Antibacterial analysis of Withanolides from *Datura innoxia*

Abstract

Objectives: This study was aimed to explore the therapeutic potentiality of *Datura innoxia* through chemoinformatic and antibacterial evaluation of withanolides extracted from it.

Methods: Pharmacokinetic and pharmacodynamic properties and drug-likeness of the withanolides; Withametelinol A, Withametelinol B, Witharifeen, Withametelin, Dinoxin B, and Daturacin of *D.innoxia* were analyzed using the SwissADME program. Shrodinger software is used to target and evaluate their antibacterial potentialities through docking studies. Penicillin-binding protein, DNA gyrase, Efflux pump protein, and Quorum sensing regulators of *S.aureus* and *E.coli* were selected as target proteins for assessing protein-ligand interaction. All observations were comparatively analyzed with the properties of withanolide A and withaferin A, the best-known withanolides. Most active Dinoxin B withanolide(12500-100000 µg/ml) extracted from leaves of *Datura innoxia* and confirmed through LC-ESI-MS; is subjected to antibacterial assay following the agar diffusion and macro broth dilution methods against Methicillin-Resistant *S.aureus* and Multi-Drug resistant *E.coli* isolated from urine sample of patients.

Results: In-silico studies reveal the therapeutical properties of various withanolides present in *D.innoxia*. Especially drug-likeness and antibacterial properties of withametelin and Dinoxin B are significantly high and remarkable due to their binding affinity towards cell membrane proteins. Docking studies show efflux pump protein of *E.coli* and penicillin-binding proteins of *S.aureus* as the target. The significant antibacterial assay revealed that the MRSA isolates were susceptible to Dinoxin B with a zone of inhibition 21±0.5mm to 24±0.5mm, and the bacteria were susceptible at a concentration rate of ≤12.5mg/ml.

Conclusion: It is to bring awareness of the therapeutical importance of *D.innoxia* and to preserve this vital plant from getting massively destroyed. As computational studies promote the effective selection of drug molecules, this research also helps to select the best compound for further clinical analysis.

Keywords

Datura innoxia; Withanolides; Chemoinformatic evaluation; Methicillin-Resistant *S.aureus;* Multi-Drug resistant *E.coli*.

1. Introduction

Withanolides have attracted the scientific community's interest in recent years, due to their structural properties and demonstration of considerable pharmacological effects such as anti-inflammatory, antitumor, immunomodulatory and antimicrobial[1]. Approximately 750 withanolides with more than twenty-two carbon skeletons are reported from various plant sources. In the Solanaceae, family withanolides are present in twenty-five genera[2]. Even then Withania and Physalis have been selected most extensively for their therapeutical analysis. Nearly 130 withanolides were extracted from various parts of Withania somnifera, a traditional Ayurvedic plant. This plant has the highest number of withanolides of any species so far, and withanolide A and withaferin A being the best antibacterial withanolides found in it[3].

As exploring studies on withanolides, this research highlights unspoken Datura species with it identified withanolides. Despite its reputation as a harmful plant due to its poisonous components, it can be purified to produce medically beneficial compounds[4]. The presence of

withanolides is seen in many species of this genus, such as *D.metel*[5], *D. innoxia*[6], *D. stramonium*[7], *D.wrightii*[8], and *D. ferox*[9].

D.innoxia (Fig.1) is native to the American Southwest, Mexico, and Central America, as far south as Belize and Guatemala, but today so common in Asian tropical regions. It is a shrubby perennial that grows to a height of 2-5 feet. Small, silky grey hairs cover the plant's stems and leaves, giving it a greyish appearance. It has an entire-edged ovate to elliptic leaves. The flowers are ten toothed and white, with a length of 12-19 cm. They grow upright at first, then incline downward. It blooms from early summer to late autumn. The fruit is an egg-shaped spiny capsule with a diameter of around 5 cm. Atropine, scopolamine, hyoscyamine, withanolides (lactones), and other tropanes are among the active factors in *D.innoxia*.

Fig.1.*D.innoxia* in its natural habitat.View from Amity Campus premise, Lucknow, India.

Even though the genus Withania is well-known for withanolide compounds, our observation of significant broad-spectrum antibacterial properties of Dinoxin b withanolide[10] from *D.innoxia* prompted us to compare drug-likeness and antibacterial properties of different withanolides obtained exclusively from *D.innoxia*, using *in-silico* methods. It is to bring awareness of the therapeutical importance of this species and to preserve this plant from getting massively destroyed. As computational studies promote the effective selection of drug molecules, this research also helps to select the best compound for further clinical analysis.

Review of literature, as well as Pubchem data[11], shows Withametelinol A[12], Withametelinol B[12], Witharifeen[13], Withametelin[14], Dinoxin B[10], and Daturacin[15], are the identified withanolides from *D.innoxia*(Fig.2).In this work, we have evaluated the inhibiting activity of these withanolides with selected target proteins of *S.aureus* and *E.coli* through docking studies as it provides a rational new approach to study the antibacterial properties of drugs. Furthermore, a pharmacological evaluation was also performed including the evaluation of the pharmacological properties as per Lipinski's rule of five, drug-likeness, bioactivity, and the drug score. Pharmacokinetics properties were evaluated to analyze the interaction of the individual from the time of administration to absorption, distribution, metabolism, excretion as well as toxicity (ADMET). A comparative assessment was carried out with pharmacological and docking scores of the known effective Withanolide A and Withaferin A to make predictions of withanolides obtained from *D.innoxia*.As chemo-informatics screening remakedly proved the effectiveness of Dinoxin B,its antibacterial proprties are evaluated using pathogenic strains of *S.aureus* and *E.coli*.

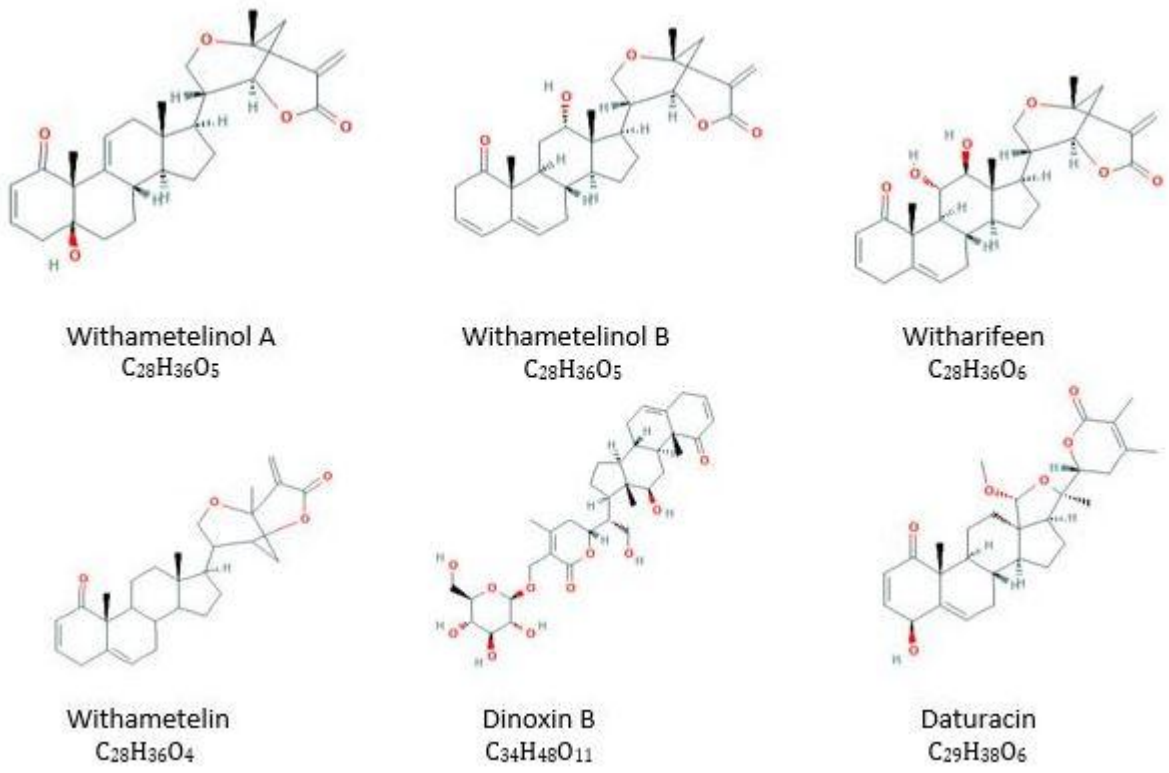


Fig.2:Molecular structure of withanolides of *D.innoxia* retrieved from Pubchem.

2. Methodology

Physicochemical properties prediction

SwissADME (https://swissadme.ch)tool is used to examine molecular properties and drug likeliness of withanolides based on Lipinski Rule. Lipinski's rule of five is used by pharmaceuticals in drug development to predict the oral bioavailability of potential lead or drug molecules[16]. These parameters include total polar surface area (TPSA), partition coefficient (water/oil) - cLogP, molecular weight, number of hydrogen acceptors, and number of hydrogen donors.

Pharmacokinetic analysis

While ADME tries to maximize the pharmacological performance of a small molecule, toxicology aims to ensure that it causes no harm in any kind of side effect[17]. Obtained scores are comparatively analyzed with the scores of prevailing broad-spectrum antibiotics Ampicillin, Gentamicin, and Cephalosporin. This program predicts based on functional group similarity of the investigated compound with the extensively in-vitro and in-vivo studied compounds present in its database.

Docking Studies

A docking study with standard precision mode using the Glide docking module of Maestro 12.5 Schrodinger[18] software was carried out to evaluate the affinity of withanolides towards *S.aureus* and *E.coli*.As shown in Table.1, different proteins responsible for resistance mechanisms are retrieved from the Protein data bank and ligands(withanolides)from PubChem(Table.2).

Proteins	**(PDB ID)**	
	S.aureus	***E.coli***

Penicillin Binding Protein	3HUM	4BJP
DNA Gyrase	2XCT	1AB4
Efflux Pump Proteins	4LLL	5ENO
Quorum Sensing Regulators	4G4K	2AVX

Table.1: Details of selected Proteins retrieved from the Protein data bank.

Ligands	**Pub Chem ID)**
Withametelinol A	15550331
Withametelinol B	101160729
Witharifeen	12135064
Daturacin	16010830
Withametelin	364746
Dinoxin B	51041991
Withanolide A	11294368
Withaferin A	265237

Table.2: Selected withanolides with its Pub Chem ID.

Extraction and Identification of Dinoxin B from D.innoxia

Following the protocol of Tandon. *et.al* [10] ethanolic leaf extract of *Datura innoxia* were fractionated using a single solvent system through column chromatography [19]. To fill up the column, Silica gel (60-120 mesh) was used and added with the leaf extract 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. For the identification of Dinoxin B, Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS) of fraction 4[10] was done from Central Drug Research Institute of India, Lucknow.

Agar Diffusion assay

Following the Kirby-Bauer diffusion technique[20], conducted an agar well plate method to assess the antibacterial property against clinical strains *S.aureus* (SU-6151) and *E.coli* (EU-6081)isolated from urine sample of patients including Methicillin-Resistant *S.aureus* (SU-6089)and Multi-Drug resistant *E.coli* (EU-6089. All the isolates were obtained from Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow. The inhibition zones were measured(ZOI). Gentamicin (85mg) and DMSO (10%)were used as positive as well as negative controls. Assays were done in triplicate, and the results were expressed as mean standard deviation.

Macrobroth Dilution for Determining MIC and MBC

Using the two-fold serial dilution procedure, different concentrations of fraction four are obtained and combined with 100µl of the test organism to achieve a final inoculum concentration of $5x10^5$. The Minimum Inhibitory Concentration(MIC) value was defined as the highest dilution that inhibited bacterial growth. The growth control was bacterial inoculum without the tested percentage, while the sterility control was bacterial inoculum itself.

Minimum Bactericidal Concentration (MBC) was determined by subculturing each tube of MIC that had no apparent growth. At 37°C, the plates were incubated for 24 hours. MBC refers to the lowest concentrations of the extract that did not result in colony formation on the solid medium.

3.Result and Discussion

Herbal drug formulation depends on the high biological potentiality and low toxicity. For oral absorption in terms of permeability, Lipinski and collaborators[16] already proposed that orally active compounds should fit at least three of observed four parameters: molecular weight < 500 g mol^{-1}, log*P* < 5; the number of hydrogen bond acceptors <10; the number of hydrogen bond donors <10; the well-known Lipinski's rule of 5 (Ro5). In other words, Ro5 stated a physicochemical space in which molecules outside its domain have a low probability to become orally active[21]. The Swiss ADME was employed to study Lipinski's rules for Withanolides and was noted that all selected compounds orally active compounds, as it follows more than three of Ro5 parameters(Table.3).

Compound	TPSA (A^0)	H bond Acceptors	H-bond donors	cLogP	nV	nROTB	MV
Dinoxin B	183.2	11	6	1.71	3	7	632.75
Withanolide A	96.36	6	2	4.15	0	2	470.61
Withametelinol-A	72.84	5	1	4.51	0	1	452.6
Withametelinol-B	72.84	5	1	4.54	0	1	452.6
Witharifeen	93.07	6	2	3.62	0	1	468.59
Withametelin	52.61	4	0	4.95	0	1	436.59
Withaferin A	116.5	7	3	3.18	0	2	486.61

Table.3:Result showing physiochemical properties to predict the drug-likeness of selected withanolides based on Lipinski's Rule of five.TPSA-Total Polar Surface Area,nV-No.of Ro5 violations,nROTB-no.of rotatable bonds,MV-Molecular weight.

The pharmacokinetic properties such as absorption, distribution, metabolism, excretion, and toxicity of withanolides were predicted by using Pre ADMET and shown in Table.4.The Caco-2 cell model and MDCK (Madin-Darby canine kidney) cell model have been accepted as reliable *in vitro* models for the assessment of oral drug absorption[22]. All observed withanolide show middle permeability as per Caco-2 and MDCK cell model with a value between 4-70 and > 0 respectively.

HIA (Human Intestinal Absorption) and skin permeability models can also forecast and discover prospective medications for oral and transdermal delivery in silico. The sum of bioavailability and absorption in humans is calculated using the ratio of excretion or cumulative excretion in urine, bile, and feces. [23]. Selected withanolides are recorded as well absorbed compounds with more than 90% of absorption rate (HIA70-100%). More HIA values indicate that the withanolides could be better absorbed from the intestinal tract upon oral administration. At the same time, the negative skin permeability of compounds predicts their poor transdermal property. As shown in Table.2, the absorption rate of Withametelin is more in all cases.

	Absorption			
Compound	Caco-2 (4 – 70 Middle permeability)	HIA (70-100%; well-absorbed compounds)	Skin Permeability (>0 Poor Skin permeability)	MDCK (> 0 shows permeability)
Withametelinol A	30.683	96.388853	-1.85921	0.059029
Withametelinol B	28.314	96.390242	-2.94982	0.103376
Witharifeen	21.682	94.789426	-3.79095	0.178691
Withametelin	46.749	97.401304	-2.21215	0.052234
Dinoxin B	20.5044	92.577878	-4.69651	0.072432
Withanolide A	22.0036	94.739698	-2.63567	0.047720

Withaferin A	20.9127	90.403397	-3.75156	0.148721

Table.4:Result of absorption property of withanolides obtained from PreADMET.

In distribution, predicting BBB(Blood-Brain Barrier) means whether compounds pass across the blood-brain barriers. CNS-active compounds must pass across it and CNS-inactive compounds must not pass across it[24]. All Withanolides with less BBB permeability are considered as CNS inactive compounds which promote its drug-likeness with fewer CNS side effects.

It is generally assumed that only the free drug can cross membranes and bind to the intended molecular target[25] and it is, therefore, important to estimate the fraction of drugs bound to Plasma Protein Binding(PPB). PPB values greater than 90% indicate that they are strongly bound to plasma proteins, and all withanolide showed remarkable PPB efficiency notably withametelin showed a hundred percentage binding affinity(Table.5).

A soluble compound promotes drug formulation as solubility is an important criterion that may alter the effectivity of a drug compound.Withametelin, Withametelinol A, and Dinoxin B with high buffer solubility (73.47,79.83,66.44mg/l) and pure water solubility (40.5,33.51,50.09 mg/l)remarkably prove their drug-likeness.

Compound	Buffer Solubility (mg/L)	Purewater Solubility (mg/L)	BBB	Plasma Protein Binding
Withametelinol A	79.8352	33.51143	0.188225	96.545118
Withametelinol B	47.511	28.41036	0.0927233	89.49667
Witharifeen	55.047	18.49928	0.143512	86.889916
Withametelin	73.4706	40.5095	0.310565	100
Dinoxin B	66.4456	50.0991	0.0483601	91.017295
Withanolide A	33.7106	35.22969	0.336643	91.590333
Withaferin A	3.31074	33.5959	0.159809	82.408739

Table.5:Result showing the distribution property of withanolides in pharmacokinetics.

For a better selection of drug compounds, knowledge about the interaction of molecules with cytochromes P450 (CYP)is very essential[26]. It has been proposed that CYP can synergistically metabolize tiny compounds to promote tissue and organism protection. Five main isoforms are thought to be the substrate of 50 to 90 percent of therapeutic compounds (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4). Inhibition of these isoenzymes is undoubtedly one of the most common causes of pharmacokinetics-related medication-drug interactions, which can result in toxic or other undesirable side effects due to decreased clearance and buildup of the drug or its metabolites (de Montellano, 2015).SwissADME enables the estimation of the withanolides to be the substrate of P-gp or inhibitor of the most important CYP isoenzymes. In-silico data estimated that the selected withanolides cannot metabolize (non-substrate) by CYP 450 2D6 and are the substrate of CYP 450 3A4 non-inhibitors for CYP 450 2C19, CYP 450 2D6, and inhibitor of CYP 450 3A4 (Table 6). The noninhibition of cytochrome P450 will help in the metabolism of these compounds.

Compound	CYP2C19 inhibition	CYP2C9 inhibition	CYP2D6 inhibition	CYP2D6 substrate	CYP3A4 inhibition	CYP3A4 substrate
Withametelinol A	Non	Inhibitor	Non	Non	Inhibitor	Substrate
Withametelinol B	Non	Inhibitor	Non	Non	Inhibitor	Substrate
Witharifeen	Non	Inhibitor	Non	Non	Inhibitor	Substrate
Withametelin	Non	Inhibitor	Non	Non	Inhibitor	Substrate
Dinoxin B	Non	Inhibitor	Non	Non	Inhibitor	Substrate

Withanolide A	Non	Inhibitor	Non	Non	Inhibitor	Substrate
Withaferin A	Non	Inhibitor	Non	Non	Inhibitor	Substrate

Table.6:Result of metabolism prediction of withanolides using SwissAdme.

The Ames test is a simple method for determining a compound's mutagenicity that was proposed by Dr. Ames. It employs several Salmonella typhimurium strains that have mutations in genes implicated in histidine synthesis, requiring histidine for growth. The mutagenic capacity to cause a reversion to growth on histidine-free media is being investigated. As shown in Table.6, *In-vitro* Ames test results in TA100 strain, TA10010, TA153510 strain, and TA1535 strain are not showed metabolic activation by rat liver homogenate. This AMES toxicity result proves that all withanolides are non-mutagenic with negative results(Table.7).

Compounds	Amestest	Oral Rat Acute Toxicity	Oral Rat Chronic Toxicity	TA10010 RLI	TA100 NA	TA153510 RLI	TA1535 NA
Withametelion A	non-mutagen	2.094	0.344	negative	negative	negative	negative
Withametelion B	non-mutagen	2.234	1.715	negative	negative	negative	negative
Witharifeen	non-mutagen	2.319	1.685	negative	negative	negative	negative
Withametelin	non-mutagen	2.045	1.171	negative	negative	negative	negative
Dinoxin B	non-mutagen	3.555	2.86	negative	negative	negative	negative
Withanolide A	non-mutagen	2.913	1.739	negative	negative	negative	negative
Withaferin A	non-mutagen	3.503	2.154	negative	negative	negative	negative

Table.7: Result of toxicity prediction of withanolides based on Ames Test using Swiss ADME.

To identify the target of these withanolides, docking interaction was observed with resistance promoting Membrane proteins and Chromosomal proteins(DNA Gyrase) of *S.aureus and E.coli* as given in Table.1.Molecular docking is an effective method to predict the binding target of protein-ligand complexes and to determine the potential mechanisms of action. Obtained results justify the antibacterial assays obtained, in which the binding affinity of withametelin[14] and Dinoxin B [10] are bacteriostatic. Docking results show that Dinoxin B possesses a significant binding affinity to membrane proteins than DNA Gyrase (1 AB4 and 2XCT) with docking score -6.504 with Efflux Pump Protein(EPP) of *E.coli* and -5.92 with Penicillin Binding Protein(PBP) of *S.aureus*. (Fig.2). Notably, this binding score is higher than Withanolide A and Withaferin A. As a common target, all these withanolide shows better affinity towards PBPs(3HUM and 4BJP) and EPP s of *E.coli* which in turn indicates membrane protein interaction as the reason for the antibacterial mode of action(Fig.3).

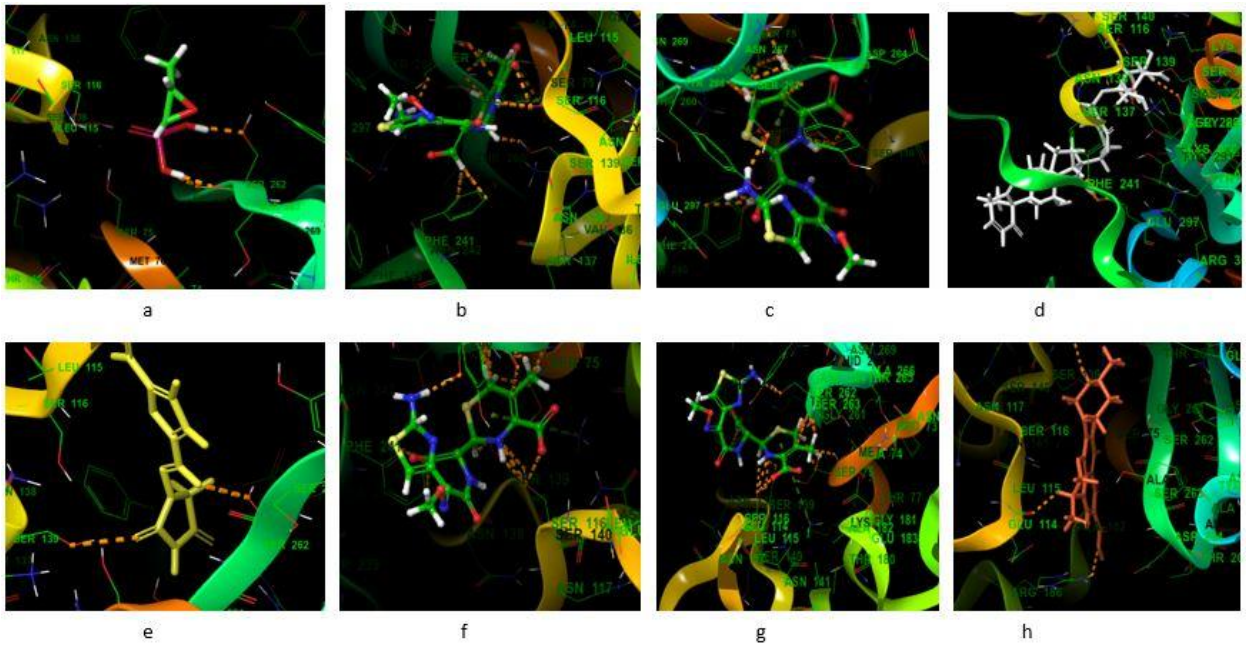
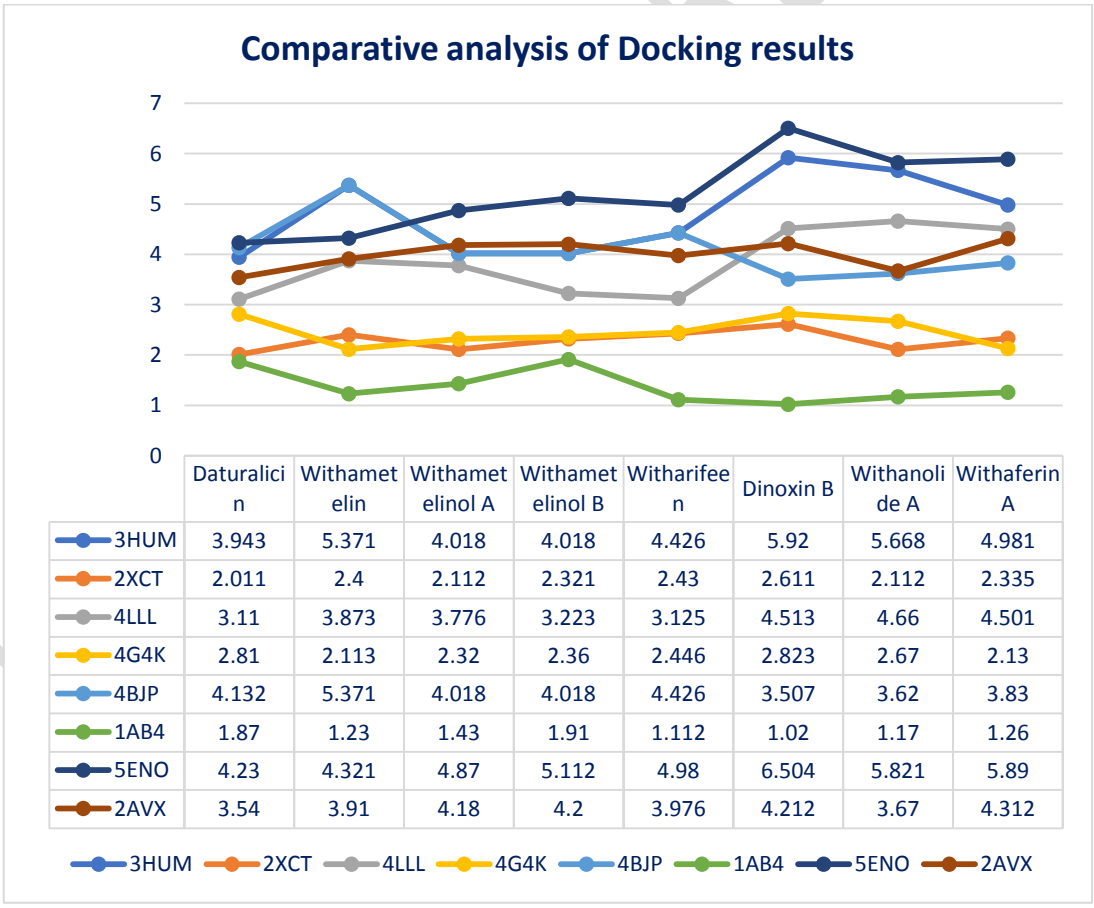


Fig.3.The result showing the best protein-ligand interaction in the docking study. (a) Withametelin(c)withanolide A and(d)dinoxin B with 3HUM(PBP) of *S.aureus*. (e)withanolide A (f)Withaferin A (g) withametelinol b (h)dinoxin b with 5ENO(EPP) of *E.coli* .(b)withametelin with 4BJ(PBP)P of *E.coli.* PBP-Penicillin binding protein, EPP-Efflux pump protein.



	Daturalicin	Withametelin	Withametelinol A	Withametelinol B	Witharifeen	Dinoxin B	Withanolide A	Withaferin A
3HUM	3.943	5.371	4.018	4.018	4.426	5.92	5.668	4.981
2XCT	2.011	2.4	2.112	2.321	2.43	2.611	2.112	2.335
4LLL	3.11	3.873	3.776	3.223	3.125	4.513	4.66	4.501
4G4K	2.81	2.113	2.32	2.36	2.446	2.823	2.67	2.13
4BJP	4.132	5.371	4.018	4.018	4.426	3.507	3.62	3.83
1AB4	1.87	1.23	1.43	1.91	1.112	1.02	1.17	1.26
5ENO	4.23	4.321	4.87	5.112	4.98	6.504	5.821	5.89
2AVX	3.54	3.91	4.18	4.2	3.976	4.212	3.67	4.312

Fig.4.Comparative analysis of docking result of withanolides of *D.innoxia*

As we reported in the previous study[10], ethanolic leaf fraction four of *D. innoxia* obtained through column chromatography is analyzed through LC-ESI-MS. This mass spectrum(Fig.5) also depicts the presence of Dinoxin B Withanolide and its aglycone. Phytoconstituents eluted in

the spectrum of fraction four depicted as M-glucose-water+H^+ (*m/z*471)and Dinoxin B Withanolide (*m/z* 633) due to the cleavage of a glycosidic bond.

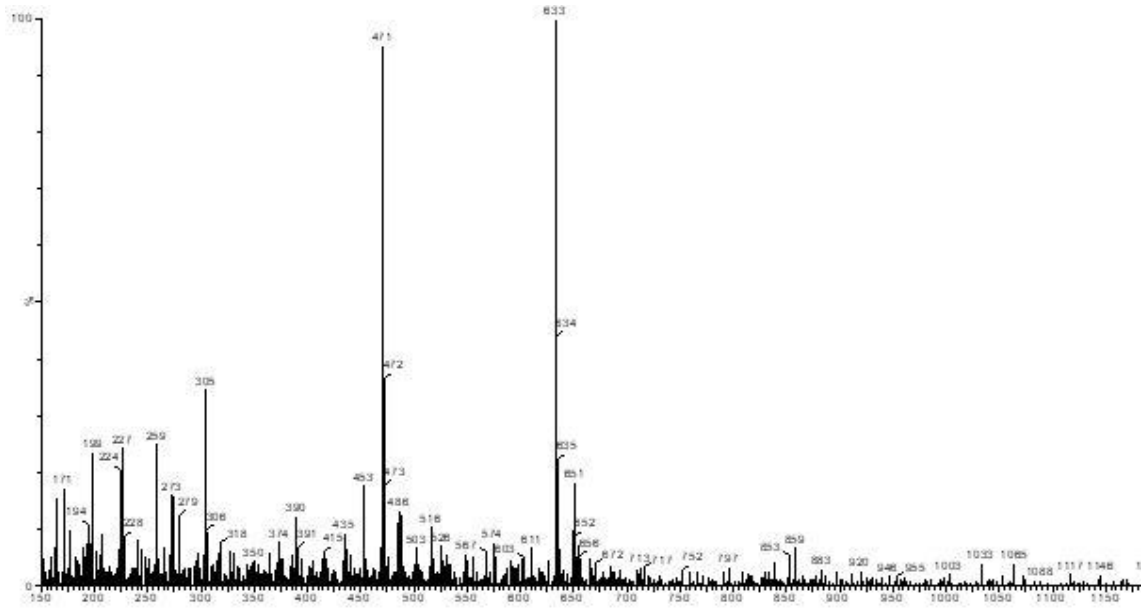


Fig.5:LC-ESI-MS Spectrum of Most Active Fraction 4. Shows the presence of Dinoxin B Withanolide and its aglycone: The phytoconstituents eluted as M-glucose-water+H^+ (*m/z*471)and Dinoxin B Withanolide (*m/z* 633).

Inhibitory potential of Dinoxin B was observed through agar well diffusion assay using different concentrations of fraction four in µg/ml (100000,50000,25000 and 12500)and compared to control(DMSO) and Gentamicin as reference antibiotic(Fig.6). As per the Kirby-Bauer test[27], *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), were showed significant activity($p < 0.05$), which was comparable to the reference antibiotic, at a higher concentration of Dinoxin B(100000 µg/ml,50000 µg/ml). Whereas MDR strain at 25000 µg/ml, 12500 µg/ml, and MRSA at 12500 µg/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.8) in range of (mm) 0-15(1250µg/ml),0-18(25000 µg/ml),9.1-20.3(50000 µg/ml),14.6-23.3(100000 µg/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.

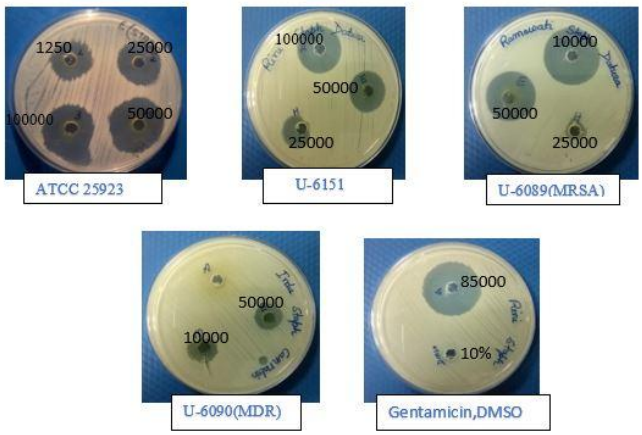


Fig.6:Zone of Inhibition (mm).*S.aureus* strains showing susceptibility to different concentrations of Dinoxin B and DMSO(control) and Gentamicin(reference antibiotic).

S.aureus	Different conc.of Fraction 4 (µg/ml)				Gentamicin (85000) (µg/ml)	DMSO (10%)
	12500	25000	50000	100000		
ATCC25923	15 ±0.5 (S)	18±1(S)	20.3±1.0(S)	23.3±0.7(S)	26.1 ±0.2(S)	0
U-6151	15.8±0.7(S)	16.2±0.5(S)	21.6±0.5(S)	24 ±0.5 (S)	25.8±0.5(S)	0
U-6081	18.6±0.5(S)	15.4±0.5(S)	21.3±0.5(S)	22.5±0.5(S)	22.8 ±0.28 (S)	0
U-6089(MRSA)	0(R)	3.4±0.5(R)	19.3±0.5(S)	22.5±0.5(S)	22.8 ±0.28(S)	0
U-6090(MDR)	0(R)	0(R)	9.1±0.28(R)	14.6±.28(S)	14.2±0.28(S)	0

Table.8: Zone of Inhibition(mm) for Dinoxin B. Datas are in triplicate and represented as mean ± SD.< 12mm (resistant); <13-14mm (intermediate)and >15mm (susceptible);shown as(R), (I)and(S)

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.9, 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 a lower MIC(12.5 ±0.00) against UTIs, Dinoxin B can be considered as a potent phytocompound. The growth of bacteria was not inhibited in the negative controls. Minimum Bactericidal Concentration(MBC) of Dinoxin B was found to be 25±0.00mg/ml by the absence of bacterial colonies on fresh Muller-Hinton agar plates.

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

Table.9 Result of MIC, MBC, and MIC/MBC. Data are in triplicate and represented as mean ± SD. nd-not determined.

The MBC/ MIC ratio ≤ 2 indicates bactericidal effects and MBC/MIC ratio ≥4 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 inTable.2.

4.Conclusion

Withanolides is a class of active compounds widely found in Solanaceae plants. In traditional applications, it has a long history and a wide range of functions. With the progress of drug structure and pharmacological research, more and more reports point to its excellent pharmacological effect. Based on the pharmacological action of inhibiting bacterial resistance, withanolides have become a research hotspot in natural medicine. In-silico results help us to conclude that Dinoxin B and Withametelin can be considered as drug candidates due to their relevant Drug-likeness and adequate pharmacokinetics features. Dinoxin B with its significant antibacterial properties emphasizes the therapeutical potentiality of D.innoxia.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Reference

1. Siddiqui BS, Arfeen S, Afshan F, Begum S. Withanolides from Datura innoxia. ChemInform 2005;36(37).
2. Singh A, Duggal S, Singh H, Singh J, Katekhaye S. Withanolides: Phytoconstituents with significant pharmacological activities. Int J Green Pharm 2010;4(4):229–37.
3. Rashmi S, Nivethitha S, Hemalatha CN, Vijay Aanandhi M. Virtual screening studies of two closely related withanolides to control cell proliferation and induction of cell senescence. Rasayan J Chem 2018;11(1):339–44.
4. Fatima H, Khan K, Zia M, Ur-Rehman T, Mirza B, Haq I ul. Extraction optimization of medicinally important metabolites from Datura innoxia Mill.: An in vitro biological and phytochemical investigation. BMC Complement Altern Med 2015;15(1).
5. Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of Datura metel against Rhizoctonia solani and Xanthomonas oryzae pv. oryzae. Physiol Mol Plant Pathol 2004;65(2):91–100.
6. Vermillion K, Holguin FO, Berhow MA, Richins RD, Redhouse T, O'Connell MA, et al. Dinoxin B, a withanolide from Datura inoxia leaves with specific cytotoxic activities. J Nat Prod 2011;74(2):267–71.
7. Fang ST, Liu X, Kong NN, Liu SJ, Xia CH. Two new withanolides from the halophyte Datura stramonium L. Nat Prod Res 2013;27(21):1965–70.
8. Zhang H, Bazzill J, Gallagher RJ, Subramanian C, Grogan PT, Day VW, et al. Antiproliferative withanolides from Datura wrightii. J Nat Prod 2013;76(3):445–9.
9. Veleiro AS, Cirigliano AM, Oberti JC, Burton G. 7-Hydroxywithanolides from Datura ferox. J Nat Prod 1999;62(7):1010–2.
10. Tandon C, Mathur P, Sen M, Kanojiya S. Identification of an antibacterial withanolide (Dinoxin b) from leaf of datura inoxia mill. Int J Phytomedicine 2016;8(1):1–12.
11. PubChem. National Library of Medicine [Internet]. Available from: pubchem.ncbi.nlm.nih.gov
12. S. Siddiqui B, Ali Hashmi I, Begum S. Two New Withanolides from the Aerial Parts of Datura innoxia. Heterocycles [Internet] 2002;57(4):715. Available from: http://www.heterocycles.jp/library/abstract.php?doi=00613
13. Siddiqui BS, Arfeen S, Afshan F, Begum S. Withanolides from Datura innoxia. Heterocycles 2005;

14. Baig MW, Nasir B, Waseem D, Majid M, Khan MZI, Haq I ul. Withametelin: a biologically active withanolide in cancer, inflammation, pain and depression. Saudi Pharm J [Internet] 2020;28(12):1526–37. Available from: https://doi.org/10.1016/j.jsps.2020.09.021

15. Siddiqui BS, Arfeen S, Begum S, Sattar FA. Daturacin, a new withanolide from Datura innoxia. Nat Prod Res 2005;19(6):619–23.

16. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2012;64(SUPPL.):4–17.

17. Shivakumar R, Venkatarangaiah K, Shastri S, Nagaraja RB, Sheshagiri A. Antibacterial property and molecular docking studies of leaf calli phytochemicals of Bridelia scandens Wild. Pharmacogn J 2018;10(6):1221–9.

18. Manual U. Schrödinger Release 2019-3: Glide, Schrödinger, LLC, New York, NY, 2019. Schrödinger Release 2018-3 LigPrep, Schrödinger, LLC, New York, NY, 2018 2018;

19. Sarker SD, Latif Z, Gray AI. Natural Products Isolation: an overview. Nat Prod Isol [Internet] 2006;864:1–25. Available from: http://link.springer.com/10.1007/978-1-61779-624-1

20. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. Am Soc Microbiol [Internet] 2012;(December 2009):1–13. Available from: https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro

21. Lin J, Sahakian D, de Morais S, Xu J, Polzer R, Winter S. The Role of Absorption, Distribution, Metabolism, Excretion and Toxicity in Drug Discovery. Curr Top Med Chem 2005;3(10):1125–54.

22. Yamashita S, Furubayashi T, Kataoka M, Sakane T, Sezaki H, Tokuda H. Optimized conditions for prediction of intestinal drug permeability using Caco-2 cells. Eur J Pharm Sci 2000;10(3):195–204.

23. Zhao YH, Le J, Abraham MH, Hersey A, Eddershaw PJ, Luscombe CN, et al. Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure - Activity relationship (QSAR) with the Abraham descriptors. J Pharm Sci 2001;90(6):749–84.

24. Ajay, Bemis GW, Murcko MA. Designing libraries with CNS activity. J Med Chem 1999;42(24):4942–51.

25. Keen P. Effect of Binding to Plasma Proteins on the Distribution, Activity and Elimination of Drugs. In: Concepts in Biochemical Pharmacology. 1971. page 213–33.

26. Tyzack JD, Kirchmair J. Computational methods and tools to predict cytochrome P450 metabolism for drug discovery. Chem. Biol. Drug Des.2019;93(4):377–86.

27. Bauer AW, Kirby WM, Sherris JC TM, Bauer A, Kirby W, Sherris J, Turck M. Susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966;45:493–6.