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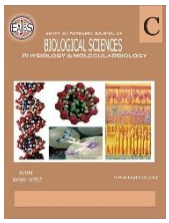

In *Vitro* and in Silico Exploration of D-Mannose: A Natural Solution for Urinary Tract Infections

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ABSTRACT

Urinary tract infections are a significant public health problem, generally caused by uropathogenic Escherichia coli (UPEC). The problem of antibiotic resistance has prompted researchers to seek natural alternatives, such as D-mannose, which inhibits the initial contact between bacteria and epithelial cells.The present work, the in vivo study, shows that D-mannose effectively treats urinary tract infections for around five days, with a dose of 0.01g, six times a day, every two hours. As an adverse effect, diarrhea was noted in R2♂ for 02 days.In silico, we studied the structure-activity relationship between FimH and Mannosidic derivatives. The various tools of Molecular Modeling are used to carry out this work and, more specifically, Molecular Docking. The study of molecular interactions in the solvent revealed that the methylated α-D-mannose derivative exhibits a lower interaction energy, indicating good efficiency. The binding site comprises the following amino acids: Phe1, Asp47, Asn46, Asp54, Asn135, Asp140, and Gln133. These amino acids interact with D-mannose hydroxyl groups, except for O5, via non-covalent hydrogen bonds. On the other hand, a simple modification in FimH significantly reduced binding. We conclude, therefore, that a simple change in the conformation of the protein or, by extension, the ligand can affect the whole system either by increasing its efficiency or decreasing its activity.

INTRODUCTION

Urinary tract infections (UTIs) are among humans' most common bacterial infections, affecting thousands yearly (Foxman, 2002). Uropathogenic Escherichia coli (UPEC) is responsible for most of these infections.

The problem of antibiotic resistance (Flores-Mireles *et al.*, 2015) has prompted researchers to find natural alternatives to treat urinary tract infections caused by uropathogenic *E. coli*; among these alternatives is D-mannose. D-mannose is a natural sugar, an epimer of Glucose, present in various foods. It binds to E. coli, which are then evacuated in the urine (Altarac and Papeš, 2014).

This work aims to find an approach to the natural treatment of urinary tract infections from a Mannopyranoside. Moreover, to study their structural, dynamic, and therapeutic properties to understand their action mechanism. To achieve these objectives, we turned to molecular modeling approaches, molecular modeling, and more specifically, the Molecular Docking method, since it has become an indispensable tool in drug molecule design (Yuriev *et al.*, 2011). In the in-silico section, we studied the interaction of FimH with α-D-mannose, β-D-mannose, and methyl α-D-mannose, comparing their efficacy. We then assessed the impact of a FimH mutation on these interactions. In the experimental part, we analyzed the efficacy of D-mannose and the duration of treatment on rats or induced urinary tract infections.

MATERIALS AND METHODS

In Vivo Study:

This study was carried out at the Mascara experimental station in Algeria on eight rats of the Wistar line (4 males and 4 females, including one control of each sex) weighing 190-210 g. The rats were housed in transparent plastic cages (50x35x20cm) with individual identification, maintained under suitable temperature and lighting conditions, and supervised by a veterinarian. Sawdust was renewed daily to ensure a sterile culture.

1. Confirmation of Rat Urine Sterilization:

To confirm the sterilization of rat urine, a urine sample was taken (after cleaning the urogenital area with Dakin), cultured on Hecktoen medium, and incubated in the oven for 24 hours at 37°C.The absence of bacterial growth confirmed the sterility of the rats.

2. Contamination of Rats:

Six rats were contaminated with uro-pathogenic E. coli strains (identified by the Yessad Khaled Hospital laboratory in Mascara).

- Rats R1♂-2R♂-R3♂ infected by ejection of E. coli solution.
- Rat R2♀ was infected by genital tract staining.
- Rats R1♀-R3♀ administered bacterial solution by gavage.

3. Bacteriological Examination of Urine and Biochemical Identification of *E. coli:*

After urine sampling, we performed a second bacteriological urine examination using the same previous steps. Biochemical tests were then carried out to identify the presence of E. coli bacteria: Mannitol, TSI (Tri Sugar Iron), Simmons Citrate, and urea-Indole.

4. Study of D-mannose Treatment in Rats:

Infected rats were given 0.01 g D-mannose orally six times a day at 2-hour intervals. The dose of D-mannose was determined to be equivalent to the human dose (3g/60kg every two hours). Bacteriological tests were carried out on urine samples from treated rats to control D-mannose action.

In Silico Study:

1. Software Used:

Hyperchem 8.0.7 **(**Macolab, 1985-2017)**,** Molegro Virtuel Docker 6.0 (Docker 2013), Molegro Virtuel Viewer 2.5(MVV) (LIB 2008) Open Babel 2.4.1 (O'Boyle *et al.*, 2011)

2. Protein and Ligand Presentation and Processing:

The *E. coli* FimH protein (representing the mannose-binding domain), the biological target of this study, was obtained from the Protein Data Bank (PDB) under the code 1uwf. Simplification was achieved by eliminating water molecules and co-crystallization inhibitors (Fig. 1).

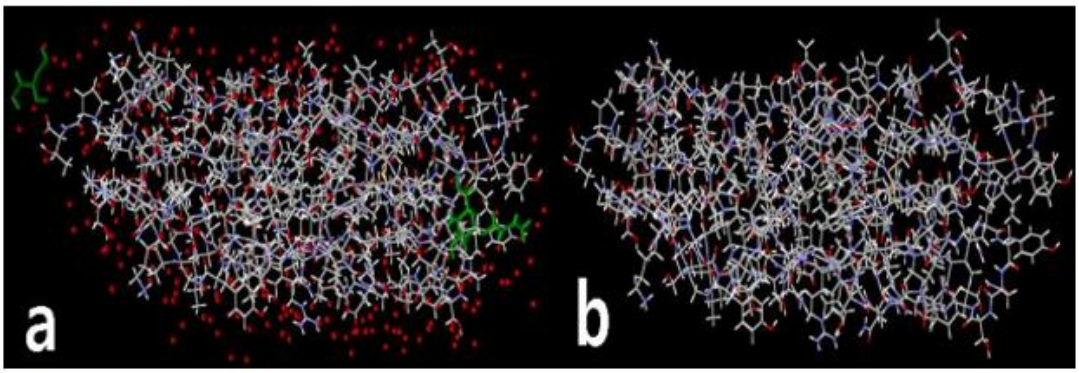

Fig. 1 (a) Unsimplified FimH. **(b)** Simplified FimH.

α-D-Mannose, β-D-Mannose and Methyl α-D-mannose were modeled using Hyperchem8.0.7 and Open Babel software (Fig. 2; Fig. 3; Fig. 4).

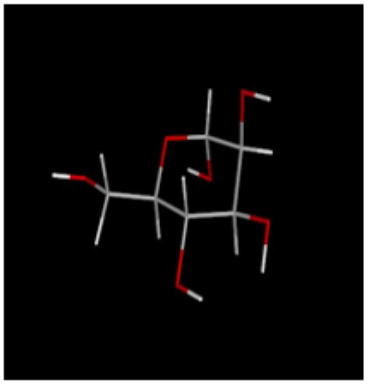

Fig. 2: α-D-Mannose.

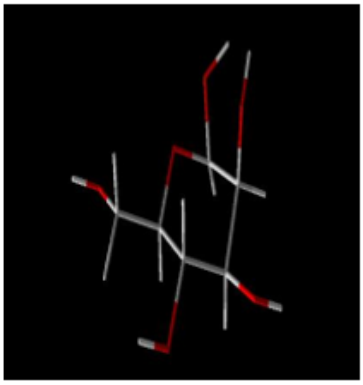

Fig. 3: β-D-Mannose.

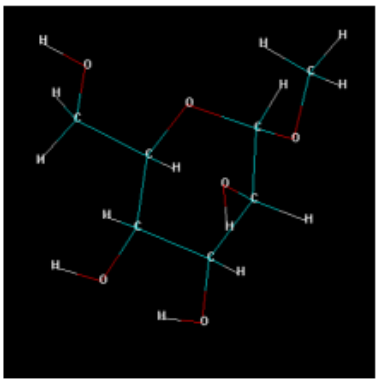

Fig. 4: Methyl α-D-Mannose.

Molecular Mechanics and Dynamics calculations were performed with Hyperchem using the ``Amber99`` Force Field and ``steepest descent`` algorithm.

3 Molecular Docking:

Molecular docking was done using the Molegro Virtual Docker software. The MolDock Optimizer search algorithm and the MolDock Score function [Grid] **(**Fig. 5**)** were used to determine the interaction sites. Three-dimensional visualization of interactions using MVV software **(**Fig. 6**).**

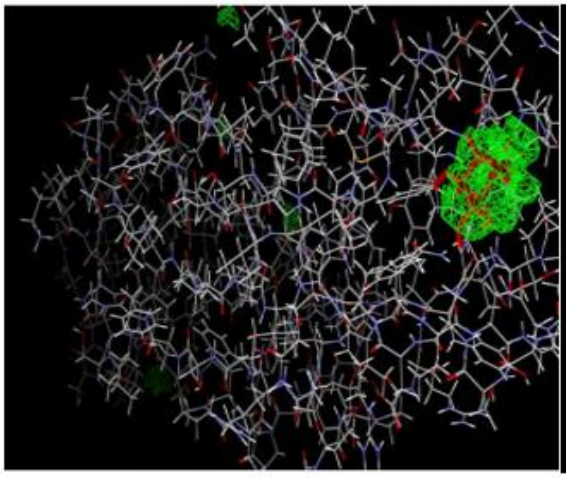

Fig. 5: Mannose ligand in the active site.

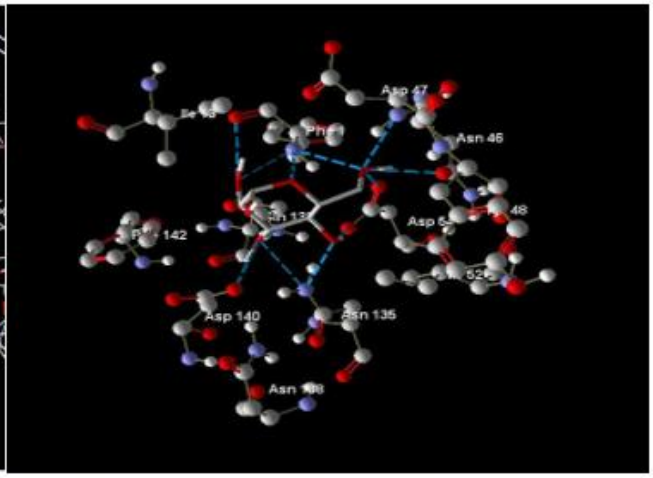

Fig. 6: Visualization of the interaction between FimH and Mannose.

Validation of the protocol is based on top-scoring obtained by "Re-Docking" (Yuriev *et al.*, 2011). This method looks at the root-mean-square deviation "RMSD" **(**Fig. 7**).**

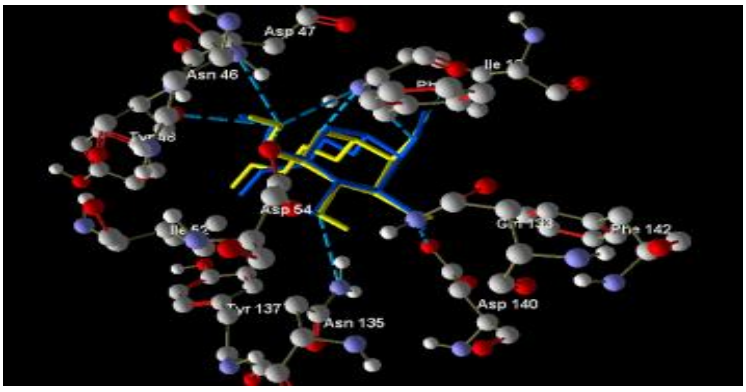

Fig. 7: The superposition of the ligand's crystallographic structure (in blue) and the pose obtained after Re-Docking (in yellow).

Solvation of molecules (Marcus, 1985) was studied explicitly in a 216-molecule water cage using Hyperchem software (Fig. 8**).**

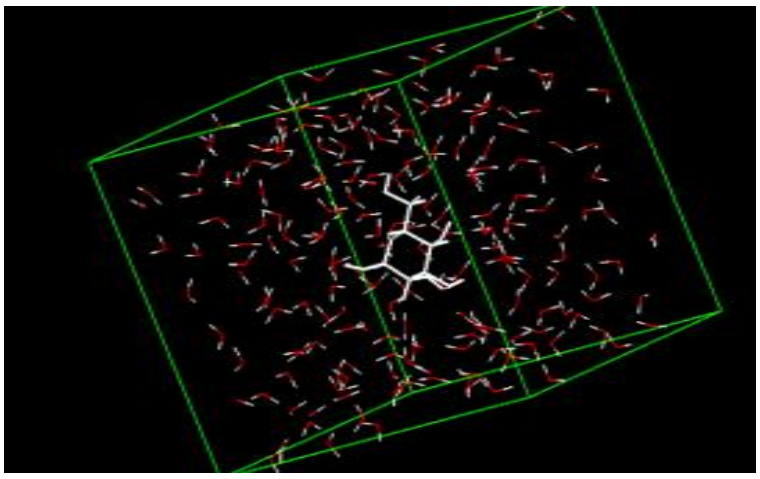

Fig. 8: Molecule solvation in a water cage.

RESULTS AND DISCUSSION

In Vivo Study:

1. Sterilization of Rat Urine:

The total sterility of the eight-culture media was noted, given the absence of bacteria on all three streaks (0 CFU/ml), thus proving that the eight rats were not affected by any pathogenic bacteria (Fig. 9; Fig. 10).

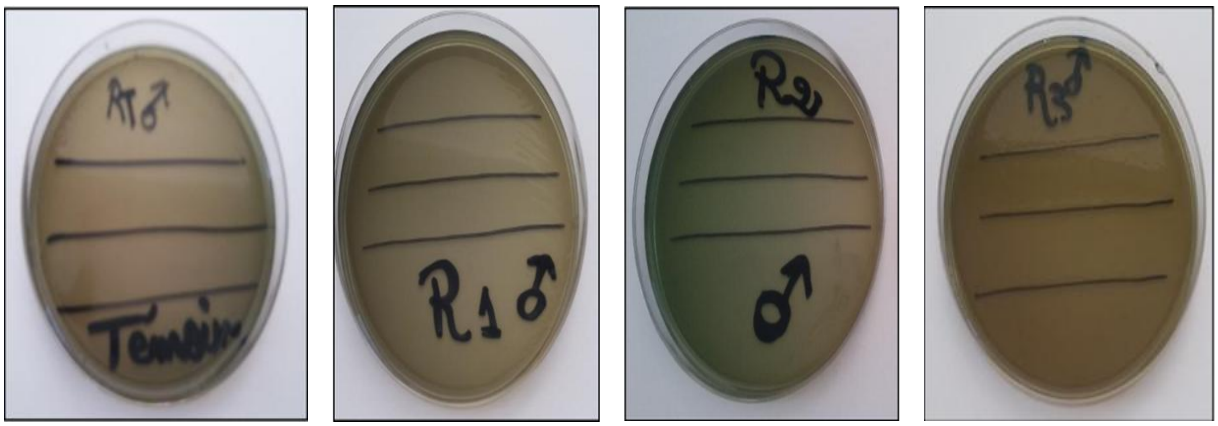

Fig. 9: RT♂ culture, R1♂, R2♂, R3♂ before contamination.

Fig. 10: RT♀, R1♀, R2♀, R3♀ culture before contamination.

2. *E-coli* Infection of Rats:

We noticed that rats R1♂-R2♂-R3♂-R2♀ were infected with *E. coli*,given the presence of bacteria on all three streaks. E. coli is detected with the naked eye by the specificity of these rounded salmon-colored colonies on the Hektoen medium (King and Metzger, 1968) **(**Fig. 11).

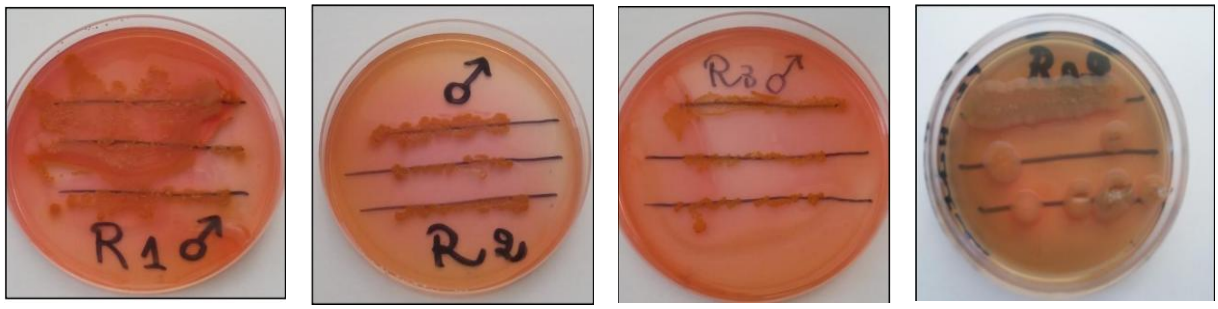

Fig. 11: R1♂, R2♂, R3♂, R2♀ culture after contamination.

Rats R1♀-R3♀ were not orally contaminated (Fig. 12)**,** perhaps for the following reasons: the unreliability of the contamination route (oral), the bacteria being neutralized by the immune system, or the gastric acidity.

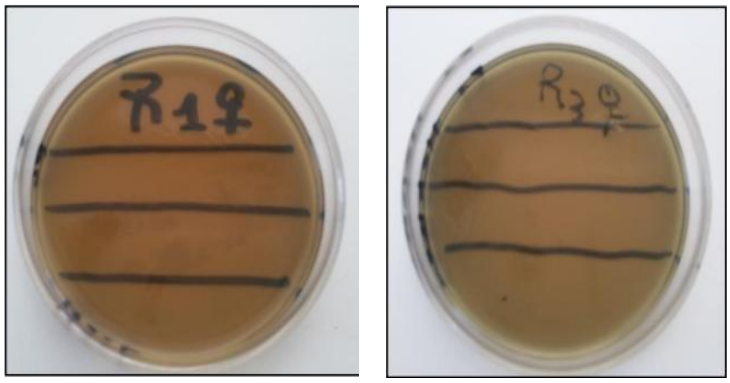

Fig. 12: R1♀, R3♀ culture after contamination.

3 Characterization of *E-coli* by Biochemical Testing:

Biochemical Test Results Confirmed the Presence of *E. coli*:

- The color change of the medium to yellow confirms mannitol fermentation **(**Nkang *et al.*, 2009), (Fig. 14).
- The color shift of the TSI medium, with yellow pellet and slope and presence of gas, shows fermentation of glucose, lactose, and sucrose, without production of H_2S (Hajna, 1945). (Fig. 15)
- The absence of a color shift in the Simmons citrate medium (Oulymata, 2007) indicates that *E.coli* cannot use Simmons citrate as a carbon source (Fig. 16).
- No color shift in the Urea-Indole (urease -) medium **(**Fig. 17**),** This confirms the absence of the urease enzyme in this bacterium (Bachmeier *et al.*, 2002).

Fig. 14: Mannitol after incubation.

Fig. 15: TSI after incubation.

Fig. 16: Simmons citrate after incubation.

Fig. 17: Urea-indole after incubation.

4. The Efficacy of D-mannose in the Treatment of Urinary Tract Infection:

A- Clinical Observation:

- Rats R3♂ and R2♀ died due to urinary retention, possibly caused by urinary tract infection (Drake *et al.*, 1998).
- The only adverse effect noted was diarrhea in R2♂ for 02 days, which D-Mannose can explain (Kranjčec *et al.*, 2014).

B- Bacteriological Analysis:

The following cultures represent the results of bacteriological examination of urine from D-mannose-treated rats (Fig. 18 for R1♂ - Fig. 19 for R2♂).

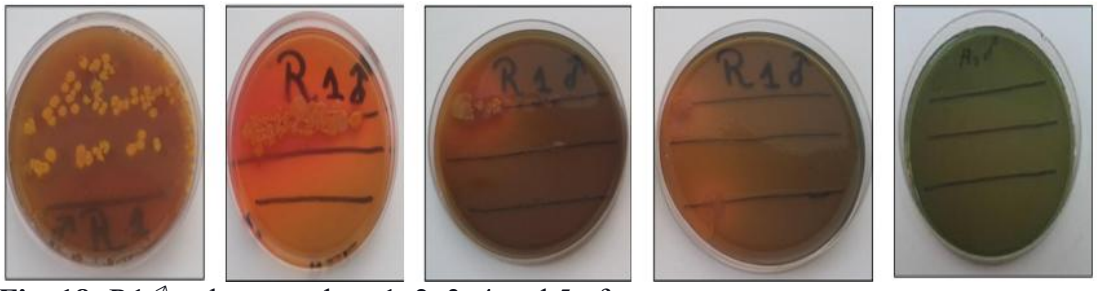

Fig. 18: R1♂ culture on days 1, 2, 3, 4 and 5 of treatment.

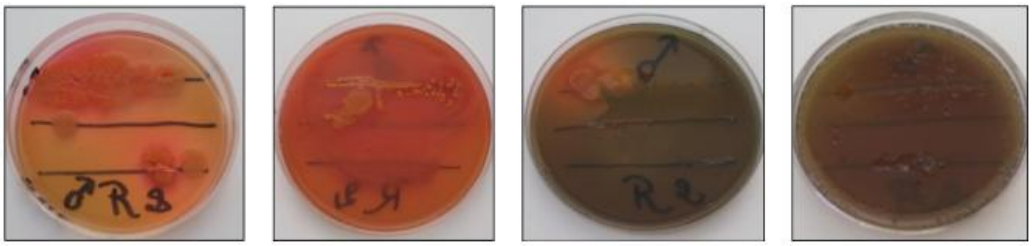

Fig. 19: Culture R2♂ on1st, 2nd, 3rd, 4th day of treatment.

The results of the bacteriological examination, used to assess the bacterial count per UFC/ml unit, are shown in the following table (Table 1)**.**

Table 1: Results of bacteriological examination

Days	R1♂	R2♂
1st Day	10^3 < Bacterial count < 10^5	Bacterial count > 10^5
2nd day	Bacterial count < 10^3	10^3 < Bacterial count < 10^5
3rd day	Bacterial count < 10^3	Bacterial count < 10^3
4th day	Bacterial count < 10^3	0
5th day	0	

A complete cure of R2♂ was observed from day 4, while for R1♂, a complete cure was only observed from day 5 of treatment.

These results explain the effect of D-mannose on the neutralization of E. coli bacteria and demonstrate that the average treatment duration is 5 days.

In Silico Study:

1. Study of Ligands Before and After Solvation:

The optimization results (Tables 2 & 3) show a decrease in energies, hence the importance of Molecular Mechanics in stabilizing the structures (α: 30,199 to 26,031 kcal/mol) (β: 705,163 to 28,211 kcal/mol). It can also be seen that the α-D-mannose structure is more stable than the β-D-mannose structure.

Table 2: Pre-optimization energy results for isolated structures (Kcal/mol).

Molecules	α-D-Mannose	β-D-Mannose
Energy	705.163	30.199
Bond	2.386	1.135
Angle	63.790	1.663
Dihedral	14.787	13.775
Van der Waals	610.842	3.860
Hydrogen	0	0
Electrostatic	13.357	9.764

Table 3: Energy values for isolated structures after optimization (Kcal/mol).

Molecules	α-D-Mannose	β-D-Mannose
Energy	28.211	26.031
Bond	0.588	0.531
Angle	0.956	1.509
Dihedral	11.484	13.376
Van der Waals	2.486	2.567
Hydrogen	0	0
Electrostatic	12.695	8.045

After the solvation of the molecules, we note a decrease in energies (Table 4) and even in molecular dynamics (α: 50.216 to -984.714kcal/mol) (β: 48.558 to-945.917kcal/mol). Hence, solvation is essential for molecular stability.

Table 4: Energies of the two anomers in solvent after optimization (Kcal/mol).

Molecules	α-D-Mannose	β-D-Mannose
Energy	-1534.009	-1580.219

2. Docking of Fimh with The Two Anomers:

After docking, the ligands interacted strongly with FimH, demonstrating good affinity (Fig. 20**)**. The energy of the FimH-α-D-Mannose complex (2590.92kcal/mol) is lower than that of the FimH-β-D-Mannose complex (2595.07kcal/mol).

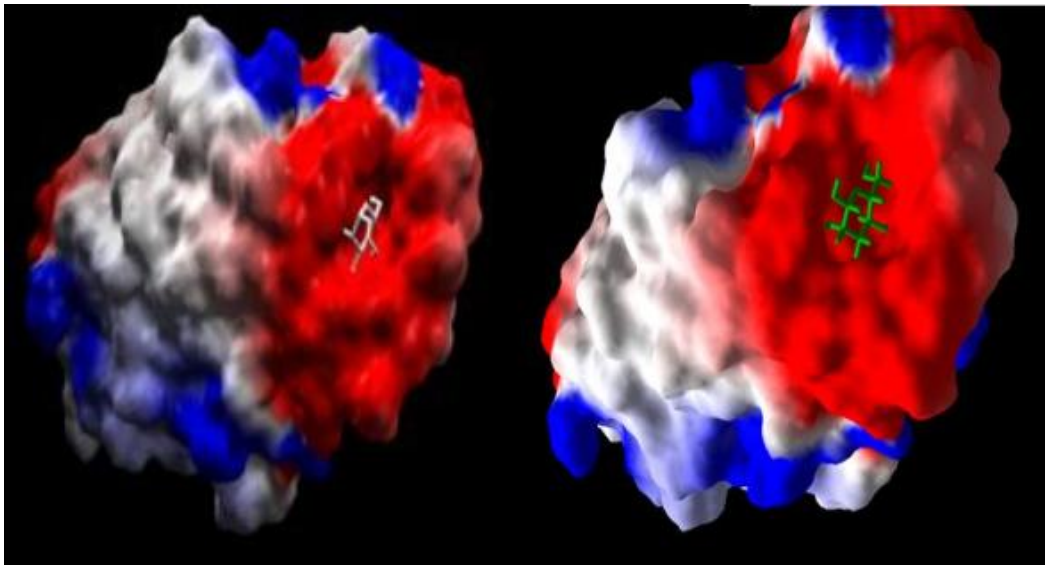

Fig. 20: Positioning of α-D-Mannose (white) and β-D-Mannose (green) in the FimH active site.

a- **Analysis of Interactions Between the Two Ligands and Fimh:**

The measured hydrogen bond distances between the two ligands and the FimH active site (Table 5) range from 2.59A° to 3.18A°. The FimH-α-D-Mannose complex shows stronger interactions **(Scott and Vanderkooi, 2010)** than the FimH-β-D-Mannose complex with the following residues: Gln133 -Asp140-Phe1-Asp47-Asp54 (Figs. 21 &22).

Table 5 : Les distances entre les deux anomers et les acids amines du site active

Distance (Â)	Phe 1	Phe 1	Phe 1	Phe 1	Asn 46	Asp 47	Asp 54	Asp 54	Asn 135	Asp 140	Gln 133
α–D-Mannose	3.04	2.75	2.67	2.77	3.00	2.76	2.70	2.61	2.94	3.05	2.59
β–D-Mannose	3.18	2.59	3.03	2.90	2.99	2.92	2.59	2.66	2.77	3.09	2.61

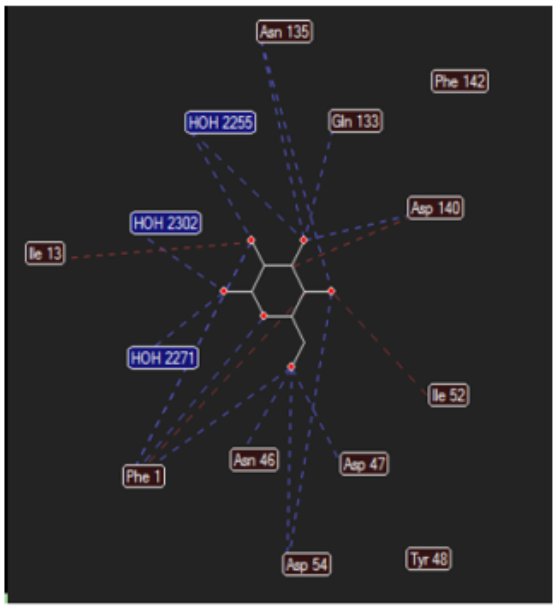

Fig. 21: α-D-Mannose binding to FimH in the presence of 2D solvent.

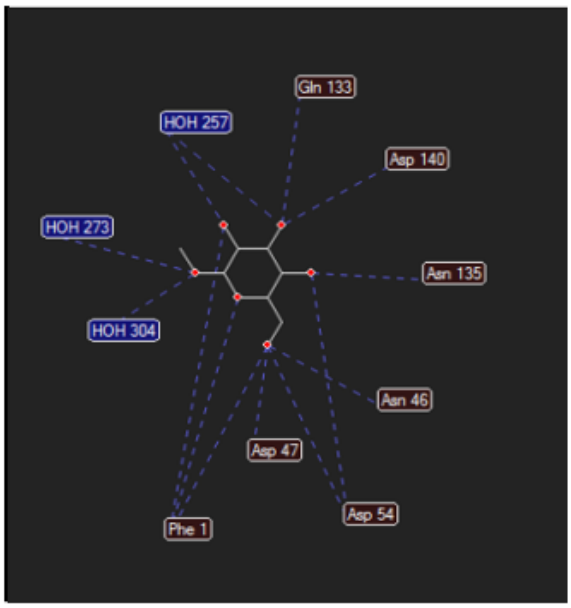

Fig. 22*:* Methyl-α-D-Mannose binding to FimH in the presence of 2D solvent.

B- Interaction Energies of the Two Complexes:

These energies are evaluated using the following relationship:

- E interactions (E total potential FimH-ligand complex) - (E total potential FimH alone +E total potential ligand).

- The FimH-α-D-Mannose complex has a lower energy interaction (1846.466Kcal/mol) than the FimH-α-D-Mannose complex than the FimH-β-D-Mannose complex (1848.958Kcal/mol) (Table 6).

Table 6: Energy balances of the two complexes in (Kcal/mol).

	Complex Energy	**Substrate Energy**	**Interaction Energy**
FimH-α-D-Mannose	2590.92	744.454	1846.466
FimH-β-D-Mannose	2595.07	746.112	1848.958

3. The Interaction of α-D-Mannose and its Methyl Derivative with Fimh:

The energies of the FimH-α-D-Mannose complex (1846.466 kcal/mol) are lower than those of the FimH-Methyl-α-D-Mannose complex (1977.627 kcal/mol)

without solvent. on the other hand, the energies of FimH- Methyl-α-D-Mannose (1607.122 kcal/mol) will become lower than those of FimH-α-D-Mannose (1812.805 kcal/mol) after Docking of the two molecules in a solvent.

Since most chemical and biological reactions occur in solutions, and solvent effects are significant in the stability of complexes, the FimH-Methyl-α-D- Mannose complex is more stable than the FimH-α-D-Mannose complex.

Measured bond distances vary between 2.59A° and 3.05A° for α-D-Mannose and between 2.5A° and 3.09A° for Methyl-α-D-Mannose. It can be seen that the FimH-Methyl-α-D-Mannose complex shows stronger interactions than the FimH-α-D-Mannose complex with the following residues: Asp140- Asn135-Phe1-Asp47-Asn46 (Fig. 23).

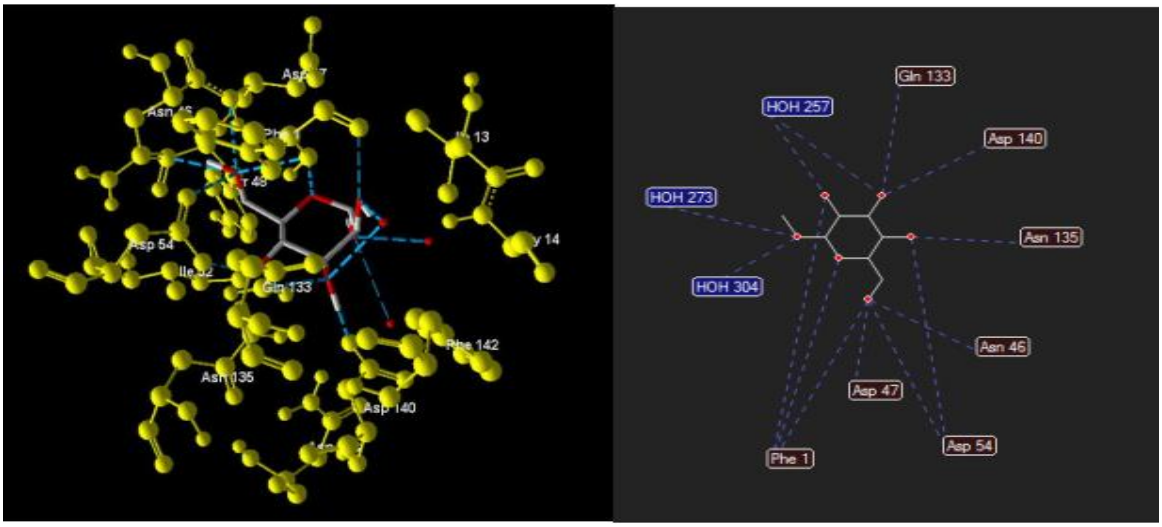

Fig. 23: The binding mode of Methyl-α-D-Mannose in the FimH active site in the presence of solvent in 3D and 2D.

4. the interaction between Methyl α-D-Mannose and mutated FimH:

The energies of the unmutated FimH-Methyl-α-D-Mannose complex (737.597 kcal/mol) are lower than those of the mutated FimH-Methyl-α-D-Mannose complex (1607.22 kcal/mol).

After a visual analysis of the interactions of the mutated FimH-Methyl-α-D- Mannose complex we can see that the mannose has lost all interaction with Asn133 and Asn135 and has retained its bond with Asp140 (Fig. 24).

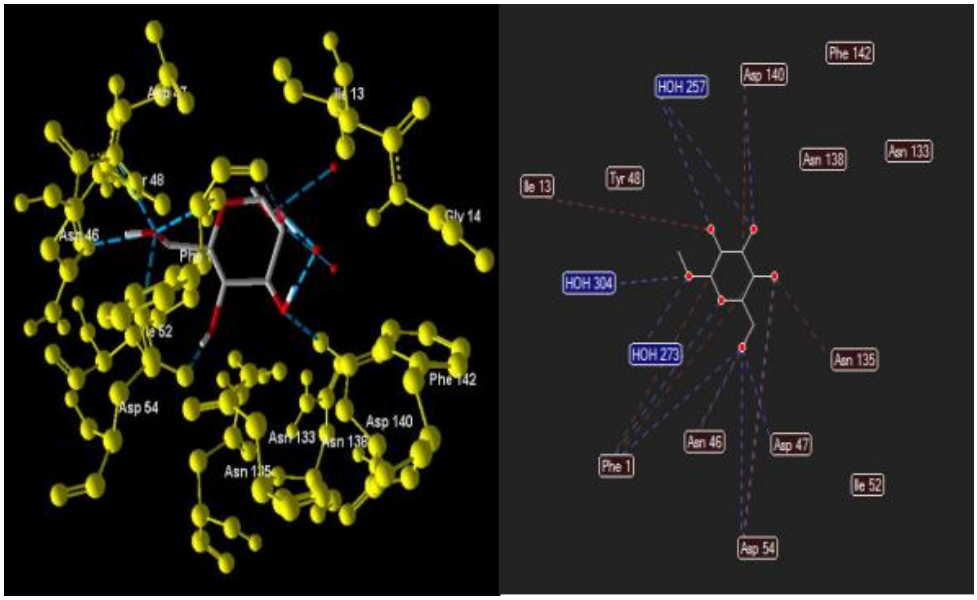

Fig. 24: Methyl-α-D-Mannose binding in the active site of 3D and 2D.

This mutation, therefore, considerably lowers biological activity and shows the extent to which a slight change in the conformation of the protein or, by extension, the ligand can affect the whole system(Hung *et al.*, 2002).

Conclusion

This work was undertaken to assess the clinical potential of D-mannose and its derivatives as a nonantibiotic alternative for the treatment of E. coli associated urinary tract infections in experimental and computational methods.

Based on this confidence shown in the stability of α-D-Mannose configuration from our in-silico analysis, we have extended it extensively across our research. And this stability is bettered through methylation of the stable α-form (see methyl α-D-Mannose in solvent medium).

The paper offers combined experimental and computational insights into the solvation effects on the molecules and highlights the important role of solvation in stabilizing molecular structures, shedding light on the complexity part.In order to provide more detail about the binding pocket and its affinity structure-function relationship, The work involved Methyl α-D-Mannose and a mutated variant of FimH, and indicated that Event hepatic alterations even within the same binding pocket can make a big difference in efficient binding.These residues in the pocket (Phe1, Asn46, Asp47, Asp54; Gln133, Asn135; and Asp140) were common to our entire test set of molecules.

From an experimental vantage point, using guinea pigs as our experimental animals same experimenter ad-libbed and in the course of several assessments, However, we ended up with another sequence that differed much from this experiment. A natural therapeutic approach thus shows a moderate degree of efficacy after being used five days, 6 times every day, say in doses of 0.01g at intervals of two hours. Condition R2♂ produced only one adverse effect case of diarrhea as a result of receiving D-mannose treatment for 2 days.

The compiled data substantiate that D-mannose and its methylated derivative hold considerable promise as alternative treatments to antibiotics for urinary tract infections, thus serving as a foundation for future therapeutic drug development.

Declarations:

Ethical Approval and Consent to Participate: The animal study protocol was approved by the Institutional Animal Ethics Committee of the Mascara experimental station. All procedures were performed in accordance with the institutional guidelines for animal care and use, and efforts were

made to minimize animal suffering and reduce the number of animals used.

Competing interests: The authors have no competing interests to declare that are relevant to the content of this article.

Availability of Data and Materials: All data generated or analyzed during this study are included in this published article.

Authors' Contributions: Authors equally shared in the data collection, wrote, revised the manuscript, and approved its publication.

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