

Dinoxin B Withanolide from *Datura innoxia* Mill as an Effective Phytochemical Against Urinary Tract Infection causing *Staphylococcus aureus*

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Dinoxin B Withanolide was isolated from *Datura innoxia* and identified with its cytotoxic activity. But its antibacterial properties are not yet evaluated. We have previously reported the broad-spectrum antibacterial property of Dinoxin B Withanolide extracted from *D.innoxia* on standard strains. Objective: This research has focused to evaluate the efficacy of Dinoxin B Withanolide against infectious *Staphylococcus aureus*, including resistant strains. Methods: Electrospray Ionization-Mass Spectrometry is used to depict the presence of Dinoxin B withanolide from the chromatographic ethanolic leaf fraction. Antibacterial activity of different concentrations of Dinoxin B(12500-100000 µg/ml) was assessed using the agar diffusion, macro broth dilution, and time-kill assay methods. Docking studies and Drug likeness properties were analyzed. Result: Electrospray Ionization-Mass Spectrometry depicted the presence of Dinoxin B. All the isolates were susceptible to Dinoxin B within the range of 15±0.5mm to 24±0.5mm, and the bacteria were susceptible at a concentration rate of =12.5mg/ml. Time-kill assay showed that 25mg/ml of Dinoxin B displayed the highest inhibitory activity after four hours. The MBC values were compatible with the cidal concentration as seen in the time-kill study's growth curve. Computer-aided techniques resulted in a good Docking score towards Quorum-signaling Sar A protein (-7.82)and Penicillin Binding Protein(-6.9). Conclusion: Dinoxin B with its bactericidal properties and significant affinity towards Quorum-signaling Sar A protein and Penicillin Binding Protein can be considered as an effective bioactive compound against Methicillin Resistance *Staphylococcus aureus*.

Keywords: Antibacterial activity; *Datura innoxia*; Dinoxin B Withanolide; Molecular docking; Methicillin-Resistant; *Staphylococcus aureus*.

Naturally occurring withanolides are steroids built on an ergostane skeleton with an oxidized form of lactone rings¹. They are mainly found in the genera of *Withania*, *Physalis*, and *Datura* of the Solanaceae family. Withanolides are often found as aglycones, but a few of them are reported as glycosides. Withanolides have

a wide range of biological activity due to their complex and special structural skeletons, including anticancer, antimicrobial, anti-inflammatory, and immunoregulatory properties.

So far studies on withanolides are mainly focused on genera of *Withania* and very few cases reported from *Physalis* and *Datura*. Many species

of *Datura* reported the presence of withanolides including *D.stramonium*², *D.ferox*³, *D. inoxia*⁴, *D. metel*⁵, and *D. fastuosa*⁶.

D.inoxia Mill(Fig.1) is a perennial herb, grow to a height of 1 to 3 meters with serrated margins on hairy leaves, funnel-shaped white flowers, and pendulous spiny fruit with brown to orange seeds and funnel-shaped white flowers⁷. Philip Miller, an English botanist, was the first to classify the species in 1768^{8,9}. In different parts of the world, the plant is cultivated commercially due to its therapeutic properties¹⁰⁻¹². As per the literature review, Dinoxin B¹³, Withametelinol A, Withametelinol B¹⁴, Withametelin¹¹, Daturalacin, and Witharifeen⁴ are the withanolides isolated from *D.inoxia*.

Urinary tract infection (UTI) caused by *Staphylococcus aureus* are a globally common infection affecting more than hundred million people per year¹⁵. They are one of the most common bacterial infection in all ages and groups with high risk in young women which results in significant morbidity and health care costs due to their Multi-Drug Resistance(MDR)^{16,17}.

Many virulence factors regulates the actions of *S.aureus* including staphylococcal accessory regulator (SarA) and the accessory global regulator (AgrA)¹⁸. Expression of Penicillin-binding proteins(PBPs) promoted with methicillin and other Beta-lactam antibiotics resistant strains^{19,20}. Methicillin-resistant *S. aureus*(MRSA) and Multiple antibiotic resistance (MAR) shows varying mechanisms such as the development of biofilm, transformation into small colony variant,

the evolvement of resistant genes, resistance to broad-spectrum efflux pumps; which limits treatment options and prompting to search for new compounds that can combat these strains^{19,21,22}.

For the first time, Vermillion *et al.*; extracted Dinoxin B(Fig.2) and identified it with cytotoxic activities¹³. But its antibacterial properties are not yet evaluated. We observed the broad-spectrum antibacterial property of Dinoxin B Withanolide extracted from *D.inoxia* on standard strains²³. Promising results of our experiment has encouraged us for studying the effect of Dinoxin B as an antibacterial, highlighting its inhibitory potentiality on Urinary tract infection causing *S.aureus*.

METHODS

Collection of Sample and Preparation of extract

Leaves of *Datura inoxia* were obtained from Amity University Campus, Lucknow. The collected leaves were cleaned with distilled water and dried in shade. Ethanolic leaf extract was made using the fine leaf powder into a final concentration of 1mg/ml.

Fractionation of Extract through Column Chromatography

For fractionation of plant extract, a single solvent system was used through column chromatography²⁴. To fill up the column, Silica gel (60-120 mesh) was used and added with the sample and the collection of the fraction was done by pouring solvent at a flow rate of 1ml/minute until silica gel became visible as colorless. The



Fig.1. *Datura inoxia* Mill

final concentration of the collected fractions was retained as 1000 μ g/ml by using 10% DMSO^[25]. Each isolated fraction was assessed for its antibacterial activity.

Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS)

Compound identification of fraction 4, which was the most active fraction in Zone of Inhibition analysis, LC-ESI-MS was performed from Central Drug Research Institute of India, Lucknow.

Test Organism used in the Study

To have a comprehensive understanding and learning, standard, as well as pathogenic

strains of *S. aureus* were used; and denoted as (ATCC 25923) and isolates from urine samples of patients (U-6151, U-6081, U-6090, and U-6089) in which U-6090 and U-6089 are MDR and MRSA strains. All the isolates were obtained from Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow.

Agar Diffusion assay

Following the Kirby-Bauer diffusion technique²⁶, conducted an agar well plate method to assess the antibacterial property against the standard as well as clinical strains of *S. aureus*. A spectrophotometer is used to check MacFarland standard turbidity. Bacterial inoculum was spread

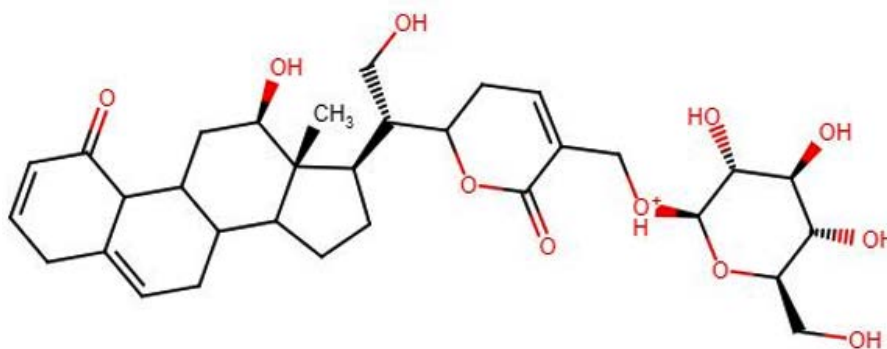


Fig. 2. Structure of Dinoin B Withanolide retrieved from PubChem(CID: 51041991)

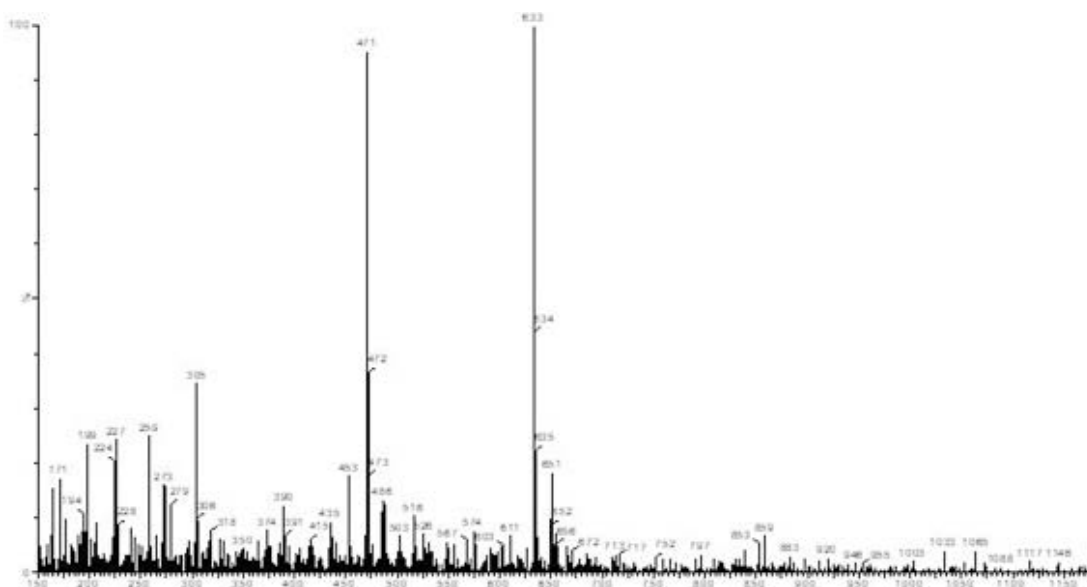


Fig. 3. LC-ESI-MS Spectrum of Most Active Fraction 4. Shows the presence of Dinoin B Withanolide and its aglycone: The phytoconstituents eluted as M-glucose-water+H⁺ (m/z471) and Dinoin B Withanolide (m/z 633)

on Muller Hinton Agar plates and inoculated with 100 μ l of fraction four of ethanolic leaf extract at different concentrations in 6mm sized wells and incubated at 37°C for 24 hrs. The inhibition zones were measured (ZOI). Gentamicin (85mg) and DMSO (10%) were used as positive as well as negative controls. All the assays were done in triplicate, and the results were expressed as mean standard deviation.

Macrobroth Dilution for Determining MICs and MBCs

Macrobroth dilution approach is used to assess Minimum Inhibitory Concentration (MIC) and Minimum bactericidal concentration (MBC), of fraction four as per the protocol followed by Chandni *et al.*²³. Different concentrations of fraction four are obtained through the two-fold serial dilution method and mixed with 100 μ l of the test organism (*Staphylococcus aureus*) to a final inoculum concentration of 5×10^5 . The maximum dilution which inhibited bacterial growth was regarded as the MIC value. Bacterial inoculum without the tested fraction was used as the growth control, whereas bacterial inoculum itself was taken as the sterility control.

Subculturing from each tube of MIC without visible growth was used to measure MBC. Plates were incubated for 24 hours at 37°C. The lowest concentrations of the extract that did not generate any colony formation on the solid medium were considered as MBC.

Antibiosis Assessment

The MBC/MIC ratio was calculated to assess the antibiosis mechanism. Ratio d^2 shows bactericidal effects and MBC/MIC ratio e^4 is usually considered to be bacteriostatic²⁷.

Time-kill Assay

The most active fraction was subjected to a time-kill assay against the MRSA clinical strain^[28]. An inoculum of approx 5×10^5 cfu/ml, used in this study. The tested fraction was then added to the inoculum suspensions with final concentrations conforming to $\frac{1}{2} \times$ MIC, MIC, and $2 \times$ MIC. Bacterial culture without a tested sample was used as a growth control in each trial. Gentamicin was used as an antibiotic control. These cultures were then incubated at 37°C. Bacterial colony-forming unit (CFU) was determined at intervals of 0, 2, 4, 6, 18, and 24 hours by taking 1.0 ml of aliquotes. The procedure was repeated three times, and the

log CFU/mL was plotted against time in a graph.

Susceptibility test of Antibiotic

The selected antibiotics for the antibiogram test comprised of three groups, namely aminoglycosides (Gentamycin); penicillins (Ampicillin), and quinolones (Ofloxacin)²⁹. ZOI of each standard antibiotic against selected strains was evaluated after 24hrs of incubation at 37 °C.

Analytical statistics

Mean \pm standard deviation is used to interpret all data. GraphPad software version 10 was used to examine statistical differences using One-way Anova. The mean is found statistically significant if the p-value is less than 0.05. Standard errors of the mean values were symbolized (\pm) and results were tabulated with standard error of the mean.

Docking studies

To analyze the antibacterial mechanism of Dinoxin B Withanolide, docking studies were conducted using the Glide docking program of Maestro 12.4 Schrodinger software³⁰. Proteins of *S.aureus* which promotes resistance to cause Urinary tract infection such as PBP^{20,31,32}, SarA Protein^{16,33-35}, multidrug efflux pump protein^{36,37}, AgrA Protein^{35,38}, Topoisomerase and DNA gyrase³⁹ were selected based on a Literature review. Protein structures were downloaded from the Protein Data Bank with PDB ID; 3HUM (Penicillin-binding protein), 2FNP (SarA Protein), 4LLL (Multidrug efflux pump protein), 4G4K (Accessory gene regulator Protein A), (4PLB) Topoisomerase and (2XCT) as DNA gyrase. Protein preparation of all selected proteins was done by using the Protein Preparation Wizard module of Glide.

Structure of ligand, Dinoxin B Withanolide (PubChem ID: 51041991) as well as antibiotics used as comparative ligands such as Ampicillin, Ofloxacin, and Gentamycin were retrieved from PubChem with PubChem ID(s) 6249, 6604200, 3467 respectively. Ligand preparation for all these molecules was done by using Lig Prep Wizard and after receptor grid generation docking was carried out.

Drug likeness properties

PreAdmet server was used to evaluate the Drug likeness of Dinoxin B based on Lipinski Rules of five^{43,44}.

ADMET property analysis

ADMET properties such as absorption,

distribution, metabolism, excretion, and toxicity of Dinoxin B withanolide were determined using the PreAdmet server to ensure its effectiveness as an oral drug compound⁴⁰.

RESULTS AND DISCUSSION

Compound Identification

As we reported in the previous study²³, ethanolic leaf fraction four of *Datura innoxia* obtained through column chromatography is analyzed through LC-ESI-MS. This mass spectrum (Fig.3) also depicts the presence of Dinoxin B Withanolide and its aglycone. Phytoconstituents eluted in the spectrum of fraction four detect the cleavage of a glycosidic bond (Fig.4).

Agar Diffusion Assay

Inhibitory potential of Dinoxin B was observed using an agar well diffusion assay using different concentrations of fraction four in $\frac{1}{4}$ g/ml (100000, 50000, 25000 and 12500) and compared to control (DMSO) and Gentamicin as reference antibiotic (Fig.5). As per the Kirby-Bauer test⁴¹, *S.aureus* susceptibility based on Zone of Inhibition was evaluated (< 12mm (resistant); <13-14mm (intermediate), and >15mm (susceptible). As shown in Fig.6 clinical strains (

U-6151, U-6081) isolated from urine samples, including MRSA (U-6089), MDR (U-6089), as well as Standard strain (ATCC 25923) of *S. aureus*, were showed significant activity ($p < 0.05$), which was comparable to the reference antibiotic, at higher concentration of Dinoxin B (100000 $\frac{1}{4}$ g/ml, 50000 $\frac{1}{4}$ g/ml). Whereas MDR strain at 25000 $\frac{1}{4}$ g/ml, 12500 $\frac{1}{4}$ g/ml, and MRSA at 12500 $\frac{1}{4}$ g/ml showed low levels of susceptibility. Susceptibility decreased with a decrease in concentration, which shows the impact of Dinoxin B in its higher concentration. Zone of inhibition varied (Table.1) in range of (mm) 0-15 (1250 $\frac{1}{4}$ g/ml), 0-18 (25000 $\frac{1}{4}$ g/ml), 9.1-20.3 (50000 $\frac{1}{4}$ g/ml), 14.6-23.3 (100000 $\frac{1}{4}$ g/ml), in which MDR (U-6090) showed higher resistance. Dinoxin B showed higher susceptibility to Methicillin-resistant strain (U-6089) than that of Gentamicin with a 22.5 mm zone of inhibition.

MIC, MBC, and MIC/MBC Ratio

Antibacterial effectiveness was evaluated through MIC assay, in which maximum dilution of Dinoxin B that slows down staphylococcal growth was noted. As shown in Table.2, Dinoxin B showed the same levels of MIC (12.5 ± 0.00) against all strains of *S.aureus* except the MDR strain (50 ± 0.00). With lower MIC (12.5 ± 0.00) against UTIs, Dinoxin B can be considered as a

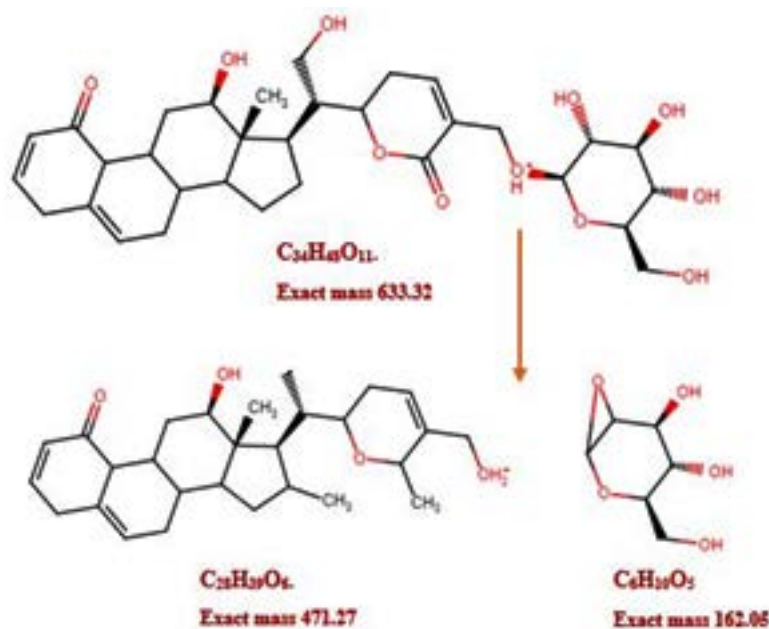


Fig. 4. Structure of phytoconstituents eluted in the spectrum of fraction four through Cleavage of a glycosidic bond

potent phytochemical. The growth of bacteria was not inhibited in the negative controls. Minimum Bactericidal Concentration(MBC) of Dinoxin B was found to be 25 ± 0.00 mg/ml (Table.2), by the absence of bacterial colonies on fresh Muller-Hinton agar plates(Fig.7)

The MBC/ MIC ratio > 2 indicates bactericidal effects and MBC/MIC ratio < 2 indicates bacteriostatic effect. Accordingly, Dinoxin B was found to be bactericidal effects against all tested isolates of *S.aureus* except MDR strain(U-6090), as shown in Table.2.

Time-kill assay

MRSA strain(U-6089) was tested for the time-kill assay using different concentrations of Dinoxin B $\frac{1}{2}$ MIC, MIC and $2 \times$ MIC (6.25 mg/ml, 12.5 mg/ml and 25 mg/ml). The results of the time-kill assay expressed changes in \log_{10} CFU/ml³⁸. The results obtained for the time-kill study shown in Fig.8. After 4 hours of incubation, the effect of MIC, and $2 \times$ MIC concentration of Dinoxin B on *S. aureus* growth inhibition was found to be almost identical. The fraction showed greater inhibitory action at 25 mg/ml followed by 12.5 mg/ml and 6.25 mg/ml after eight hours of incubation. However, the bactericidal action of $2 \times$ MIC (25 mg/ml) was observed after 18h incubation and of MIC (12.5 mg/ml) after 22h of incubation. The bactericidal effect of Dinoxin B to MRSA was

confirmed by the substantial reduction of bacterial colonies between 4 and 8 hours of incubation. Apart from that, the number of bacterial colonies on control plates increased as time increased, suggesting that the growth of *S.aureus* entered into the log phase. Present findings indicate that the fraction could yield a better inhibitory activity with an increase in the concentration and incubation time. The MBC values were compatible with the cidal concentration as seen in the time-kill study's growth curve.

Results of antibiogram evaluated based on Kirby-Bauer scaling of Zone of inhibition; < 12 mm (resistant); < 13 -14 mm (intermediate) and > 15 mm (susceptible);⁴¹ and shown that all isolates possess comparatively similar susceptibility to Dinoxin B ($100 \frac{1}{4}$) compared with the standard drugs as shown in Fig.9. MDR strain showed resistance to Ampicillin and Ofloxacin while susceptible to Gentamicin and Dinoxin B. Effectiveness of Dinoxin B as an MRSA inhibitor is notable as it with a higher zone of inhibition (22.5 mm) than that of referenced antibiotics.

Docking Results

Resistant proteins of *S.aureus* that promote Multi-Drug resistance, which results in urinary tract infection were selected for docking studies. The results of docking interactions between Dinoxin B withanolide and targeted receptor

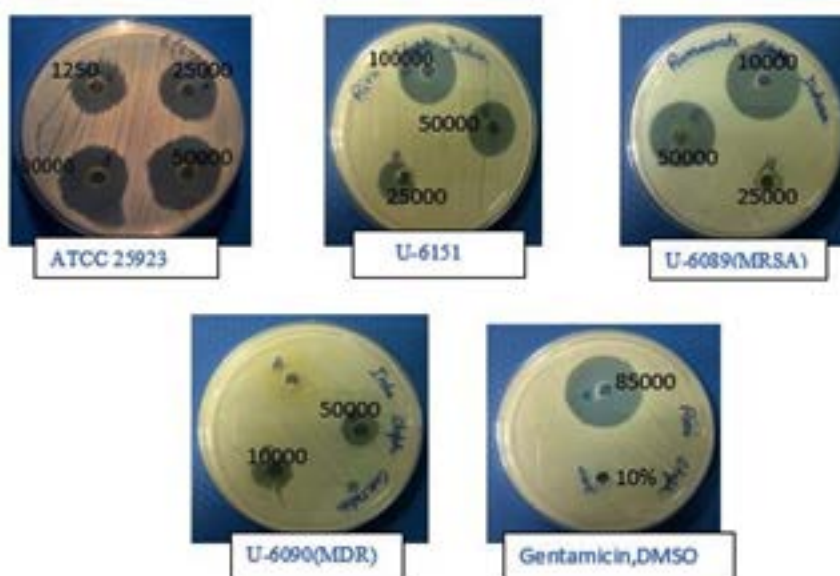


Fig. 5. Zone of Inhibition (mm). *S.aureus* strains showing susceptibility to different concentrations of Dinoxin B and DMSO(control) and Gentamicin (reference antibiotic)

proteins were shown in Table 3. Comparative docking results between Dinoxin B and selected antibiotics help to focus on its mechanism of action(Fig.10).

It was found that(Fig.10) Dinoxin B Withaolide showed a significant docking score with 2FNP (-7.82). Staphylococcal accessory regulator A (SarA) Protein(PDB ID:2FNP)in *S.aureus*, controls the modulation of the virulence factors¹⁸. SarA a known master controller of biofilm formation by regulating Quorum-signaling and

promotes MRSA⁴². Dinoxin B can be considered as a Sar A selective therapeutic candidate, as its docking score is higher than all other selected antibiotics. Also, the review shows inhibition of Sar A proteins leads to bactericidal results⁴³, which in turn indicates that methicillin resistance is shown by Dinoxin B possibly by destroying the biofilm.

Varying expressions of PBPs are another reason for resistance to methicillin and other Beta-lactam antibiotics¹⁹. Drugs that regulate PBP activities are used to manage MRSA strains.

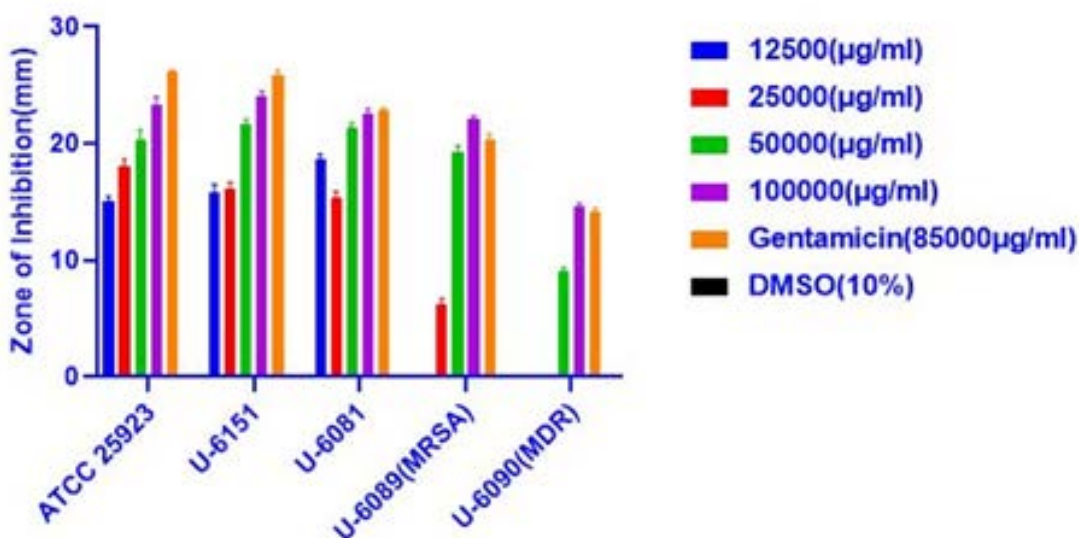


Fig. 6. Comparison of Zone of Inhibition of Dinoxin B at different concentration((µg/ml) against ATCC 25923, U-6151, U-6081, U-6089 (6089) U-6090(MDR), DMSO (control) and Gentamicin (reference antibiotic)

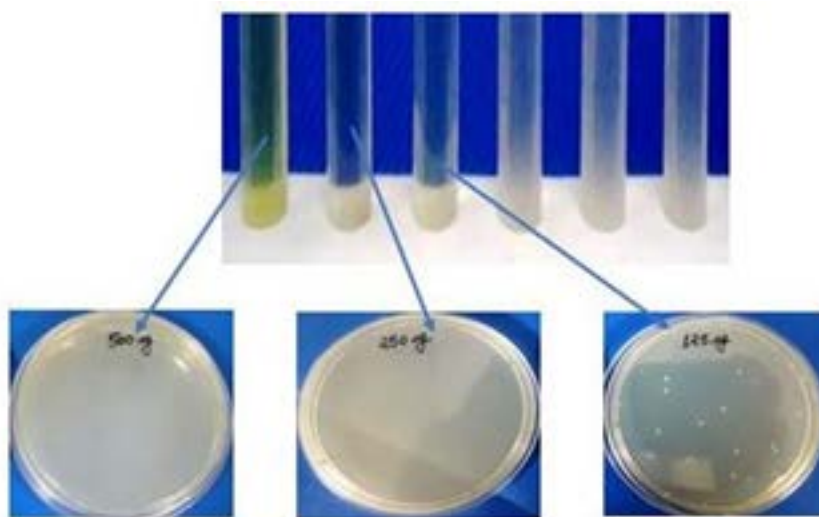


Fig. 7. Minimum bactericidal concentration against Methicillin-Resistant *S.aureus* as 25mg/ml

Docking score(-6.987) of Dinoxin B Withanolide and Penicillin Binding protein (PDB ID:3HUM); (Table.3 and Fig.10) is higher than the docking score of Ampicillin(-6.912), a beta-lactam antibiotic, Gentamicin(-6.151) and Ofloxacin(-5.301). The capacity to bind with PBPs inhibits the synthesis of the cell wall and thus promotes the bactericidal activity of ampicillin. The binding potentiality

of Dinoxin B Withanolide towards PBPs and similarity in docking score with that of Ampicillin highlights the efficacy of Dinoxin B Withanolide as a PBP inhibitor.

Docking score of Dinoxin B, as shown in Fig.10 with Multidrug efflux pump protein(4LLL) AgrA protein(4G4K), resulted in a moderate score (-4.513 and-2.823).In the case of Topoisomerase

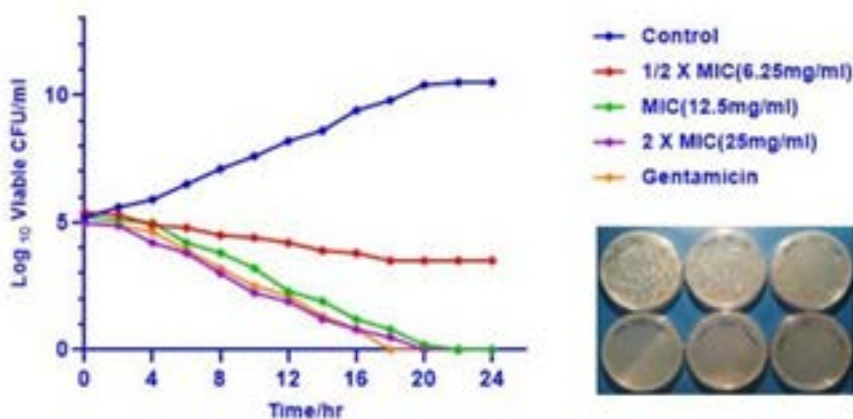


Fig. 8. Time-kill kinetics curve showing bacteriostatic action of Dinoxin B against Methicillin-Resistant *S.aureus*

Table 1. Zone of Inhibition(mm) for Dinoxin B. Datas are in triplicate and represented as mean \pm SD. < 12mm (resistant); <13-14mm (intermediate) and >15mm (susceptible); shown as (R), (I) and (S)

<i>S.aureus</i>	Different conc. of Fraction 4 (μ g/ml)				Gentamicin (85000) (μ g/ml)	DMSO (10%)
	12500	25000	50000	100000		
ATCC25923	15 \pm 0.5 (S)	18 \pm 1(S)	20.3 \pm 1.0(S)	23.3 \pm 0.7(S)	26.1 \pm 0.2(S)	0
U-6151	15.8 \pm 0.7(S)	16.2 \pm 0.5(S)	21.6 \pm 0.5(S)	24 \pm 0.5 (S)	25.8 \pm 0.5(S)	0
U-6081	18.6 \pm 0.5(S)	15.4 \pm 0.5(S)	21.3 \pm 0.5(S)	22.5 \pm 0.5(S)	22.8 \pm 0.28 (S)	0
U-6089(MRSA)	0(R)	3.4 \pm 0.5(R)	19.3 \pm 0.5(S)	22.5 \pm 0.5(S)	22.8 \pm 0.28(S)	0
U-6090(MDR)	0(R)	0(R)	9.1 \pm 0.28(R)	14.6 \pm 0.28(S)	14.2 \pm 0.28(S)	0

Table 2. Result of MIC, MBC, and MIC/MBC. Data are in triplicate and represented as mean \pm SD. nd-not determined

<i>S.aureus</i> Strains	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC Ratio	Bactericidal(+) Bacteriostatic(-)
ATCC 25923	12.5 \pm 0.00	25.0 \pm 0.00	2	+
U-6151	12.5 \pm 0.00	25.0 \pm 0.00	2	+
U-6081	12.5 \pm 0.00	25.0 \pm 0.00	2	+
U-6090(MDR)	50 \pm 0.00	>100	nd	nd
U-6089(MRSA)	12.5 \pm 0.00	25.0 \pm 0.00	2	+
Gentamicin	12.5 \pm 0.00	25.0 \pm 0.00	2	+
DMSO	0	0	0	0

(4PLB) and DNA gyrase(2XCT), Dinoxin B Withanolide showed comparatively less docking scores(-1.034 and -1.501. This in turn indicates that Dinoxin B Withanolide shows better inhibition with cell wall proteins. Fig.11 shows the docking interaction of Dinoxin B to all selected proteins. It demonstrates the role of hydroxyl groups⁴⁴ in protein-ligand interactions, which promote Dinoxin B's inhibitory potential.

Drug likeness of Dinoxin B

Lipinski and colleagues suggested in 1997(52) that medically active compounds should

match at least three of the observed criteria such as molecular weight less than 500 g mol⁻¹, log*P* less than 5; the number of hydrogen bond acceptors less than 10 and the number of hydrogen bond donors less than 10. The PreAdmet software was employed to study Lipinski's rules for Dinoxin B and Withanolide and was found (Table 4) as an orally active compound, as it follows three parameters.

ADMET properties of Dinoxin B

ADMET properties of Dinoxin B were predicted using the PreADMET tool. *In vitro*

Table 3. Docking Result Of Dinoxin B Withanolide: Docking result of Dinoxin B with Sar A protein(2FNP), Multi-Drug Efflux pump Protein (4LLL), AgrA Protein(4G4K), Penicillin-binding Protein(3HUM), Topoisomerase(4PLB), and DNA Gyrase(2XCT)

PDB ID	Docking score	Glide ligand efficiency	Glide score	Glide e-score	Glide energy
2FNP	-7.821	0.132	-7.82	-79.603	-57.179
4LLL	-4.513	-0.100	-4.513	-56.378	-44.233
4G4K	-2.823	-0.063	-2.823	-36.214	-33.290
3HUM	-6.987	-0.124	-5.920	-70.642	-55.525
4PLB	-1.034	-0.021	-1.034	-31.113	-29.491
2XCT	-1.501	-0.023	-1.501	-32.653	-30.129

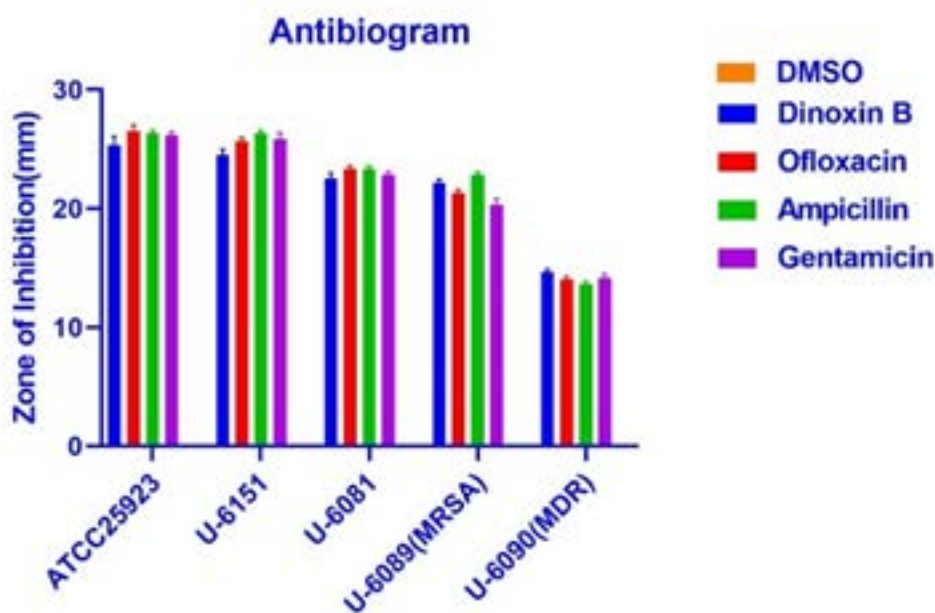


Fig. 9. Antibiogram graph showing zone of inhibition of Dinoxin B with different antibiotics-Gentamicin, Ampicillin, Ofloxacin and DMSO(control) .< 12mm (resistant); <13-14mm (intermediate) and >15mm (susceptible)

model of an oral drug, absorption was carried out by evaluating permeability through Caco-2 and MDCK (Madin-Darby canine kidney) cell model⁴⁵. Dinoxin B shows (Table 4) moderate permeability with its predicted result (20.504).

The potentiality of drugs for oral delivery and transdermal delivery can be assessed through

HIA (Human Intestinal Absorption) model and skin permeability model⁴⁶. PreADMET can predict the percent of human intestinal absorption (%HIA). Obtained data shows that Dinoxin B Withanolide with 82.577% of HIA is a well-absorbed compound (HIA 70-100%). A high intestinal absorption rate promotes its possibility as

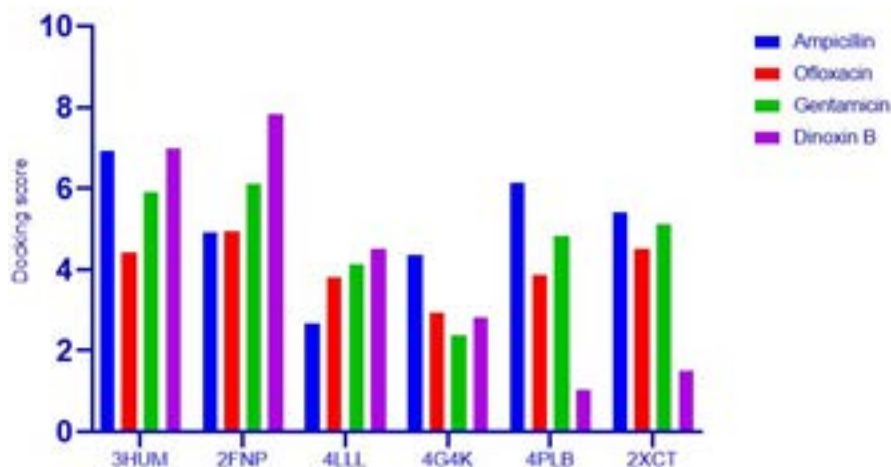


Fig. 10. Comparative Docking result of Dinoxin B Withanolide with Ampicillin, Ofloxacin, and Gentamicin with that of Sar A protein(2FNP), AgrA Protein(4G4K), Multi-Drug Efflux pump Protein(4LLL), Penicillin-binding Protein(3HUM), Topoisomerase(4PLB), and DNA gyrase(2XCT)

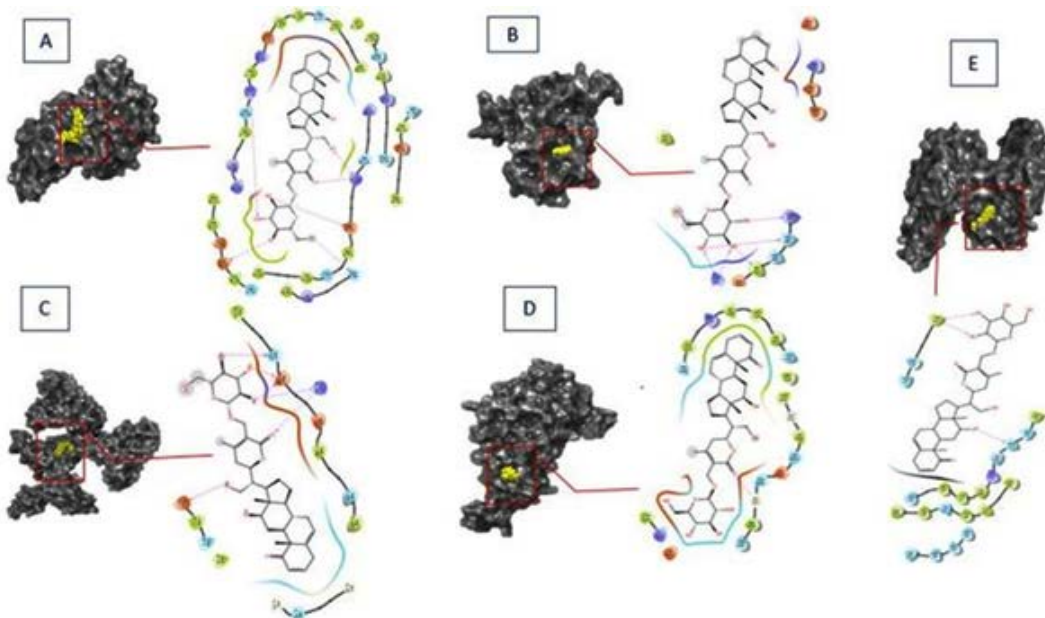


Fig. 11. Interaction of Dinoxin B Withanolide with Proteins. 3D surface view and 2D view of interaction with amino acids. [A] Sar A protein(2FNP) [B] Agr A protein(4G4K) [C] Efflux pump protein (4LLL) [D] Topoisomerase (4PLB) [E] Penicillin binding protein(3HUM)

Table 4. Result showing physiochemical properties to predict the drug-likeness of Dinoxin B Withanolide as per Lipinski's Rule

Physiochemical Properties		Lipinski Rule violation
cLogP	1.71	No
Molecular weight	632.75	Yes
Hydrogen bond acceptors	10	No
Hydrogen bond donors	5	No

an oral drug. Negative skin permeability of Dinoxin B Withanolide predicts its poor transdermal property.

BBB(Blood-Brain Barrier) prediction helps to identify the distribution potentiality of compounds, which may affect the Central Nervous system(CNS) [47]. Dinoxin B Withanolide with 0.004 BBB permeability(less than 0), can be considered as a CNS inactive compound.

As only free drugs can cross membranes

Table 5. Result showing ADMET Properties of Dinoxin B using and Pre-ADMET Prediction

ADMET Properties	Dinoxin B	Pre-ADMET Prediction
CaCO2	20.5044	4 – 70 Middle permeability
MDCK	0.072	< 4 Good permeability
HIA	82.5778	70-100%; well absorbed compounds
Skin permeability	-2.212	< 0 Poor Skin permeability
BBB	0.004	< 0 CNS inactive compound
PPB	91.01	>90 Chemicals strongly bound
CYP 2C19 inhibition	Non-inhibitor	Promote metabolism
CYP 2C9 inhibition	Non Inhibitor	Promote metabolism
CYP 2D6 inhibition	Non Inhibitor	Promote metabolism
CYP 3A4 inhibition	Non Inhibitor	Promote metabolism
CYP 3A4 substrate	Substrate	Promote metabolism
Ames test	Non-mutagen	Non-Carcinogenic

and link to their desired targets, determining the amount of drug bound to plasma proteins (PPB) is important in drug discovery⁴⁸. PPB values greater than 90% indicate that they are strongly bound to plasma proteins, and Dinoxin B Withanolide showed remarkable(91.01%) PPB efficiency.

Fifty to ninety percent of therapeutic molecules are the substrate of five major isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) of P450⁴⁹. Inhibition of these isoforms is undoubtedly one of the most common causes of pharmacokinetics-related undesirable side effects. Result indicate Dinoxin B as a substrate or non-inhibitor of most of the evaluated isoforms. The Ames test is a method for determining a compound's mutagenicity that was proposed by Dr. Ames⁵⁰ and the result showed Dinoxin B Withanolide as a non-mutagen.

CONCLUSION

Dinoxin B Withanolide was found in ethanolic leaf extract of *Datura innoxia*, which had strong antibacterial properties, according to our previously published research and current research. Dinoxin B was confirmed to be bactericidal to UTI-causing *S.aureus* using different methods. Its significant inhibition to methicillin-resistant strains has been proven in both dry and wet test results. In silico findings revealed that it has a high binding potential against Sar A proteins (biofilm regulators) and Penicillin-binding proteins, which are modern-day threats as they promote Methicillin Drug Resistance. The interaction of dinoxin B with cell wall proteins is proven to be the mechanism of action, according to observed results. As the experiments demonstrated, if this phytochemical can compete with the currently used antibiotics, it

can undoubtedly be considered as a drug candidate (green antibiotics) for treating UTI-causing *S.aureus*.

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None.

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