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R E S E A E C H A R T I C L E

Identification of Candidate Inhibitory Ligands from Allium cepa and Molecular Docking Against Trypanosma brucei Phosphoglycerate Kinase; The In-Silico Structure-Activity Relationship

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A B S T R A C T

Trypanosoma brucei is a unicellular parasite causing African sleeping sickness in cattle and humans. Infection occurs when a vector tsetse fly bites a mammalian host. The fly injects the metacyclic trypomastigotes into the skin tissue. The trypomastigotes enter the lymphatic system and into the bloodstream. The initial trypomastigotes are short and stumpy. Once inside the bloodstream, they grow into long and slender forms. Then, they multiply by binary fission. Onion (Allium cepa) contains a high level of dietary flavonoids with evidences indicating they exhibit antiparasitic effects. A molecular docking study was carried out on five structurally similar Allium cepa flavonoids against Trypanosoma brucei phosphoglycerate kinase using the Autodock Vina software. Extensive structure activity relationship study was also carried out with these molecules. The physicochemical analysis, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of Apigenin, Kaempferol, Luteolin, Myricetin and Quercetin were evaluated. These molecules were designed using the ChemAxon software. The scoring function (empirical binding free energy) was used to estimate the inhibitory activity of the protein-ligand complex. The binding energy of apigenin, kaempferol, luteolin, myricetin and quercetin were -7.7, -7.6, -7.7, -7.5 and -7.6Kcal/mol respectively while the number of hydrogen bonds formed with the target enzyme were 8, 6, 10, 7, 7 respectively. The low values (negative) of free binding energies displayed by the bioactive components of Allium cepa means that they show a high level of antiparasitic activity, while the higher number of hydrogen bonds formed by the luteolin component indicates a higher binding affinity with the target enzyme. While the myricetin component showed a poor GI absorption rate, all the flavnoids do not cross the blood brain barrier (BBB) showing that they cannot cause problem to the brain. These results clearly indicated that the luteolin substituents may be a better antiparasitic agent, having exhibited the best binding affinity with the Trypanosma brucei Phosphoglycerate kinase. Laboratory synthesis and pre-clinical studies of the luteolin component with Trypanosoma brucei phosphoglycerate kinase is recommended in order to confirm its potentials as a better antiparasitic agent than the other Allium cepa flavonoids.

Keywords: Infection, Flavonoids, Molecular docking, Pharmacokinetics, Lipophilicity.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Trypanosoma brucei is a species of parasitic kinetoplastid belonging to the genus Trypanosoma. The parasite causes a vector-borne disease of vertebrate animals, which includes humans, carried by tsetse fly in sub-Saharan Africa [3]. In humans T. brucei is the cause of African trypanosomiasis, or sleeping sickness. It causes animal trypanosomiasis in animals also. This is known as nagana in cattle and horses [7]. T. brucei is transmitted between mammal hosts by an insect vector belonging to different species of tsetse fly (Glossina). Biting during the insect's blood meal leads to the transmission. The mammalian bloodstream forms are notable for their cell surface proteins, variant surface glycoproteins, which undergo remarkable antigenic variation, enabling persistent evasion of host adaptive immunity leading to chronic infection [14].

T. brucei is one of the few pathogens known to cross the blood brain barrier. The need for the development of new drug therapies is imminent, as current treatments can have severe side effects and can prove fatal to the patient.When trypanosoma organism lives in the mammalian bloodstream, it depends completely on glycolysis for its supply of ATP because it does not have a functional Krebs cycle nor oxidative phosphorylation. It also does not store any carbohydrate. Glycolysis in trypanosomes and in other members of the Kinetoplastida family is not the same as the corresponding pathway in other organisms.

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin; they are extremely important because of their health benefits cannot be over emphasized [10]. It has been predicted that average intake of all flavonoids is several grams per day. Flavonoids occur in foods as O-glycosides with bound sugars at the C_3 position [9]. With the conducted studies on the flavonoids content of some edible tropical plants, it was observed that the highest total flavonoids content (quercetin, kaempferol, apigenin, myricetin, luteolin) was observed in onion (Allium cepa) leaves (quercetin (1497.50 mg/kg), kaempferol (832.0 mg/kg), luteolin (391.0 mg/kg) [11, 12].

In this study, selected bioactive components of Allium cepa were computationally evaluated for therapeutic potentials in relevance to trypanosomiasis, by predicting the binding energies and various pharmacokinetics parameters necessary for computational drug design. A bioinformatics investigation of the Trypanosoma brucei phosphoglycerate kinase was directed at the prediction of the enzyme stability and its percentage sequence identity with the Homo sapien Trypanosoma brucei phosphoglycerate kinase.

2. Materials and Methods

Sequence retrieval: The Trypanosoma brucei and Homo sapien Phosphoglycerate kinase amino acid sequence were obtained from the National Center for Biotechnological Information database (NCBI). The proteins were assigned an accession number of P07378.1 and P27362.1 respectively.

Multiple sequence alignment

Multiple sequence alignment was carried out using ClustalW, at default settings .

Physiological–biochemical characterization: The Expasy Protparam server was used for the physicochemical characterization and to know the molecular weight, theoretical isoelectric point (pI), total number of negative and positive residues, aliphatic index, extinction coefficient, instability index, and grand average hydropathicity (GRAVY) of Trypanosoma brucei Phosphoglycerate kinase.

Protein preparation

The crystal structure of T. brucei and H. sapien, were obtained from the Protein Data Bank, PDB 16PK and 3OZA (Figure 12 and 13). The refinement of the protein structures was done using the Pymol viewer [8].

Designing of 2D Structure of Flavonoids

The 2D structure of the flavonoids (Figure 2-6) was sketched using the ChemAxon software. The structures were minimized using the Chimera software.

Molecular docking

The docking was performed using the AutoDock Vina Software. Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of the flavonoids were determined using Swiss ADME Server [6].

3. Results and Discussions

Trypanosoma brucei Phosphoglycerate kinase amino acid sequence (FASTA)

>sp|P07378.1|PGKC_TRYBB RecName:
Full=Phosphoglycerate kinase, glycosomal;
Short=Phosphoglycerate kinase C
MTLNEKKSINECDLKGKKVLIRVDFNVPVKNGKITD
YRIRSALPTLKKVLTEGGSCVLMSHLGRPKGIPMAA
GKIRSTGGVPGFQQKATLKPVAKALSELLLRPVTFAP
DCLNAADVVSKMSPGDVVLLENVRFYKEEGSKKAK
DREAMAKILASYGDVYISDAFGTAHRDSATMTGIPKI
LGNGAAGYLMEKEISYFAKVLGNPPRPLVAIVGGAK
VSDKIQLLDNMLQRIDYLLIGGAMAYTFLKAQGSIG
KSKCEESKLEFARSLLKKAEDRKVQVILPIDHVCHTE
FKAVDSPLITEDQNIPEGHMALDIGPKTIEKYVQTIGK
CKSAIWNGPMGVFEMVPYSKGTFAIAKAMGRGTHE
HGLMSIIGGGDSASAAELSGEAKRMSHVSTGGGSLE

LLEGKTLPGVTVLDEKSAVVSYASAGTGTLSNRWSS
L

Homo sapien Phosphoglycerate kinase amino acid sequence (FASTA)

>sp|P27362.1|PGK_PLAF7 RecName:
Full=Phosphoglycerate kinase
MLGNKLSISDLKDIKNKKVLVRVDFNVPIENGIIKDT
NRITATLPTINHLKKEGASKIILISHCGRPDGLRNEKY
TLKPVAETLKGLLGEEVLFLNDCVGKEVEDKINAAK
ENSVILLENLRFHIEEEGKGVDANGNKVKANKEDVE
KFQNDLTKLADVFINDAFGTAHRAHSSMVGVKLNV
KASGFLMKKELEYFSKALENPQRPLLAILGGAKVSD
KIQLIKNLLDKVDRMIIGGGMAYTFKKVLNNMKIGT
SLFDEAGSKIVGEIMEKAKAKNVQIFLPVDFKIADNF
DNNANTKFVTDEEGIPDNWMGLDAGPKSIENYKDVI
LTSKTVIWNGPQGVFEMPNFAKGSIECLNLVVEVTK
KGAITIVGGGDTASLVEQQNKKNEISHVSTGGGASLE
LLEGKELPGVLALSNK

Figure 1: Multiple sequence alignment.(*) red color denotes identical amino acids, (:) green color denotes strongly similar amino acids and (.) blue color denotes weakly similar amino acids.

The pI of the T. brucei PGK by the biochemical characterization analysis has predicted the protein to be slightly acidic with a value of 9.25[33]. The hydrophobicity scale produced values that define relative hydrophobicity of amino acid residues. The more positive the value, the more hydrophobicthe amino acids located in that region of the protein [23]. The GRAVY calculator used in predicting the hydrophobicity assigned to the protein a value of -0.110. The instability index provides an estimate of the stability of a protein in a test tube. A protein whose instability index is greater than 40 is predicted as unstable and a value below 40 predicts the protein may be stable [23]. The T. brucei PGK is therefore a stable protein with an instability value of 32.77.

The multiple sequence alignment of T. brucei PGK with the H. sapien PGK revealed the variable sites of the parasitic enzyme's amino acid sequence. These regions are potential target sites for therapeutic agents [4]. The percentage sequence identity is 46.25%. This indicates a high level of identity and as such makes T. brucei PGK an ideal target for therapeutic agents [22]. The tertiary structure comparison of the T. brucei and Homo sapien PGK suggests a functional similarity between the two enzymes [16, 30]T. brucei PGK contains 440 amino acid residues. The docking pose exhibited all the flavonoids showed that their binding pattern with the active site of T. brucei PGK is in a similar orientation, as is evident from the superposition of the flavonoids in Figure 9-13.

The flavonoids also show a steric interaction with the amino acid residues of T. brucei PGK. The calculated free energy of the flavonoids (apigenin, kaempferol, luteolin, myricetin and quercetin) were -7.7, -7.6, -7.7, -7.5 and -7.6Kcal/mol respectively. This proved the reliability of the docking results going by the structural similarities exhibited by the flavonoids.Hydrogen-bonds play a crucial role in the determination the specificity of ligand binding [37].

The luteolin substituent forms the highest number of hydrogen bond interaction with T. brucei PGK. This shows it has the highest binding affinity with the parasitic enzyme.The biotransformation and elimination of a druglike compound is a function of its solubility. All the experimental flavonoids were soluble in water which makes them likely to be drugs (figure 14-17). A compound can be considered drug-like if it is characterized by high lipophilicity (less than 5) [1] and low molecular weight (less than 500g/mol) [2]. Lipophilicity is expressed as Log Po/w. The lipophilicity value of all the flavonoids are less than 5 and are most likely to be drugs (figure 14-17). Distinguishing between drug-like and non drug-like molecules requires the application of the lipinski's rule of 5 [26].

The probability of success or failure is dependent on the compliance with 2 or more of the following rules: Molecular mass less than 500g/mol; High lipophilicity (expressed as Log Po/w less than 5); Less than 5 hydrogen bond donors; Less than 10 hydrogen bond acceptors; Molar refractivity should be between 40-130. For the avoidance of costly late-stage preclinical and clinical failures, application of these filters could help in the early stages of the preclinical drug development [20]. All the five flavonoids violated none of the lipinski's rule and therefore are likely to be drugs (figure 14-17). Drugs targeting the central nervous system (CNS) must have a characteristic high penetration rate, whereas penetration of the blood brain barrier (BBB) should be limited for non-CNS drugs to avoid undesired side-effects [5].

The gastrointestinal drug absorption of all the flavonoids but myricetrin was high and none could cross the blood brain barrier (BBB). This indicates that the flavonoids pose no threat to the CNS. For synthetic accessibility, values of 1 to 5 means that the drug could easily be synthesized. Falcarindiol and all it analogues showed values less than 5. This means that the compounds can easily be synthesized in the laboratory. Synthetic studies followed by preclinical test of the flavonoids are further recommended.

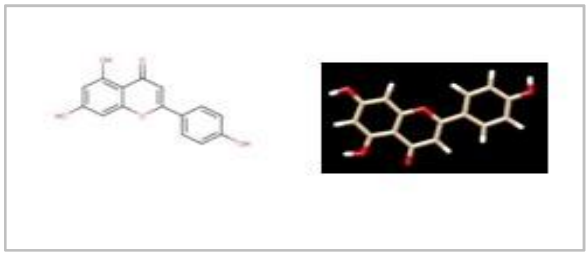

Fig 2:2D and 3D Structure of Apigenin

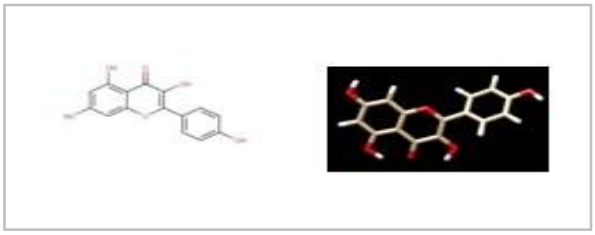

Fig 3: 2D and 3D Structure of Kaempferol

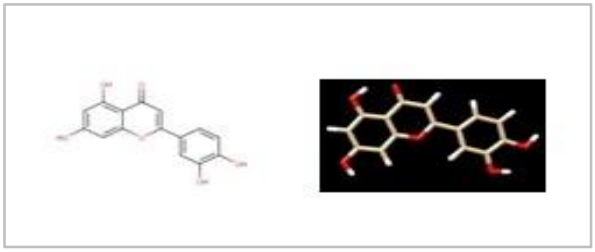

Fig 4:2D and 3D Structure of Luteoline

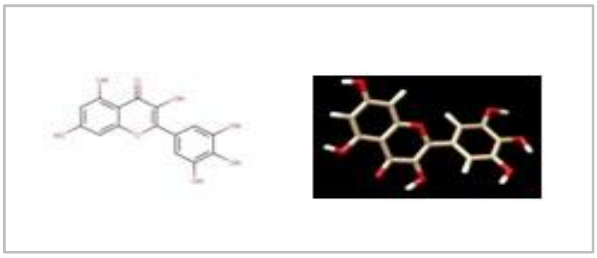

Fig 5:2D and 3D Structure of Myricetin

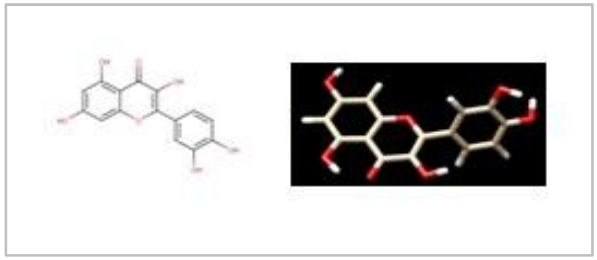

Fig 6:2D and 3D Structure of Quercetin

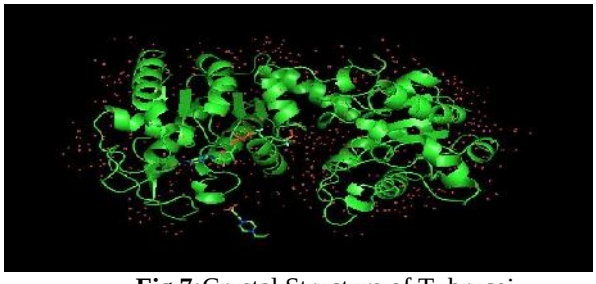

Fig 7: Crystal Structure of T. brucei

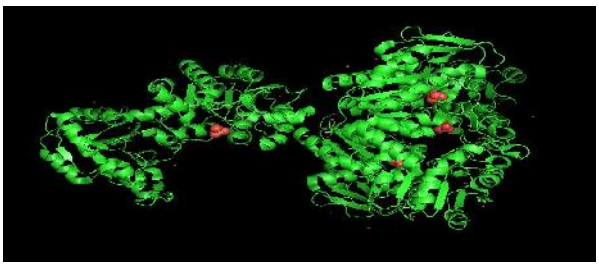

Fig 8: Crystal Structure of H. sapien

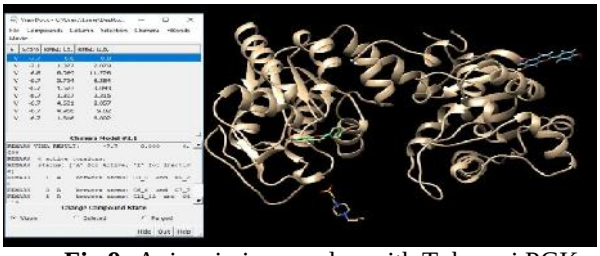

Fig 9: Apigenin in complex with T. brucei PGK

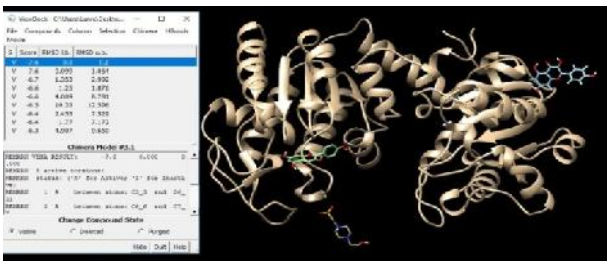

Fig 10: Kaempferol in complex with T. brucei PGK

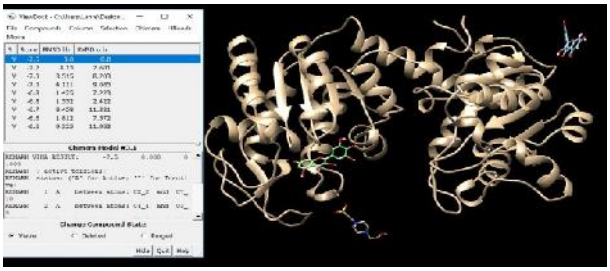

Fig 11: Myricetin in complex with T. brucei PGK

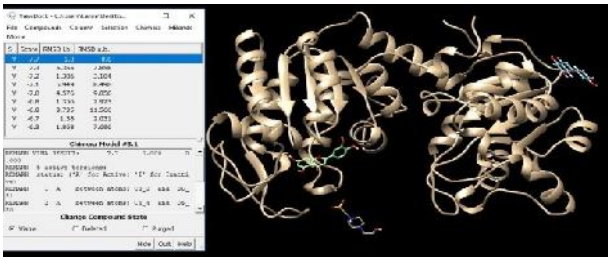

Fig 12: Luteolin in complex with T. brucei PGK

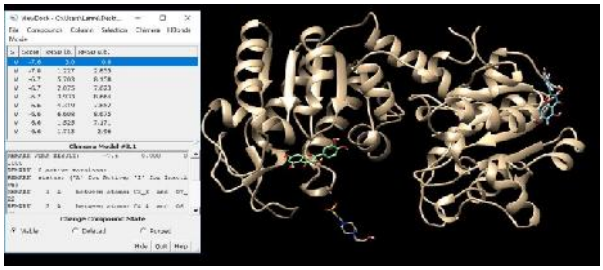

Fig 13: Quercetin in complex with T. brucei PGK

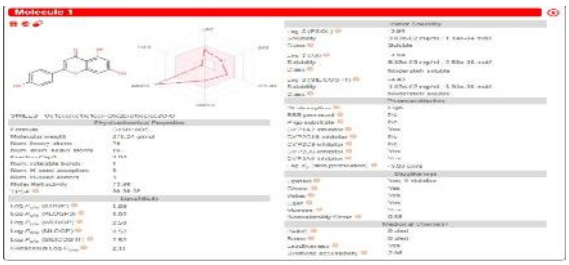

Fig 14: Structure-Activity Relationship of Apigenin

Fig 14: Structure-Activity Relationship of Luteolin

Fig 15: Structure-Activity Relationship of Kaempferol

Fig 16: Structure-Activity Relationship of Myricetin

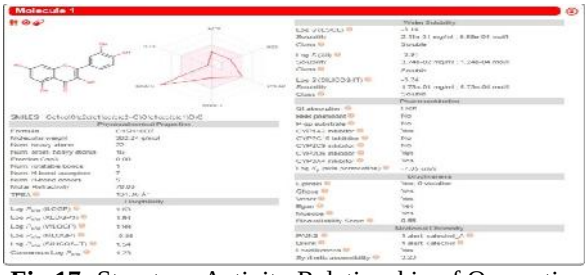

Fig 17: Structure-Activity Relationship of Quercetin

4. Conclusion

An In-Silico structure activity relationship and molecular docking study was carried out on isolated flavonoids from Onion (Allium cepa) against Trypanosoma brucei phosphoglycerate kinase. The obtained result showed that the luteolin component showed the best therapeutic prospect against the parasite as it is characterized by a high binding energy of -7.7Kcal/mol and the highest number of hydrogen bond interaction with the parasitic enzyme. The Myricetin component showed the lowest binding energy among the five experimented flavonoids with a value of -7.5Kcal/mol. It also exhibited a low gastrointestinal absorption rate. This makes it an unsuitable therapeutic candidate for drug development against Trypanosoma brucei phosphoglycerate kinase which has been indicated to be a stable parasitic enzyme. We recommend the laboratory synthesis of luteolin and its monosubstituted analogues for further preclinical trial against the Trypanosoma brucei phosphoglycerate kinase.

Abbreviations: PDB: Protein Data Bank; BBB: Blood Brain Barrier; CNS: Central Nervous System; PGK: Phosphoglycerate kinase; pI: Isoelectric Point; GI: Gastrointestinal.

5. References

[1] JR Baker. The subspecific taxonomy of Trypanosoma brucei. Parasite, 1995, **2**(1):3–12.

[2] S Deborggraeve, M Koffi, V Jamonneau, FA Bonsu, R Queyson, PP Simarro, P Herdewijn, P Büscher. Molecular analysis of archived blood slides reveals an atypical human Trypanosoma infection. Diagnostic Microbiology and Infectious Disease, 2008, **61** (4): 428–33.

[3] W Gibson . Resolution of the species problem in African trypanosomes. International Journal for Parasitology, 2007, **37** (8-9): 829–38.

[4] W Masocha, K Kristensson. Passage of parasites across the blood-brain barrier. Virulence, 2012, **3** (2):202-12.

[5] D Legros, G Ollivier, M Gastellu-Etchegorry, C Paquet, C Burri, J Jannin, P Büscher. Treatment of human African trypanosomiasis--present situation and needs for research and development. The Lancet. Infectious Diseases, 2002, **2** (7): 437–40.

[6] V Hannaert, PAM Michels. J. Bioenerg. Biomembr, 1994, **26**:205–212.

[7] Pam Michels, V Hannaert. J.Bioenerg. Biomembr. 1994, **26**:213–219.

[8] FR Opperdoes. Annu. Rev. Microbiol. 1987, **41**:127–151.

[9] Formica J.V and Regelson W, Food Chem. Toxicol., 33, 1061 (1995)

[10] KR Markham, Methods Plant Biochem., 1989, 1, 197

[11] H Fujiki, T Horiuchi, K Yamashita, H Hakii, M Suganuma, H Hishino, A Iwashima, Y Hirata, and T Sugimura, in: V. Cody, E. Middleton, and J.F. Harborne (Eds) Proc. Symp. Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, Structure–Activity Relationships, Buffalo, NY, July 22–26, 1986, Liss, NewYork, 1986, pp. 213, 429–440.

[12] R-Closaas Garcia, CA Gonzalez, A Aguda, and E Riboli, Cancer Causes Contr., 1999, 10, 71.

[13] M Goujon, H McWilliam, W Li, F Valentin, S Squizzato, J Paern, R Lopez. Nucleic acids research 2010 Jul, 38 Suppl: W695-9

[14] MA Larkin, G Blackshields, NP Brown, R Chenna, PA McGettigan, H McWilliam, F Valentin, IM Wallace, A Wilm, R Lopez, JD Thompson, TJ Gibson and DG Higgins.Bioinformatics 2007 23(21):2947-2948.

[15] E Gasteiger, C Hoogland, A Gattiker, S Duvaud, MR Wilkins, RD Appel, A Bairoch A.Protein Identification and Analysis Tools on the ExPASy Server; (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press (2005). pp. 571-607.