Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2022; 11(2): 24-32
Received: 18-03-2022
Accepted: 29-03-2022

Dana Isabelle A Segui
Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, España Blvd., Sampaloc, Manila, Philippines

Ryne James P Gandia
Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, España Blvd., Sampaloc, Manila, Philippines

Ramil Joseph P Pizarro
Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, España Blvd., Sampaloc, Manila, Philippines

Julian P Soriano
Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, España Blvd., Sampaloc, Manila, Philippines

Clemonne John S Madarang
Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, España Blvd., Sampaloc, Manila, Philippines

Andrea G Vargas
Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, España Blvd., Sampaloc, Manila, Philippines

Corresponding Author:
Dana Isabelle A Segui
Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, España Blvd., Sampaloc, Manila, Philippines

Potential inhibitory properties of selected plant secondary metabolites from local plant families in the Philippines against AcrAB-TolC drug efflux pump system of *E. coli*: An *In silico* analysis

Dana Isabelle A Segui, Ryne James P Gandia, Ramil Joseph P Pizarro, Julian P Soriano, Clemonne John S Madarang and Andrea G Vargas

DOI: https://doi.org/10.22271/phyto.2022.v11.i2a.14372

Abstract
Antibiotics heralded an approach to controlling and ending infectious diseases caused by bacteria. However, these life-saving drugs have begun to lose their efficacy as various bacteria have gained a significant level of antibiotic resistance over the years. Amidst these bacterial survival mechanisms, plant secondary metabolites provide a possible countermeasure against this phenomenon because of their defense mechanisms. Through *in silico* analytic procedures, nine selected plant secondary metabolites from *Ixora coccinea*, *Mimosa pudica*, and *Origanum vulgare*, in the Philippines were molecularly docked using AutoDock simulation software and Biovia Discovery Studio against the RND efflux pump system, AcrAB-TolC of *E. coli*. All of the selected metabolites showed negative binding energies implying high ligand-receptor affinity and good stability, especially the secondary metabolites of *I. coccinea*. Metabolites that have remarkable properties similar to the existing efflux pump inhibitors include lupeol, quercetin, galangin, kaempferol, and ursolic acid.

Keywords: Plant secondary metabolites, AcrAB-TolC drug efflux pump, *E.* coli, efflux pump inhibitors, phytochemistry

Introduction
Nearly a century has passed since the discovery of antibiotics, which revolutionized the medical world and led to significantly reduced number of disease-related fatalities during the early years of its discovery. However, these drugs and antibiotics that once saved countless lives have started losing efficacy against the bacteria they were made to combat. Antibiotic resistance, particularly multidrug resistance (MDR), has become a public health problem [1]. Multidrug-resistant infections are correlated to poor clinical outcomes, and there is a growing concern that pan-resistant strains will lead to some ailments becoming completely untreatable. Resistance to antibiotics typically occurs due to drug modification/inactivation, target site mutation, and reduced accumulation due to decreased permeability and increased efflux pump activity [2].

Efflux pumps are membrane-spanning proteins situated in the cytoplasmic membrane of eukaryotic and prokaryotic cells. These have been reported to contribute significantly to the issue of antibiotic resistance [2, 3]. Furthermore, it is described as a key mechanism in antibiotic resistance, particularly in gram-negative bacteria. These efflux pumps essentially allow microorganisms to regulate their internal environment by removing toxic substances, as well as metabolites, quorum sensing molecules, and antimicrobial agents [3]. The efflux pumps can be composed of either a single component or multiple components, with the latter being only exclusive to gram-negative bacteria. These pumps are classified into six families based on the number of components, transmembrane spanning regions, energy source, and the types of molecules they specifically export [3, 4]. The six families classify into: (1) the ATP-binding cassette (ABC) superfamily, (2) the major facilitator superfamily (MFS), (3) the multidrug and toxic compound extrusion (MATE), (4) the small multidrug resistance (SMR) family, (5) the resistance-nodulation-division (RND) superfamily, and (6) the drug metabolite transporter (DMT) superfamily [4]. Initially observed as a mechanism of resistance to tetracycline in *Escherichia coli*, the activities of the efflux pumps have been observed within many organisms, wherein they have become increasingly treated as important determinants to antimicrobial resistance [4].

Gram-negative bacteria possess an outer membrane that protects them from a wide range of antibiotics and detergents that would typically damage the bacteria itself [5]. Most antibiotics pass the outer membrane to lock onto their respective targets, such as hydrophilic drugs passing through the porins or hydrophobic drugs entering through the diffusion pathway. However, its outer membrane can alter its hydrophobic properties or cause a mutation within its porins to grant the bacteria resistance to various antibiotics, which boosts the gram-negative bacteria's capabilities of fighting against antibiotics [6]. Furthermore, the pathogenesis of gram-negative bacteria rely on the assemblies of tripartite protein spanning their double membrane to extrude the antibiotics from the cell. This tripartite complex consists of a periplasmic membrane fusion protein, outer membrane protein, and inner membrane protein of the RND family [7]. The recent successes in determining the structure and analyzing the functions of MexB and AcrB components of the MexAB-TolC and AcrAB-TolC drug efflux pump systems have significantly contributed to understanding the mechanism for efflux inhibition [7].

As the modern means of defense against bacteria weaken, it is only natural to search for other pathways to negate the rise of antibiotic resistance. Since ancient times, people have always employed various plants and their derivatives for medicinal purposes, including treating infectious diseases. In eastern medicine, the plants' role in medicinal treatment has always been prevalent throughout the years, and only until recently, western medicine started using herbal extracts as potential therapeutic agents [8]. In addition to low cost, high accessibility, and availability, herbal extracts are abundant sources of various plant secondary metabolites with high therapeutic value. This would give reason to the rising attention medicinal plants have been gaining as about 40% of modern medicine was derived from phytochemicals. The remedial effects of these medicinal plants are highly contributed by a mixture of substances called plant secondary metabolites. Plant secondary metabolites are a diverse group of biochemical substances produced by the plant cell through secondary metabolic pathways derived from the primary metabolic pathways [9]. Compared to their direct counterparts that mainly focus on survival, plant secondary metabolites play the role of a defender against different kinds of threats, including microbes [8, 9].

To this day, there are about 200,000 different plant secondary metabolites that have been isolated [10]. A variety of plants use plant secondary metabolites as a defense mechanism against pathogens, which means that they can either partially or entirely completely inhibit some microorganisms' proliferation. Plant-derived compounds have also been known to directly interfere with the main pathogenic process, potentially decreasing the bacteria's chances of developing drug resistance. Thus, using these compounds in association with traditional antibiotics like methicillin, which have lost their efficacy, is promising as it brings the idea of possibly reusing these old antibiotics that they may be able to overcome the bacteria's MDR pump systems [10]. This *in silico* study investigated the feasibility of secondary plant metabolites from local plant sources in the Philippines as the main sources of inhibitors for *E. coli*'s AcrAB-TolC drug efflux pump system. Specifically, it explored the inhibitory properties of the selected secondary metabolites through *in silico* analysis.

Materials and Methods

Numerous plants in nature possess various secondary metabolites. These plant secondary metabolites could effectively disrupt the efflux pump systems, thereby contributing to the growing case of antibiotic resistance. The local plant species used as samples for this study are the following species: *Ixora coccinea, Mimosa pudica, and Origanum vulgare.* These are known for their medicinal uses. Furthermore, the basis for these selected widespread plants is because of their reported bioactivities and availability. Selected secondary metabolites from these plant species were studied for further analysis, to ascertain their antibacterial capacity, chemical structure, and purity. Upon blind molecular docking through AutoDock simulation software, the binding energies and inhibition constants of the analyzed data were also compared to existing efflux pump inhibitors, artesunate and phenylalanyl arginyl β-naphthylamide (PAβN).

Ligands

Information about the various plant secondary metabolites used in this study were gathered from databases containing a large collection of chemical information, particularly PubChem. Such information mainly includes their three-dimensional structure, conformation, and their sequence. The metabolites in Table 1 were chosen based on the reported bioactivities from other references [11, 12, 13, 14].

Table 1: Selected metabolites of the subjects in the study

M. pudica		*I. coccinea*		*O. vulgare*	
Ligand	**PubChem CID**	**Ligand**	**PubChem CID**	**Ligand**	**PubChem CID**
Mimosine [15]	3862	Ursolic acid [18]	64945	Kaempferol [21]	5280863
Orientin [16]	5281675	Quercetin [19]	5280343	Caffeic acid [22]	689043
Galangin [17]	5281616	Lupeol [20]	259846	Rosmarinic acid [23]	5281792

As for the existing efflux pump inhibitors, artesunate and PAβN, these were chosen to further discuss the data gathered from the selected metabolites of the subjects in the study [24, 25].

Table 2: Selected existing efflux pump inhibitors in the study.

Ligand	PubChem CID
Artesunate [26]	6917864
PaβN [27]	443301

Subsequently, these metabolites were minimized through UCSF Chimera before removing heteroatoms and water molecules using Biovia Discovery Studio.

Receptors

The receptors used for the molecular docking of the metabolites in the study were obtained from Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB) [28]. The AcrAB-TolC drug efflux pump system of *E. coli* was divided into three sections: (1) AcrA, (2) AcrB, and (3) TolC, due to software limitations. These sections were minimized through Avogadro prior to visualization using Biovia Discovery Studio.

Results and Discussion

Among the three plants, the secondary metabolites of *I. coccinea* exhibited the lowest potential energies using the

AcrAB-TolC efflux pump system of *E. coli*. Lupeol had the lowest binding energy, making it the most stable metabolite. Moreover, the results for stability of lupeol in the molecular docking agree with Muktar *et al.* (2018) [29]. The isolated lupeol in the root bark of *Ficos sycomorus* as a potential antibacterial agent is stable and effectively works against ciprofloxacin. Furthermore, the values of binding energy for the metabolites of *M. pudica* and *O. vulgare* were close to each other. The nine selected metabolites showed negative binding energies implying good stability using *E. coli*'s TolC efflux pump system.

Table 3: Binding energy and kl of selected plant secondary metabolites using AcrA efflux pump of *E. coli*

Plant	Secondary metabolite	Bind energy	kl
I. coccinea	Ursolic acid	-6.83	9.89 uM
I. coccinea	Quercetin	-6.42	107.14 uM
I. coccinea	Lupeol	-8.23	932.83 nM
M. pudica	Mimosine	-3.51	2.65 mM
M. pudica	Orientin	-3.4	3.22 mM
M. pudica	Galangin	-6.1	33.6 uM
O. vulgare	Kaempferol	-5.67	69.41 uM
O. vulgare	Caffeic acid	-4.39	609.1 uM
O. vulgare	Rosmarinic acid	-3.2	4.51 mM

Table 4: Binding energy and kl of selected plant secondary metabolites using AcrB efflux pump of *E. coli*

Plant	Secondary metabolite	Bind energy	kl
I. coccinea	Ursolic acid	-7.33	4.25 uM
I. coccinea	Quercetin	-5.37	115.73 uM
I. coccinea	Lupeol	-8.7	421.55 nM
M. pudica	Mimosine	-3.17	4.75 mM
M. pudica	Orientin	-4.1	996.09 uM
M. pudica	Galangin	-5.6	78.67 uM
O. vulgare	Kaempferol	-5.83	53.29 uM
O. vulgare	Caffeic acid	-4.09	1.0 mM
O. vulgare	Rosmarinic acid	-2.89	7.62 mM

Table 5: Binding energy and kl of selected plant secondary metabolites using TolC efflux pump of *E. coli*

Plant	Secondary metabolite	Bind energy	kl
I. coccinea	Ursolic acid	-7.33	4.25 uM
I. coccinea	Quercetin	-4.47	525.29 uM
I. coccinea	Lupeol	-8.23	930.28 nM
M. pudica	Mimosine	-3.42	3.11 mM
M. pudica	Orientin	-4.97	227.39 uM
M. pudica	Galangin	-4.1	996.09 uM
O. vulgare	Kaempferol	-4.7	360.88 uM
O. vulgare	Caffeic acid	-3.94	1.29 mM
O. vulgare	Rosmarinic acid	-2.89	7.62 mM

Table 6: Binding energy and kl of existing EPIs using AcrAB-TolC efflux pump of *E. coli.*

EPIs	AcrA		AcrB		TolC	
	Bind energy	kI	Bind energy	kI	Bind energy	kI
Artesunate	-5.28	134.33 uM	-5.61	76.62 uM	-5.13	173.58 uM
PaβN	-4.4	590.75 uM	-7.24	4.9 uM	-6.72	11.84 uM

Table 7: Favorable (F) and less-favorable (LF) binding energies of selected plant secondary metabolites based on Artesunate's values.

Selected Secondary Metabolite	AcrA		AcrB		TolC	
	Artesunate: -5.28		Artesunate: -5.61		Artesunate: -5.13	
	Bind energy	F/L	Bind energy	F/L	Bind energy	F/L
Ursolic acid	-6.83	F	-7.33	F	-7.33	F
Quercetin	-6.42	F	-5.37	L	-4.47	L
Lupeol	-8.23	F	-8.7	F	-8.23	F
Mimosine	-3.51	L	-3.17	L	-3.42	L
Orientin	-3.4	L	-4.1	L	-4.97	L
Galangin	-6.1	F	-5.6	F	-4.1	L
Kaempferol	-5.67	F	-5.83	F	-4.7	L
Caffeic acid	-4.39	L	-4.09	L	-3.94	L
Rosmarinic acid	-3.2	L	-2.89	L	-2.89	L

Table 8: Favorable (F) and less-favorable (LF) binding energies of selected plant secondary metabolites based on PAβN's values.

Selected Secondary Metabolite	AcrA		AcrB		TolC	
	PAβN: -4.4		PAβN: -6.82		PAβN: -6.72	
	Bind energy	F/L	Bind energy	F/L	Bind energy	F/L
Ursolic acid	-6.83	F	-7.33	F	-7.33	F
Quercetin	-6.42	F	-5.37	L	-4.47	L
Lupeol	-8.23	F	-8.7	F	-8.23	F
Mimosine	-3.51	L	-3.17	L	-3.42	L
Orientin	-3.4	L	-4.1	L	-4.97	L
Galangin	-6.1	F	-5.6	L	-4.1	L
Kaempferol	-5.67	F	-5.83	L	-4.7	L
Caffeic acid	-4.39	F	-4.09	L	-3.94	L
Rosmarinic acid	-3.2	L	-2.89	L	-2.89	L

The secondary metabolites were divided into two groups: favorable and less favorable. The binding energies of the existing EPIs, artesunate and PAβN, were used to classify them, and their values were set as the standard for comparison. A metabolite is classed as less favorable if its observed values are higher than the standards, whereas it has been labeled as favorable if lower. The researchers fixed a parameter such that when results are -0.01 below the standard, it can still be labeled as favorable since binding has already been established and the difference is too little to be disregarded. This form of classification was conducted to further demonstrate that all selected secondary metabolites have binding energies but only differ by a certain degree.

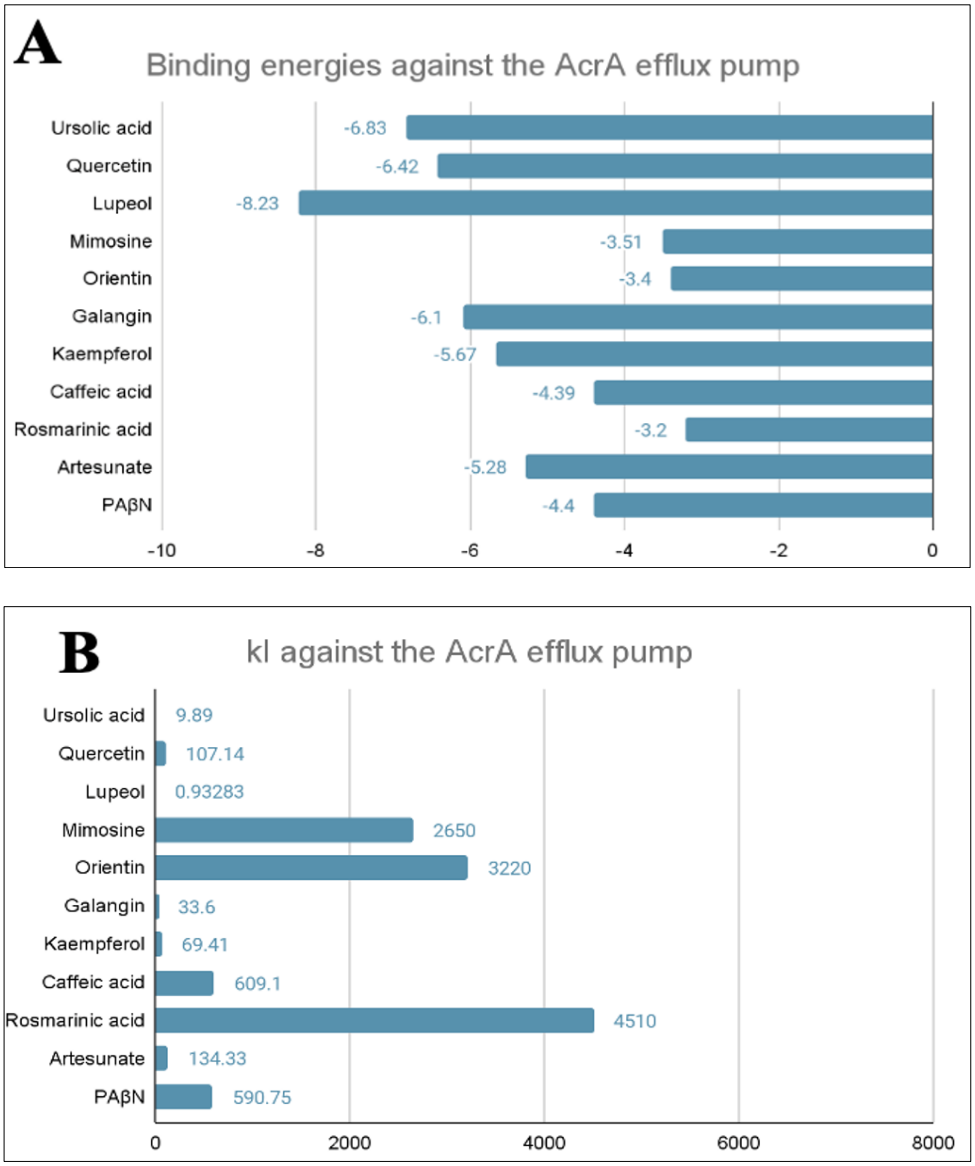


Fig 1: Comparison of (A) binding energy and (B) kI of selected plant secondary metabolites with existing EPIs using AcrA efflux pump of *E. coli.*

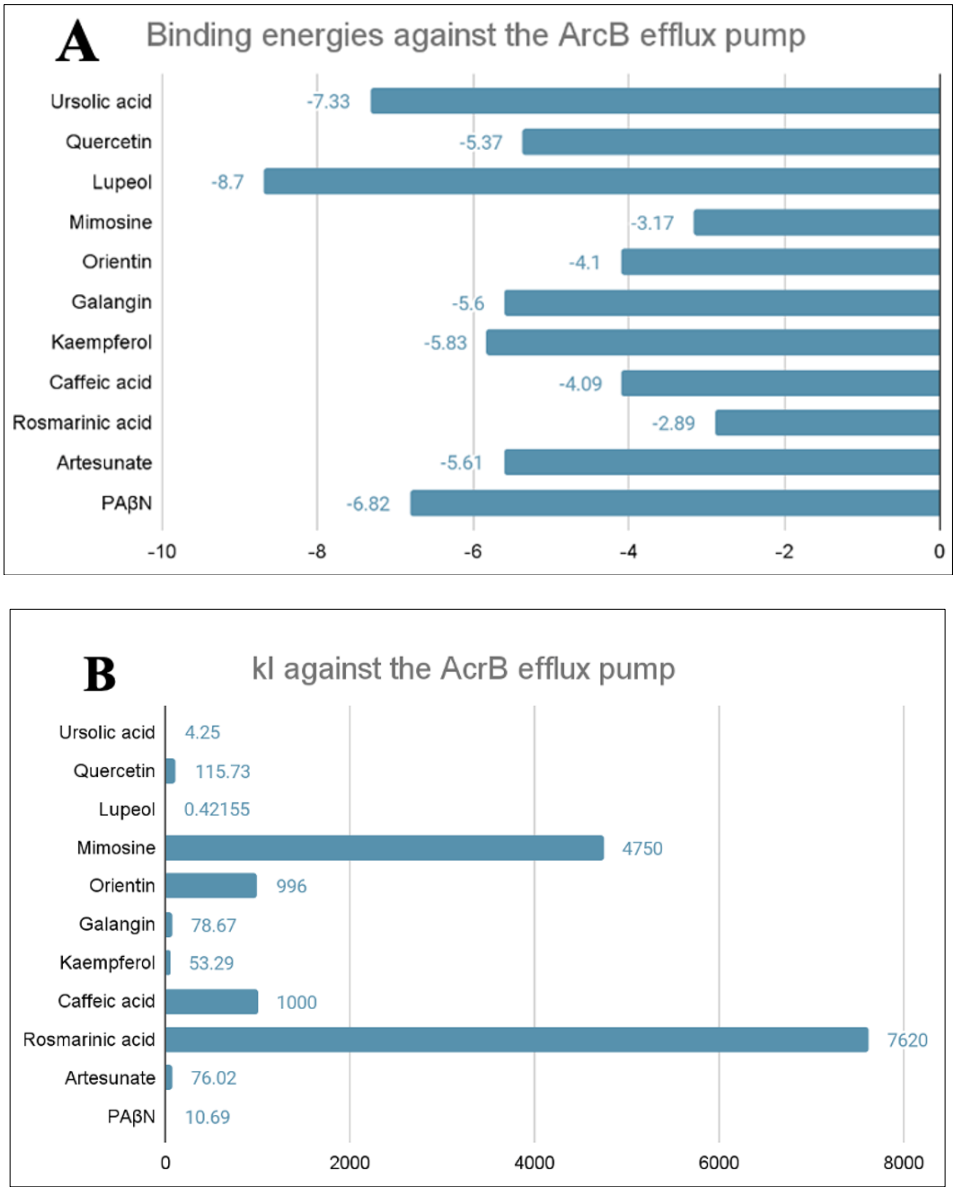
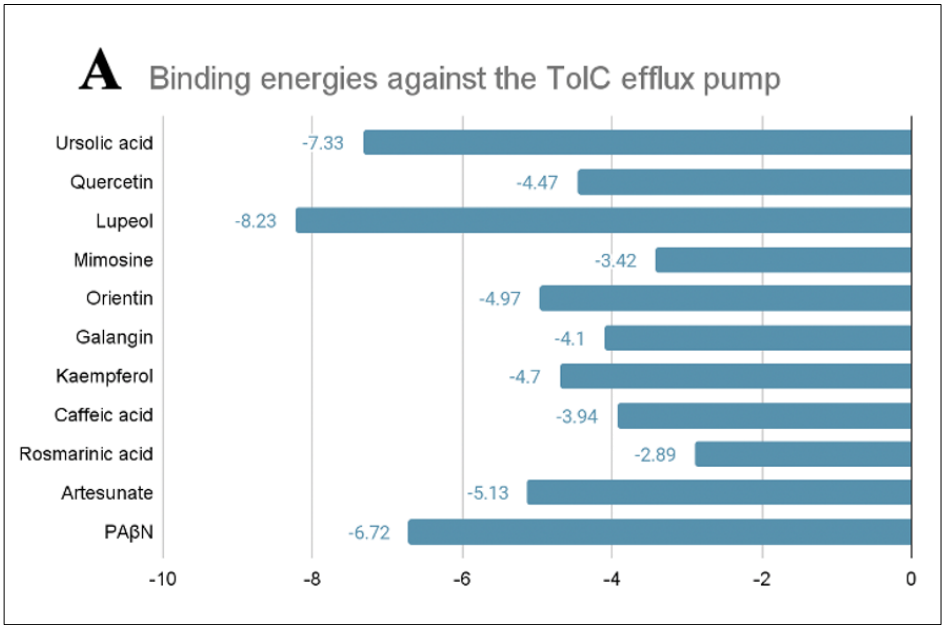


Fig 2: Comparison of (A) binding energy and (B) kI of selected plant secondary metabolites with existing EPIs using AcrB efflux pump of *E. coli.*



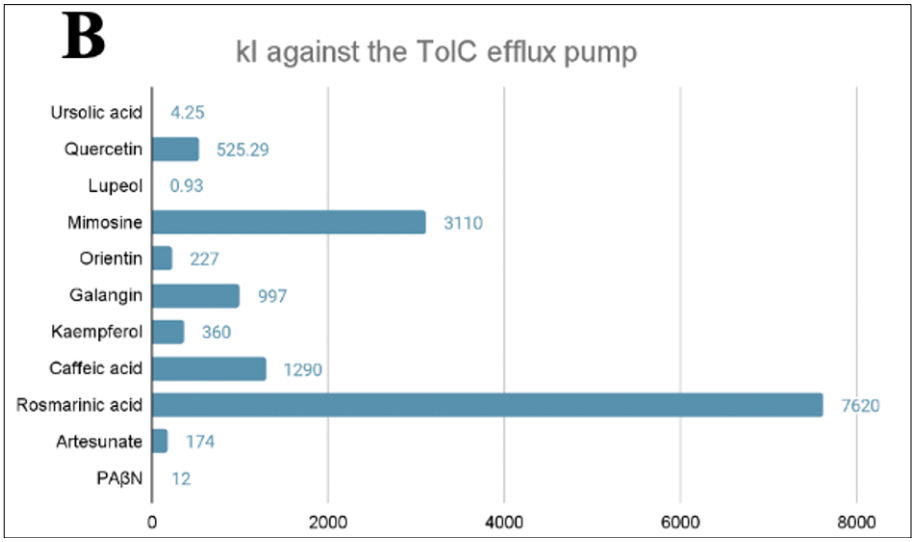
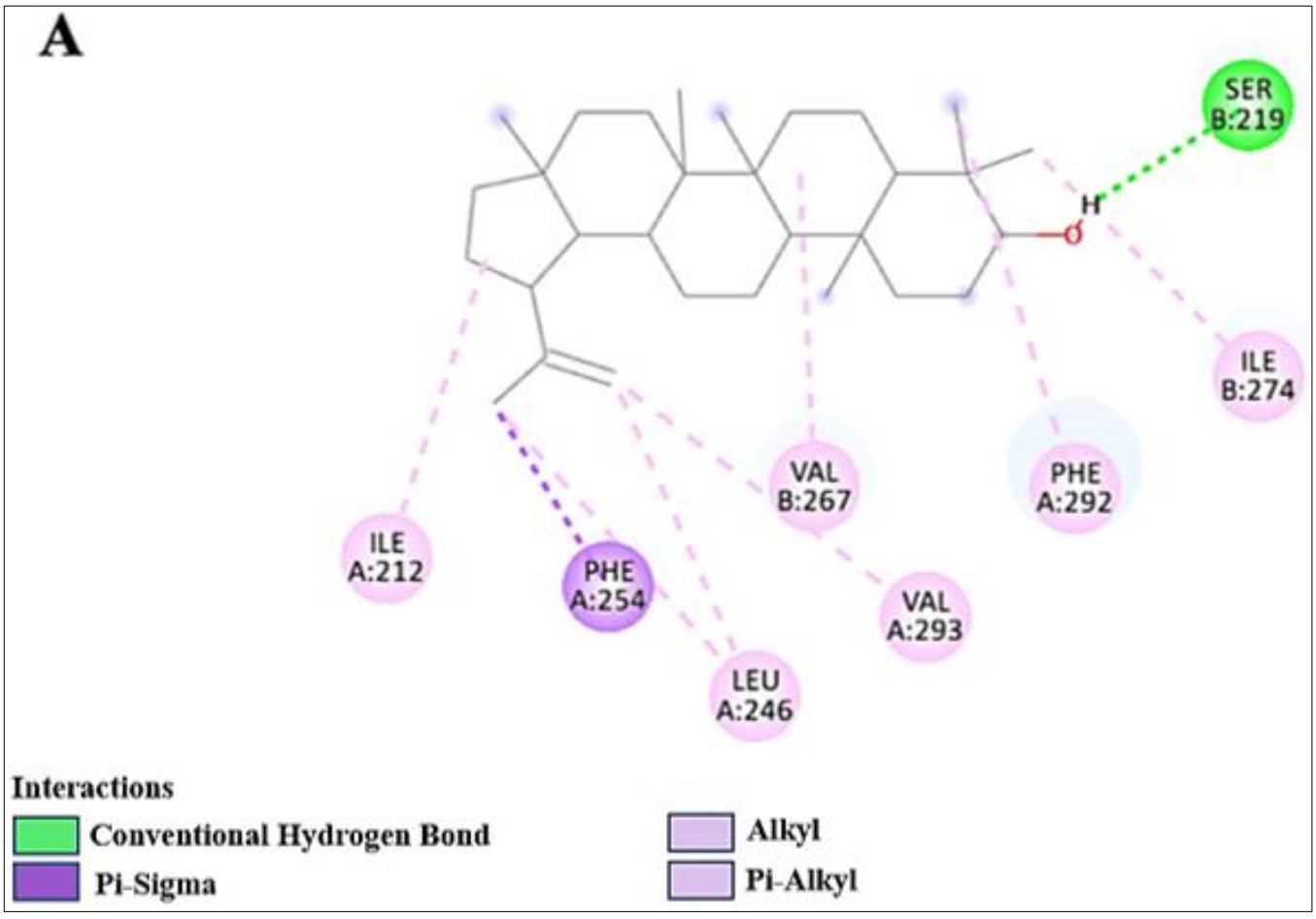


Fig 3: Comparison of (A) binding energy and (B) kI of selected plant secondary metabolites with existing EPIs using TolC efflux pump of *E. coli.*

In contrast, inhibition constant (kI) indicates the potency of an inhibitor wherein the lower the kI value, the higher its inhibition activity [30]. In the case of the interactions of the secondary metabolites with the AcrAB-TolC efflux pump system of *E. coli*, lupeol produced the lowest inhibition constant. Therefore, it suggests an increased likelihood of the metabolite inhibiting the efflux pump system.

The respective inhibition constants of all metabolites varied depending on the efflux pump system. Some metabolites presented low binding energy towards one efflux pump system while displaying a high inhibition constant. This would suggest that although the metabolite's interaction with the efflux pump system is stable due to the low binding energy, there are some secondary metabolites whose inhibition activity may not be strong enough to inhibit the activities of the efflux pump successfully.

To further screen the metabolites, their respective values were compared to the binding energy and inhibition constants of existing efflux pump inhibitors, artesunate and PAβN, as seen in Table 6. Artesunate is associated with suppressing AcrAB-TolC by significantly increasing β-lactam antibacterial effect against *E. coli* clinical strain [31]. On the other hand, PAβN was the first inhibitor for RND efflux pumps [24].



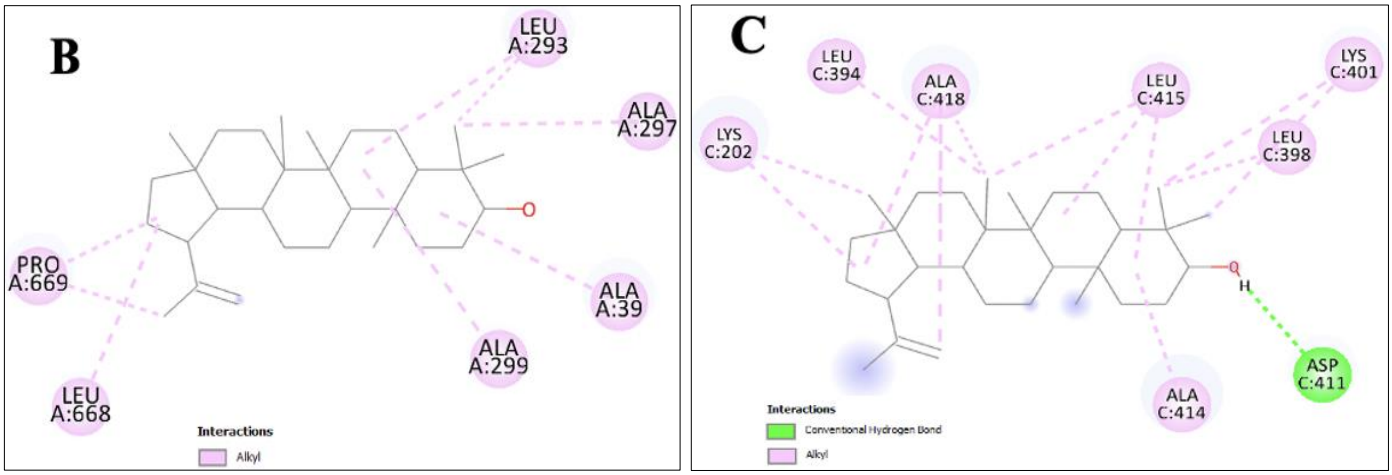
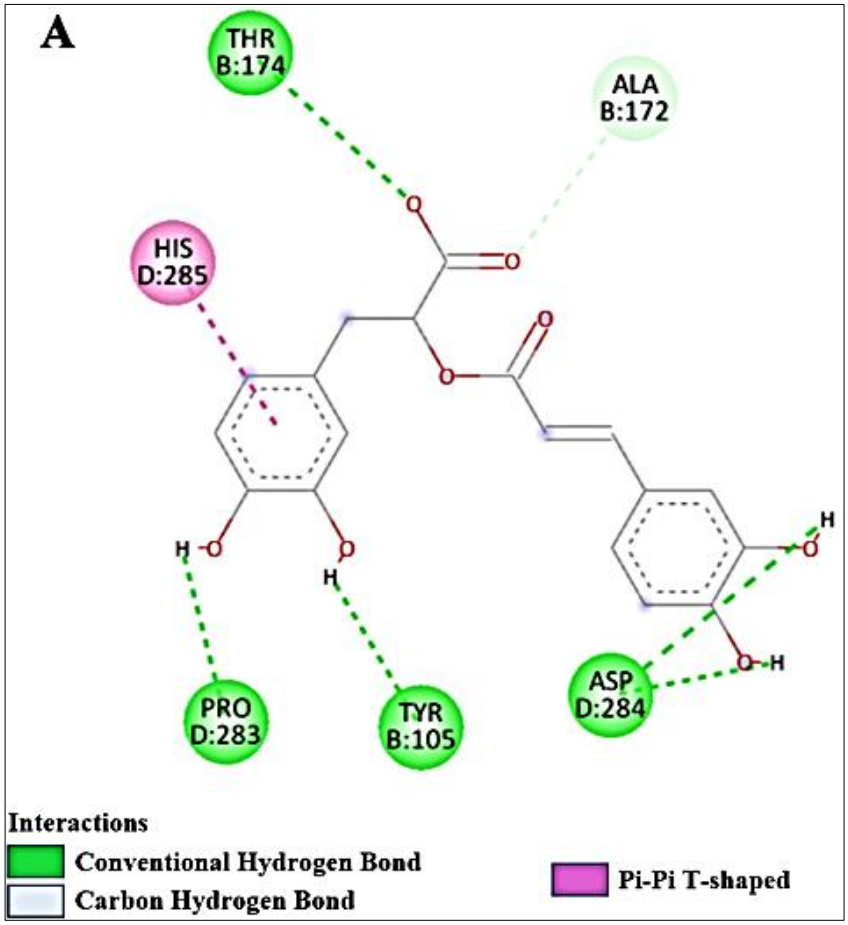


Fig 4: 2D diagram of molecularly docked lupeol with (A) AcrA, (B) AcrB, and (C) TolC.



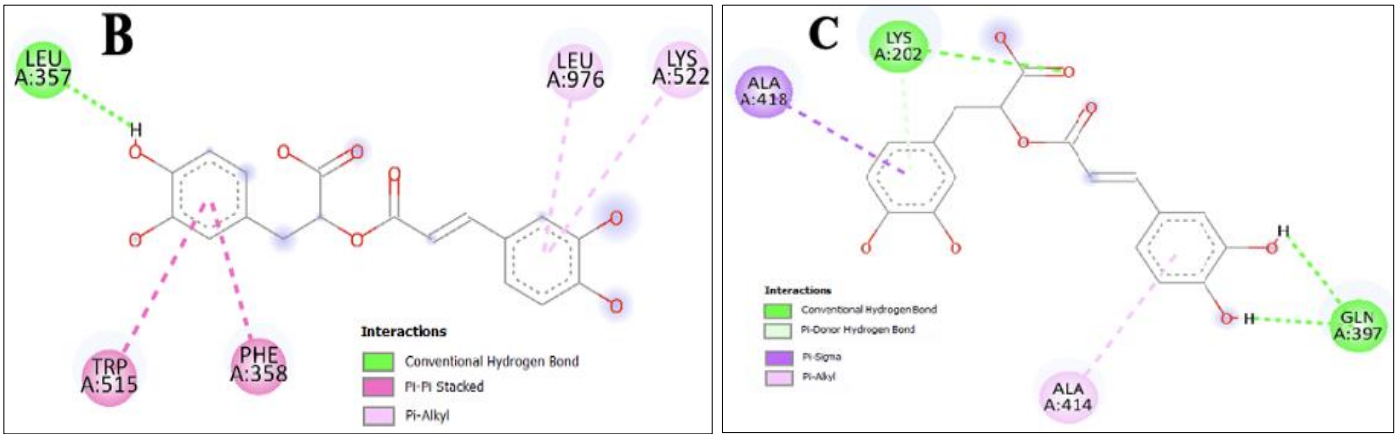


Fig 5: 2D diagram of molecularly docked rosmarinic acid with (A) AcrA, (B) ArcB, and (C) TolC.

Lupeol showed the greatest stability and strongest inhibition activity among all the selected plant secondary metabolites across the AcrAB-TolC efflux pump system. Comparing the results obtained from docking the known EPIs against the metabolites, lupeol presents more favorable inhibition as it has lower binding energy and kI. It also surpasses the binding energy of artesunate and PAβN with regards to the efflux pump system. Lupeol shows alkyl, pi-alkyl, conventional hydrogen bonds, and pi-sigma bonds for AcrA, alkyl groups for AcrB, and conventional hydrogen bond and alkyl bond for TolC.

Compared to rosmarinic acid, which showed less binding energy and inhibition constant, conventional hydrogen bond, carbon hydrogen bond, and Pi-Pi T-shaped were observed for AcrA, conventional hydrogen bond, Pi-Pi stacked bonds, and Pi-alkyl bonds for AcrB, and conventional hydrogen bonds, Pi-Donor hydrogen bond, pi-sigma bond, and Pi-alkyl bonds for TolC.

Among the other metabolites that have remarkable properties similar to the existing EPIs are quercetin, galangin, kaempferol, and ursolic acid. Based on the 2D diagrams of the metabolites, the five metabolites and the existing EPIs have similar interactions. Particularly, these included conventional hydrogen, alkyl, and pi-alkyl interactions. The results obtained also agree with Sharma *et al.* (2019) and Waditzer and Bucar (2021). Thus, these five plant secondary metabolites can be stable inhibitors of the RND efflux pump system, AcrAB-TolC of *E. coli*.

To further support the results, the phytochemicals of *I. coccinea*, such as quercetin, lupeol, and ursolic acid, are abundant in many medicinal plants and have a broad spectrum of pharmacological activities. These include antimicrobial and antibacterial properties [33, 34]. Other properties of *I. coccinea* that have been reported are anti-inflammatory and antimitotic activities from the leaves, cytotoxic and antitumor properties from the flowers [35].

In contrast, *M. pudica* is a popular ornamental plant among folk healers valued for its antispasmodic, anti-inflammatory, analgesic, diuretic, and hypoglycemic properties (Gupta *et al*., 2019). Phytochemical studies on this plant species revealed that the presence of non-protein amino acid (mimosine), flavonoids (galangin) flavone (orientin), sterols, tannins, terpenoids, and alkaloids were reported of antibacterial activities against human pathogens, *E. coli, B. subtilis, S. pyogenes, P. mirabilis*, and *P. fluorescens* [36]. Galangin alone caused a 100,000 fold decrease in the viability of *S. aureus* [37].

Finally, *O. vulgare* is known for its phytochemicals that can inhibit gram-positive and gram-negative bacteria by disrupting the integrity and permeability of the cell membrane [38, 39]. Kaempferol, a natural phenolic compound, is known for its antibacterial and bacteriostatic effects. Through the broth microdilution method, Wu *et al.* (2013) concluded a significant positive correlation between its antibacterial capacity and membrane rigidification.

Conclusion and Recommendation

It has been established through the molecular docking results that the selected plant secondary metabolites can be efficient EPIs against the AcrAB-TolC efflux pump system of *E. coli*. All of the docked secondary metabolites had relatively low binding energies and inhibition constants. Five of the nine selected plant secondary metabolites, namely lupeol, quercetin, galangin, kaempferol, and ursolic acid, showed the potential to be stable inhibitors of the drug efflux pump system. Based on the 2D diagrams, the five plant secondary metabolites and existing EPIs shared similar interactions, particularly conventional hydrogen, alkyl, and pi-alkyl bonds. It is also worth noting that all the selected metabolites from the plant species *I. coccinea* exhibited excellent binding energy and inhibition constant, suggesting that *I. coccinea* would contain other metabolites that would serve as potential EPIs.

In terms of the future direction of this study, it would be prudent to screen and discover other local plant families such as crown-of-thorns (*Euphorbia milii*), gumamela (*Hibiscus rosa-sinensis*), and white kalachuchi (*Plumeria obtusa*), which could contain the selected metabolites used in the docking procedure. In addition, it would be beneficial to test other metabolites found in *I. coccinea*, *M. pudica*, and *O. vulgare* and determine if they could also become EPIs for the development of cost-effective drugs via *in vivo* methods.

Conflict of Interest

The authors declare no conflict of interest, financial, or otherwise.

References

1. Lehtinen S, Blanquart F, Lipsitch M, Fraser C. On the evolutionary ecology of multidrug resistance in bacteria. PLoS pathogens. 2019;15(5):e1007763.
2. Ferrer-Espada R, Wang Y, Goh XS, Dai T. Antimicrobial blue light inactivation of microbial isolates in biofilms. Lasers in Surgery and Medicine. 2019;52(5):472-478.
3. Blanco P, Sanz-García F, Hernando-Amado S, Martínez JL, Alcalde-Rico M. The development of efflux pump inhibitors to treat Gram-negative infections. Expert opinion on drug discovery. 2018;13(10):919-931.
4. Poole K. Efflux pumps as antimicrobial resistance mechanisms. Annals of Medicine. 2007;39(3):162-176.
5. Breijyeh Z, Jubeh B, Karaman R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molecules (Basel, Switzerland). 2020;25(6):1340.
6. Exner M, Bhattacharya S, Christiansen B, Gebel J, Goroncy-Bermes P, Hartemann P, *et al*. Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria?. GMS hygiene and infection control. 2017;12:5.
7. Venter H, Mowla R, Ohene-Agyei T, Ma S. Rnd-type drug efflux pumps from gram-negative bacteria: Molecular mechanism and inhibition. Frontiers in Microbiology, 2015, 06.
8. Sameiyan E, Hayes AW, Karimi G. The effect of medicinal plants on multiple drug resistance through autophagy: A review of *in vitro* studies. European Journal of Pharmacology. 2019;852:244-253.
9. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. Journal of pharmaceutical analysis. 2019;10(4):277-290.
10. Gorlenko C, Kiselev H, Budanova E, Zamyatnin A, Ikryannikova L. Plant Secondary Metabolites in the Battle of Drugs and Drug-Resistant Bacteria: New Heroes or Worse Clones of Antibiotics? Antibiotics. 2020;9(4):170.
11. Martins D, Nunez C. Secondary Metabolites from *Rubiaceae* Species. Molecules. 2015;20(7):13422-13495.
12. Muhammad G, Hussain MA, Jantan I, Bukhari S. *Mimosa pudica L.*, a High-Value Medicinal Plant as a Source of

Bioactives for Pharmaceuticals. Comprehensive reviews in food science and food safety. 2016;15(2):303-315.
13. Özer Z, Çarıkçı S, Yılmaz H, Kılıç T, Dirmenci T, Gören AC. Determination of secondary metabolites of *Origanum vulgare subsp. hirtum* and *O. vulgare* subsp. *vulgare* by LC-MS/MS. Journal of Chemical Metrology. 2020;14(1):25-34.
14. Suresh K, Manivannan R, Nivetha B. *In silico* docking analysis of phytochemicals from *Mimosa pudica* L. Leaves as an antiviral agent against herpes simplex virus type I. IntJ Biomed. NanoLet. 2021;1(1):1-9.
15. PubChem Compound Summary for CID 3862, Mimosine. https://pubchem.ncbi.nlm.nih.gov/compound/Mimosine. 19 January, 2022.
16. PubChem Compound Summary for CID 5281675, Orientin. https://pubchem.ncbi.nlm.nih.gov/compound/Orientin. 19 January, 2022.
17. PubChem Compound Summary for CID 5281616, Galangin. https://pubchem.ncbi.nlm.nih.gov/compound/Galangin. 19 January, 2022.
18. PubChem Compound Summary for CID 64945, Ursolic acid. https://pubchem.ncbi.nlm.nih.gov/compound/Ursolic-acid. 19 January, 2022.
19. PubChem Compound Summary for CID 5280343, Quercetin. https://pubchem.ncbi.nlm.nih.gov/compound/Quercetin. 19 January, 2022.
20. PubChem Compound Summary for CID 259846, Lupeol. https://pubchem.ncbi.nlm.nih.gov/compound/Lupeol. 19 January, 2022.
21. PubChem Compound Summary for CID 5280863, Kaempferol. https://pubchem.ncbi.nlm.nih.gov/compound/Kaempferol. 19 January, 2022.
22. PubChem Compound Summary for CID 689043, Caffeic acid. https://pubchem.ncbi.nlm.nih.gov/compound/Caffeic-acid. 19 January, 2022.
23. PubChem Compound Summary for CID 5281792, Rosmarinic acid. https://pubchem.ncbi.nlm.nih.gov/compound/Rosmarinic-acid. 19 January, 2022.
24. Sharma A, Gupta VK, Pathania R. Efflux pump inhibitors for bacterial pathogens: From bench to bedside. The Indian journal of medical research. 2019;149(2):129-145.
25. Djeussi DE, Noumedem JA, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, *et al*. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC complementary and alternative medicine. 2013;13:164.
26. PubChem Compound Summary for CID 6917864, Artesunate. https://pubchem.ncbi.nlm.nih.gov/compound/Artesunic-acid. 19 January, 2022.
27. PubChem Compound Summary for CID 443301. https://pubchem.ncbi.nlm.nih.gov/compound/443301. 19 January, 2022.
28. Asymmetric AcrABZ-TolC. https://www.rcsb.org/structure/5O66. 5 June, 2017.
29. Muktar B, Bello IA, Sallau MS. Isolation, characterization, and antimicrobial study of lupeol acetate from the root bark of fig-Mulberry Sycamore (*Ficus sycomorus* linn). Journal of Applied Sciences and Environmental Management. 2018;22(7):1129.
30. Arumugam M, Umamaheswari M, Kuppusamy A, Thirumalaisamy S, Subhadradevi V, Jagannath P. Docking studies*: In silico* aldose reductase inhibitory activity of commercially available flavonoids. Bangladesh Journal of Pharmacology. 2012;7:266-271.
31. Li B, Yao Q, Pan XC, Wang N, Zhang R, Li J, *et al*. Artesunate enhances the antibacterial effect of {beta}-lactam antibiotics against *Escherichia coli* by increasing antibiotic accumulation via inhibition of the multidrug efflux pump system AcrAB-TolC. The Journal of antimicrobial chemotherapy. 2011;66(4):769-777.
32. Waditzer M, Bucar F. Flavonoids as inhibitors of bacterial efflux pumps. Molecules. 2021;26(22):6904.
33. Baliga MS, Kurian PJ. *Ixora coccinea* Linn.: traditional uses, phytochemistry and pharmacology. Chinese journal of integrative medicine. 2012;18(1):72-79. https://doi.org/10.1007/s11655-011-0881-3
34. Ramadwa TE, Awouafack MD, Sonopo MS, Eloff JN. Antibacterial and antimycobacterial activity of crude extracts, fractions, and isolated compounds from leaves of sneezewood, ptaeroxylon obliquum (Rutaceae). Natural Product Communications. 2019;14(11).
35. Annapurna J, Amarnath PVS, Amar Kumar D, Ramakrishna SV, Raghavan KV. Antimicrobial activity of *Ixora coccinea* leaves. Fitoterapia. 2003;74(3):291-293.
36. Vijayalakshmi K, Udayakumar R. Antibacterial Activity of Leaf and Root of *M. pudica* L. against Selected Human Pathogenic Microorganisms. Journal of Biomedical and Pharmaceutical Sciences. 2018;01(2).
37. Cushnie TPT, Hamilton VES, Lamb AJ. Assessment of the antibacterial activity of selected flavonoids and consideration of discrepancies between previous reports. Microbiological Research. 2003;158(4):281-289.
38. Thapa D, Losa R, Zweifel B, Wallace RJ. Sensitivity of pathogenic and commensal bacteria from the human colon to essential oils. Microbiology (Reading, England). 2012;158(Pt 11):2870-2877.
39. Lambert RJ, Skandamis PN, Coote PJ, Nychas GJ. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of applied microbiology. 2001;91(3):453-462.
40. Wu T, He M, Zang X, Zhou Y, Qiu T, Pan S, *et al*. A structure–activity relationship study of flavonoids as inhibitors of E. coli by membrane interaction effect. Biochimica Et Biophysica Acta (BBA) - Biomembranes, 2013;1828(11):2751-2756.