrobial agents are derived from plants, either directly or indirectly. The Indian Vedas, Chinese literature, Ebers Papyrus mentions treatment using plants and their parts [7]. The shift from single target to multidrug target approach for complex diseases offers a platform for herbal formulations. In both developed and developing countries because of biodegradability, easy availability, and no adverse side effects, plant-based products is gaining importance [8]. Isolation of bioactive compounds from the plant and their role in disease management has sought attention towards the usage of plants. Researchers, while screening medicinal plants, reported *Terminalia chebula* to be one of the important plants exhibiting high medicinal activities [9]. *T.chebula*, commonly known as haritaki, exhibits a wide range of medicinal properties since they contain huge amount of essential phytochemicals. Each part of the plant exhibits high medicinal value which is traditionally used for the treatment of different human ailments. It has been shown that the plant has several pharmacological and medicinal functions, such as antioxidant, antimicrobial, antidiabetic, hepatoprotective, anti-inflammatory and wound healing [10].

Molecular docking being one of the *in-silico* approach is more efficient because of its efficacy in virtually evaluating the interaction of bioactive compound (ligands) present in medicinal plants that targets the protein (receptor). Protein-ligand characterization aids in predicting the binding energy expressed in kilocalories per mole (kcal/mol) of a ligand to a known 2D and 3D

structure of effector protein [11]. Binding energy is one of the methods to calculate the energy associated with ligand binding. Better stability of the protein ligand complex is achieved with higher negative binding energy.Thus indicating the lower the energy the higher the stability of the complex [12].Protein-ligand interaction provides a virtual prediction minimizing the time consumption and expense for the drug discovery process [13]. Thus *in-silico* analysis helps to speed up the process in a cost-effective way by using software and gives a clear image of where exactly the ligand binds to the target (effector protein). Our approach is novel since it aims in studying the phytochemical compounds present in the crude methanolic extracts of *T. chebula* fruit through HR-LCMS (SAIF IIT Bombay), followed by molecular docking to explore the interaction of the ligand that contribute to antibacterial property against the effector proteins of *X. campestris* pv *vesicatoria*, the causative agent of bacterial spot in tomato.

MATERIALS AND METHODS

Preparation of Plant Extract

Healthy *T.chebula* fruit was collected from Mysore region. *The outer coat of seed was removed and* was grounded using a sterilized mortar and pestle followed by soxhlet extraction. 50g of grounded powder was placed in the soxhlet and extraction was carried out by using 300ml of methanol. The extraction process lasted for 6 -7 hrs and repeated until the solution was clear. The resultant extract was subjected for evaporation to obtain dried product under reduced pressure at 60^0C. Further, the dried extract was stored in sterilized tubes to carry out phytochemical analysis [14].

High Resolution- Liquid Chromatography Mass Spectroscopy (HR-LCMS)

HR-LCMS (LCMS using database plant extract, impurity profiling, and metabolite identification) were performed at IIT Bombay's Sophisticated Analytical Instrument Facility (SAIF), Mumbai for the methanolic extract of *T. chebula* fruit. Liquid chromatography/Mass Spectroscopy is an analytical separation tool that utilizes high-performance liquid chromatography with mass spectroscopy that aid in detection of bioactive compounds [15]. G6550A model (Agilent technologies) which posses good resolution LC/MS with 0.01 percent resolution has been used in analysing the chemical contents of the methanolic extract qualitatively. MS was chosen as the acquisition method, with a minimum range of 100 (m/z) and a maximum range of 1000 (m/z) having a scanned rate of one spectrum per sec. The temperature of the gas chromatography system was kept at 250°C, with a gas flow rate of 13 psi/min. For HR-LCMS, a sampler with the model named G4226A with an average speed of 100 l/min, an ejection speed of 100 l/min, a flush out factor of 5 l, and a 5 l injection volume was utilized. The HR-LCMS acquisition method is used for 30 minutes, with a 2 minute start flow of 95:5 solvent compositions A: B. For the analysis, 100% water and 100% acetonitrile were employed [16]. Using HR-LCMS, the chemical constituents of the bioactive compounds from the methanolic extract of *T.chebula* fruit was determined.

In-silico studies

Preparation of the Ligand and ADMET Studies

In the present study among 10 bioactive compounds identified from *T.chebula* methanolic fruit extract by HR-LCMS analysis, three bioactive compounds namely gluconic acid, elagic acid and 4-methoxycinnamic acid have been chosen based on the peaks obtained from the chromatograph to conduct molecular docking analysis since these bioactive chemicals are well known for its activity and also effectively present in the extracts of *T.chebula*. The selected compounds were two dimensionally (2D) sketched using Chemsketch software and further OpenBabel software was used to convert the 2D structure (PDB format) to three dimension (3D) by adding hydrogens and 3D coordinates and geometrical cleaned using the ArgusLab software. Thus, the obtained PDB files of the ligands were used for further molecular docking procedure.

ADMET (Absorption, Distribution, Metabolism, Elimination and Toxicity) studies were performed to access the toxicity of the phytoconstituents. The analysis was conducted with the sdf formated structure of the ligands to check its toxicity level

and satisfaction of Lipinski "Rule of five" using Data Warrior [17].

Preparation of protein (receptor) molecule

The proteins those are highly responsible for the virulent nature of the bacteria has been selected in the present study [18, 19]. The three dimensional structure of the selected proteins were downloaded from PDB (Protein Data Bank) database with PDB ID- 201X and 4FC9. The downloaded protein structures were edited accordingly to delete the attached water molecules and other non-standard amino acid residues. Finally, the interaction between the identified bioactive compounds were analysed against the protein of *Xanthomonas campestris* pv vesicatoria. The binding pocket forming residues in the respective protein were obtained through CASTp (Computer Atlas of Surface topography of Proteins) online tool [20].

Molecular Docking (MD)

In the present study Molecular docking was carried out using PyRx software. The three bioactive compounds gluconic acid, ellagic acid and 4-methoxycinnamic acid of *T chebula* fruit methanolic extract selected were formatted to 2D and 3D structure using chemsketch and Openbabel software and used as ligand. All these compounds were subjected to molecular docking studies against the selected protein receptors PDB ID-201X and 4FC9 to know its ability in inhibiting the target receptor. Before proceeding with the molecular docking procedure, the binding site residues of each protein were selected and a grid box was generated around the residues [17].

RESULTS

Preparation of Plant Extract

Methanolic extraction of *T.chebula* fruit was done using soxhlet extractor. The yield obtained for 50g of *T.chebula* fruit sample in 300ml of methanol solvent was 1.867g.

HR-LCMS Analysis

The crude methanolic extract of *T. chebula* fruit was evaluated using HR-LCMS. The combination of MS/MS (Mass Spectroscopy/Mass Spectroscopy) with collision-induced dissociation (CID) has been demonstrated to allow for the precise identification of phenolic compounds and tannins in complex extracts with co-eluting peaks. Thus indicating MS/MS is an effective tool for identifying the active components in Methanolic extract of *T.chebula* fruit extract. HR-LCMS analysis of methanolic extract *T. chebula* fruit showed the presence of ten phytoconstituents. HR-LCMS analysis with compound name, retention time (RT) in min. Molecular formula, molecular weight, structure and DB (PPM) are represented in Table 1 and the chromatograph of phytoconstituents in Fig. 1. Among them three phytoconstituents viz gluconic acid, ellagic acid, 4-methoxycinnamic acid are known for its antibacterial properties.

Table 1. Bioactive compounds of *T. chebula* fruit methanolic extract obtained from HR-LCMS

Sl No	Compound name	RT (MIN)	Molecular weight	Molecular formula	DB (PPM)
1	Gluconic acid	0.905	196.05731	$C_6H_{12}O_7$	87.9
2	Proline	0.96	113.08441	$C_5H_9NO_2$	84.6
3	Ellagic acid	0.986	371.10695	$C_{14}H_6O_8$	62.3
3	D-(-)-Quinic acid	0.965	362.03898	$C_7H_{12}O_6$	73
4	4-Methoxycinnamic acid	20.046	180.00752	$C_{10}H_{10}O_3$	89.6
5	Dodecyl sulfate	23.313	266.15401	$C_{12}H_{26}O_4S$	82.2
6	1-Methylhistidine	24.502	121.08942	$C_7H_{11}N_3O_2$	95.4
7	Bromhexine	25.245	114.12396	$C_{14}H_{20}Br_2N_2$	82.6
8	Chloramben	25.247	363.92366	$C_7H_5Cl_2NO_2$	80.5
9	1-Methylhistidine	28.019	122.04843	$C_7H_{11}N_3O_2$	85.3
10	4-Dodecylbenzenesulfonic acid	28.845	326.19026	$C_{18}H_{30}O_3S$	78.6

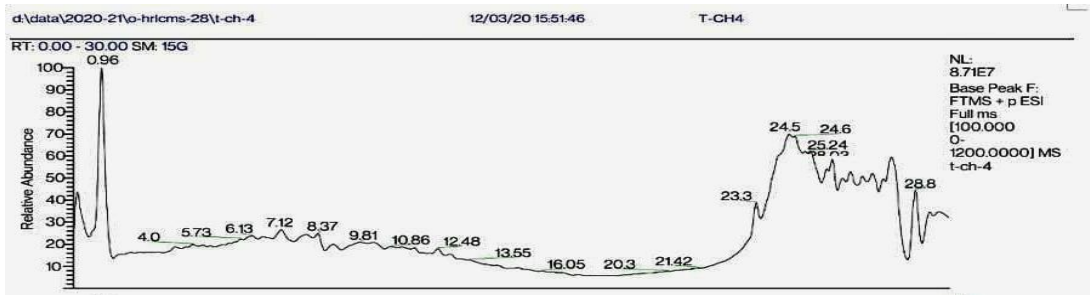
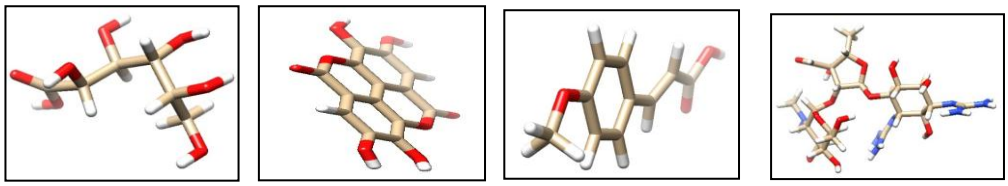


Fig. 1. HR-LCMS chromatograph of *T. chebula* fruit methanolic extract



(a) (b) (c) (d)

Fig. 2. The 3D representation of optimized ligands a) Gluconic acid b) Ellagic acid c) 4-Methoxycinnamic acid and standard drug d) Streptomycin

Table 2. ADMET study and drug likeliness prediction using Data Warrior

Compounds	cLogP	cLogS	H-acceptors	H-donors	Drug likeliness	TPSA
Gluconic acid	-2.42	3.02	7	6	0.56	138.45
Ellagic acid	1.00	-3.35	8	4	0.55	141.34
4-Methoxycinnamic acid	1.87	-1.99	3	1	0.85	46.53

In silico Studies

Ligand preparation and ADME studies

The 3D structural file of the ligand molecules Gluconic acid, Ellagic acid, 4-Methoxycinnamic acid and standard drug Streptomycin used to carry out the protein docking interaction (Fig. 2).

ADMET studies of three compounds (Gluconic acid, Ellagic acid and 4-Methoxycinnamic acid) obtained from HR-LCMS studies is shown in Table 2. Based on the DataWarrior result all the three compounds satisfied Lipinski's rule, Log S and Log P along with good drug score and less toxicity.

Preparation of protein (receptor) molecule

The 3D structural format of the proteins 20IX and 4FC9 was isolated using PyMOL software (Fig. 3).

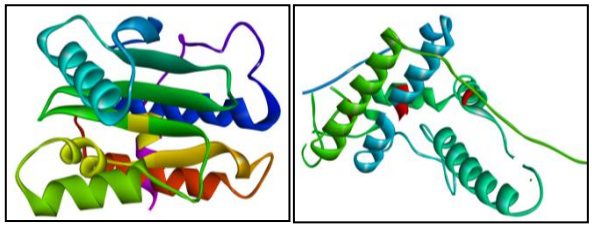
Molecular docking

Molecular docking was carried out to predict the orientation and binding affinity at the active site of the receptor. The molecular docking of HR-LCMS identified ligand molecules- gluconic acid, ellagic acid and 4-methoxycinnamic acid with receptor 201X, 4FC9 is shown in Fig. 4. Among these three bioactive compounds, ellagic acid exhibited better docking score against both proteins. Ellagic acid forms three hydrogen bonds with amino acids in the active site of the target protein, with the least binding affinity of -6.5 and -7.9 respectively and hence it is considered as the best dock conformation. The next good docking score was obtained for compound 4-methoxycinnamic acid that formed two and one hydrogen bonds with amino acids in the active site of the target proteins 20IX and 4FC9 with the least binding affinity -5.4 and -5.7 respectively. Gluconic acid showed less

dock score comparatively with the other two bioactive compounds by forming 4 hydrogen bonds with the least binding affinity -4.7 and -4.8 respectively (Table 3). Compared to the standard streptomycin, the three bioactive compounds have shown greater interaction and least binding affinity against 20IX protein.

DISCUSSION

Tomatoes are prone to numerous bacterial diseases. Since bacterial pathogens double their population under favourable environmental conditions and colonize in the internal spaces of plants, it is not easy to control bacterial diseases [6]. One of the important diseases in Tomato is bacterial spot caused by species of *Xanthomonas*, that causes lesions on fruits and leaves, which decreases the quality of fruit and seedling stage infection, also leads to decreased quality of fruit and finally resulting in significant yield loss [5]. Since the usage of chemicals releases toxic substances that are hazardous to the environment, an eco-friendly approach to gain pathogen-free plants is essential. Phytochemical studies on various medicinal plants show the presence of various secondary metabolites that possess a wide range of pharmacological activities. Aqueous, organic extracts prepared from leaves, stems, and fruits of *Withania somnifera L* showed maximum antifungal activity against the pathogen *Fusarium oxysporum (F.oxysporum) f. sp. radicis- lycopersici* - causal agent of *Fusarium crown* and root rot in tomato [7]. The potential of *T.chebula* fruit extract was screened by mixing methanolic, aqueous and ethyl acetate extracts with a range of conventional antibiotics. Strong antimicrobial activity was seen in the extracts against the bacterial triggers of all autoimmune inflammatory diseases [21].

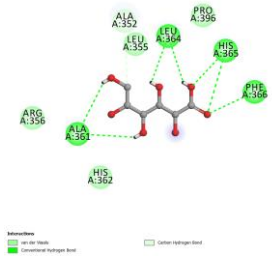


(a) **(b)**

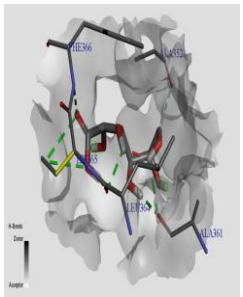
Fig. 3. 3D view of a) 20IX and b) 4FC9 protein captured in PyMOL

Table 3. Molecular docking values of bioactive compounds obtained from HR-LCMS analysis

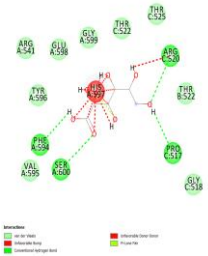
Sl.No	Protein (Receptor)	Compounds	Binding affinity (kcal/mol)	Number of hydrogen bonds	Hydrogen bond forming amino acid residues
		Gluconic Acid	-4.7	04	ALA 361, LEU364, HIS 365, PHE 366
1	201X	Ellagic Acid	-6.5	03	PHE 366, HIS 365, ASP 394
		4-Methoxycinnamic Acid	-5.4	02	HIS 365, PHE 366
		Streptomycin	-4.2	04	ALA 361, PHE 366, THR 348, ASP 349
		Gluconic Acid	-4.8	04	PHE 594, SER 600, PRO 517, ARG 520
2	4FC9	Ellagic Acid	-7.9	02	GLU 510, LYS 519
		4-Methoxycinnamic Acid	-5.7	01	LYS 519
		Streptomycin	-7.9	04	LYS 519, ASP 523, GLY 528, ARG 505



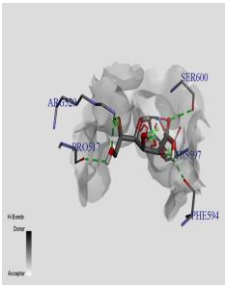
2D: 20IX Protein - Gluconic acid



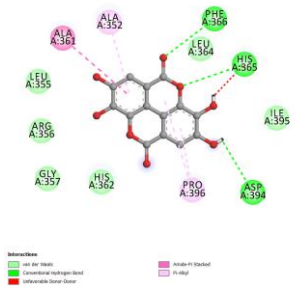
3D:20IX Protein - Gluconic acid



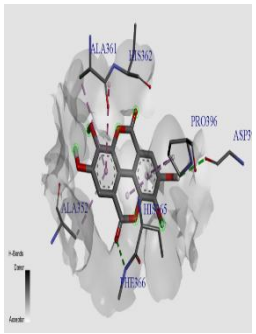
2D:4FC9Protein-Gluconic acid



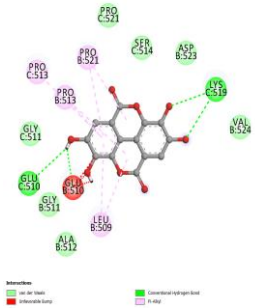
3D:4FC9 Protein - Gluconic acid



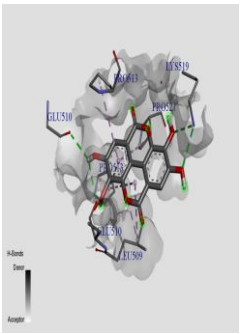
2D:20IX Protein - Ellagic acid



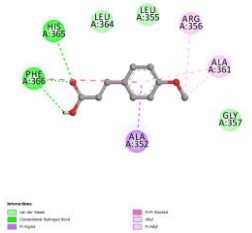
3D:20IX Protein - Ellagic acid



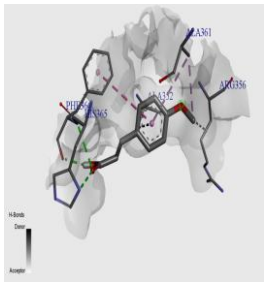
2D: 4FC9 Protein -Ellagic acid



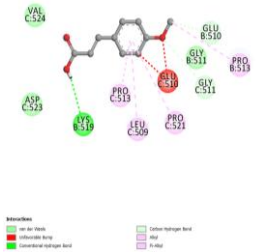
3D:4FC9 Protein -Ellagic acid



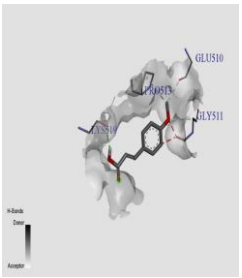
2D:20IX Protein - 4-methoxycinnamic acid



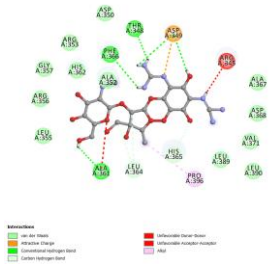
3D:20IX Protein – 4-methoxycinnamic acid



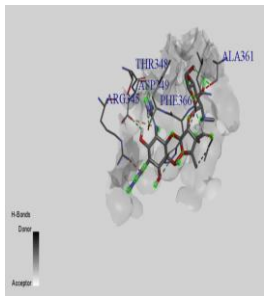
2D:4FC9Protein- 4-methoxycinnamic acid



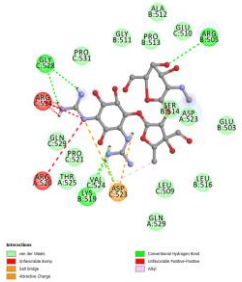
3D:4FC9Protein- 4-methoxycinnamic acid



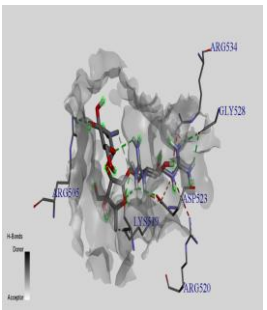
2D:20IX Protein - Streptomycin



3D:20IX Protein - Streptomycin



2D:4FC9Protein- Streptomycin



3D:4FC9Protein- Streptomycin

Fig. 4. 2D and 3D structural representation of interaction of proteins 20IX and 4FC9 with ligands Gluconic acid, ellagic acid, 4-methoxycinnamic acid and the standard ligand Streptomycin

Plant based phytoconstituents Viz. alkaloids, tannins, steroids, phenolic compounds and terpenoids, due to its antibacterial, antifungal, antioxidant, antiviral, anticancer activity has gained attention and offers an effective drug action against targeting the effector protein. In the present study, HR-LCMS analysis showed the presence of various compounds in the methanolic extract of *T.chebula* fruit. Among them ellagic acid, gluconic acid and 4-methoxycinnamic acid posses good antimicrobial activity [22, 23, 24]. Structure based drug imaging using phytocompounds can be used to speed up the process, also direct focus on the target site can be achieved at low cost and thereby reducing the risk factor.

In consideration of rapid growth of molecular docking, many studies have been conducted using phytochemicals to evaluate and prove its efficacy. Previous studies [25] conducted on *In-silico* analysis to evaluate the efficacy of *T.chebula* phytochemicals against toxic alpha haemolysin protein of MDRUPEC (Multiple Drug Resistance Uropathogenic *Escherichia coli*) strains, had showed good scoring of -5.44kcal/mol in ellagic acid. In the present study also, among all the three bioactive compounds of methanolic extract of *T.chebula* fruit ellagic acid showed least binding affinity of -6.5kcal/mol and -7.9kcal/mol, thus showing greater interaction against the effector proteins of *X. campestris* pv *vesicatoria* 20IX and 4FC9 respectively in comparison to the standard streptomycin. Streptomycin is bactericidal and broad spectrum antibiotic, active against gram negative and gram positive bacteria ,which has the ability to block 30S ribosomal subunits to make proteins that results in the death of the bacteria and hence streptomycin was chosen has standard drug in our study[26]. This innovative approach to use active phytocompounds obtained from methanolic extracts of *T.chebula* fruit in insilico studies is helpful for mankind for the development of effective agents against phytopathogens.

CONCLUSION

The present work mainly focused on using *in-silico* analysis for the management of bacterial spot in tomato by targeting the effector protein of *X.campestris* pv *vesicatoria*. Also the methanolic extract of *T.chebula* fruit was subjected for HR-LCMS analysis to select the ligands for interaction with proteins. HR-LCMS analysis showed the presence of many phytochemicals. Based on the percentage of the compound present in the extract, which was depicted in the peaks of chromatography, three compounds were selected Gluconic acid, ellagic acid and 4-methoxycinnamic acid for molecular docking to understand the interaction of ligands with effector proteins 20IX, 4FC9 and 5JP1 of *X.campestris* pv *vesicatoria*, retrieved from PDB. From the results obtained, ellagic acid proved its efficacy by showing good interaction and least binding affinity against all the bacteria. This molecular docking studies aided in the virtual prediction of understanding the role of bioactive compound.

DISCLOSURES

The authors declare that there is no conflict of interest in this article's content.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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