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Insilico Interaction Profile of *Terminalia chebula* Phytocompounds with Alpha Haemolysin of Multiple Drug Resistant Uropathogenic *Escherichia coli*

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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, phytocomopounds, *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 (Chaaopadhyay & 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

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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 ellagitannic 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. Insilco 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 to1.016.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 614,Ala 615,Ile 616, Ile 628, Asp 630,Glu 631,Ala 635,Leu 636,Asp 637, Glu 639, Ser 640, Ile 660, Ala 661, Ala 662, Ile 674, Val 675, Met 676, Glu 677, Lys 678, Gly 679 And Lys 680.The PDB Sum results shows the best binding site residues as follows Ser 504,Lys 508,Gly 507,Ser 506,Ser 509,Gln 610,Thr 510,Ser 607 and Gly 505,which adds to the fact that the best binding site is filtered for the study. The sitemap results shows the best binding site and the selected best site based on the site score further studied for the interaction with the ligand (Fig 1 and Table 1).

The prominence in computational drug design has been extended from the time-honoured QSAR techniques used to unearth biologically energetic molecules to added quantitative structure-property relationship (QSPR)-oriented estimates of the ADME properties of molecules. As fraction of this shift in accent several groups have attempted to delineate the "drug-likeness" of molecules subsequent the pioneering "rule of five" exertion by Lipinski rule (Lipinski et al., 1997). Lipinski's rule of five employed to assess the drug likeliness and durability of a phytocompounds (Benet et al., 2016). Ellagic acid and gallic acid satisfies the drug-like physicochemical properties, structural properties and selectivity of the compound as well as to ensure drug-like physicochemical properties are maintained as described by Lipinski's rule. Tannic acid cannot be considered pharmacologically active lead due to its high molecular weight and doesn't satisfy the Lipinski rule. Phytochemical compounds which satisfy the Lipinski's rule are subjected to docking studies (Meenambiga et al., 2018).

B. Characterization of the Interaction of GA (CID 370) with Hly A

The molecular docking results including the type of amino acids, glide score and binding energy involved in the interaction between ligand and the target receptor protein are elucidated below (Figure 2 and Table 2). It was observed that the two generated conformers of GA is docked and shows a glide score of -6.525 and the glide energy of -22.965Kcal/mol. The interaction of GA was favoured by four hydrogen bond interactions. One hydrogen bond interaction was observed with the amino acid residue Gln516 and Ser 509 with a bond length of 1.88 and 2.21 Aº respectively and two other H bond interaction among the amino acid residues Asp630with bond length of 1.75 and 1.48 Aº respectively. The binding of GA with α haemolysin was also favoured by 8 amino acids residues Ser 509, Thr 512,Lys 513,Phe 518,Ile 660,Arg 611,Val 547 and Leu549 (shown in Fig 2) ,which in turn includes the most discussed and the best binding residues of the protein as obtained from PDB Sum (as discussed in 1.4).The hydrogen bond length and the interacting residues elucidate the effective binding of GA with the protein.

1) Binding mode of EA (CID 5281855) with HlyA

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 four hydrogen bond interactions. One hydrogen bond interaction was observed with the Asp 551, Asp 551, Gly 603 and Ala 604 with bond lengths of 1.87, 2.20, 2.20 and 1.63 respectively.

The binding of EA with HlyA was also favoured by seven amino acids Gly 600,Gln 602,Gly 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.

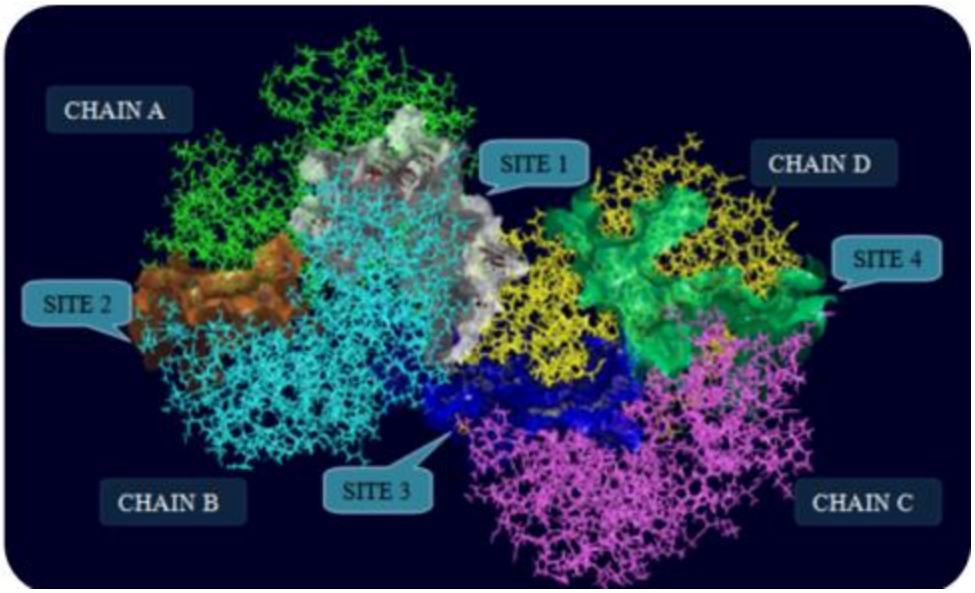

Fig. 1: α - haemolysin showing the four binding site

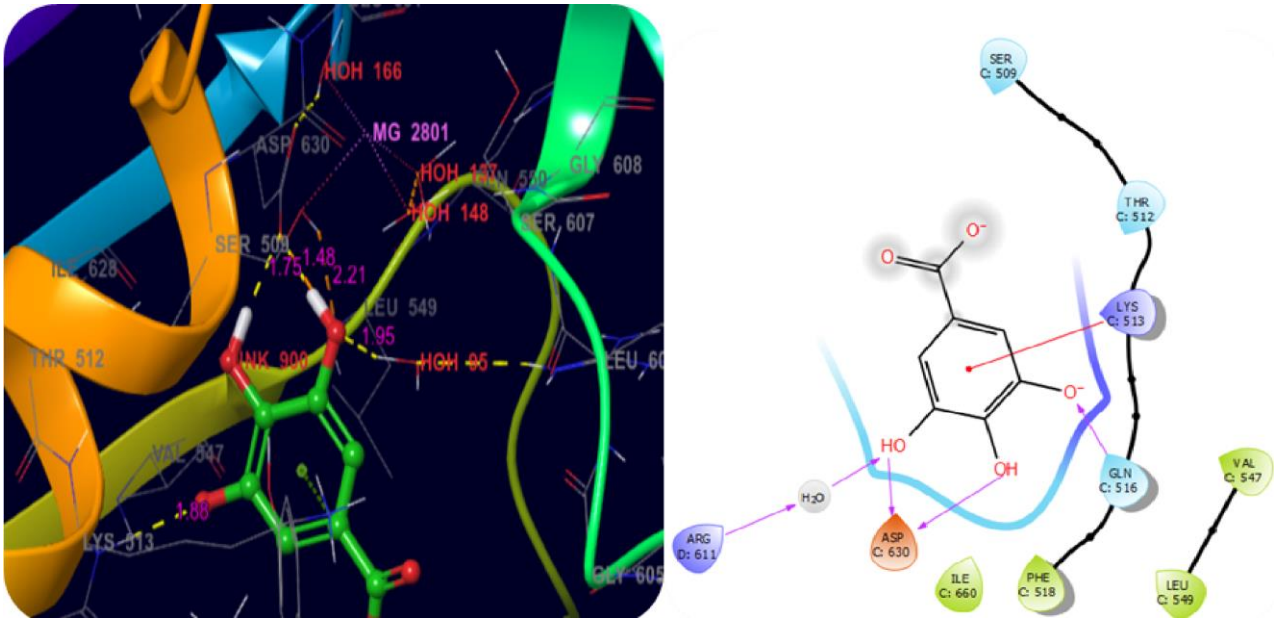

Fig. 2: Docking complexes and interaction of gallic acid (CID 370) with α haemolysin

Table .1: The drug likeliness characterisation of the three phytocompounds

S. No	Compound name	Mol_ MW	Donor HB	Acceptor HB	QPlogP oct	Rotatable bonds	No of hydrogen bonds
1	Ellagic acid	302.197	0	4	1.137	4	4
2	Gallic acid	170.121	1	2	6.27	4	3
3	Tannic acid	1701.206	25	46	6.2	31	14

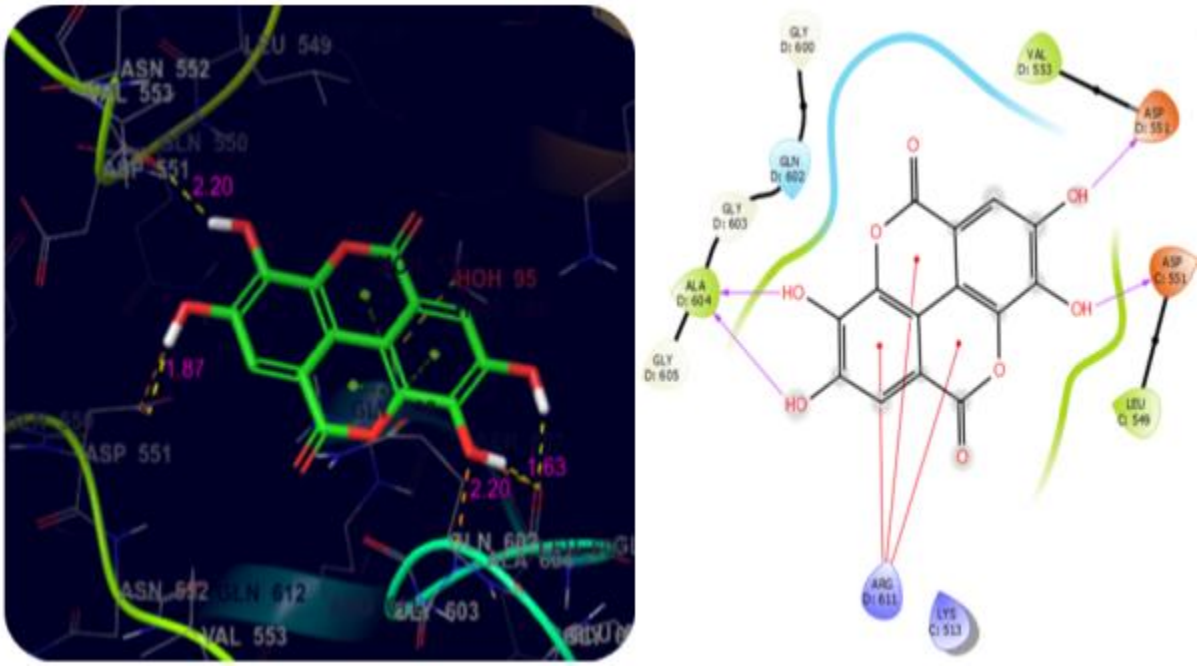

Fig. 3: Docking complexes and interaction of ellagic acid (CID 5281855) with α haemolysin

Table 2: *Insilico* interaction studies of α haemolysin with gallic acid (CID 370)

Amino acid residues interacting and favouring the interaction	Glide score	Glide energy (Kcal/mol)
Ser 509,Thr 512,Lys 513,Gln 516,Phe 518,Ile 660,Arg 611,Val 547,Leu549,Asp 630,Ser 509	-6.525	-22.965

Table 3: *Insilico* interaction studies of α haemolysin with ellagic acid (CID 5281855)

Amino acid residues interacting and favouring the interaction	Glide score	Glide energy (Kcal/mol)
Gly 600,Gln 602,Gly 605, Val 553,Leu 549,Arg 611, Gly 603,Ala 604, Asp 551 and Lys 513	-5.443	-37.274

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 phytocompound 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

UTI: Urinary Tract Infection, MDRUPEC: Multiple Drug Resistant Uropathogenic *Escherichia coli*, WHO: World Health Organization, HlyA : α – haemolysin, TA - tannic acid, GA – Gallic acid, EA – Ellagic acid, HPTLC: High Performance Thin Layer Chromatography, CLSM - Confocal Laser Scanning Microscopy, PDB - Protein Data Bank, SDF - Structural Data Format, QSAR - Quantitative structure-activity relationship, ADME - Absorption, distribution, metabolism, and excretion.

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REFERENCES

Aishwarya, V., Kanimozhi, M., & Sridhar, S. (2013). Correlation of *insilico* and *invitro* analysis of Lux S receptor with the crude extracts of *Terminalia chebula* Retz. *Advanced Bio Tech*, 12(10), 1- 4. ISSN: 2319 – 6750. Article ID 125247, 7. doi :10.1155/2012/125247.

Bag, A., Bhattacharyya, S.K., & Chattopadhyay, R.R. (2013). The development of *Terminalia chebula* Retz. (Combretaceae) in clinical research. *Asian Pac J Trop Biomed*, 3(3), 244 – 252. PMC 3631759; PMID: 23620847. doi: 10.1016/S2221 – 1691 (13)60059 -3.

Bag, A., & Chattopadhyay, R.R.(2014). Efflux-pump inhibitory activity of a gallotannin from *Terminalia chebula* fruit against multidrug-resistant uropathogenic *Escherichia coli*. *Nat Prod Res*, 28(16), 1280-1283. doi: 10.1080/14786419.2014.895729.

Benet, L.Z., Hosey, C.M., Ursu, O., & Oprea, T.I. (2016). BDDCS, the rule of 5 and drugability. *Adv Drug DeliveryRev*, 101, 89-98.

Blair, J.M., Webber, M.A., Baylay, A.J., Ogbolu, D.O., & Piddock, L.J. (2015). Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol*, 31, 42-51.

Chan, C.L., & Lin, C.S. (2012). Phytochemical composition, anti oxidant activity and neuro protective effect of *Terminalia chebula* Retzius extracts. *Evid-based Compl Alt Med*, 1-8.

Chang, H.H., Cohen, T., Grad, Y.H., Hanage,W.P., O'Brien, T.F., & Lipsitch, M. (2015). Origin and proliferation of multiple-drug resistance in bacterial pathogens. *Microbiol Mol Biol Rev*, 79, 101-116.

Chattopadhyay,R.R., & Bhattacharya,S.K. (2007). Plant review: *Terminalia chebula* : an update. *Pharmacogn. Rev*, 1(1), 151 – 156.

Chawdhary, G., Goyal, S., & Poonia, P. (2010). *Lawsonia inermis* Linnaeus: A Phytopharmacological review. *Int J Pharm Sci Drug Res*, 2(2), 91 – 98. ISSN 0975 – 248X.

Gaidhani, S.N., Lavekar, G.S., Juvekar, A., Sen, S., Arjun, S., & Suman, K. (2009). *Invitro* anticancer activity of standard extracts used in Ayurvedha. *Pharmacogn Mag: Research Article*, 5(20), 425 – 429.

Janifer, J., Geethalakshmi, S., Satyavani, K., & Viswanathan, V. (2009). Prevalence of lower urinary tract infection in South Indian type 2 diabetic subjects. *Indian J Nephrol*, 19(3), 107–111.doi:10.4103/0971–4065.57107.PMID: 20436730.

Kim, H.G., Cho, H.G., Jeong, E.Y., Lim, J.H., Lee, S.H., & Lee, H.S. (2006). Growth inhibitory activity of active component isolated from *T. chebula* fruits against intestinal bacteria. *J food Prot*, 69(9), 2205 – 2209.

Lee, D., Boo, K.H., Woo, J.K., Duan, F., Lee, K.H., Kwon, T.K., Lee, H.Y., Riu, K.Z., & Lee, D.S. (2011). Antibacterial and antiviral activities of extracts from *Terminalia chebula* barks. *J. Korean Soc. Appl. BI*, 54(2), 295-298. DOI: 10.3839/jksabc.2011.046.

Lipinski, C.A., Lombardo, F., Dominy, B.W., & Feeney, P.J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev*, 23, 3-25.

McPhillie, M.J., Cain, R.M., Marramore, S., Fishwick, C.W., & Simmons, K.J. (2015). Computational methods to identify new antibacterial targets. *Chem Biol Drug Des*, 85, 22-29.

Meenambiga, S.S., Venkataraghavan, R., & Biswal, A. (2018). *Insilico* analysis of plant phytochemicals against secreted aspartic proteinase enzyme of *Candida albicans*. *J App Pharm Sci*, 8(11), 140-150.

Mohanty, I.R., Borde, M., Kumar, S., & Maheswari, U. (2018). Dipeptidyl peptidase IV Inhibitory activity of *Terminalia arjuna* attributes to its cardio protective effects in experimental diabetes: *Insilico*, *invitro* and *invivo* analyses. *Phytomedicine*, 57, 158-165. doi: https://doi.org/10.1016/j.phymed.2018.09.195.

Narayanan, A.S., Raja, S.S.S., Ponmurugan, K.C., Kandekar, S.C., Natarajaseenivasan, K., Maripandi, A., & Mandeel, Q.A. (2011). Antibacterial activity of selected medicinal plants against Antibiotic resistant uropathogens: A study from Kolli hills, Tamilnadu, India. *Benef Microbes*, 2(3), 235-243. doi: 10.3920/BM2010.0033.

Nhu, N.T.K., Phan, M-D., Forde, B.M., Murthy, A.M.V., Peters, K.M., Day, C.J., Poole, J., & Kidd, T.J., et al. (2019).

Complex multilevel control of haemolysin production by uropathogenic *Escherichia coli*. *Molecular biology and physiology*, mBio10: e02248-19. https://doi.org/10.1128/mBio.02248-19.

Nitha, B., Remashree, A.B., & Balachandran, I. (2012). Antibacterial activity of some selected Indian medicinal plants. *Int. J Pharma. Sci Res*, 3(7), 2038 – 2042. ISSN: 0975 – 8232. PMID: 16995525. doi: 10.4315/0362 – 028x – 69.9.2205.

Priya, E.S., Selvan, P.S., & Ajay, B. (2017). Tannin rich fraction from *Terminalia chebula* fruits as Anti-inflammatory agent. *Journal of Herbs, Spices and Medicinal plants*, 1-14. doi: 10.1080/10496475.2017.1399953

Pugazhendhi, A., Shafreen, R.B., Devi, K.P., & Suganthi, N. (2018). Assessment of antioxidant, anticholinesterase and antiamyloidogenic effect of *Terminalia chebula*, *Terminalia arjuna* and its bioactive constituent 7-methyl gallic acid – An *in vitro* and *in silico* studies. *J Mol Lipids*, 257, 69-81. doi:10.1016/j.molliq.2018.02.081.

Raut, J.S., & Karuppavil, S.M. (2016). Phytochemicals as inhibitors of Candida biofilm. *Curr Pharm Des*, 22, 4111-34.

Reichhardt, C., & Parsek, M.R.(2019). Confocal Laser Scanning Microscopy for analysis of *Pseudomonas aeruginosa* biofilm architecture and matrix localization. *Front Microbiol*, 10 (Article 677), 1 – 9. doi: 10.3389/fmicb.2019.00677.

Savitha, T., & Arrivukkarasu, R (2014). Determination of phytocompounds from T*erminalia chebula Retz* by HPTLC densitometric method. *Int J Pharm Pharmaceut Sci*, 6(7), 516-520. ISSN: 0975 – 1491.

Savitha, T. (2017). Quantitative evaluation of pytocompounds from *Terminalia chebula* by High Performance Thin Layer Chromatography (HPTLC) method and its antibiofilm activity. *Int. J. Pharma Bio Sci*, 8(4), (B) 286 – 291. ISSN: 0975 – 6299. doi: http://dx.doi.org/10.22376/ijpbs.2017.8.4.b286-291.

Savoia, D. (2012). Plant-derived antimicrobial compounds: Alternatives to antibiotics. *Future Microbiol*, 7, 979-990.

Setzer, M.S., Sharifi-Rad, J., & Setzer, W.N. (2016). The search for herbal antibiotics: An *insilico* investigation of antibacterial phytochemicals. *Antibiotics*, 5, 30. doi:10.3390/antibiotics5030030.

Wang, C., Li, Q., Lv,J., Sun, X., Cao, Y., Yu, K., Maio, C., Zhang, Z.S., Yao, Z., & Wang, Q. (2020). Alpha-haemolysin of uropathogenic *Escherichia coli* induces GM-CSF-mediated acute kidney injury. *Mucosal Immunology*, 13, 22-33. https://doi.org/10.1038/s41385-019-0225-6.

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