Abstract: To evaluate *Insilico* interaction efficacy of *Terminalia chebula* phytocompounds with alpha haemolysin of Multiple Drug Resistance Uropathogenic *Escherichia coli* (MDRUPEC) strains. In the present investigation, *Insilico* interaction profile of the phytocompounds retrieved from PubChem amalgam database such as tannic acid, ellagic acid and gallic acid from Haritaki were evaluated against toxic protein Haemolysin (HlyA) of MDRUPEC strains. The glide module of Schrodinger was used to study the docking interaction of the compounds. Based on the *Insilico* research findings, ellagic acid found to be the best docking phytocompound with the scoring efficiency of -5.44. This innovative approach formed a framework for the development of new pharmacologically active phytocompounds as therapeutic agents for the effective aid for the illness of human population by the MDR strains.

Index Terms: *Terminalia chebula*, uropathogens, antibiotic, resistance, phytocompounds, *Insilico* analysis.

I. INTRODUCTION

UTIs are of serious health concern in the Indian community. Even though quite a lot of microorganisms cause UTIs, bacteria ensue to be the foremost contributing pathogens which are accountable for above 95% of UTI cases (Janifer et al., 2009) and MDRUPEC is the fore runner among the bacteria. Due to indiscriminate use of various antimicrobials, plant extracts worn as impending resource for the improvement of a novel phytomedicine to be active against infectious bacteria. The antimicrobial activities can be improved if the phytoactive compounds are purified and ample dose indomitable for appropriate administration. Development of contemporary drugs from plants should be emphasizing for the control of contagious diseases (Nitha et al., 2012). There is substantiation of herbs have been used in the healing of diseases and for restorative body systems in round about all prehistoric civilizations such as Indian, Egyptian, Chinese, Greek and the Roman civilizations. Herbal plants were the foremost twig of medicine and endorsed with extraordinary and almost weird powers of healing. They have an immense potential for their use as therapeutic medicines (Gaidhani et al., 2009). Plants emerged as fascinating source of new medicines and provide lead compounds for drug development (Savia, 2012). Medicinal plants typically restrain numerous biologically energetic ingredients and are used principally for treating placid or persistent ailments. WHO report evidenced, 80% of the population relies mainly on the plant based traditional medicinal practices for their healthcare needs. Herbal medicines are in immense demand in the urbanized as well as budding countries for primary healthcare as of their wild biological and medicinal activities, elevated protection boundaries and lesser expenses (Chawdhary et al., 2010).

*T. chebula* is acknowledged to have bizarre power of curative with an extensive continuum of biological activity (Chhaopadhyay & Bhattacharya, 2007). *T. chebula* belonging to Combretaceae family is the inhabitant plant of India, South East Asia and has been reported to parade an array of biological

DOI: 10.37398/JSR.2021.650121
activities including antiviral, antibacterial and anticancer (Lee et al., 2011). Chemical constituents like anthraquinones, 4,2,4 – chebulyl – D – glucopyranose and flavonoids such as tannic acid, ellagic acid, gallic acid and ellagittannic acid encompass be well-known in this species (Kim et al., 2006). Various scientific research studies on medicinal plants revealed that some new antimicrobial compounds from plant extracts were elucidated to combat various infectious diseases.

Virulence factors of UPEC contributes to pathogenesis of urinary tract infection mainly include fimbriae, which involved in adherence and invasion to host cells, toxins affecting host cells, like α- haemolysin of UPEC, and iron – acquisition systems for bacterial growth. HlyA, cytotoxic to a wide range of cells and causes serious tissue damage during urinary tract infection and found to be an important factor in pyelonephritis (Wang et al., 2020). Nhu et al., (2019) Reported that, 50% of all UPEC strains produce the potent pore forming toxin, its expression is associated with enhanced virulence. This serious threat was not yet focused in the aspect of insilico studies up to the author’s knowledge. Even though numerous research studies emphasized on various aspects of this pathogenic protein interaction with the phytocompounds of herbal origin, insufficient reports are framed in this present study, which highlights pathogenic protein interaction with specific phytocompounds of selected plant extract.

The efficacy of phytocompounds such as TA and EA from dried fruits of T. chebula Retz was evaluated against MDRUPEC by using HPTLC (Savitha & Arrivukkarasu, 2014) and by CLSM (Reichhardt & Parsek, 2019) techniques. The research findings have shown that, amongst an assortment of concentrations of the plant methanol extracts used, 40μg/ml has exhibited the best antibacterial activity against the test strains. The evaluation of phytocompounds GA, TA and EA were quantitatively estimated as 279.42 ng (nanogram), 16.13 μg (microgram) and 22.00 μg respectively. In this context, many ethno medicinal properties like neuro protective effect (Chan & Lin, 2012), anti inflammatory properties (Priya et al., 2017), Efflux-pump inhibitory activity (Bag & Chattopadhyay, 2014), anticholinesterase and antiamyloidogenic effect (Pugazhendhi et al., 2018), cardioprotective effect (Mohanty et al., 2018). However none of the earlier attempts are made on the line of MDRUPEC with insilico analysis of phytocompounds with α-haemolysin. Based on this footstep, the researchers are interested to carry out Insilico interactions of these bioactive principles with the pathogenic protein binding moieties. These newer approaches have formed a frame work for the development of new phytochemical therapeutic agents for the effective treatment of illnesses of human population by the multi drug resistant microbes (Savitha, 2017).

Present research focused on the insilico binding mechanism of secondary metabolites of Terminalia chebula, with α Hemolysin. The three compounds gallic acid, ellagic acid and tannic acid where analyzed for having higher binding affinity for α - Haemolysin, which is one of the target protein. The α - Haemolysin (1XEF) was retrieved from PDB data resources based on the ruling of structure, experimental practicability by prevailing conditions data, virtual value of crystallographic sculpt and X-ray diffraction data and 10% amputation of data for the structure pragmatic and 90% of structure compared through crystallographic representation to get the module configuration. From our study and previous literature references, ellagic acid shows better interaction with α - haemolysin thereby bringing this phytocompound to limelight for further analysis. Further, these studies might also lead a path to design the novel antibiotics that can inhibit the activity of α - haemolysin.

II. METHODS

A. Insilico interaction of α - Haemolysin of UPEC with bioactive compounds of T.chebula (gallic acid, ellagic acid and tannic acid)

Due to the emergence of multiple drug resistant pathogenic microbes, extended concern needed for alternative chemotherapeutic agents (Blair et al., 2015; Chang et al., 2015) to combat these microbes. To overcome this dreadful crisis, higher plants serve as boon for new antimicrobials to replace with existing therapeutic measures (Setzer et al., 2016). The secondary metabolites derived from plant origin having various pharmacological properties such as antimicrobial, anticancer and antidiabetic activities (Raut and Karuppavil, 2016).

The PubChem amalgam database contains the validated chemical portrayal in sequence provided to illustrate substances in PubChem substance. Structures stored within PubChem compounds are pre-clustered and cross-referenced throughout uniqueness and resemblance groups. PubChem is structured as three correlated databases within the NCBI's Entrez information retrieval system.

The structure of selected phytocompounds, gallic acid (CID 370), ellagic acid (CID 5281855) and tannic acid (CID 16129778) were retrieved as SDF from PubChem database to study their binding efficiency with the α haemolysin of an uropathogenic virulence factor from E.coli. The glide module of Schrodinger was used to study the docking interaction of the compounds. The binding surface of the receptors and the ligand compounds in SDF format were used to dock with in the binding pockets of the α haemolysin with uropathogenic virulence factor and Maestro Ligand interaction 2-D diagram was used to understand the in-depth interaction pattern to the ligands and α haemolysin. The binding energies of these three phytocompounds with possible active targets of virulent protein were calculated and ranked to identify the better target in order to predict their various modes of action and their results were recorded.
III. RESULTS AND DISCUSSION

A. Insilico interaction profile of virulence protein with the bioactive phytocompounds of dried fruit methanol extract of *T. chebula*

Traditional medicinal plants and herbs has been a pivotal part of treatment and cure for many centuries including multi drug resistant (MDR) pathogens (Narayanan et al., 2011). Literature has reported that the various pharmacological activities of dried fruits of *T. chebula* were due to the presence of numerous secondary metabolites. These metabolites have found to have a lot of potential application in primary healthcare, where 80% of Asian population depends upon traditional medicine. As per the report (Bag & Bhattacharyya, 2013), among the above mentioned metabolites, gallic acid, ellagic acid and tannic acid were found to be potential anti bacterial metabolites. In the present study, computational tools were used to investigate the mode of molecular interaction of the three selected metabolites (gallic acid and ellagic acid and tannic acid) and bacterial target protein (α Haemolysin), in turn assisting us to predict the insights of antibacterial mechanism.

1) α – Haemolysin

α-Haemolysin is synthesized as a 1024-amino acid polypeptide followed by intracellular activation by specific fatty acylation and it contains four chains and shows four binding sites. Protein or glycoprotein receptors for α haemolysin might exist on the cell surface, but the toxin is also dynamic on pure lipid bilayers.

2) Binding sites in α haemolysin

α - haemolysin protein has four possible binding sites from the sitemap results. The best site among the four sites is selected based on the site score ~ 1.050 tol.016. The best binding site with a best score of 1.050 is selected for further analysis. The best binding site as per the site score has 64 residues. The binding site residues are Phe 470, Phe 475, Arg 476, Try 477, Ile 484, Leu 485, Ile 500, Val 501, Gly 502, Arg 503, Ser 504, Gly 505, Ser 506, Gly 507, Lys 508, Ser 509, Thr 510, Leu 511, Thr 512, Lys 513, Leu 514, Ile 515, Gln 516, Phe 518, Ala 615, Ile 616, Ile 628, Asp 630, Ghu 631, Ala 635, Leu 636, Asp 637, Gla 639, Ser 640, Ile 660, Ala 661, Ala 662, Ile 674, Val 675, Met 676, Glu 677, Lys 678, Gly 679, Lys 680.

The docking interactions between the ligand EA (CID 5281855) and HlyA showed in Figure 4.24. It was observed that EA was docked with a glide score of -5.443 and the glide energy of -37.274 Kcal/mol (Fig 3 and Table 3).

The interaction of EA was favoured by seven amino acids Gly 600, Gln 602, Gla 605, Val 553, Leu 549, Arg 611 and Lys 513 apart from the hydrogen bond forming amino acid residues (Table 3). All the four hydrogen bond formed and other amino acid residues favouring the interaction between EA and HlyA makes EA a better lead for the biofilm study.
Institute of Science, BHU Varanasi, India
Earlier study by Aishwarya et al., (2013) evidenced that, the potential antibacterial metabolites GA (CID 370) and EA (CID 5281855) with the bacterial target protein (Lux S receptor) molecular docking by using Auto dock module of PyRx version 0.8 software. They analyzed that, GA formed two hydrogen bonds with the target receptor protein (SER6, LYS35), while EA formed single hydrogen bonds with the target protein (GLU57) within the active site. The efficient binding energy of GA was found to be -1.79Kcal/mol where as for EA -4.33 Kcal/mol. It was quite interesting to analyze that, the less binding energy and more hydrogen bonds may be due to lack of other type of interactions. Thus the binding energies of these two selected metabolites by insilico interaction support good antibacterial activity of these phytocompounds. Virtual screening methods such as cheminformatics, pharmacophore or ligand and structure based target protein prediction are employed as an advantageous alternative high-throughput identification protocols (McPhillie et al., 2015) for the identification of potential novel lead structures or biological targets for anti-infective drug discovery.

CONCLUSION

The insilico analysis of the interaction between α haemolysin and the phyto compounds from the Terminalia chebula retz extracts elucidates the effective implementation of the compounds in the bio film formation. The analysis of the EA, GA and TA shows that the tannic acid does not satisfy the drug likeliness rule even though it shows good interaction with the protein. It is observed that both the compounds GA and EA interaction with the active site of α haemolysin with the formation of H bonds with the best binding site residues. The lesser the energy the better the interaction thereby EA shows lesser binding energy and good interaction with the protein. These docking studies suggested that the phyto compound EA strongly interacts with the HlyA with the formation of four
hydrogen bonds with good glide score of -5.443 and the glide energy of -37.274 Kcal/mol. From the present study and previous literature references, EA shows better interaction with the alpha haemolysin thereby bringing this phytocompound to limelight for further analysis. Further, these studies might also lead a path to design the novel antibiotics that can inhibit the activity of HlyA.

ABBREVIATIONS

ACKNOWLEDGEMENT
The authors are thankful to Vice – Chancellor, Bharathiyar University, Coimbatore of Tamil Nadu state, Provost, Uka Tarsadia University, Surat of Gujarat state, Management and Principal of Tiruppur Kumaran College, Tiruppur of Tamil Nadu state for providing necessary basic facilities and support to carry out the work.

REFERENCES


***