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Urease inhibition and DPPH radical scavenging potential of phytoconstituent from *Alstonia scholaris* and molecular docking interactions of bioactive luteolin with target proteins

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Abstract: A polyphenolic flavone Luteolin (3′,4′,5,7-tetrahydroxyflavone) is found in various plants and is traditionally used in Chinese medicine. It is obtained from *Alstonia scholaris* (L.) R.Br Flower belonging to the family Apocynaceae while investigation. Various studies have been demonstrated the antioxidant or antiulcer potential of luteolin from different plant sources. In the present investigation the antioxidant or antiulcer effect of the Luteolin has been carried out using molecular docking simulations. The objective of this study was to analyze the antioxidant and antiulcer potential of luteolin obtained during isolation. The *in vitro* biological evaluation has been supported by the *in silico* studies using Autodock vina 4 shows the ligand-protein interaction of lute olin with 1HD2, 4GY7 and 3O1Q. Luteolin showed significant DPPH scavenging and urease inhibition activity i.e., 23.4 ± 0.87, 6.21±0.45 IC50 (uM) respectively as compared to the standard BHA and thiourea 44.2±0.45, 22.4±0.29 IC50 (uM) respectively. The docking simulations showed significant binding pocket sites with the respective proteins1HD2, 4GY7 and 3O1Q with the least binding energy -6.8, -8.0 and -8.2 kcal/mol respectively. Thus, Strong evidence has been presented with their confirmation structural interaction via molecular docking with proteins that serve as binding sites for available Luteolin molecule. The findings justify the application of the compound as a novel antioxidant and antiulcer agent.

Keywords: *Alstonia scholaris,* luteolin, DPPH scavenging, urease inhibition; molecular docking.

INTRODUCTION

Alstonia scholaris, generally known as “saptaparna” or “devil's tree”. It belongs to the Apocynaceae family and has been used to treat a variety of human ailments (Adotey *et al*., 2012; Antony *et al*., 2011). According to the latest literature survey, Alstonia is a popular folklore treatment and found effective in the treatment of stomach diseases, dyspepsia, leprosy, skin conditions, tumors, chronic and severe ulcers, malaria, asthma, bronchitis, Antimicrobial, antioxidant, anti-inflammatory, hepatoprotective and anticancer properties (Adotey *et al*., 2012; Anubha and Rai, 2015; Arulmozhi *et al*., 2007; Baliga, 2012).

Luteolin (3',4',5,7-tetrahydroxyflavone), the flavonoid is found in a wide variety of plants, including citrus, plants, and medicinal herbs. Lutein-rich plants have been utilized in traditional Chinese medicine to treat a variety of ailments, including hypertension, inflammations and carcinoma. Biochemically, luteolin acts as an antioxidant or a pro-oxidant (Lin *et al*., 2008). Preclinical studies have demonstrated that this flavone exhibits a range of pharmacological properties, including antioxidant, anti-inflammatory, antibacterial and antiulcer properties (Lopez-Lazaro, 2009). In the latest study, the quantitative determination indicates that Luteolin had the most protective effect against DNA damage of all flavonoids tested in the presence of Hydrogen peroxide (Romanova *et al*., 2001). Luteolin boosted the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase synergistically (GR) (Ashokkumar and Sudhandiran, 2008; Leopoldini *et al*., 2004; Ozgen *et al*., 2011). Luteolin is a known compound that is safe and possesses a multiplicity of effects against gastric ulceration. Various clinical studies indicate the antiulcer potential of this flavonoid (de la Lastra *et al*., 1994; Izzo *et al*., 1994; Ode and Asuzu, 2014; Zahran *et al*., 2021)

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MATERIALS AND METHODS

Collection of Plant collection

Plant material (*Alstonia scholaris* (L.) R.Br) Flower was collected from the field of HEJ Research Institute of Chemical and Biological Center (ICCBS). A taxonomist from the University of Karachi's Department of Botany identified the plant. A voucher sample of the flowers of *Alstonia scholaris* has been preserved Voucher number G.H. 94482. The reference sample has been deposited at the herbarium.

Extraction and isolation

Plant extracts (ASF-EtA) are prepared via solvent extraction using ultrasonic waves. 95 percent ethanol (20 L 3 times, AR grade, Thailand) was used to extract *A. scholaris* (flower bud) with at room temperature for half hour with ultrasonic assistance. The *A. scholaris* ethanolic extract (ASF-EtOH) was kept refrigerated until it was used. Ethyl acetate was used to achieve liquid-liquid partitioning of ASF-EtOH. It was labeled ASF-EtA (ethyl acetate layer) and kept in the fridge until needed. Isolation of ASF-EtA (ethyl acetate layer) was performed. Luteolin is isolated by column chromatography, eluent using hexane, hexane: dichloromethane (DCM), DCM and DCM: Methanol (MeOH) mixtures (CC, silica gel, HEX, DCM, and MeOH in order of increasing polarity). 11 fractions designated as F1-F11, respectively. Fraction (F10) was triturated with methonal to generate off-white precipitates that were filtered. After elution with MeOH, the residue was treated to gel chromatography using Sephadex, resulting in the separation of luteolin (18.5 mg).

Characterization of compound

NMR (Nuclear Magnetic Resonance), EI-MS (Electron Ionization- Mass Spectrometry), and HREIMS (High-Resolution Electron Ionization- Mass Spectrometry) techniques were designed to estimate the structures of isolated molecules. The molecular weights were determined using MS spectra. Infrared spectroscopy was used to assess the functional groups in the compound.

In vitro DPPH radical scavenging activity

The scavenging activity of 1,1-diphenyl-2-picryl-hydrazil (DPPH) was determined using the method published by Gulcin *et al* (1). BHA was employed as a control. The following formula was used to determine the scavenging activity, DPPH scavenging effect (%) = Ac-As/Ac × 100.
Where
Ac = Absorbance of Control (DMSO treated)
As = Absorbance of Sample

In vitro urease inhibition activity

Urease activity was estimated by measuring ammonia generation using Weather burn's indophenol technique. SoftMax Pro software was used to process the data (change in wavelength per minute) (Molecular Device, USA). All experiments were carried at a pH of 8.2. (0.01 M K2HPO4.3H2O, 1mM EDTA and 0.01M LiCl2). Thiourea was utilised as the usual urease inhibitor. The following formula was used to compute the percentage of inhibitions, 100 - ($OD_{testwell}/OD_{control}$) x100.

STATISTICAL ANALYSIS

The Inhibition concentration IC50 values were calculated using the GraphPad Prism software. Unless othenvise specified, these values are expressed as mean ± standard deviation of triplicate as shown in table 2.

Molecular docking simulations

Molecular docking, of the isolated pure compound with significant in-vitro antioxidant and antiulcer activities, was performed with the Auto dock vina (Trott *et al*, 2010) program. The first conformation with the least energy was taken for further analysis. The interaction analyses were carried out by Discovery studio visualizer (Dassault systemes, 2016).

PDB structure files of target proteins namely, Human per oxiredoxin 5, Helicobacter pylori Urease Accessory Protein UreF and Urease from Jack bean were downloaded from the RCSB repository with PDB codes 1HD2, 4GY7 and 3O1Q respectively. All protein structures were analyzed in VMD (Humphrey *et al* 1996; Waterhouse *et al*, 2018). Pdbqt receptor file was created using AutoDockTools4 (Morris *et al*, 2009). The ligand's 3D structure file of Luteolin was downloaded from Pubchem (code: 5280445) and polar hydrogen atoms and Gasteiger partial charges were added to the ligand. AutoDockTools4 was used to generate. Pdbqt files.

RESULTS

NMR spectroscopy

Luteolin isolated as a yellow powder (Lin *et al.*, 2015) presented the following data in table 1 depicted the structure of isolated compound fig. 1.

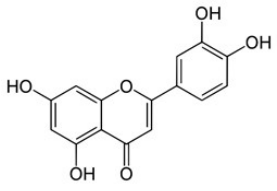


Fig. 1: Structure of Luteolin Compound

In vitro DPPH radical scavenging activity and urease inhibition activity

The pure compound was tested for antioxidant and antiulcer activity using DPPH scavenging and urease inhibition methodology. Luteolin showed some significant results which have been presented in table 2.

Table 1: NMR spectral analysis of luteolin.

S. No	Spectral Parameters	Analysis
1.	EI- MS	M/z 286 [M]+ (100), 270, 258, 229, 153, 134, 96 and 43.
2.	HREI- MS	M/z 286.0437 (Calcd. 286.0477 for C15H10O6).
3.	1H NMR	(d6-DMSO, 400 MHz): δ 7.35 (1H, dd, J=8.3, 1.8 Hz, H-6'), 7.30 (1H, d, J=1.8 Hz, H-2'), 6.76 (1H, d, J=8.3 Hz, H-5'), 6.52 (1H, s, H-3), 6.31 (1H, d, J=1.2 Hz, H-8) and 6.05 (1H, d, J=1.2 Hz, H-6).

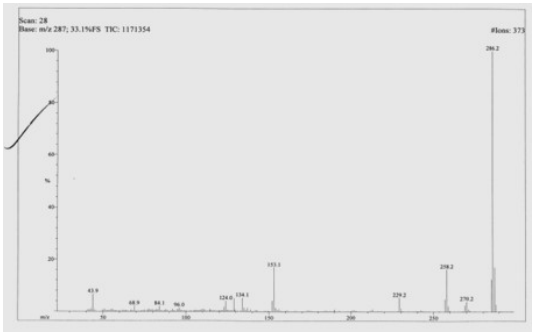
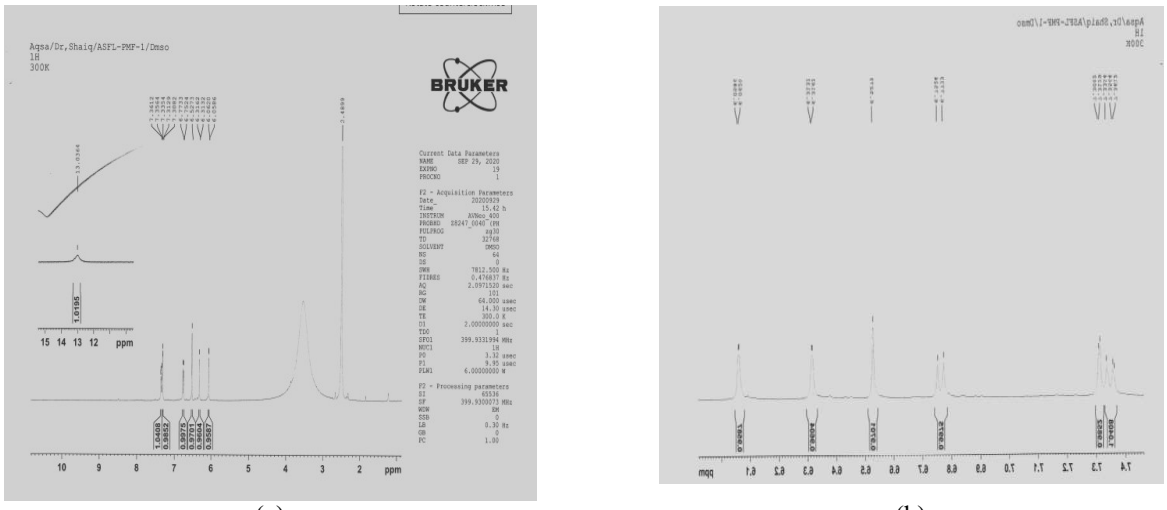


Fig. 2: NMR- EI+ MS Spectra of Luteolin.



(a) (b)

Fig. 3: Interpretation of NMR- EI+ MS Spectra of Luteolin (a) and (b)

Table 2: Antioxidant and urease inhibition activity (IC_{50}) of luteolin from *Alstonia scholaris* flower extract.

S. No.	Compound/ Standard	Antioxidant Activity IC_{50} value (uM)	Urease Inhibition Activity IC_{50} value (uM)
1.	Luteolin	23.4 ± 0.87	6.21 ±0.45
2.	BHA(Butylated Hydroxyanisole)	44.2 ± 0.45	
3.	Thiourea		22.4 ± 0.29

Table 3: Ligand-protein interaction of luteolin with human peroxiredoxin Protein

S. No.	Ligand	Human peroxiredoxin 5 (1HD2)
1.	The binding energy of 1st docked conformation of Luteolin in kcal/mol	-6.8
2.	Interacting Residues	Glu16, Thr81, Gly82, Gly85, Ala90, Gly92, Lys93, Arg95, Leu96
3.	Hydrogen bonds interactions	Thr81 (3.08Å), Gly82 (2.51Å), Gly85 (2.96Å)
4.	Hydrophobic interactions	Ala90, GLy92, Lys93, Arg95, Leu96

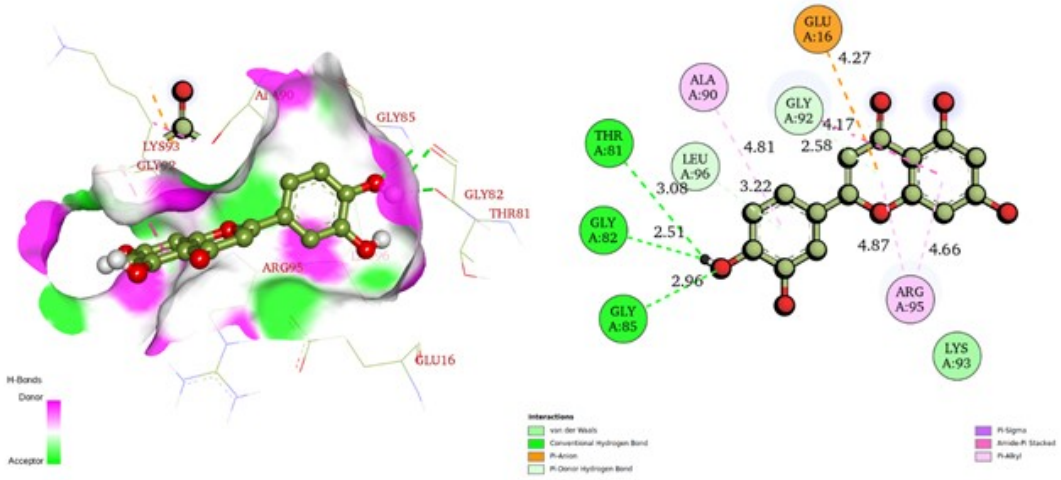


Fig. 4: 3D and 2D interactions of Human peroxiredoxin 5 (1HD2) with Luteolin.

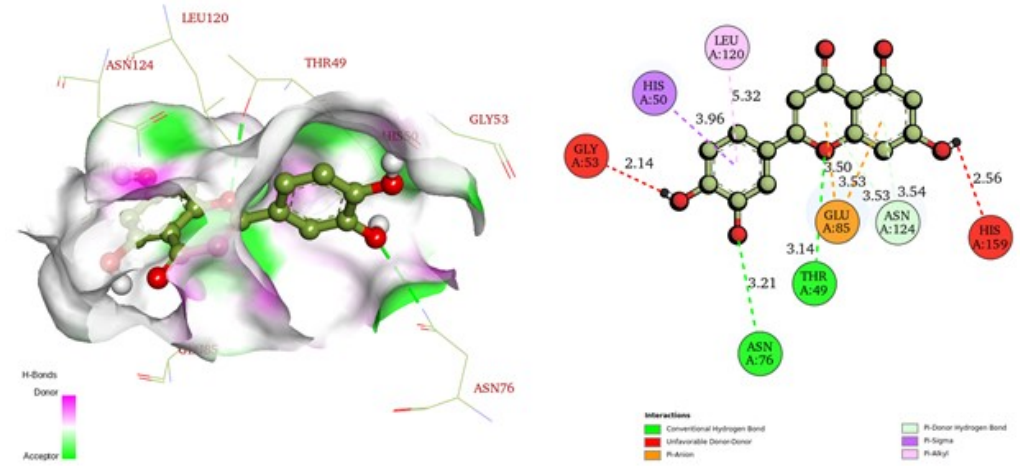


Fig. 5: 3D and 2D interactions of Helicobacter pylori Urease Accessory Protein UreF (3O1Q) with Luteolin.

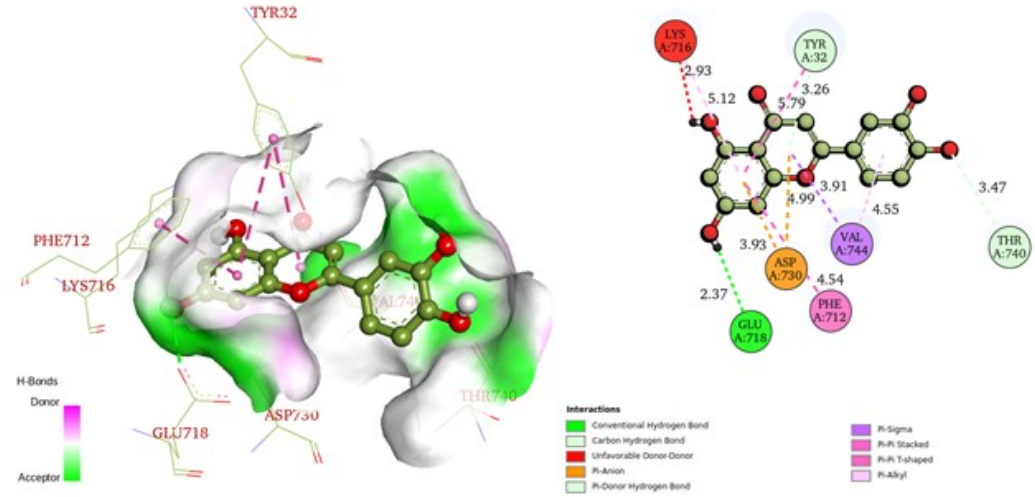


Fig. 6: 3D and 2D interactions of Urease from Jack bean (4GY7) with Luteolin.

Table 4: Ligand-protein interaction of luteolin with urease from jack bean protein

S. No.	Ligand	Urease from Jack bean (4GY7)
1.	The binding energy of 1st docked conformation of Luteolin in kcal/mol	-8.2
2.	Interacting Residues	Tyr32, Phe712, Lys716, Glu718, Asp730, Thr740, Val744
3.	Hydrogen bonds interactions	Glu718 (2.37Å)
4.	Hydrophobic interactions	Tyr32, Val744, Phe712

Table 5: Ligand-protein interaction of luteolin with helicobacter pylori urease accessory protein UreF protein

S. No.	Ligand	Helicobacter pylori Urease Accessory Protein UreF (3O1Q)
1.	The binding energy of 1st docked conformation of Luteolin in kcal/mol	-8.0
2.	Interacting Residues	Thr49, His50, Gly53, Asn76, Glu85, Leu120, Asn124, His159
3.	Hydrogen bonds interactions	Thr49 (3.14Å), Asn76 (3.21Å)
4.	Hydrophobic interactions	His50, Leu120

Docking and simulations

The top docked conformation with their energy, interacting residues, Hydrogen bond formation with distance and hydrophobic interaction with all four proteins have been present in tables 3-5.

The binding site interacting residues of Human per oxiredoxin 5 (1HD2) with Luteolin. The binding energy of the first conformation was found to be -6.8 kcal/mol. The interacting residues included Glu16, Thr81, Gly82, Gly85, Ala90, Gly92, Lys93, Arg95 and Leu96. Of these residues, Thr81 (3.08A), Gly82 (2.51A), and Gly85 (2.96Å) are involved in hydrogen bond formation, while Ala90, GLy92, Lys93, Arg95 and Leu96 are involved in various types of hydrophobic as shown in fig. 4.

The binding site interacting residues of Helicobacter pylori Urease Accessory Protein UreF (3O1Q) with Luteolin. The binding energy of the first conformation was found to be -8.0kcal/mol. The interacting residues included Thr49, His50, Gly53, Asn76, Glu85, Leu120, Asn124 and His159. Of these residues, Thr49 (3.14A), and Asn76 (3.21A) are involved in hydrogen bond formation, while His50 and Leu120 are involved in various types of hydrophobic interactions as indicated in fig. 5. Gly53 and His159 are involved in unfavorable donor-donor hydrogen bond formation. However, the overall binding energy is favorable.

The binding site interacting residues of Urease from Jack bean (4GY7) with Luteolin. The binding energy of the first conformation was found to be -8.2kcal/mol. The interacting residues included Tyr32, Phe712, Lys716, Glu718, Asp730, Thr740 and Val744. of these residues, only Glu718 (2.37Å) is involved in hydrogen bond formation, while Tyr32, Val744 and Phe712 are involved in various types of hydrophobic interactions as indicated in fig. 6. Lys716 is involved in unfavorable donor-donor hydrogen bond formation however, the overall binding energy is favorable.

DISCUSSION

The pure compound Luteolin, isolated from the flowers of *Alstonia scholaris* were characterized using NMR spectroscopic techniques. The compound was subjected to *in-vitro* and *in-silico* studies including antioxidant activity, urease inhibition, and molecular docking simulation. Luteolin showed significant results as an antioxidant, and urease inhibitor, thiourea and BHA (Butylated Hydroxyanisole) were used as a positive control. Subsequently, Luteolin was adopted to molecular docking simulations because of the potential for novel drugs in various illnesses such as ulcers, and oxidative stress-mediated disease, etc. Luteolin was docked with human per oxiredoxin 5 (1HD2). Per oxiredoxin 5 (PRDX5) is a new thioredoxin per oxidase that has recently been discovered in a range of human cells and tissues and is assumably vital in oxidative stress defense mechanisms (Dubuisson *et al*., 2004; Mohammad *et al*., 2020; Wang *et al*., 2001). Luteolin docked with per oxiredoxin 5 with binding energy -6.8kcal/mol with hydrogen bond formation that indicates a good binding response.

Thus, the *in-vitro* results of luteolin as an antioxidant were found to be promising for *in-vivo* protein binding capacity against any oxidative stress management (Auniq *et al*., 2021; Bankeu *et al*., 2019). Wei *et al* (2018) investigated the effects of luteolin on myocardial ischemia/reperfusion injury were investigated in vivo (Sprague-Dawley rats) and *in vitro*, as well as the underlying processes, with a particular emphasis on peroxiredoxin II signalling. Thus, Luteolin significantly increased peroxiredoxin II expression. Molecular docking analysis demonstrated that luteolin can definitely attach to the active site of peroxiredoxin II. However, docking luteolin with peroxiredoxin 5 (1HD2) revealed an important binding pocket that has not been explored previously.

Moreover, *Helicobacter pylori* Urease Accessory Protein UreF (3O1Q), Urease from Jack bean (4GY7) have also been interacted with Luteolin and showed significant interaction with lower binding energy -8.0 kcal/mol and -8.2 kcal/mol respectively (table 5 and 6). Both the proteins 3O1Q and 4GY7 were from different sources were assessed to confirm the possible efficacy of luteolin *in-vivo* as an antiulcer agent. Recently, several synthetic derivatives were assessed for their ability to inhibit Jack Bean urease using a well-established *in vitro* assay, and molecular docking was used to determine the manner of binding of compounds to urease (Barakat *et al*., 2020; Gholivand *et al*., 2019; Hamad *et al*., 2020; Rauf *et al*., 2015). Bashir *et al*. (2017) identified three flavanonol chemicals from Picea Smithiana (wall) Boiss: quercetin (1), dihydroquercetin (2), and dihydromyricetin (3). Docking studies were conducted on compounds 1-3. According to the docking statistics, compound 3 has a binding affinity of -8.5 kcal/mol for 4GY7.However, the ligand-protein interaction of luteolin with 3O1Q and 4GY7 has been done the first time which has revealed potent activity against urease and can be used for future drug discovery of novel medicine for the treatment of ulcers (Xiao *et al*., 2012; Zahran *et al*., 2021). On the contrary, it supports the *in-vitro* investigation which showed the highly potent result as compared to thiourea used as a positive control.

CONCLUSION

In conclusion, Luteolin has strong antioxidant and urease inhibition activity. It has been presented with their confirmation structural interaction via molecular docking with target proteins that serve as a binding site for available Luteolin molecules. The novelty of work is to study the molecular docking for modeling the antioxidant activity of compounds against ligand-protein interaction with human peroxiredoxin 5 (1HD2). Similarly, the enzyme inhibition activity (urease) has also been studied first time for Helicobacter pylori Urease Accessory Protein UreF (3O1Q), urease from Jack bean (4GY7). The findings justify the application of the compound as a novel antioxidant and antiulcer agent.

REFERENCES

Adotey JPK, Adukpo GE, Opoku Boahen Y and Armah FA (2012). A review of the ethnobotany and pharmacological importance of Alstonia boonei De Wild (Apocynaceae). *ISRN* Pharmacol. doi:10.5402/2012/587160

Antony M, Menon DB, Joel J, Lipin D, Arun K and Thankamani V (2011). Phytochemical analysis and antioxidant activity of *Alstonia scholaris. Pharmacog. J.,* **3**(26): 13-18.

Anubha A, Rai Y (2015). A review: Phytochemictry, ethanobotanical and pharmacological activities of *Alstonia scholaris* R. Br (Apocynaceae). *Int. J. Adv. Res.,* **3**(8): 584-590.

Arulmozhi S, Mazumder PM, Ashok P, Narayanan LS (2007). Pharmacological activities of *Alstonia scholaris* Linn. (Apocynaceae)-A review. *Pharmacogn. Rev*. **1**(1): 163-170

Ashokkumar P and Sudhandiran G (2008). Protective role of luteolin on the status of lipid peroxidation and antioxidant defense against azoxymethane-induced experimental colon carcinogenesis. *Biomed. Pharmacother.,* **62**(9): 590-597.

Auniq RBJ, Chy MdNU, Adnan Md, Chowdhury MdR, Dutta T, Ibban SS, Jahan I, Tona MR, Shima M, Sawon MIH, Chakrabarty N, Hossain S and Paul A (2021). Evaluation of anxiolytic, sedative and antioxidant activities of *Vitex peduncularis* Wall. leaves and investigation of possible lead compounds through molecular docking study. *Adv. Tradit. Med*. **21**(3): 507-518.

Baliga MS (2012). Review of the phytochemical, pharmacological and toxicological properties of *Alstonia scholaris* Linn. R. Br (Saptaparna). *Chin. J. Integr. Med.*pp.1-14.

Bankeu JJ, Sattar H, Fongang YS, Muhammadi SW, Simoben CV, Ntie-Kang F, Feuya GR, Tchuenmogne MA, Lateef M and Lenta BN (2019). Synthesis, urease inhibition and molecular modelling studies of novel derivatives of the naturally occurring β-amyrenone. *Nat. Prod. Bioprospect.,* **9**(1): 49-59.

Barakat A, Soliman SM, Ali M, Elmarghany A, Al-Majid AM, Yousuf S, Ul-Haq Z, Choudhary MI and El-Faham A (2020). Synthesis, crystal structure, evaluation of urease inhibition potential and the docking studies of cobalt (III) complex based on barbituric acid Schiff base ligand. *Inorganica Chimica. Acta.* **503**: 119405.

Bashir K, Bashir A, Rauf A, Bawazeer S, Urrehman K, Rehman T, Saleem M, Ahmed R, Linfang H, Ikram R and Khan G (2017). Urease inhibition potential and molecular docking of dihydroquercetin and dihydromyricetin isolated from *Picea smithiana* (wall) Boiss. *Biomed. Res.* **28**(22): 10026-10032.

Dassault Systemes BIOVIA, Discovery Studio Modeling Environment, Release (2017). Dassault Systèmes, San Diego.

De la Lastra A, Martin MJ and Motilva V (1994). Antiulcer and gastroprotective effects of quercetin: A gross and histologic study. *Pharmacol.,* **48**(1): 56-62.

Dubuisson M, Vander Stricht D, Clippe A, Etienne F, Nauser T, Kissner R, Koppenol WH, Rees JF and Knoops B (2004). Human peroxiredoxin 5 is a peroxynitrite reductase. *FEBS Letters,* **571**(1-3): 161-165.

Gholivand K, Pooyan M, Mohammadpanah F, Pirastefar F, Junk PC, Wang J, Ebrahimi Valmoozi AA and Mani-Varnosfaderani A (2019). Synthesis, crystal structure and biological evaluation of new

phosphoramide derivatives as urease inhibitors using docking, QSAR and kinetic studies. *Bioorg. Chem,* **86**(1): 482-493.

Gulcin I, Alici HA and Cesur M (2005). Determination of *in vitro* antioxidant and radical scavenging activities of propofol. *Chem. Phar. Bull.,* **53**(3): 281-285.

Hamad A, Abbas Khan M, Ahmad I, Imran A, Khalil R, Al-Adhami T, Miraz Rahman K, Quratulain Zahra N and Shafiq Z (2020). Probing sulphamethazine and sulphamethoxazole based Schiff bases as urease inhibitors; synthesis, characterization, molecular docking and ADME evaluation. *Bioorg. Chem.,* **105**: 104336.

Humphrey W, Dalke A and Schulten K (1996). VMD-Visual molecular dynamics. *J. Molec. Graphics* **14**(1): 33-38.

Izzo AA, Carlo GD, Mascolo N, Capasso F and Autore G (1994). Antiulcer effect of flavonoids. Role of endogenous PAF. *Phytother Res,* **8**(3): 179-181.

Leopoldini M, Pitarch IP, Russo N and Toscano M (2004). Structure, conformation and electronic properties of apigenin, luteolin and taxifolin antioxidants. A first principle theoretical study. *J. Phys. Chem. A.,* **108**(1): 92-96.

Lin L C, Pai Y F and Tsai TH (2015). Isolation of luteolin and luteolin-7-*O*-glucoside from *Dendranthema morifolium* Ramat Tzvel and their pharmacokinetics in rats. *J. Agric. Food Chem.,* **63**(35): 7700-7706.

Lin Y, Shi R, Wang X and Shen HM (2008). Luteolin: A flavonoid with potential for cancer prevention and therapy. *Curr. Cancer Drug Targets* **8**(7): 634-646.

Lopez-Lazaro M (2009). Distribution and biological activities of the flavonoid luteolin. *Mini Rev Med. Chem.* **9**(1): 31-59.

Mohammad A, Saini RV, Kumar R, Sharma D, Saini NK, Gupta A, Thakur P, Winterbourn CC and Saini AK (2020). A curious case of cysteines in human peroxiredoxin I. *Redox Biol.,* **37**(10): 101738.

Morris GM, Huey R and Lindstrom W (2009). AutoDock4 and Auto Dock Tools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.,* **30**(16): 2785-2791.

Ode OJ and Asuzu IU (2014). Luteolin isolate from the methanol extract identified as the single-carbon compound responsible for broad antiulcer activities of *Cassia singueana* leaves. *IOSR J. Pharm.,* **4**(10): 17-23.

Ozgen U, Mavi A, Terzi Z, Kazaz C, Asci A, Kaya Y and Secen H (2011). Relationship between chemical structure and antioxidant activity of luteolin and its glycosides isolated from *T. sipyleus* subsp. sipyleus var. sipyleus. *Rec. Nat. Prod.,* **5**(1): 12.

Rauf A, Shahzad S, Bajda M, Yar M, Ahmed F, Hussain N, Akhtar MN, Khan A and Jonczyk J (2015). Design and synthesis of new barbituric- and thiobarbituric acid derivatives as potent urease inhibitors: Structure activity relationship and molecular modeling studies. *Bioorg. Med. Chem.* **23**(17): 6049-6058.

Romanova D, Vachalkova A, Cipak L, Ovesna Z and Rauko P (2001). Study of antioxidant effect of apigenin, luteolin and quercetin by DNA protective method. *Neoplasma.,* **48**(2): 104-107.

Trott O, & Olson AJ (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.,* **31**(2): 455-461.

Wang MX, Wei A, Yuan J, Clippe A, Bernard A, Knoops B and Murrell GAC (2001). Antioxidant enzyme peroxiredoxin 5 Is upregulated in degenerative human tendon? *Biochem. Biophys. Res. Commun.* **284**(3): 667-673.

Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R and Schwede T (2018). SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Res.,* **46**(1): 296-303

Wei B, Lin Q, Ji YG, Zhao YC, Ding LN, Zhou WJ, Zhang LH, Gao CY and Zhao W (2018). Luteolin ameliorates rat myocardial ischaemia-reperfusion injury through activation of peroxiredoxin II. *Br. J. Pharmacol.* **175**: 3315-3332.

Xiao ZP, Wang XD, Peng ZY, Huang S, Yang P, Li QS, Zhou LH, Hu XJ, Wu LJ and Zhou Y (2012). Molecular docking, kinetics study and structure–activity relationship analysis of quercetin and its analogous as *Helicobacter pylori* urease inhibitors. *J. Agric. Food Chem.,* **60**(42): 10572-10577.

Zahran EM, Abdelmohsen UR, Hussein AS, Salem MA, Khalil HE, Yehia Desoukey S, Fouad MA and Kamel MS (2021). Antiulcer potential and molecular docking of flavonoids from *Ocimum forskolei* Benth., family Lamiaceae. *Nat. Prod. Res.,* **35**(11): 1933-1937.