urnaljpriJournal of Pharmaceutical Research International

33(56B): 43-54, 2021; Article no.JPRI.77943
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Biological Activities of Withanolides from *Datura innoxia*

Ruby George [a] and Priti Mathur [a*]

[a] Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Malhaur, Gomti Nagar Extension, Lucknow – 226 028, Uttar Pradesh, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i56B33924

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/77943

Original Research Article

Received 02 September 2021
Accepted 07 December 2021
Published 13 December 2021

ABSTRACT

Aims: This study aimed to explore the therapeutic potential of *Datura innoxia* through the chemoinformatic and antibacterial evaluation of withanolides extracted from it.
Study Design: The pharmacokinetic and pharmacodynamic properties and drug-likeness of the withanolides—withametelinol A, withametelinol B, witharifeen, withametelin, dinoxin B, and daturalicin—of *D. innoxia* were analyzed using the SwissADME program. Schrodinger software was used to target and evaluate their antibacterial potentialities through docking studies. The 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 interactions. 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;* was subjected to antibacterial assay against methicillin-resistant *S. aureus* (MRSA) and multi-drug resistant(MDR) *E. coli* isolated from the urine samples of urinary tract infected patients.
Results: *In-silico* studies revealed the therapeutical properties of various withanolides present in *D. innoxia*. In particular, the drug-likeness and antibacterial properties of withametelin and dinoxin B were significantly and remarkably high due to their binding affinity toward cell membrane proteins. Docking studies have shown that the efflux pump protein of *E. coli* and penicillin-binding proteins of *S. aureus* to be the ligand -interaction targets. A significant antibacterial assay revealed that the MRSA isolates were susceptible to dinoxin B, with a zone of inhibition of 21±0.5 mm to 24±0.5 mm, and the bacteria were susceptible at a concentration rate of ≤ 12.5 mg/ml.

**Corresponding author: E-mail: pmathur@lko.amity.edu;*

Conclusion: It is crucial to bring awareness of the therapeutical importance of *D. innoxia* and to preserve this vital plant from being largely 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 properties [1]. Approximately 750 withanolides with more than twenty-two carbon skeletons have been reported from various plant sources. In the *Solanaceae* family, withanolides are present in twenty-five genera [2]. Of these, *Withania* and *Physalis* have been selected most extensively for therapeutic analysis. Nearly 130 withanolides have been extracted from various parts of Withania somnifera, a traditional Ayurvedic plant. This plant has the highest known number of withanolides of any species, and withanolide A and withaferin A have been found to be the best antibacterial withanolides [3].

In exploring studies on withanolides, the present research highlights unstudied *Datura* species and their identified withanolides [4,5]. Despite its reputation as a harmful plant due to its poisonous components, it can be purified to produce medically beneficial compounds [6]. The presence of withanolides is seen in many species of this genus, such as *D. metel* [7], *D. innoxia* [1], *D. stramonium* [2], *D. wrightii* [3], and *D. ferox* [6].

D. innoxia (Fig. 1) is native to the American Southwest, Mexico, and Central America, as far south as Belize and Guatemala, but today is common in tropical Asian regions. *D. innoxia* is a shrubby perennial that grows to a height of 0.5m-1.5m. Small, silky gray hairs cover the plant's stems and leaves, giving it a grayish appearance. It has an entire-edged ovate to elliptic leaves. The flowers are ten toothed and white, with a length of 12–19 cm. The plants grow upright at first, then incline downward, and bloom from early summer to late autumn. The fruit is an egg-shaped spiny capsule with a diameter of approximately5 cm. Atropine, scopolamine, hyoscyamine, withanolides (lactones), and other tropanes are among the active factors of *D. innoxia*.

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

Although the genus *Withania* is well-known for withanolide compounds, our observation of significant broad-spectrum antibacterial properties of dinoxin B withanolide [8] from *D. innoxia* prompted us to compare the drug likeness and antibacterial properties of different withanolides obtained exclusively from *D. innoxia* using *in-silico* methods. This was undertaken to bring awareness to the therapeutical importance of this species and to preserve this plant from being destroyed on a large scale [9,10]. As computational studies promote the effective selection of drug molecules, this research also helps to select the best compound for further clinical analysis.

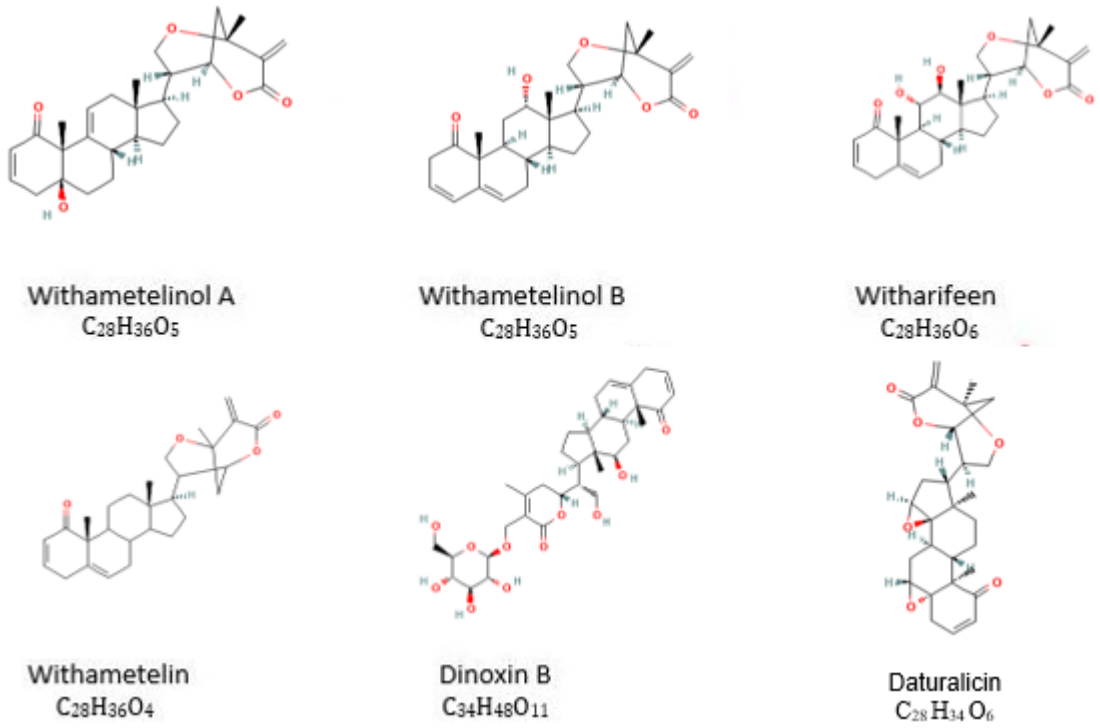


Fig. 2. Molecular structure of withanolides of *D. innoxia* retrieved from PubChem

A review of the literature, as well as Pubchem data [11], showed that withametelinol A [12], withametelinol B [12], witharifeen [13], daturalicin [13], withametelin [14], dinoxin B [8] and daturacin [15] are the identified withanolides of *D. innoxia* (Fig. 2). Since the structure of daturacin is not available in Pubchem data ,this withanolide is not considered for this study.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 doing so provides a rational new approach to study the antibacterial properties of drugs. Furthermore, evaluations of the pharmacological properties, as per Lipinski's rule of five, drug likeness, bioactivity, and drug score were all performed. In drug discovery, pharmacokinetic properties were evaluated to analyze the interaction of the bioactive compound from the time of administration to absorption, distribution, metabolism, excretion, and 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 the chemo-informatics screening notably proved the effectiveness of dinoxin B, its antibacterial properties were evaluated using pathogenic strains of *S. aureus* and *E. coli*.

2. METHODOLOGY

2.1 Physicochemical Properties Prediction

The *SwissADME* (https://swissadme.ch) tool was used to examine the molecular properties and drug likeness of withanolides based on Lipinski's rule of five. This rule 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.

2.2 Pharmacokinetic Analysis

In pharmacokinetic analysis(ADMET),while ADME seeks to maximize the pharmacological performance of a small molecule, toxicology(T) aims to insure that it causes no harm in any kind of side effect [17]. In the present study, the obtained scores were 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 extensive *in vitro* and *in vivo* studied compounds present in its database.

2.3 Docking Studies

A docking study undertaken 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 toward *S. aureus* and *E. coli*. As shown in Table 1, different proteins responsible for resistance mechanisms were retrieved from the protein data bank, and ligands (withanolides) were retrieved from PubChem (Table 2).

2.4 Extraction and Identification of Dinoxin B from *D. innoxia*

Following the protocol of Tandon et al. [8], ethanolic leaf extracts of *Datura innoxia* were fractionated using a single solvent system through column chromatography [7]. 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.From the collected sequence of fractions,fraction four [8] was taken for the identification of Dinoxin B through Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS) . LC-ESI-MS was done from the Central Drug Research Institute of India, Lucknow.

2.5 Agar Diffusion Assay

Following the Kirby–Bauer diffusion technique [19], we conducted an agar well plate method to assess the antibacterial property against clinical strains of *S. aureus* (U-6151)and *E. coli* (U-6081) isolated from urine samples of patients, including methicillin-resistant *S. aureus* (U-6089)and multi-drug resistant *E. coli* (U-6090). All isolates were obtained from the Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India, and the inhibition zones (ZOI) were then measured. Gentamicin (85 mg) and 10% Dimethylsulfoxide (DMSO) were used as positive and negative controls. Assays were done in triplicate, and the results were expressed as mean ± standard deviation.

2.6 Macrobroth Dilution for Determining MIC and MBC

Using the two-fold serial dilution procedure, different concentrations of chromatographically purified fraction four of ethanolic leaf extract were obtained and combined with 100 µl of the test organism to achieve a final inoculum concentration of 5×10^5. The minimum inhibitory concentration (MIC) value was defined as the highest dilution that inhibited bacterial growth. The growth control was the bacterial inoculum without a tested percentage, while the sterility control was the bacterial inoculum itself.

Table 1. Details of selected proteins retrieved from the protein data bank

Proteins	(PDB ID)	
	S. aureus	***E. coli***
Penicillin Binding Protein	3 HUM	4BJP
DNA Gyrase	2XCT	1AB4
Efflux Pump Proteins	4 LLL	5ENO
Quorum Sensing Regulators	4G4K	2AVX

Table 2. Selected with anolides with PubChem ID.

Ligands	PubChem ID
Withametelinol A	15550331
Withametelinol B	101160729
Witharifeen	12135064
Withametelin	364746
Dinoxin B	51041991
Daturalicin	12135065
Withanolide A	11294368
Withaferin A	265237

Minimum bactericidal concentration (MBC) was determined by subculturing each MIC tube 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. RESULTS AND DISCUSSION

Herbal drug formulation depends on its high biological potentiality and low toxicity. For oral absorption in terms of permeability, Lipinski and collaborators [20] have proposed that orally active compounds should fit at least three of the observed four parameters—molecular weight < 500 g mol^{-1}, log*P* < 5; number of hydrogen bond acceptors <10; number of hydrogen bond donors <10; and the well-known Lipinski's rule of 5 (Ro5). That is, Ro5 indicates a physicochemical space in which molecules outside its domain have a low probability of becoming orally active [21]. The SwissADME was employed to study Lipinski's rules for withanolides, and it was noted that all selected compounds were orally active compounds, as they satisfy more than three Ro5 parameters (Table 3).

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

Human intestinal absorption (HIA) 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 when they have an absorption rate of over 90% (HIA 70–100%). 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 properties. As shown in Table 4, the absorption rate of with ametelin was higher in all cases.

In distribution, predicting blood–brain barriers (BBB) is done to indicate whether compounds pass across blood-brain barriers. Central Nervous System(CNS)-active compounds must pass across the BBB, while CNS-inactive compounds must not pass across this barrier [24]. All withanolides with less BBB permeability are considered CNS inactive compounds, which promote its drug likeness with fewer CNS side effects.

It is generally assumed that only a free drug can cross membranes and bind to the intended molecular target [25], and thus it is important to estimate the fraction of drugs bound throughplasma protein binding (PPB). Drugs with greater PPB values than 90% indicate that they are strongly bound to plasma proteins. All withanolides in the present study showed remarkable PPB efficiency; notably, withametelin showed a 100% binding affinity (Table 5).

Table 3. Results showing physiochemical properties to predict the drug likeness of selected withanolides based on Lipinski's Ro5. TPSA: total polar surface area; nV: No. of Ro5 violations; ROTB: no. of rotatable bonds; MV: molecular weight.

Compound	TPSA (A^0)	(H bond Acceptors	H-bond Donors	cLogP	nV	nROTB	MV
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
Dinoxin B	183.2	11	6	1.71	3	7	632.75
Daturalicin	77.67	6	0	4.13	0	1	466.6
Withanolide A	96.36	6	2	4.15	0	2	470.61
Withaferin A	116.5	7	3	3.18	0	2	486.61

Table 4. Results of absorption properties of withanolides obtained from PreADMET

	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	30.68	96.38	-1.86	0.05
Withametelinol B	28.31	96.39	-2.95	0.10
Witharifeen	21.68	94.78	-3.79	0.18
Withametelin	46.74	97.40	-2.21	0.05
Dinoxin B	20.50	92.58	-4.69	0.07
Daturalicin	23.51	97.62	-2.38	0.06
Withanolide A	22.00	94.74	-2.63	0.05
Withaferin A	20.91	90.40	-3.75	0.15

Table 5. Results showing the distribution property of withanolides in pharmacokinetics

Compound	**Buffer Solubility (mg/L)**	**Pure water Solubility (mg/L)**	**BBB**	**Plasma Protein Binding**
Withametelinol A	79.83	33.51	0.19	96.54
Withametelinol B	47.51	28.41	0.09	89.49
Witharifeen	55.05	18.49	0.14	86.89
Withametelin	73.47	40.51	0.31	100
Dinoxin B	66.44	50.09	0.05	91.02
Daturalicin	52.54	32.14	0.17	93.54
Withanolide A	33.71	35.23	0.34	91.59
Withaferin A	3.31	33.59	0.16	82.41

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) significantly proved their drug likeness.

To improve the selection of drug compounds, knowledge about the interaction of molecules with cytochrome P450 (CYP) is essential [26]. It has been proposed that CYP can synergistically metabolize minute compounds to promote tissue and organism protection. Five main isoforms are thought to be the substrate of 50–90% of therapeutic compounds (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4). The 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 a drug or its metabolites [27]. SwissADME enables the estimation of the withanolides to the substrate of P-gp or the inhibitor of the most important CYP isoenzymes. *In silico* data estimated that the selected withanolides could not metabolize (non-substrate) by CYP 450 2D6 and were the substrate of CYP 450 3A4 non-inhibitors for CYP 450 2C19 and CYP 450 2D6, and the inhibitor of CYP 450 3A4 (Table 6). The noninhibition of cytochrome P450 was shown to help in the metabolism of these compounds.

The Ames test is a simple method for determining a compound's mutagenicity that was proposed by Dr. Bruce.N.Ames;1979 [28]. 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, the *in vitro* Ames test results in the TA100, TA10010, TA153510, and TA1535 strains did not show metabolic activation using rat liver homogenate. The Ames toxicity results proved that all withanolides in the present study were non-mutagenic, with negative results (Table 7).

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 shown 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. The results justified the antibacterial assays obtained, in which the binding affinity of withametelin [14] and dinoxin B [8] were shown to be bacteriostatic. Docking results showed that dinoxin B possessed a significant binding affinity to membrane proteins compared to DNA gyrase (1 AB4 and 2XCT) with docking score 6.504 with Efflux Pump Protein(EPP) of E.coli(5ENO) and 5.92 with Penicillin Binding Protein(PBP) of S.aureus (3HUM). (Fig. 2). Notably, this binding score was higher than withanolide A and withaferin A. As a common target, all withanolides in the present study showed better affinity toward PBPs (3HUM and 4BJP) and EPPs of *E. coli,* which in turn indicated membrane protein interaction as the reason for the antibacterial mode of action (Fig. 3).

Table 6. Result of metabolism prediction of withanolides using SwissADME

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

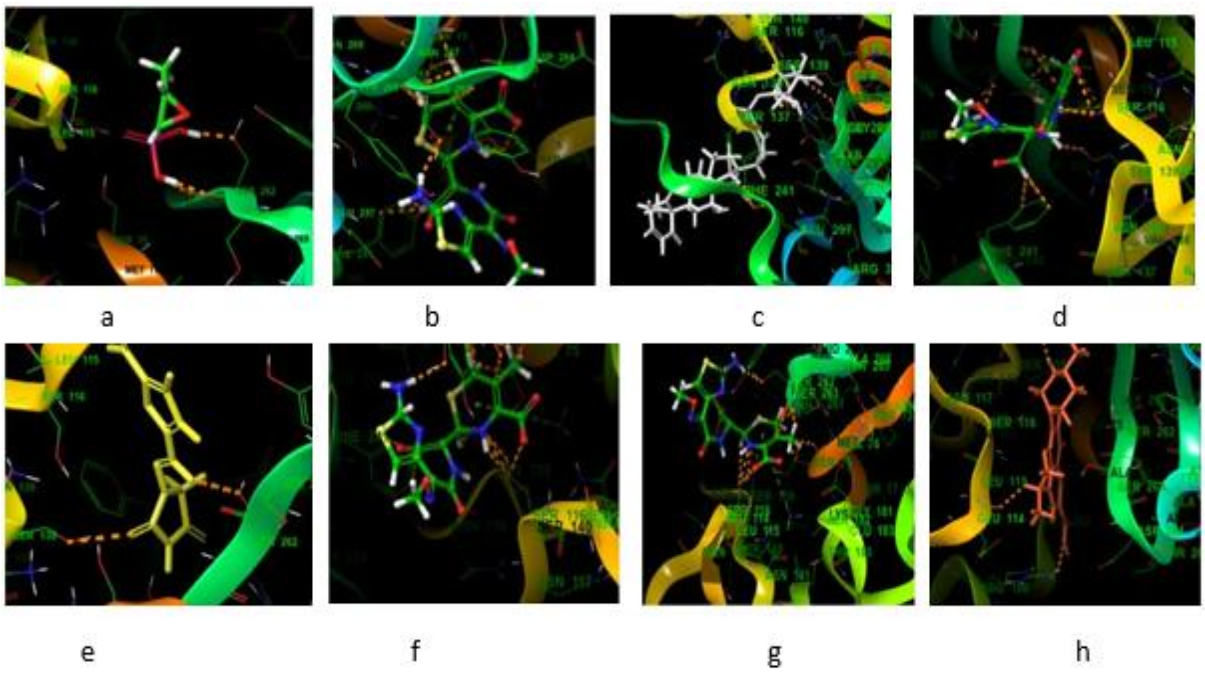
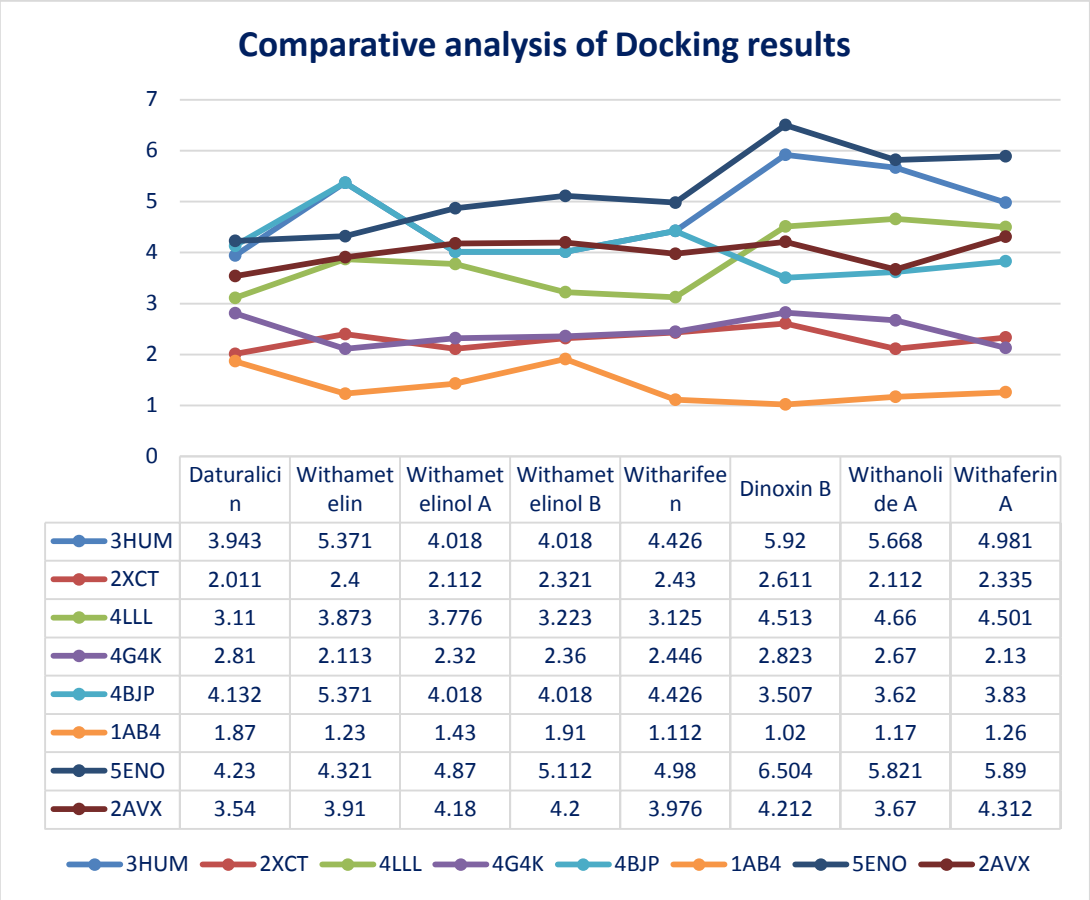


Fig. 3. The results show the best protein–ligand interaction in the docking study: (a)Withametelin (b)Withanolide A and (c) Dinoxin B with 3 HUM(Penicillin Binding Protein of *S. aureus*). (d)Withametelin with 4 BJP (Penicillin Binding Protein of of E.coli). (e)Withanolide A (f)Withaferin A (g)Withametelinol B and (h) Dinoxin B with 5ENO(Efflux Pump Protein of *E. coli*)



	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 results of withanolides of *D. innoxia*

Table 7. Result of toxicity prediction of withanolides based on Ames test using SwissADME

Compounds	Ames Test	Oral Rat Acute Toxicity	Oral Rat Chronic Toxicity	TA10010 RLI	TA100 NA	TA153510 RLI	TA1535 NA
Withametelion A	non-mutagen	2.09	0.34	negative	negative	negative	negative
Withametelion B	non-mutagen	2.23	1.71	negative	negative	negative	negative
Witharifeen	non-mutagen	2.31	1.68	negative	negative	negative	negative
Withametelin	non-mutagen	2.04	1.17	negative	negative	negative	negative
Dinoxin B	non-mutagen	3.55	2.86	negative	negative	negative	negative
Daturalicin	non-mutagen	2.72	1.84	negative	negative	negative	negative
Withanolide A	non-mutagen	2.91	1.74	negative	negative	negative	negative
Withaferin A	non-mutagen	3.50	2.15	negative	negative	negative	negative

As we reported in a previous study [8], ethanolic extraction of *D. innoxia* leaves were fractionated through column chromatography. And fraction four was analyzed using LC-ESI-MS. This mass spectrum (Fig. 5) also depicted the presence of dinoxin B withanolide and its aglycone. Phytoconstituents were 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.

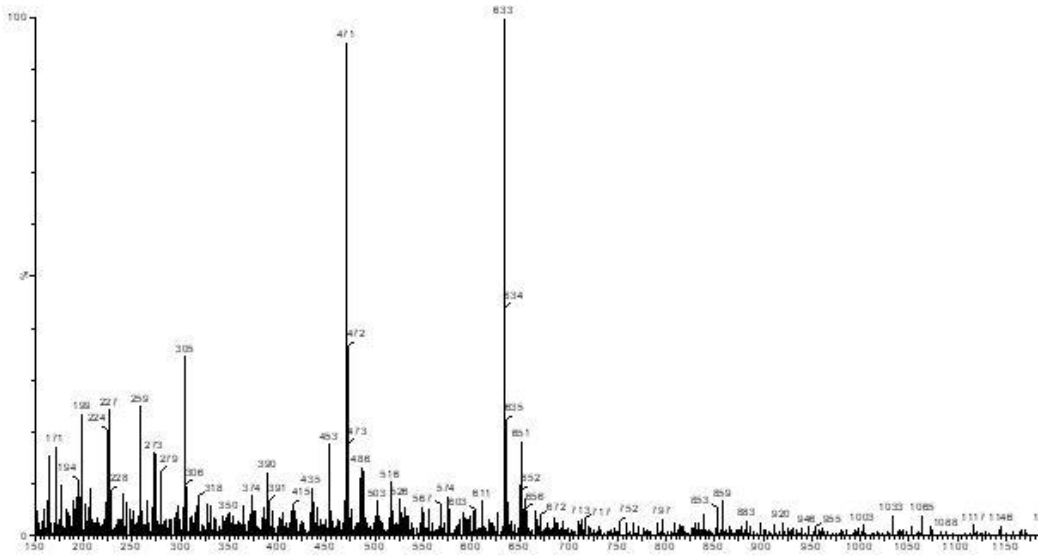


Fig. 5. LC-ESI-MS spectrum of most active fraction four showing the presence of dinoxin B withanolide and its aglycone. The phytoconstituents were eluted as M-glucose-water+H^+ (*m/z*471) and dinoxin B withanolide (*m/z* 633)

Table 8. ZOI (mm) for dinoxin B. Data is in triplicate and represented as mean ± SD. < 12mm (resistant); < 13–14mm (intermediate), and > 15mm (susceptible); shown as (R), (I), and (S)

S. aureus	Different conc. Of Fraction 4 (µg/ml)				Gentamicin	DMSO
	12,500	25,000	50,000	100,000	(85,000) (µg/ml)	(10%)
U-6151(*S.aureus*)	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(*E.coli*)	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*E.coli*)	0(R)	0(R)	9.1±0.28(R)	14.6±.28(S)	14.2±0.28(S)	0

Table 9. Results of MIC, MBC, and MIC/MBC. Data is in triplicate and represented as mean ± SD. nd: not determined

S. aureus Strains	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC Ratio	Bactericidal(+) Bacteriostatic(-)
U-6151(*S. aureus*)	12.5±0.00	25.0±0.00	2	+
U-6081(*E.coli*)	12.5±0.00	25.0±0.00	2	+
U-6090 (MDR-*E. coli*)	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

In our recent paper,antibacaterial potentiality of Dinoxin B against pathogenic S.aureus was published [29]. In this study,the broad -spectrum inhibitory potential of dinoxin B was observed through agar well diffusion assay using different concentrations of fraction four in µg/ml (100,000, 50,000, 25,000, and 12,500) and compared to the control (DMSO) and gentamicin as reference antibiotics. As per the Kirby–Bauer test [30], *S. aureus* susceptibility based on the ZOI was evaluated (< 12 mm [resistant]; <13–14 mm [intermediate]; and > 15 mm [susceptible]). As shown in Table 8, clinical strains of *S.aureus* (U-6151) and *E.coli* (U-6081) isolated from urine samples, including MRSA (U-6089) and MDR strain of *E.coli* ((U-6090), showed significant activity ($p < 0.05$), which was comparable to the reference antibiotic at a higher concentration of dinoxin B (100,000 µg/ml, 50,000 µg/ml). Whereas the *E.coli* strains at 25,000 µg/ml and 12,500 µg/ml, as well as MRSA at 12,500 µg/ml showed low levels of susceptibility. Susceptibility

decreased with a decrease in concentration, which showed the impact of dinoxin B in higher concentrations. The ZOI varied (Table 8) in the range of (mm) 0–15 (1250 µg/ml), 0–18 (25,000 µg/ml), 9.1–20.3 (50,000 µg/ml), and14.6–23.3 (100,000 µg/ml), in which MDR (U-6090) showed higher resistance. Dinoxin B showed higher susceptibility to the methicillin-resistant strain (U-6089) than did gentamicin, with a 22.5 mm ZOI.

Antibacterial effectiveness was evaluated through MIC assay, in which the maximum dilution of dinoxin B that slowed 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 of *E.coli* (50±0.00). With a lower MIC (12.5±0.00) against UTIs, dinoxin B can be considered a potent phytocompound. The growth of bacteria was not inhibited in the negative controls. The minimum bactericidal concentration (MBC) of dinoxin B was found to be 25±0.00 mg/ml by the absence of bacterial colonies on fresh Muller–Hinton agar plates.

The MBC/MIC ratio ≤ 2 indicated bactericidal effects, and the MBC/MIC ratio ≥ 4 indicated bacteriostatic effects. Accordingly, dinoxin B was found to have bactericidal effects against all tested isolates of *S. aureus* except the MDR strain (U-6090), as shown in Table 2.

4. CONCLUSION

Withanolides are a class of active compounds widely found in *Solanaceae* plants. In traditional applications, they have a long history and a wide range of uses. With the progress of drug structure and pharmacological research, reports increasingly point to their excellent pharmacological effects. Based on the pharmacological action of inhibiting bacterial resistance, withanolides have become a research hotspot in natural medicine. The use of *in silico* results allowed us to conclude that dinoxin B and withametelin can be considered drug candidates due to their relevant drug likeness and adequate pharmacokinetic features. Dinoxin B, with its significant antibacterial properties, emphasizes the therapeutic potential of *D. innoxia*.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors are thankful to Pro-Vice-Chancellor, Amity University Uttar Pradesh, Lucknow Campus; Prof (Dr.) J K Srivastava, Head of Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Dr. Manoodeep Sen, Associate professor, Ram Manohar Lohia Institute of Medical Science, Lucknow; and Mr.Vinod Devaraj, Application Scientist, Schrodinger Inc. for providing the necessary facilities for experiments and their constant support and encouragement.

DISCLAIMER

The products used for this research are commonly and predominantly used in our area of research and in India. 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. In addition, the research was not funded by the producing company; rather, it was funded by the personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Siddiqui BS, Arfeen S, Afshan F, Begum S. Withanolides from *Datura innoxia*. Chem Inform 2005;36(37).
2. Singh A, Duggal S, Singh H, Singh J, Katekhaye S. Withanolides: phyto-constituents with significant pharma-cological 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. Sarker SD, Latif Z, Gray AI. Natural products isolation: an overview. Nat Prod Isol [Internet]. 2006;864:1–25. Available:http://link.springer.com/10.1007/978-1-61779-624-1.
5. 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.

6. 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).
7. 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.
8. 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.
9. Hudzicki J. Kirby–Bauer disk diffusion susceptibility test protocol author information. Am Soc Microbiol [Internet] 2012;1–13.
   Available:https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro.
10. 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.
11. PubChem. National Library of Medicine [Internet].
    Available:pubchem.ncbi.nlm.nih.gov.
12. Siddiqui SB, Ali Hashmi I, Begum S. Two new withanolides from the aerial parts of *Datura innoxia*. Heterocycles [Internet] 2002;57(4):715.
    Available: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: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.
19. 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.
20. 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.
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. 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.
23. Veleiro AS, Cirigliano AM, Oberti JC, Burton G. 7-Hydroxywithanolides from *Datura ferox*. J Nat Prod. 1999;62(7): 1010–2.
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. Concepts in Biochemical Pharmacology. 1971;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. de Montellano PRO. Cytochrome P450: Structure, mechanism, and biochemistry, fourth edition. 2015;1-905
28. Föllmann W, Degen G, Oesch F, Hengstler JG. Ames Test. Elsevier Inc. 2013;1:104-107.
29. George R, Mathur P, Tandon C. Dinoxin B Withanolide from Datura inoxia Mill as an Effective Phytocompound Against Urinary Tract Infection causing Staphylococcus aureus 2021(14):1707–20
30. 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.

© 2021 George and Mathur; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/77943