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PURE CHEMICAL COMPOUNDS OBTAINED FROM TANZANIAN MEDICINAL PLANTS COMPETITIVELY BLOCK ATP BINDING IN DNA GYRASE AS ANTIMICROBIAL TARGET

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ABSTRACT: The current study investigated possible molecular target for the antimicrobial activities of pure chemical compounds isolated from various Tanzanian medicinal plants and verified the efficacy of the medicinal plants as sources of potent natural antibacterial agents. The plants are locally employed in the treatment of bacterial and fungal infections as one of the commonest diseases in Tanzania. With the aid of *in silico* tools, twenty pure chemical compounds were screened via docking against DNA gyrase; a key enzyme that is essential for negative supercoiling of DNA in bacteria. The protein has emerged as a clinical target for antimicrobial agents. Out of all the compounds studied, six compounds with best binding affinity to ATP-binding pocket on the enzyme were further analysed for molecular interaction. With binding energy values ranging from -8.2Kcal/mol to -9.8Kcal/mol, the selected compounds favourably docked at the targeted site on the protein and established hydrogen bonds with amino acid residues which are normally essential for ATP binding. These residues include Gly-102, Gly-117, Asn-46, Thr-165, and Asp-73. Thus, the compounds competitively block ATP from binding to the enzyme which results in their potent antimicrobial effects. The enzyme-ligand complexes were also stabilized by hydrophobic interactions and π-stacking association. The results obtained from this study identified DNA gyrase as a possible antimicrobial target for the compounds with ATP-competitive inhibition mechanism and confirmed that these chemical compounds are mainly responsible for the antimicrobial activities of the medicinal plants. The results also authenticate reported medicinal potential of the plants and justify the use of the plants for treatment of infection. In addition, the compounds fulfilled drug-likeness conditions based on Lipinski's rule of 5. Therefore, they provide chemical scaffolds that may help in design and development of effective antimicrobial agents.

Peer review under responsibility of Life Science Informatics Publications

KEYWORDS: Tanzanian medicinal plants, antimicrobial, DNA gyrase, molecular interaction, docking

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1.INTRODUCTION

The use of locally available medicinal plants in the treatment of numerous diseases plighting human's health has been known for ages. Such medicinal plants provide natural and alternative medicine especially for people in the rural areas all over the World. Till date, many plants have been reported for their therapeutic potentials including anti-inflammatory, anticancer, anti-diabetes, antimicrobial, antiviral, antioxidant, etc [1- 4]. Most of these plant materials are used in their crude form (extracts). Over the years, researchers have developed interest in isolating and characterizing the chemical compounds available in various medicinal plant extracts and this has enhanced the identification of natural chemical compounds for their specific biological properties. In Tanzania, the people have been observed for the act of using different parts (leaves, root, stem, flower and bark) of medicinal plants in their vicinity to cure and treat ailments [5]. Various extracts, such as aqueous and ethanolic extracts, are obtained and applied for management of diverse diseases. Common among the ascribed reasons for the choice of local medicinal plants over standard drugs are the cheaper cost and reduced side effects. Among the valuable plants that are often used are Garcinia species, Annona spp, Mammea spp, Baphia spp, Dalbergia spp, Harrisonia spp, Allanblackia spp and Teclea spp. The known classes of phytochemicals isolated from these plants include xanthones, alkaloids, benzophenone, coumarins, flavonoids, limonoids, terpenoids and other compounds [6]. It has been reported that bacterial infections and malaria actually dominated the list of diseases in the country and they are managed with the use of traditional medicines [7]. Due to the wide claim of antimicrobial properties of these plants and their effectiveness in management of diseases caused by bacteria and fungi, some of the chemical compounds present in the plants have been isolated in their pure form [6,8]. However, their molecular target for antimicrobial potency as well as their mechanism of action for the observed biological properties still remain unknown. In this research, the possible molecular

target for the reported antimicrobial efficacy of these pure chemical compounds isolated from Tanzanian medicinal plants was investigated. The mechanism of action was also elucidated. The study revealed DNA gyrase as a potential target for the antimicrobial compounds. The protein, DNA gyrase, is a unique protein that is involved in coupling the free energy of ATP hydrolysis to formation of negative supercoils into DNA [9]. The protein has emerged as a clinical target for antimicrobial agents. Usually, gyrases are known to be present only in prokaryotes, hence making them an attractive target for anti-bacterial drugs. The known classes of gyrase inhibitors are effective but their side effects, such as cytotoxicity, is a great concern. This has reduced the use of some of the most potent gyrase inhibitors like fluoroquinolones. The gyrase inhibitors directed against ATP binding site in bacteria, for instance novobiocin, have also be reported for their harmful effects because they bind to a number of other human proteins like Heat Shock Protein 90, F1 ATP-synthetase, histones etc. This prevents novobiocin use as an antibacterial drug [10]. Therefore, research of new gyrase inhibitors is still encouraged. Modern microbe-targeting drugs development largely depends on rational design of compounds directed against specific targets. Such therapeutically active compounds may be of natural origin or synthetically derived [10]. Most natural compounds are obtained from medicinal plants and Ethno-pharmacologists all over the World have explored some of the beneficial phytochemicals that are present in various locally available medicinal plants. Hence in this study, the inhibitory potential of pure chemical compounds obtained from Tanzanian medicinal plants on the antimicrobial target protein was studied. The compounds were also examined for their ADME characteristics according to Lipinski's rule of five.

2. MATERIALS AND METHODS

Selection and preparation of protein structure through homology modeling

The "FASTA" files (Accession: Accession: 4PRX_A GI:694270172) for the protein was obtained from GenBank and used in homology modeling of the protein done on the Swiss Model Server (http://swissmodel.expasy.org). The coordinate file of templates from protein data bank was used to model the 3D structure DNA gyrase. All water molecules and ligand (ADP and Pi) crystallized with the protein were deleted before molecular docking procedures.

Evaluation and validation of the predicted model

The modeled protein was assessed by PROCHECK for structural analysis. The Ramachandran plot and Z score values were generated using the protein structure and model assessment tools of Pdbsum database of the European Bioinformatic instute (EMBL-EBI) (http://www.ebi.ac.uk/) and the Swiss-Model workspace (http://swissmodel.expasy.org). The protein structures were also loaded on ProQ web server to evaluate the PDB structure [11]. The values for LG-score, Maxsub as well as Qmean

score were obtained.

Preparation of ligands

A total of twenty-one (21) ligands used for docking study were selected from the literature. Out of these compounds, twenty are natural compounds isolated from various Tanzanian medicinal plants while one (1) was a known DNA gyrase inhibitor (novobiocin) which was used as reference compound. The chemical structure of novobiocin was obtained from NCBI PubChem compound database (http://www.ncbi.nlm.nih.gov/pccompound) while the plant-derived compounds were prepared using Marvin sketch. Three-dimensional optimizations of the ligand structures were done before use in docking studies. The ligands were saved as MOL SD format after optimization. These were docked into refined DNA gyrase model using "LigandFit" in the AutoDock 4.2.

ADME Screening

ADME (Absorption, Distribution, Metabolism and Excretion) screening for the polyphenolic compounds was done using available online servers (www.chemicalize.org). ADME screening helps in detecting drug likeness of compounds. According to Lipinski's rule of five, the number of rotatable bond, compound molecular weights (MW), calculated logarithm of the partition coefficient between n-octanol and water (LogP), molar refractivity, number of hydrogen bond acceptors (HBA) and number of hydrogen bond donors (HBD) were used to assess the "drug-likeness" [12].

Molecular docking

For molecular docking analysis, AutoDock 4.2 was used in this study [13, 14]. All optimized ligand molecules were docked into the active site of refined DNA gyrase protein.

The rotatable bonds of the ligands were set to be free however the protein molecules were treated as a rigid structure [15]. The grid parameters were set at x=60, y=60 and z=60 while the coordinate of origin was set at -9.44, -48.48 and -9.73 (x, y and z) to include all the amino acid residues at the binding site while the spacing between grid points was kept at 0.375Å. Ten (10) docking runs were performed for each compound with the number of modes set to 10 so as to achieve more accurate and reliable results.

Data analysis

All protein snapshots were taken using PYMOL while receptor-ligand molecular interaction was analysed using Ligplot [16].

3. RESULTS AND DISCUSSION

Availability of structures of ligand-protein complexes of gyrase B fragments for inhibitors such as cyclothialidine, ADPNP, chlorobiocin and novobiocin have shown the structural background for ATP-competitive inhibition which have enhanced the structure-based design of novel inhibitors [10, 17].

This research reports the molecular target and basis of action for the earlier observed antimicrobial activities of pure chemical compounds isolated from Tanzanian medicinal plants. The research features *in silico* experiments where docking was carried out by AutoDock 4.2 in ATP-binding pocket of the DNA gyrase [18]. Molecular docking studies of Baphikinone (BAPHI), 12-hydroxy-des-D-garcigerrin A (12HDGA), 3-Methoxy-8,9-mehylenedioxypterocarpene (3MMDC), Baphikixanthone A (BKXA), Baphikixanthone B (BKXB) and Teclamaniensine B (TCMB) showed favorable binding with the ATP-binding site of DNA gyrase (Figure 6). This indicates effective antimicrobial potential of these compounds. The obtained results showed that the selected compounds bind to the 24kDa fragment of *Escherichia coli* DNA gyrase B with binding energy values ranging from -8.2Kcal/mol to -9.8Kcal/mol and blocked ATPase activity of DNA gyrase B. Their binding site overlapped with ATP binding pocket and hence competitively inhibit the coupling of ATP hydrolysis with negative DNA supercoiling in the bacteria [19, 20]. The protein normally utilizes the free energy from ATP hydrolysis to catalyze topological alterations in the microbial genome as the essential function of DNA gyrase is the introduction of negative DNA supercoils into the genome [21]. According to this study, baphikinone was observed establishing four hydrogen bond with DNA gyrase at residues Asp-73 (3.28Å), Gly-102 (2.99Å), Tyr-109 (2.91Å) and Gly-119 (2.72Å). The compound also formed hydrophobic interaction with Val-118, Gly-117, Lys-103, Val-120 and Ile-78 (Table 1). The very low binding energy value obtained for this compound indicates its high binding affinity to the protein while the ligand-receptor complex was stabilized by the hydrogen bonds with minimum bond length 2.72Å as well as the hydrophobic interactions (Figure 6a). The amino acid Asp-73 of DNA gyrase is an essential residue which is known to form a direct hydrogen bond with inhibitors of the protein. It is important for ATP binding and its replacement by other amino acids, specifically asparagine or alanine, is known to result in abolition of both ATPase and DNA supercoiling activities. The amino acid residue Asp-73, Gly-77, Gly-178 and Thr-165 in DNA gyrase have been identified as critical residues for DNA binding and thus its enzymatic activities in E. Coli [22]. The replacement of these residues is known to cause resistance to novobiocin in bacteria. This indicates their significance in the enzymatic activity and are therefore targeted by inhibitors to the protein. Both 3MMDC and BKXB have binding energy value of -9.8Kcal/mol (Table 1). However, BKXB interacted via three hydrogen bonds with residues Asn-46, Thr-165 and Asp-73 while 3MMDC formed four hydrogen bonds with amino acid residues Gly-102, Gly-119, Lys-115 and Gly-117 of varying bond length. They also interacted with other residues at the binding site through hydrophobic interaction (Figure 6b and 6d). The hydrogen bonds coupled with the hydrophobic interactions may be responsible for the strong binding affinity of these compound to DNA gyrase. BKXA relatively showed a reduced affinity for

the protein with a binding energy value of -9.5Kcal/mol and formed only two hydrogen bonds with the residues at the ATP-binding site. The fewer number of hydrogen bonds may likely contribute to this reduced effect. With an energy value of -8.2Kcal/mol, 12HDGA exhibited the least binding affinity to the receptor and established hydrogen bonds with residues Gly-102, Asn-46 and Tyr-109. However, the compound showed fewer hydrophobic interactions with the residues at the binding pocket on DNA gyrase (Figure 6c). TCMB, having binding energy -9.2Kcal/mol, formed just two hydrogen bonds with residue Lys-103 and Gly-77 but hydrophobic interactions with many amino acid residues (Table 1). Based on the results gotten from this study, all the selected compounds are considered as specific ligand of DNA gyrase with high docking scores (Figure 3), hence they possess antimicrobial properties. This is in agreement with reported results of previous studies [6, 23, 24]. The importance of the interacting residues in the ATP-binding pocket is well known for E. coli DNA gyrase. For instance, the lack of ATPase activity has been ascribed to mutation that changed Asp-73 to asparagine while the re-conversion of asparagine to aspartate on this location restored both ATPase and DNA supercoiling activities wet experiments [9]. This emphasizes the extreme relevance of Asp-73 residue for catalytic activity of DNA gyrase. In the same vein, mutant proteins whose residues at Glu-42, Asn-46, Glu-50, Asp-73, Arg-76, Gly-77, and Ile-78 were changed to alanine were observed to possess reduced or no detectable ATPase activity, indicating an essential role for these residues in ATP hydrolysis [22]. It is also very interesting to note that the selected compounds are relatively small in size, therefore they are easy to accommodate in the ATP-binding pocket of DNA gyrase. It is believed that further derivation of these natural compounds could serve as novel templates for effective antibacterial drugs [25]. In addition, the results for ADME screening proved the compounds as drug candidates since they satisfied all necessary criteria for drug likeness based on Lipinski's rule of 5. The values for molecular weight are less than 500 and the number of rotatable bond is below 5 for all the compounds (Table 2). This suggests that the analysed compounds are good therapeutic candidates. Taken together, these data indicate that the pure chemical compounds could be used as antimicrobial agents. They can also serve as good chemical scaffolds toward the synthesis and development of novel antibacterial drugs [25]. The Ligplot analysis was carried out to reveal the in-depth interaction pattern that exist between docked ligand and amino acid residues in the active site of the protein target [26]. One of the importance of Ligplot analysis was its potential to show the hydrophobic interactions as well as hydrogen bonding pattern between the ligand-protein complex (Figure 3). Assessment of a protein model, as carried out in this study, is also essential because the structure of any protein serves a key role in determining the function of such protein. Thus, the protein used in this study was structurally assessed and validated (Figure 4). From the Ramachandran analysis,

93% residues were found in the most favoured region, 6.6% residues were located in the additional allowed regions while only 0.3% of the total residues was present in the disallowed regions (Table 3). PROCHECK checks the stereochemical quality of a protein structure via analyzing residue-to-residue geometry as well as the overall structure geometry. This is actually based on two criteria, torsion angles (Phi, Psi and Chi) and hydrogen bonding energy [27]. The PROCHECK results therefore showed that the overall quality of the modeled protein was good, reliable and therefore, acceptable for the docking study.

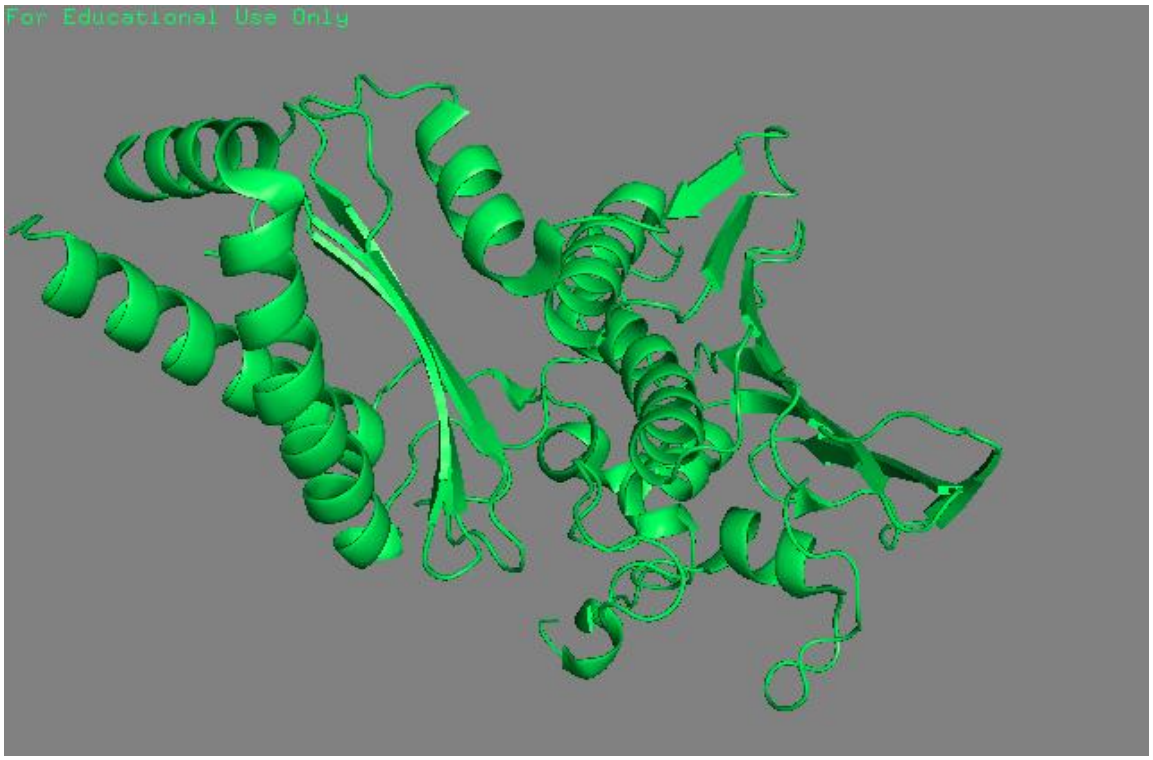
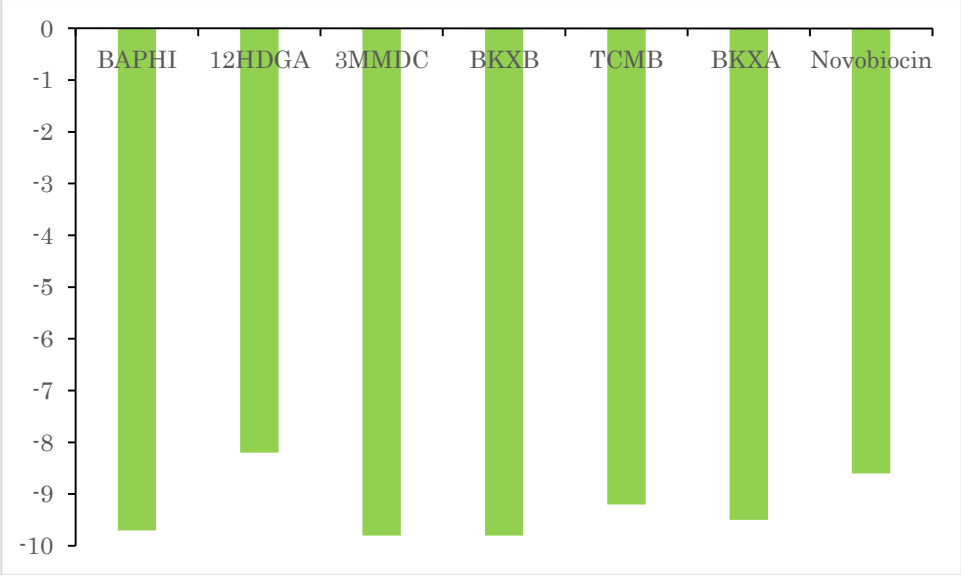


Figure 1. 3D model of E. Coli DNA gyrase B

MHHHHHHSNSYDSSSIKVLKGLDAVRKRPGMYIGDTDDGTGLHHMVFEV
VDNAIDEALAGHCKEIIVTIHADNSVSVQDDGRGIPTGIHPEEGVSAAEVIM
TVLHAGGKFDDNSYKVSGGLHGVGVSVVNALSQKLELVIQREGKIHRQIYE
HGVPQAPLAVTGETEKTGTMVRFWPSLETFTNVTEFEYEILAKRLRELSFL
NSGVSIRLRDKRDGKEDHFHYEGGIKAFVEYLNKNKTPIHPNIFYFSTEKD
GIGVEVALQWNDGFQENIYCFTNNIPQRDGGTHLAGFRAAMTRTLNAYMD
KEGYSKKAKVSATGDDAREGLIAVVSVKVPDPKFSSQTKDKLVSSEVKSAVE
QQMNELLAEYLLENPTDAKIVVGKIIDAARAREAARRAREMT

Fig. 2. Amino acid sequence of E. Coli DNA gyrase B



Test ligands; Novobiocin serves as a control

Fig. 3: Binding energy values of the test compounds with the control

Table 1: Docking results and hydrogen bond interaction between DNA gyrase and ligands

Compound name	E-value (Kcal/mol)	No. of H-Bond	Residues involved in hydrogen bonding	Distance (Å)	Residues involved in hydrophobic interaction
Baphikinone	-9.7	4	Asp-73 (O-H--O) Gly-102 (N-H--O) Tyr-109 (O-H--O) Gly-119 (N-H--O)	3.28 2.99 2.91 2.72	Ile-78, Val-120 Val-118, Gly-117 Lys-103

12HDGA	-8.2	5	Gly-102 (N-H--O) Asn-46 (N-H--O) Asn-46 (N-H--O) Tyr-109 (O-H--O) Tyr-109 (O-H--O)	3.25 3.08 2.58 3.01 2.80	Gly-117, Ile-78, Val-120
3MMDC	-9.8	4	Gly-102 (N-H--O) Gly-119 (N-H--O) Lys-115 (N-H--O) Gly-117 (N-H--O)	3.14 2.96 3.12 2.82	Pro-79, Glu-50, Asn-46, Gly-77, Tyr-109
BKXB	-9.8	3	Asn-46 (N-H--O) Thr-165 (O-H--O) Asp-73 (O-H--O)	2.94 2.91 3.25	Val-120, Lys-103, Tyr-109, Pro-79, Ile-78
TCMB	-9.2	2	Lys-103 (N-H--O) Gly-77 (N-H--O)	3.18 3.16	Glu-50, Tyr-109, Tyr-109, Gly-77, Gly-117
BKXA	-9.5	2	Asn-46 (N-H--O) Asp-73 (O-H--O)	2.87 2.70	Val-167, Ile-94, Val-120, Pro-79, Ile-78, Gly-119

Table 2: ADME result for the compounds based on the rule of five formulations

Compound	Molecular Weight	LogP	HBD	HBA	Rotatable Bond	Molar refractivity
Baphikinone	380.396	4.23	2	5	4	104.72
12HDGA	312.321	4.38	3	4	2	86.07
3MMDC	296.278	2.58	0	5	1	76.70
BKXB	394.423	5.33	3	5	2	111.40
TCMB	328.364	3.25	1	4	4	89.34
BKXA	396.439	5.85	4	5	4	113.23

HBA = Hydrogen bond acceptor, HBD = Hydrogen bond donor, LogP = The logarithm of the partition coefficient between n-octanol and water

Table 3. Ramachandran plot statistics of DNA gyrase

Ramachandran Plot statistics	DNA gyrase	
	No of residues	**Percentage**
Most favoured regions [A,B,L]	312	93.1%*
Additional allowed regions [a,b,l,p]	22	6.6%
Generously allowed regions [~a,~b,~l,~p]	0	0.0%
Disallowed regions [XX]	1	0.3%
Non-glycine and non-proline residues	335	100.0%
End-residues (excl. Gly and Pro)	2	
Glycine residues	34	
Proline residues	12	
Total number of residues	383	

Ramachandran Plot analysis of receptor n487 (DNA gyrase)

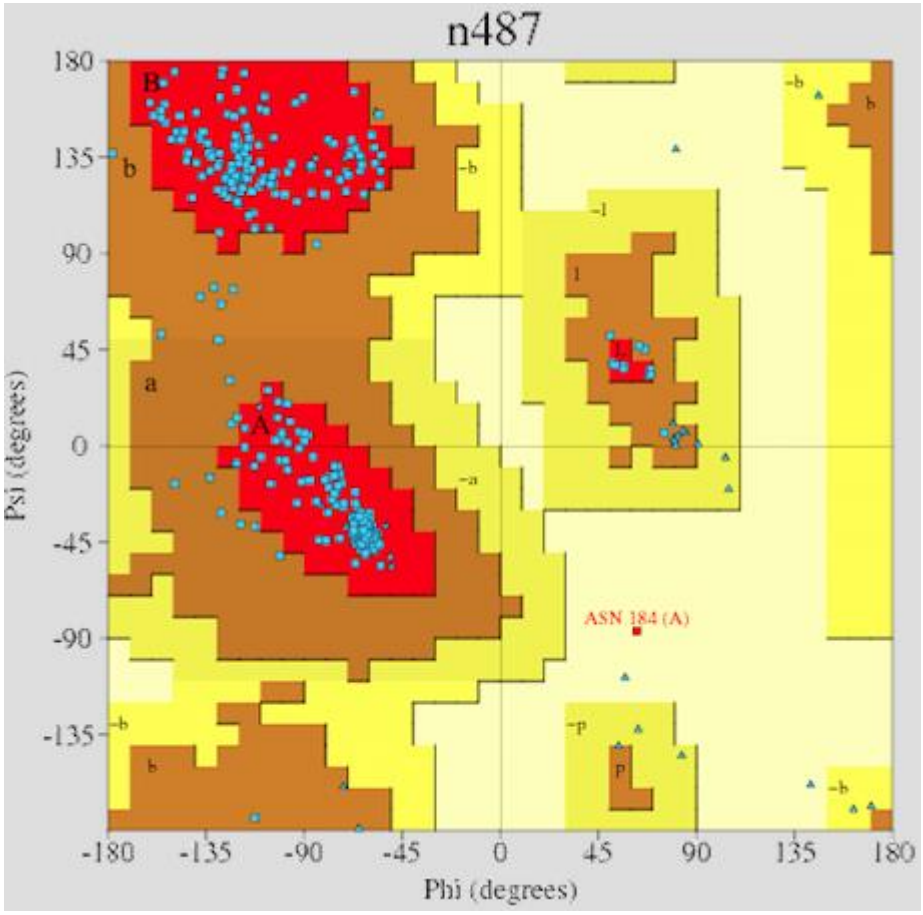
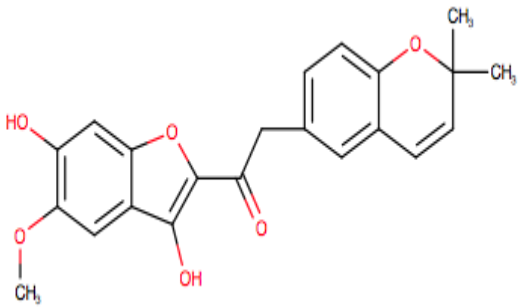
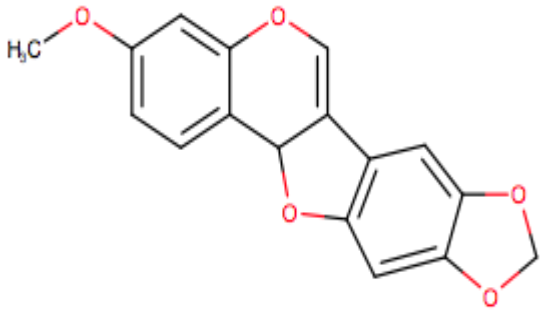


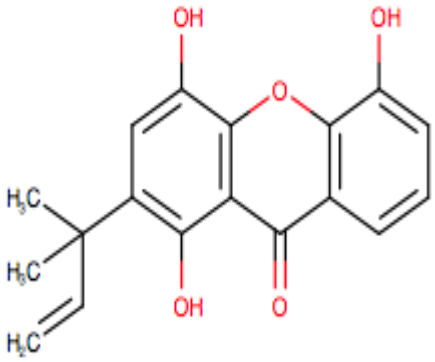
Fig. 4: PROCHECK-Ramachandran plot and summary for the model**.** Red areas correspond to favored region, allowed region are presented in yellow while light yellow areas correspond to generously allowed region and disallowed region is shown in white.



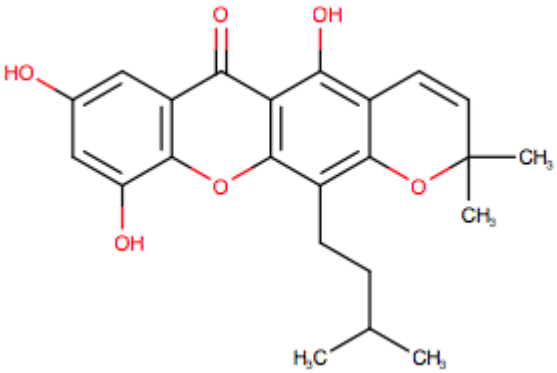
Baphikinone



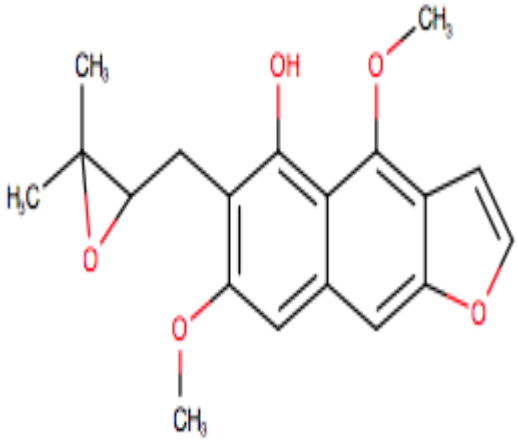
3MMDC



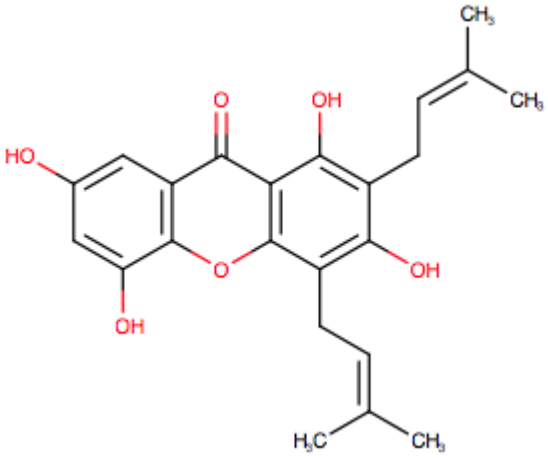
12HDGA



Baphikixanthones B

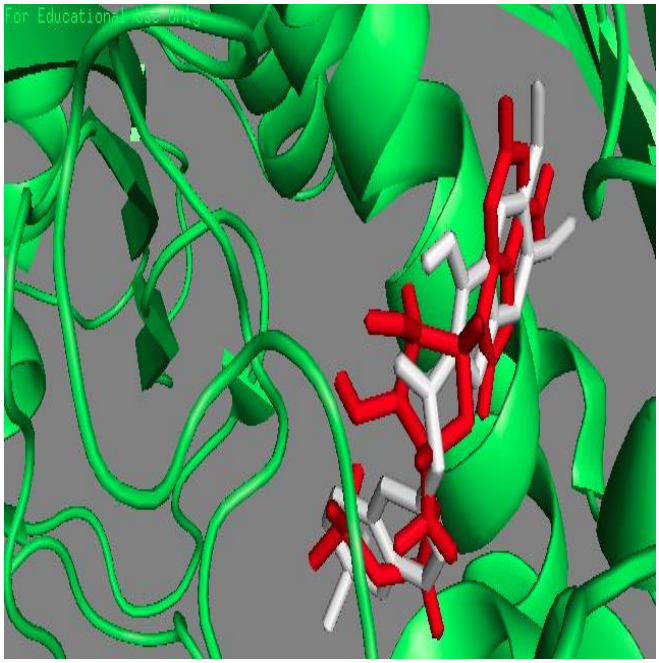


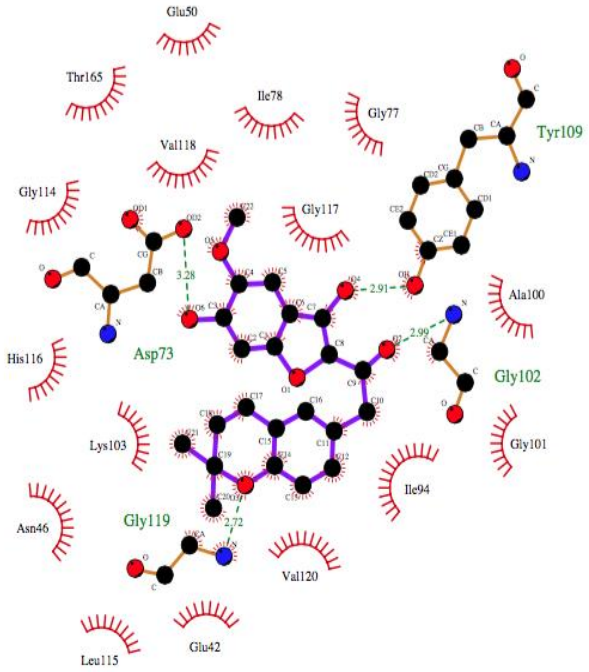
Tecleamaniensine B



Baphikinoxanthones A

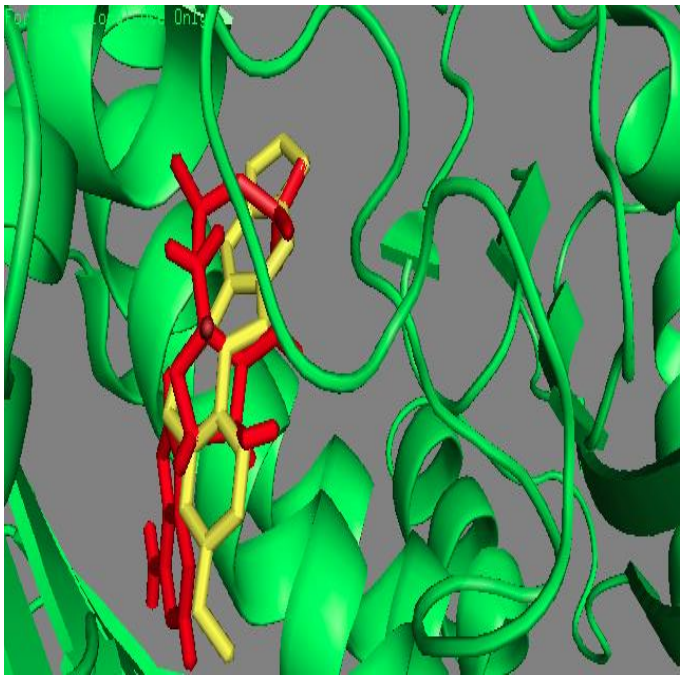
Figure 5: 2D structures of selected natural compounds used as ligand in this study.

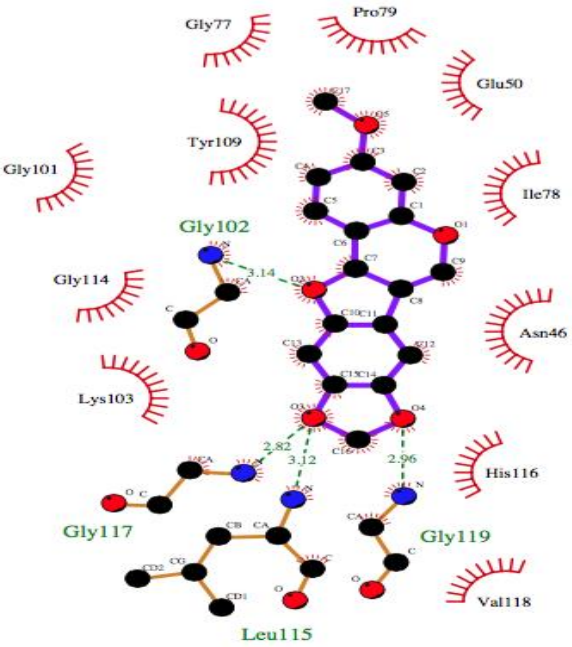




Baphikinone(white) and ADP(red) docked to Gyrb LigPlot of docked Baphikinone to Gyrb

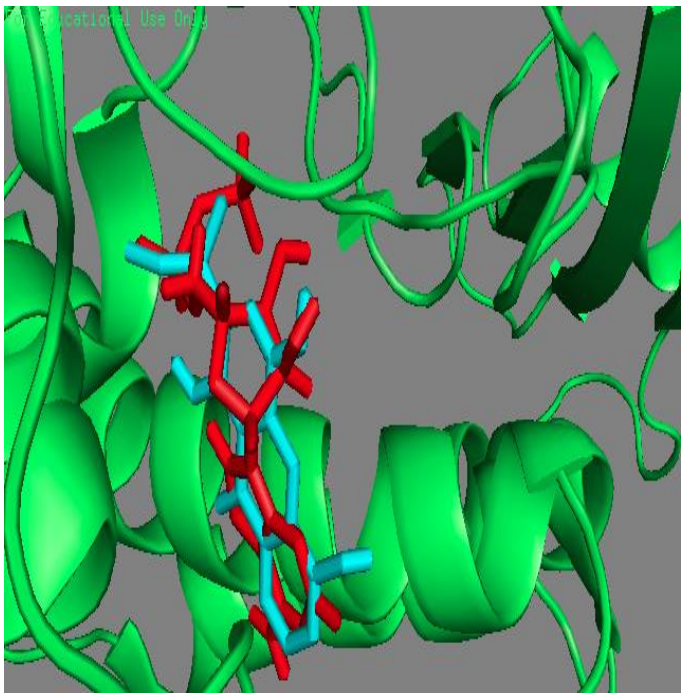
Fig. 6a. Baphikinone docked to Gyrb; specific residues in contact

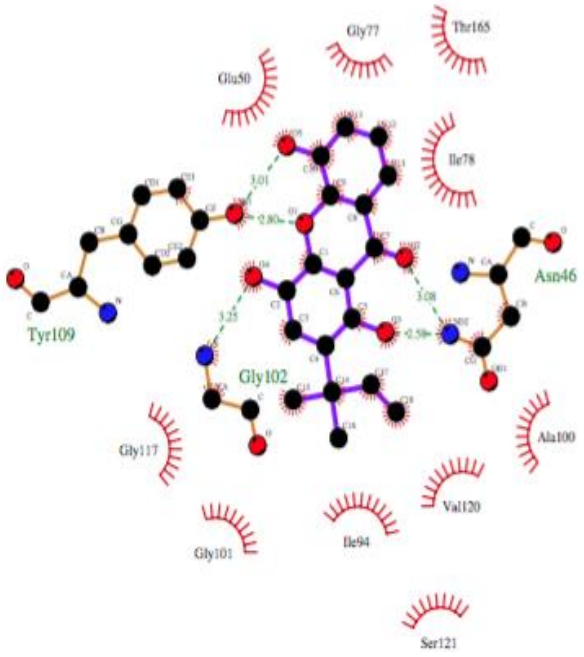




3MMDC(yellow) and ADP(red) docked to Gyrb LigPlot of docked 3MMDC to Gyrb

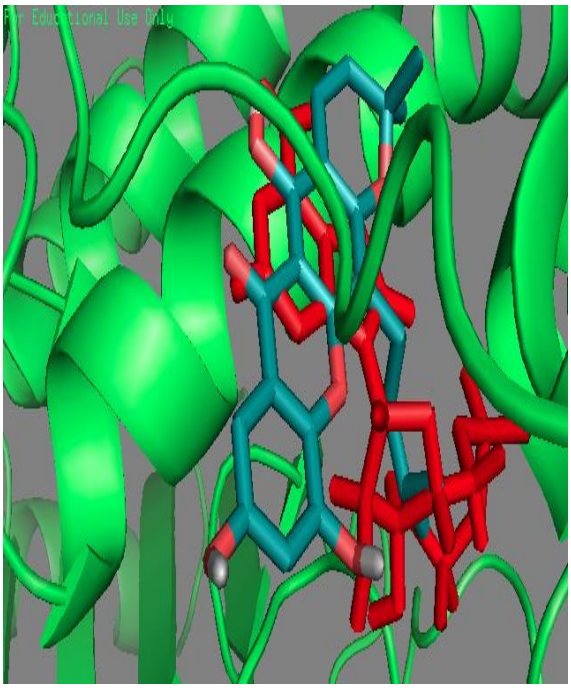
Fig. 6b. 3MMDC docked to Gyrb; specific residues in contact

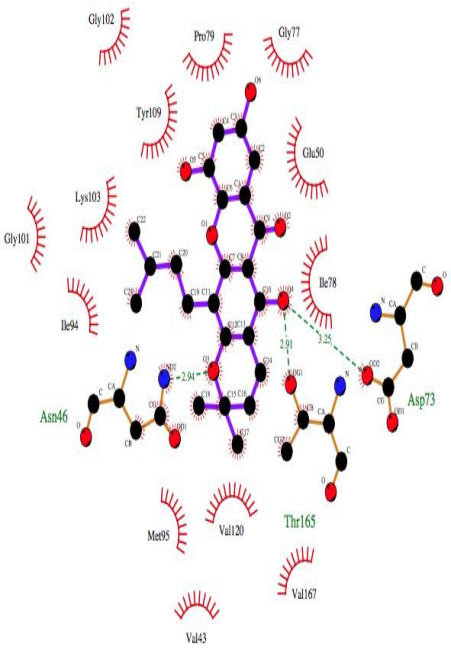




12HDGA(cyan) and ADP(red) docked to Gyrb LigPlot of docked 12HDGA to Gyrb

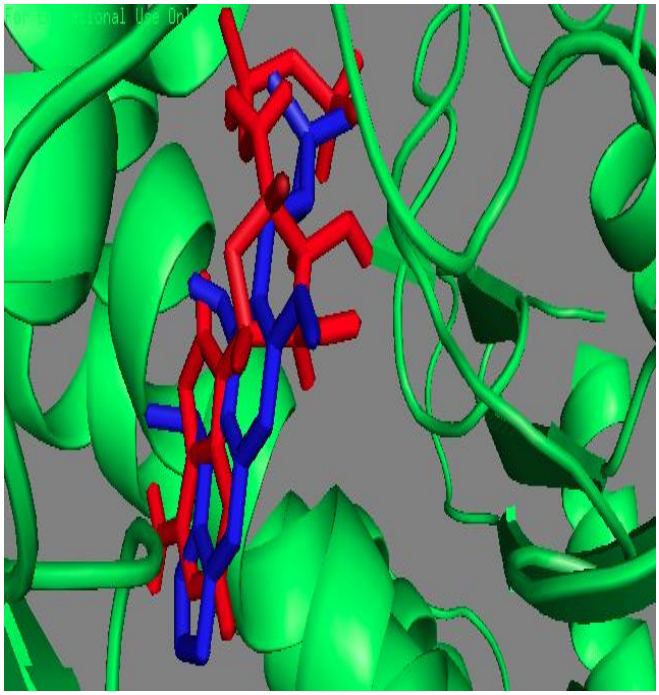
Fig. 6c. 12HDGA docked to Gyrb; specific residues in contact



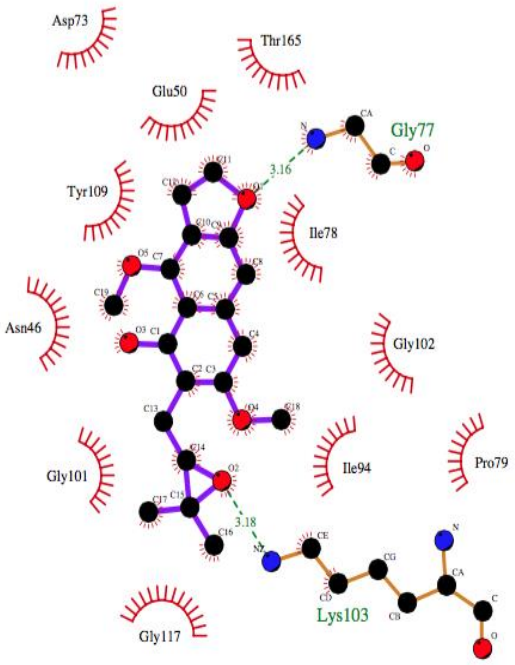


BKXB(green) and ADP(red) docked to Gyrb LigPlot of docked BKXB to Gyrb

Fig. 6d. Baphikixanthones B docked to Gyrb; specific residues in contact

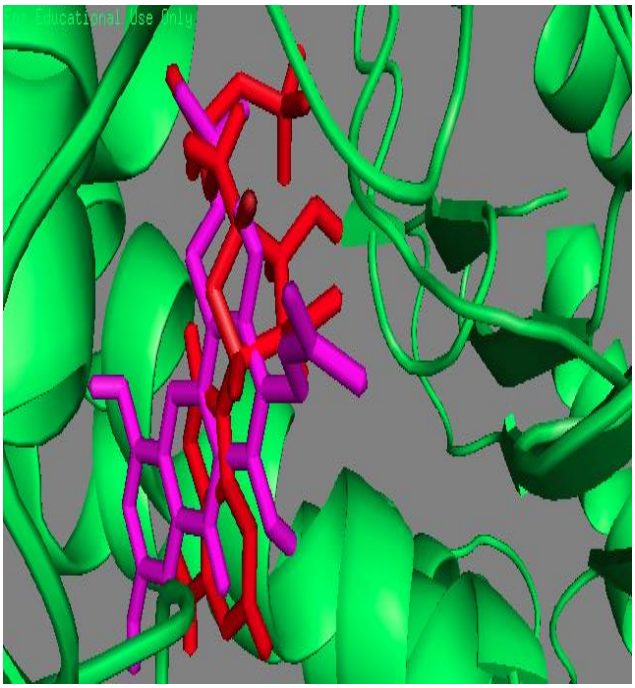


TCMB(blue) and ADP(red) docked to Gyrb

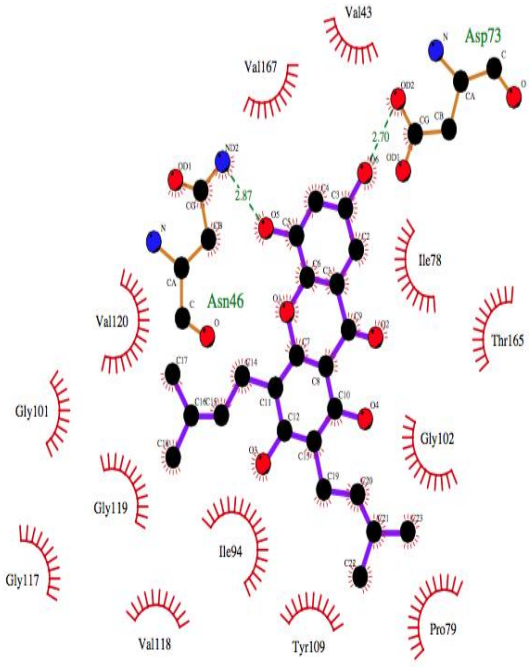


LigPlot of docked TCMB to Gyrb

Fig. 6e. Tecleamaniensine B docked to Gyrb; specific residues in contact



BKXA(magenta) and ADP(red) docked to Gyrb



LigPlot of docked BKXA to Gyrb

Fig. 6f. Baphikixanthones A docked to Gyrb; specific residues in contact

Over all, this work has investigated the mechanism of action and ascertain the possible molecular target for antimicrobial effect of compounds derived from Tanzania medicinal plants. The findings of this study validate the antibacterial and antifungal potential of the medicinal plant source for the compounds.

4. CONCLUSION

Out of twenty pure chemical compounds isolated from Tanzanian medicinal plants, the best six compounds with highest binding affinity to DNA gyrase are reported in this study. Analysis of the ligand-target molecular interactions revealed that the compounds competitively blocked ATP binding on DNA gyrase and formed hydrogen bonds as well as hydrophobic interactions with residues required for ATP binding at the binding pocket, thereby exerting antimicrobial effect on the organisms. Based on the results obtained from this study, the plant-derived chemical compounds isolated from Tanzanian medicinal plants target DNA gyrase for inhibition and are clearly responsible for the antimicrobial effects of the plants. The results therefore verify the reported medicinal potential of the plants and justify the use of these plants in treatment of microbial infection. Meanwhile, the pure chemical compounds selected for this research obviously provide chemical scaffolds that may aid the design and development of not only effective but also novel antimicrobial agents targeting DNA gyrase.

CONFLICT OF INTEREST

The author declares that no competing interests exist.

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