Therapeutic activity ofphyto ligands from Honey and standard drugs against *Staphylococcus aureus*PBP2a (5M19)

Abstract

Staphylococcus aureus is the most dangerous of all of the many common staphylococcal bacteria. These gram-positive, sphere-shaped (coccal) bacteria often cause skin infections but can cause pneumonia, heart valve infections, and bone infections and may be resistant to treatment with some antibiotics. Phenols were the most abundant phytochemical compounds and accounted for 25.8% of the phytochemical composition of the honey. The presence of these phytochemicals implied that the honey could have therapeutic properties like antimicrobial, anticancer, antioxidant, diuretic, and anti-inflammatory activities. two out of the six tested compounds faulted the Lipinski rule of 5 which states that Hydrogen Bond Donor (HBD) <5 Hydrogen –Bond Acceptor (HBA) < , 10 Molecular Weight <500 < 500 Molecular weight, < 5 H-bond donors, <10 H-bond acceptors.< 10 number of rotatable bonds, and < 140 $Å^2$ PSA value In this study. favourable overall docking score was observed in the range of -3.085to -8.724. Chlorogenic acid had the best docked score (lowest binding energy)when compared to the synthetic inhibitors and other phytoligands. The binding manners and geometrical orientation of the studied phyto ligand from Honey and standard drugs against *Staphylococcus aureus*PBP2a (5M19).The results revealed that, Chlorogenic acid showed pivotal binding interactions with PBP2a, as it exhibited the best binding energy (-8.724 kcal/mol) compared with to standard drugs (Cefoxitin; -5.985 kcal/mol, Oxacillin; -5.222 kcal/mol and methiclin -3.08 kcal/mol) and other phytoligands (protocatechuic acid -4.473 kcal/mol and Hesperetin -4.191 kcal/mol) tested. Hence, Chlorogenic acid could be studied as promising lead candidates acting as allosteric effectors of PBP2a that might be subjected to further structural optimization to enhance their biological activity and hence been synthesized and tested to evaluate their actual biological activity.

Keywords:Phyto-Ligand, *Staphylococcus aureus*, Honey and Chlorogenic acid

Introduction

Phyto (plant) ligands are naturally occurring compounds found in plants that can bind to specific proteins, receptors or enzymes in living organisms, including humans. These ligands are typically small molecules, such as flavonoids, alkaloids, terpenes, and phenolic acids, that are synthesized by plants for various purposes such as defence against herbivores, attraction of pollinators, and regulation of plant growth and development. Phyto ligands have been found to have a wide range of biological activities and health benefits, including anti-inflammatory,

antioxidant, anti-cancer, anti-microbial, and neuroprotective effects. For example, the flavonoid quercetin, which is abundant in fruits and vegetables, has been shown to have anti-inflammatory and anti-cancer properties, while the terpenecurcumin, found in turmeric, has potent antioxidant and anti-inflammatory effects.

Due to their diverse pharmacological activities, phyto ligands have gained significant interest as potential therapeutic agents for the prevention and treatment of various diseases. However, more research is needed to fully understand the mechanisms of action and potential side effects of these compounds before they can be used as medications.

Honey has been used for medicinal purposes for centuries due to its antibacterial and anti-inflammatory properties. Here are some of the most common medicinal uses of honey:

Wound healing: Honey can help to promote wound healing and prevent infection due to its antibacterial properties. It can be applied topically to wounds and burns (Al-Waili, 2011; Jull *et al.,* 2015).Cough and sore throat: Honey has been shown to be effective in relieving cough and sore throat symptoms. It can be taken by mouth alone or mixed with tea or warm water.Digestive issues: Honey can help to soothe digestive issues, such as diarrhea and stomach ulcers, due to its anti-inflammatory properties (Eteraf-Oskouei, and Najafi, 2013).Allergy relief: Some people believe that consuming local honey can help reduce allergy symptoms, as it may contain small amounts of pollen that can desensitize the body to allergens (Khoo*et al.,* 2013).Skin conditions: Honey can be used topically to help with various skin conditions, such as acne, eczema, and psoriasis, due to its anti-inflammatory and antibacterial properties.Sleep aid: Honey can help to promote relaxation and improve sleep quality. It can be consumed alone or mixed with warm milk or tea before bedtime (Samarghandian, *et al.,* 2017).It is important to note that honey should not be given to infants under one year old, as it may contain botulism spores that can cause serious illness. Additionally, people with diabetes should consume honey in moderation, as it is high in sugar. It is always best to consult with a healthcare provider before using honey for medicinal purposes.Staph infections are caused by staphylococcus bacteria. These types of germs are commonly found on the skin or in the nose of many healthy people. Most of the time, these bacteria cause no problems or cause relatively minor skin infections. But staph infections can turn deadly if the bacteria invade deeper into your body, entering your bloodstream, joints,

bones, lungs or heart (Taormina, 2011). A growing number of otherwise healthy people are developing life-threatening staph infections.

Treatment usually involves antibiotics and cleaning of the infected area. However, some staph infections no longer respond, or become resistant, to common antibiotics. To treat antibiotic-resistant staph infections, health care providers may need to use antibiotics that can cause more side effects.

Methodology

Molecualr Docking

Docking studies was done using methodologies from the study of Mo et al. (2015) Auto-Dock version 4.0.MarvinSketch software (Chen *et al.*, 2005).PyMOL molecular graphics system (DeLano, 2012)

Pharmacokinetics profile

The Drug likeness profile of selected phyto-ligands from honey and standard drugs were analysed based on their compliance to the Lipinski's rule of five using SwissADMET free online sever and are presented in Table 1. From the result, two out of the six tested compounds faulted the Lipinski rule of 5 which states that Hydrogen Bond Donor (HBD) <5 Hydrogen –Bond Acceptor (HBA) < , 10 Molecular Weight <500 < 500 Molecular weight, < 5 H-bond donors, <10 H-bond acceptors.< 10 number of rotatable bonds, and < 140 $Å^2$ PSA value In this study, Hesperetin, protocatechuic acid, methicillin and oxacillin obeyed the entire Lipinski rule. However the best docked compound cholingenic and the standard drugs (cefoxitin) met all the rule but one having a topological polar surface area values of 165 $Å^2$, 202 $Å^2$ respectively which should be normally be <140 $Å^2$.

Table 1: Drug likeness profile of phyto-ligands from honey and standard drugs

Compound Name	PubChem ID	Molecular formula	Molecular weight MW g/mol	HB donor	HB acceptor	PSA	No of rotatable bonds	Rule Of Five
Chlorogenic acid	1794427	$C_{16}H_{18}O_9$	354.3	6	9	165 Å²	5	1
Cefoxitin	441199	$C_{16}H_{17}N_3O_7S_2$	427.5	3	9	202 Å²	8	1
Oxacillin	6196	$C_{19}H_{19}N_3O_5S$	401.4	2	7	138 Å²	4	0
Methicillin	6087	$C_{17}H_{20}N_2O_6S$	380.4	2	7	131 Å²	5	0
protocatechuic acid	72	$C_7H_6O_4$	154.12	3	4	77.8 Å²	1	0
Hesperetin	72281	$C_{16}H_{14}O_6$	302.28	3	6	96.2 Å²	2	0

TPSA- Topological Polar Surface Area **nrtB-** Number of rotableAtoms**nViolation-** Violation of Lipinski's rule ≤ 5 **Hydrogen Bond Donor (HBD)** <5 **Hydrogen –Bond Acceptor (HBA) < ,** 10 **Molecular Weight** <500

< 500 Molecular weight, < 5 H-bond donors, <10 H-bond acceptors.< 10 number of rotatable bonds, and < 140 $Å^2$ PSA value

Molecular docking results

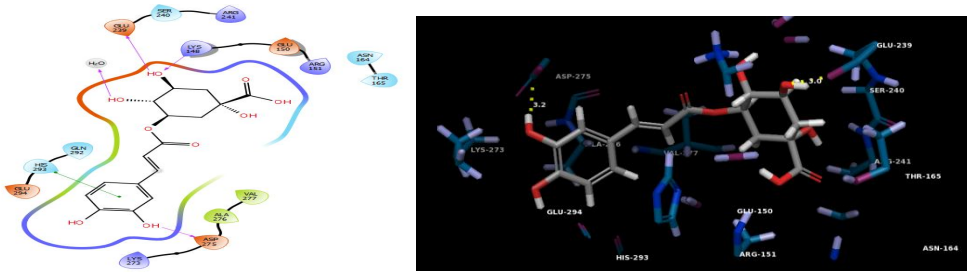
Table 2reveals the docking score of binding energies of the studied phyto ligand from Honey and standard drugs against *Staphylococcus aureus*PBP2a (5M19). The binding score represented in negative value indicating that the compounds were correctly docked into the crystal structure of their target protein. As shown in table 2 favourable overall docking score was observed in the range of -3.085to -8.724. Chlorogenic acid had the best docked score (lowest binding energy)when compared to the synthetic inhibitors and other phytoligands as shown in table 1

Protein-Ligand Molecular Interactions of selected phyto ligand from Honey and standard drugs against *Staphylococcus aureus*PBP2a (5M19)

The binding manners and geometrical orientation of the studied phyto ligand from Honey and standard drugs against *Staphylococcus aureus*PBP2a (5M19) is presented in Figure 1 a-d. The result shows that all the ligands docked, occupied a common space in the binding pocket and conform in a manner similar to the co-crystalized ligands. Phyto-ligands (Chlorogenic acid) and all three standard drugs with the lowest binding affinities were highlighted to further analyse their interactions.

Table 2: Binding energies of the studied phyto ligand from Honey and standard drugs against *Staphylococcus aureus*PBP2a (5M19)

Compound Name	PubChem ID	Molecular formula	Docking score Kcal/mol	Glide energy Kcal/mol
Chlorogenic acid	1794427	$C_{16}H_{18}O_9$	-8.724	-55.336
Cefoxitin	441199	$C_{16}H_{17}N_3O_7S_2$	-5.985	-32.924
Oxacillin	6196	$C_{19}H_{19}N_3O_5S$	-5.222	-47.801
Methicillin	6087	$C_{17}H_{20}N_2O_6S$	-3.085	-34.633
protocatechuic acid	72	$C_7H_6O_4$	-4.473	-38.355
Hesperetin	72281	$C_{16}H_{14}O_6$	-4.191	-41.626



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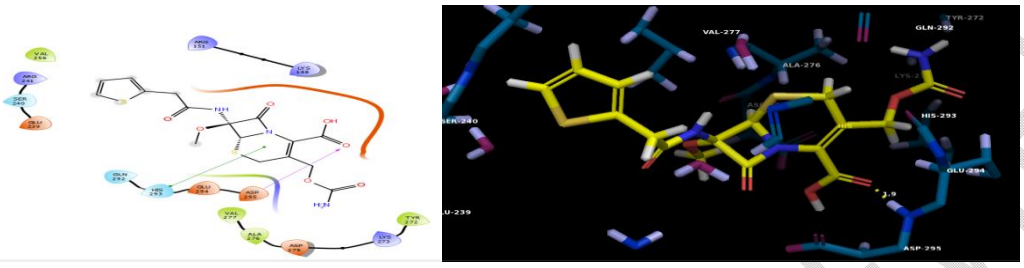

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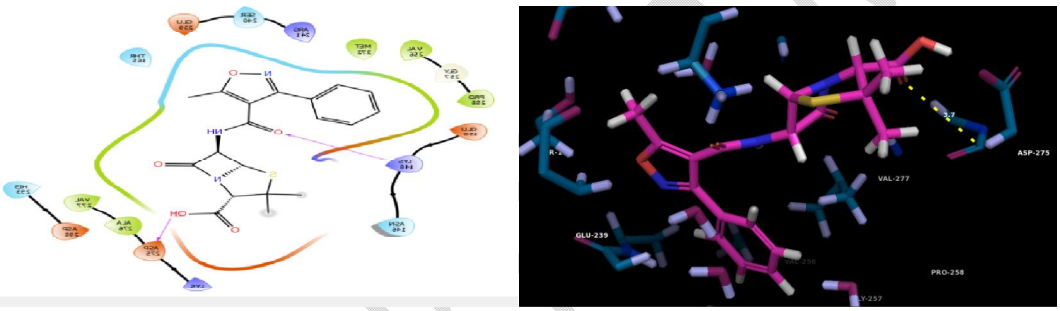

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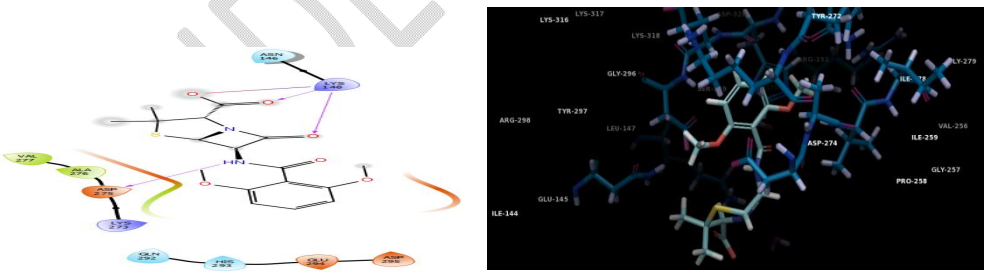

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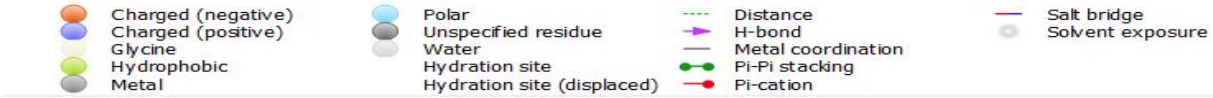

Figure 1: 2 and 3D representation of the lowest binding energies of phyto ligand of Honey and standard drugs against *Staphylococcus aureus*PBP2a(5M19) (a).Chlorogenic acid + PBP2a (b).Cefoxitin,+ PBP2a (c). Oxacillin + PBP2a

Discussion

For any molecule to be screened as a drug, it must to obey Lipinski's rule of five (Lipinski, et al., 2001) which stipulates that poor absorption or permeation are more likely when there are< 5 H-bond donors,< 500, and there are< 10 H-bond acceptors. Small compounds classes that are substrates for biological transporters are exceptions to the rule. The listed phytochemicals obtained from Honey and the standard drugs which showed an affinity toward PBP2a were analysed for their compliance to the Lipinski's rule of five using SwissADMET free online sever (Table 1). In our study, Hesperetin, protocatechuic, methicillin acid and oxacillin obeyed the entire Lipinski rule. However the best docked compound cholinegenic and the standard drugs (Cefoxitin) met all the rule but one having a topological polar surface area values of 165 Å², 202 Å² respectively which should be normally be >140 Å² . The polar surface area (PSA) and the molecular volume components are the most important descriptors for brain barrier permeability and is described as the surface area ($Å^2$) occupied by nitrogen and oxygen atoms and the polar hydrogens attached to them and is strongly reflective of hydrogen bonding capacity and polarity (Pajouhesh and Lenz, 2005).The findings of this study reveals that cholingenic and the standard drugs (Cefoxitin) haven had good binding energies may have poor brain barrier permeability.

Molecular docking analysis was employed to examine the variation in binding efficiency of Cefoxitin, Oxacillin and three phyto ligands from honey (Chlorogenic acid, protocatechuic acid and Hesperetin) against the PBP2a (5M19) allosteric binding site.The binding affinity of Cefoxitin, Oxacillin and three phyto ligands from honey at allosteric site was analysed based on their binding energy (kcal/mol) and molecular interaction (Table 2). H-bond and hydrophobic interactions in Figure 1 a-d. The results revealed that, Chlorogenic acid showed pivotal binding interactions with PBP2a, as it exhibited the best binding energy (-8.724 kcal/mol) compared with to standard drugs (Cefoxitin; -5.985 kcal/mol, Oxacillin; -5.222 kcal/mol and methiclin -3.08 kcal/mol) and other phytoligands (protocatechuic acid -4.473 kcal/mol and Hesperetin -4.191 kcal/mol) tested. Chlorogenic acid was observed to interact with the allosteric site residue of the enzyme through a network of three hydrogen bonds (Asp 275, Glu 239, Lys 148) and 2 hydrophobic interactions (Val 277 and Ala 276). Also a pi-pi interaction was observed with His 293 (Figure 1a). Similar to the phyto ligands, the standard drugs docked exhibited similar molecular interactions (Feng, 2002). Cefoxitin was found to participate in one hydrogen bond interaction (Asp 295), a pi-pi interaction with His 295 and 5 hydrophobic interactions (Ala 276, Val 277, Tyr 272, Val 256)(Figure 1b). Oxacillin was observed to participate in two hydrogen bond

interaction (Lys 148 and Aps 275) and five hydrophobic interactions (Ala 276, Val 277, Met 327, Pro 258))(Figure 1c) Also, Methicillin formed hydrogen bond interaction (Asp 275 Lys 148) and hydrophobic interaction (Val 277 and Ala 276)(Figure 1d). Interestingly, the key amino acid (Aps 275 and Lys 148, Thr165, Ser, 240, Val 277, Glu 239), which plays the paramount role in the allosteric mechanism of PBP2a, was found to be attached to the hydroxyl group of the Chlorogenic acid via a strong hydrogen bond interaction (Mahasenan et al. 2017). The binding of these ligands at the allosteric favours a conformational state of the protein wherein the active site is more accessible and thus enable acylation of the catalytic Serine 403 residue. Mahasenan*et al.* (2017) demonstrated that other β-lactams bind either exclusively to the active site (covalently) or to both the allosteric (non-covalent) and the active sites (covalent) confirming that allosteric site is the first step in the activation process of PBP2a.Ligand binding, whether antibiotics or peptidoglycan, at the allosteric site has been shown to favour a conformational state of the protein wherein the active site is more accessible. The generality of this allosteric hypothesis was proven with several β-lactam antibiotics as mimetics of the peptide stem of the peptidoglycan in a previous study by Fishovitz et al. (2015). Similarly, Rani et al. (2015) has shown the crucial role of allosteric effectors of PBP2a . Hence, Chlorogenic acid could be studied as promising lead candidates acting as allosteric effectors of PBP2a that might be subjected to further structural optimization to enhance their biological activity and hence been synthesized and tested to evaluate their actual biological activity.

Conclusion

In conclusion, the results of this study suggest that phytochemicals from honey exhibit inhibitory activity against Staphylococcus aureus PBP2a (5M19) comparable to that of standard drugs. The findings provide insight into the potential use of honey phytochemicals as an alternative or complementary therapy for S. aureus infections. Further studies are necessary to investigate the mechanisms of action of these phytochemicals and to determine their safety and efficacy in clinical settings. Overall, this research highlights the importance of exploring natural products as potential sources of new antimicrobial agents.

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