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Natural urease inhibitors from *Aloe vera* resin and *Lycium shawii* and their structural-activity relationship and molecular docking study

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ABSTRACT

Bio-assay guided fractionation of the methanolic extract of *Aloe vera* resin and *Lycium shawii* stem successively afforded twenty three compounds; fourteen (**1–14**) from *A. vera* and nine (**15–23**) from *L. shawii*. All these compounds were characterized by 1D and 2D NMR spectroscopic techniques viz., ^{1}H, ^{13}C, DEPT, HSQC, HMBC, and COSY, and NEOSY, ESI-MS and compared with the reported literature. These compounds were assessed for their potential as urease inhibitors targeted in peptic ulcer. Among crude extracts and fractions of *A. vera* resin, *n*-butanol fraction (23.5 ± 1.7 μg·mL^{-1}) showed the most potent urease inhibition followed by methanol (30.9 ± 0.3 μg/mL) and ethyl acetate (31.7 ± 0.5 μg·mL^{-1}). In case of *L. shawii*, ethyl acetate fraction exhibited the highest urease activity (41.0 ± 1.4 μg/mL) trailed by dichloromethane (55.2 ± 1.5 μg/mL) fraction. Among the isolates, compounds 7, **11** and **23** were found to be excellent urease inhibitors with IC_{50} values of 14.5 ± 0.90 μM, (16.7 ± 0.16 μM) and 14.0 ± 0.8 μM, respectively. To the best of our knowledge, this is the first report on the urease enzyme inhibitory activity of the said compounds excluding compound **18**. In addition, the urease activity of different fractions of *L. shawii* stem was also reported for the first time. The molecular docking studies showed that all the active compounds well accommodate in the active site of the urease enzyme by interacting with key amino acids.

1. Introduction

Urease (EC 3.5.1.5) is the enzyme that catalyzes the hydrolysis of urea to yield ammonia and carbon dioxide, which spontaneously hydrolyzes to produce carbonic acid and a second molecule of ammonia at a rate approximately 10^{14} times the rate of the uncatalyzed reaction [1–3]. In recent years, urease has gained much attention in research field as a significant virulence factor implicated in the pathogenesis of several clinical conditions such as pyelonephritis, urolithiasis, ammonia and hepatic encephalopathy, hepatic coma and urinary catheter encrustation, and formation of infection-induced urinary stones [4,5]. Moreover, urease is the major cause of pathologies induced by Helicobacter pylori as this allows bacteria to survive at the low pH of the stomach and hence plays a vital role in producing gastritis and peptic ulcer, which in some cases can ultimately lead to cancer [6,7]. Urease inhibitors (UIs) are known to be potent antiulcer drugs that control the damaging effect of ureolytic bacterial infections in humans and animals [8]. Although many UIs have been described in the past decades, parts of them were prevented from being used in vivo because of their toxicity or instability. Therefore, the search for new sources of natural UIs that provide low toxicity, reduced side effects, bioavailability, and greater stability has gained major priority to overcome the problem [9–12].

For several decades, plants remain the most common source for the treatment of various diseases that provide a great pharmacophore template for new drugs [13]. A notable progress has been observed in the investigation of natural products in recent years, especially plants. Discovery of new bioactive compounds and the detection of highly efficient providers of such substances are the main aspects that have attracted the researcher's consideration to plant metabolites [14,15].

A. vera (L.) Burm. F. (Liliaceae) belongs to genus *Aloe* (600 species) and is currently being employed for the treatment of arthritis, asthma, anti-inflammatory, cosmetics, Crohn's disease, skin irritations, hypoglycemic, antiseptic, ulcerative colitis, ulcers, acne, burns, wound healing, cold, and sores [16–19]. It has also been reported that *A. vera* gel filtrate can be used as an inhibitor of urease, and has good effect on

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treating gastric ulcer or duodenal ulcer [20]

L. shawii Roem and Schult (native plant of the Arabian Gulf region; Solanaceae) belong to genus *Lycium* (90 Species) and is employed as a hypotensive [21], anti-diabetic [22], and antiplasmodial [23]. It is used in traditional medicine to treat mouth sores, constipation, coughs, backache, stomach ache, and fever [24–26]. Furthermore, *L. shawii* extracts are employed to treat jaundice, stomach, mouth sores, and coughs [27]. Several crude extracts from the fruits of *L. shawii* displayed promising antioxidant, anti-plasmodial, anti-inflammatory, anticancer and an antimicrobial activity against different strains [26,28–30].

Previous findings investigated the biological activities of different parts (leaves and fruits) of *L. shawii* but, to the best of our knowledge, this is the first report on the urease inhibition of *L. shawii* stem and *A. vera* resin fractions. The present study aims to investigate the phytochemical composition of these plants which resulted in the isolation of twenty three compounds and were further screened for urease inhibition.

2. Experimental section

2.1. General instrumentation

NMR spectra were recorded on a NMR spectrometer (BRUKER) operating at 600 MHz with cryoprobe prodigy (150 MHz for ^{13}C; chemical shift (δ) = ppm; coupling constants (J) = Hz). Infrared (IR) spectra were recorded on Attenuated Total Reflectance (ATR-Tensor 37 spectrophotometer, Bruker). ESI-MS spectra were recorded on a Mass Spectrometer (Waters Quattro Premier XE, Waters, Milford, MA). For Thin Layer Chromatography (TLC, silica gel 60F-254, E. Merck), precoated aluminum sheets were used. TLC plates were visualized under the UV light at 254 and 366 nm and mostly sprayed with the ceric sulfate ($Ce(SO_4)_2$) reagent followed by heating.

2.2. Plant collection and identification

The stem of *L. shawii* and resin of *A. vera* were purchased from Souq and identified by the plant taxonomists Mr. Saif Al-Hatmi (Oman Botanical Garden, Muscat (OBGM)) and Dr. Syed Abdullah Gillani (Department of Biological Sciences and Chemistry (DBSC), University of Nizwa, the Sultanate of Oman, respectively. Voucher specimens of *A. vera* (No. AFS-08/2016) and *L. shawii* (No: BSHR-05/2015) were deposited in the herbarium of OBGM and DBSC, respectively.

2.3. Fractionation and isolation

The air dried powdered resin of *A. vera* was exhaustively extracted with methanol at room temperature. Evaporation of the MeOH in *vacuo* at 45 °C yielded a crude methanol extract, which after suspension in water was successively fractionated into *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH [18]. The combined material of CH_2Cl_2 and EtOAc, similar in chemical nature, was applied to a silica gel chromatographic column (70–230 mesh; Merck) and eluted with *n*-hexane, *n*-hexane–EtOAc, EtOAc, EtOAc/MeOH, and pure MeOH with 10% increments in polarity to afford several fractions which were subsequently subjected to further repeated column chromatography with mixtures of *n*-hexane-EtOAc-MeOH as eluent to obtain eleven compounds **1–14**.

The air-dried stem powder of *L. shawii* was exhaustively extracted with MeOH for two weeks. The resulting methanol extract was suspended in water and successively portioned to provide *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH and H_2O fractions. The *n*-hexane fraction was subjected to column chromatography which was resulted in the isolation of two compounds **15** and **16** [26]. Similarly the EtOAc fraction was loaded on column chromatography (CC) and eluted with an increasing polarity, viz., *n*-hexane–EtOAc, EtOAc, EtOAc–MeOH and pure MeOH to isolate seven compounds **17–23** [31].

2.4. Urease enzyme inhibition

The reaction were performed in 96-well plates with total volume of of 200 µL, which consisted of 25 µL of urease enzyme of Jack bean (*Canavalia ensiformis*) source, incubated with 5 µL of test compounds (0.5 mM) and 55 µL of substrate urea (100 mM) in phosphate buffer of 4 mM at pH 6.8. Incubation was carried out in 96-well plates for 15 min at 30 °C. The conjugates such as, 45 µL phenol reagent (0.005% w/v sodium nitroprussside and 1% w/v phenol), and 70 µL of alkali reagent (0.1% w/v NaOCl and 0.5% w/v NaOH) were added to each well. Urease activity was monitored through the indophenols method by measuring ammonia production, as described by Weatherburn [32]. The absorbance was continuously measured for 50 min at 630, in a microplate reader (SpectraMax M2, Molecular Devices, CA, USA). Thiourea was used as the standard inhibitor of urease [33,34]. Finally the results were processed by SoftMax Pro (Molecular Devices, CA, USA). The IC_{50} values were calculated by using different concentration of test compound. The results were processed by MS-Excel and Ez-fit software programs. The Percent inhibition was calculated from their optical density (OD) using the formula given below:

$$\% \text{ Inhibition} = 100 - (\text{OD test}/\text{OD control}) \times 100.$$

2.5. Molecular docking

Molecular docking study was performed in order to predict the binding mode of the active isolated compounds in the active site of urease enzyme using MOE (Molecular Operating Environment) [35]. The three dimensional structures of the isolated compounds were built using the builder tool implemented in MOE software (www.chemcomp.com). The generated compounds were 3D protonated and energy minimized using the default parameters of the MOE (gradient: 0.05, Force Field: MMFF94X). 3D structure of the target protein was retrieved from the protein databank (PDB ID 4UBP), the solvent molecules were removed and 3D protonation was carried out. To get a stable conformation of the protein molecule, 3D protonation of the protein was energy minimized using the default parameters of MOE. For docking studies the parameters of MOE used were Placement: Triangle Matcher, Rescoring 1: London dG, Refinement: Forcefield, Rescoring 2: GBVI/WSA. For each ligand 10 conformations were allowed to be formed and the top ranked conformations on the basis of docking score were selected for further analysis.

3. Results and discussion

3.1. Isolation of compounds 1–23

Previous phytochemical investigation of *A. vera* resin led to the isolation of fourteen compounds including 10-hydroxy aloin A (**1**) [36], aloinoside B (**2**) [37], one coumarin, 7-demethylsiderin (**3**) [38], 6′-*O*-coumaroylaloesin (**4**) [39], 7-methoxy-6′-*O*-coumaroylaloesin (**5**) [40], feroxidin (**6**) [41], feralolide (**7**) [40,42], Aloe emodin 11-*O*-rhamnoside (**8**) [43], 3-(4-hydroxyphenyl)propanoic acid (**9**), methyl 3-(4-hydroxyphenyl)propionate (**10**) [44], 1-(2,4-dihydroxy-6-methylphenyl) ethanone (**11**) [45], *p*-anisaldehyde (**12**), salicylaldehyde (**13**) [46], and *p*-cresol (**14**) [47,48] (Fig. 1). Similarly, phytochemical investigation of *L. shawii* stem provided nine compounds; dehydrocostus lactone (**15**), costunolide (**16**), lyciumate (**17**), catechin (**18**), aloe emodin (**19**), emodin (**20**), chrysophanol-8-*O*-*β*-D-glucoside (**21**), aloe emodine 11-*O*-D-rhamnoside (**22**) and lyciumaside (**23**) [27,32] (Fig. 2).

3.2. Urease enzyme inhibition

All the crude extracts and isolated compounds (**1–23**) were subjected to urease enzyme inhibition potential, in order to know the anti-ulcer activity of these plants. All the results are presented in Table 1 and

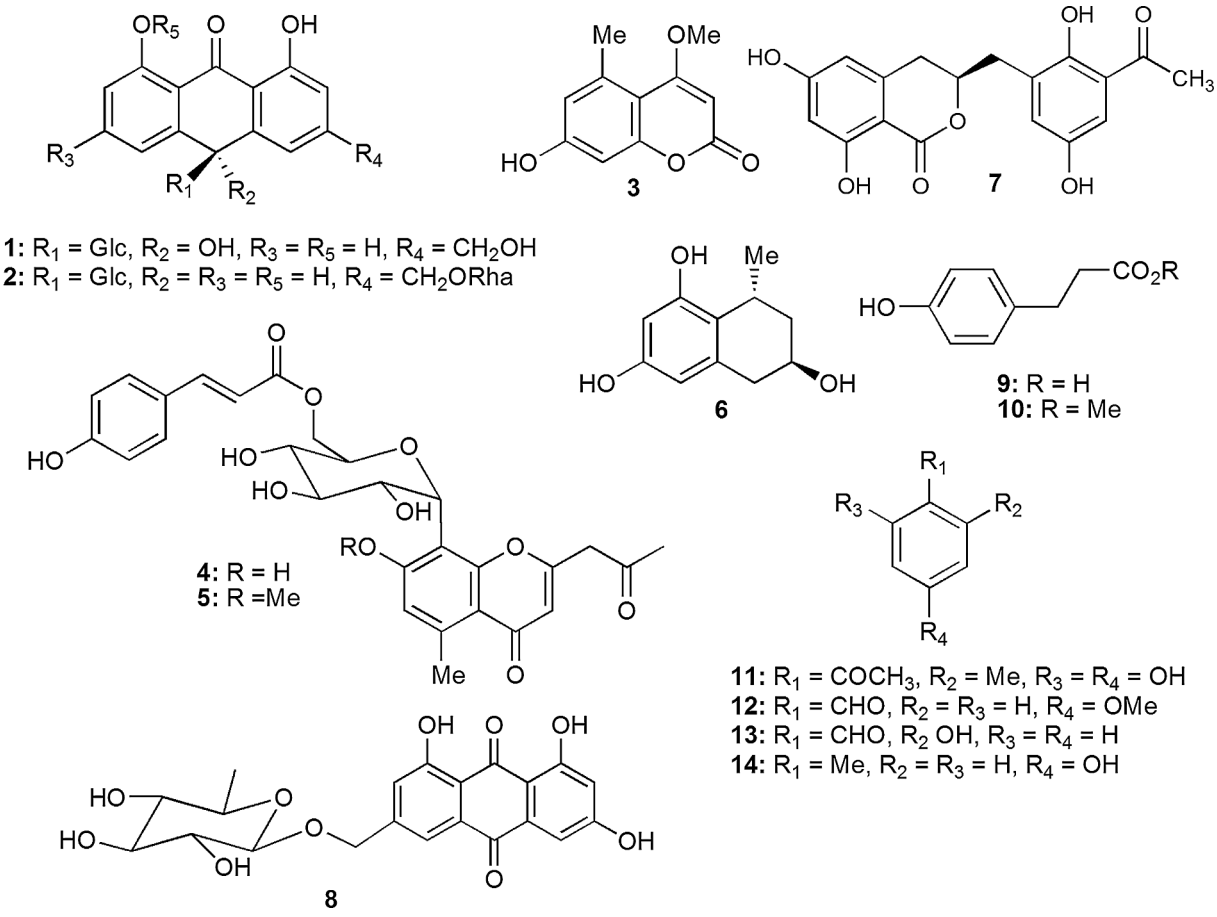

Fig. 1. Structures of the compounds 1–14 isolated from *A. vera* resin.

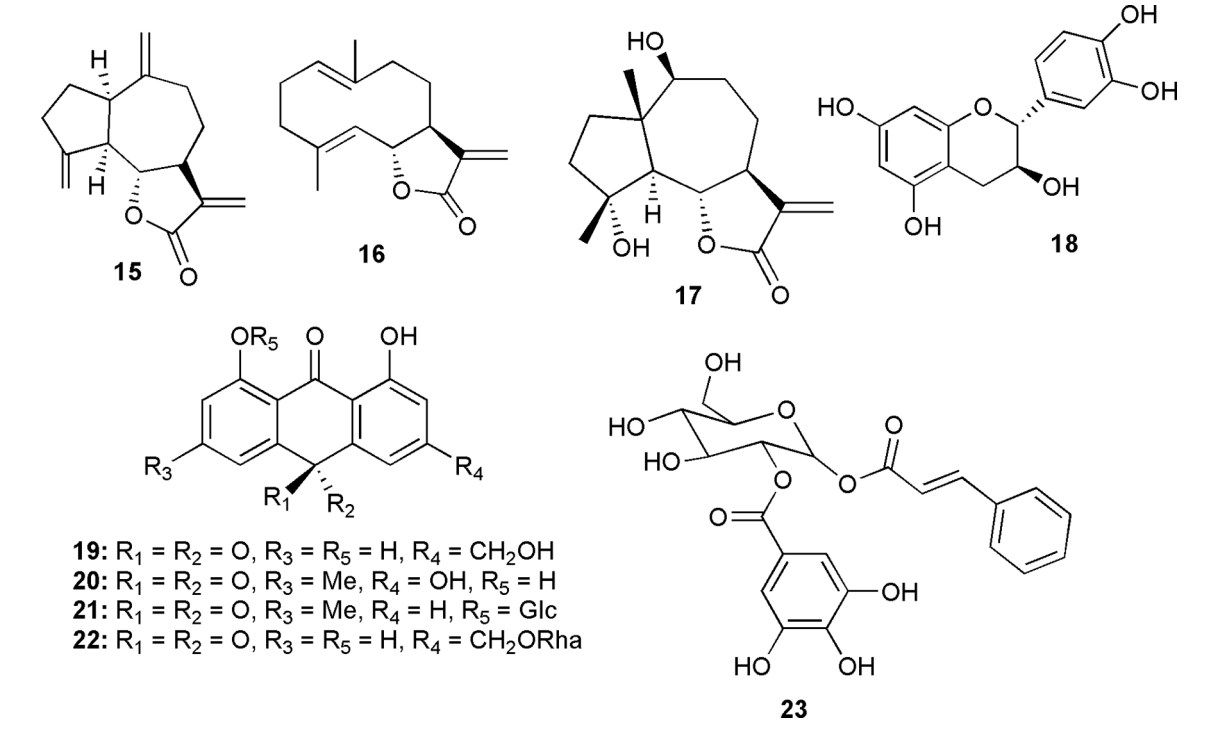

Fig. 2. Structures of the compounds 15–23 isolated from *L. shawii.*

Table 1
Urease inhibition activity of different fractions of *A. vera* and *L. shawii*.

Code	% inhibition	IC_{50} ± SEM
A. vera resin		
MF	91.3	30.9 ± 0.33
EF	85.6	31.7 ± 0.57
DF	94.2	37.4 ± 1.25
WF	68.2	NA
HF	43.6	NA
BF	85.6	23.5 ± 1.72
L. shawii		
MF	55.5	168.2 ± 3.52
EF	92.7	41.0 ± 1.49
DF	87.0	55.2 ± 1.52
WF	30.5	NA
HF	16.0	NA
BF	58.0	158.2 ± 4.23

IC_{50} = μg mL^{-1}; concentration = 0.5 mM; NA = not active; BF = *n*-butanol, MF = methanol, WF = aqueous, HF = *n*-hexane, EF = ethyl acetate, DF = dichloromethane.

Table 2
Urease inhibition of active constituents from *A. vera* resin and *L. sahwii*.

Code	% Inhibition	IC_{50} = μM ± SE
1	46.9 ± 1.19	NA
2	45.7 ± 0.98	NA
4	91.3 ± 0.95	25.6 ± 0.96
5	93.5 ± 1.54	37.3 ± 0.99
6	91.3 ± 0.87	39.3 ± 0.82
7	88.7 ± 0.98	14.5 ± 0.90
8	53.6 ± 1.23	416.4 ± 7.5
11	91.5 ± 1.25	16.7 ± 0.16
16	56.5 ± 1.34	435.1 ± 1.4
18	81.5 ± 1.36	36.8 ± 0.6
19	83.5 ± 1.05	114.9 ± 1.74
20	46.9 ± 1.84	NA
23	83.5 ± 0.54	14.0 ± 0.8
Thiourea	98.0 ± 0.52	21.0 ± 0.01

NA = not active.

2. During preliminary screening the crude extracts and fractions of *A. vera*, *n*-butanol fraction (*n*-BeOH, IC_{50} = 23.5 ± 1.7 μg mL^{-1}) exhibited promising urease inhibition followed by methanol (MeOH, IC_{50} = 30.9 ± 0.3 μg/mL) and ethyl acetate (EtOAc, IC_{50} = 31.7 ± 0.5 μg mL^{-1}). In case of *L. shawii*, EtOAc fraction exhibited highest activity (IC_{50} = 41.0 ± 1.4 μg/mL) trailed by dichloromethane (CH_2Cl_2, IC_{50} = 55.2 ± 1.5 μg mL^{-1}) (Table 1). Among the isolated compounds from *A. vera* resin, compound **7** exhibited significantly potent inhibition (14.5 ± 0.90 μM), against urease enzyme followed by **11** (16.7 ± 0.16 μM), even better than standard (Thiourea, IC_{50} = 21.0 ± 0.01 μM). Compounds **4–6** also displayed significant (IC_{50} = 25.6 ± 0.96 μM, 37.3 ± 0.99 μM, and 39.3 ± 0.82 μM, respectively) urease inhibitory activities comparable to standard, while compound **8** displayed weak activity having IC_{50} value of 416.4 ± 7.5 μM. Similarly, in case of *L. shawii*, compound **23** displayed excellent urease inhibition with IC_{50} value of 14.0 ± 0.8 μM, superior than standard thiourea followed by **18** (IC_{50} = 36.8 ± 0.6 μM) and **19** (114.9 ± 1.74 μM) (Table 2). Compound **16** showed weak activity with IC_{50} value of 435.1 ± 1.4 μM almost similar to compound **8**.

3.3. Structural-Activity Relationship (SAR)

The possible SAR study showed that both compounds **7** and **11** have acetone group attached to benzene ring at the *ortho* position of hydroxyl group. The higher activity of compound **7** when compared with **11** may be due to the presence of extra cyclic ester group and OH groups (four) present in the molecule. Compound **23** has six numbers of OH groups, two straight chain esters and one sugar moiety. The potency of **23** can be attributed to the presence of one sugar moiety and OH groups compared to **7**. Compound **4** displayed relatively higher activity than compound **5** which can be due to the presence of one extra −OH group attached to benzene ring at C-7 position instead of $-OCH_3$ in compound **5**. Among anthraquinones, compound **8** has weaker activity compared to **1** and **2** possibly due to the presence of extra OH group directly attached to benzene ring at C-3 position. The presence of −OH groups attached in *ortho* and *para* positions with respect to the acetyl group accounts for the high activity of compound **11**.

3.4. Molecular docking

To predict the inhibition mechanism of isolated compounds shown by the kinetics study, molecular docking of the active compounds was carried out with the crystal structure of urease enzyme. The most favorable docking conformations of all compounds were observed inside the active site with proper orientation. The active site consists of both the hydrophobic and hydrophilic amino acids.

The hydrophilic amino acids included Glu166, 223, Arg339, His323, 324, Asp224, 363, and Asp494 while hydrophobic part was composed of Lys169, Ala170, 366, Leu319, Cys322 and Met 637. The two Ni ions also played a vital role by linking the key amino acid and ligands. It has been observed that almost all the conformations of all the ligands showed interactions with key residues inside the pocket. The docked poses were ranked by the scores from the GBVI/WSA binding free energy calculation. The most promising docked conformation of

Table 3
Docking scores (S) and interactions detail of the active compounds.

Code No.	Docking Score	Interactions Detail								
		Ligand			Receptor			Interaction	Distance	E (kcal/mol)
4	−8.7508	O2	33	SG	CYS	322	(C)	H-donor	3.31	−1.2
		O	37	CA	ALA	366	(C)	H-acceptor	2.85	−1.0
5	−7.8270	O2	33	SD	MET	318	(C)	H-donor	2.55	0.4
		O	9	NI	Ni	798	(C)	metal	1.70	−1.0
6	−7.6474	O2	27	NI	NI	799	(C)	metal	2.29	−1.9
7	−9.6954	O	4	O	ALA	279	(C)	H-donor	3.38	−0.8
		O	11	OD2	Cys	322	(C)	H-donor	2.48	1.8
		O	23	NI	NI	799	(C)	metal	2.71	−0.9
11	−8.8547	O	13	ND1	HIS	249	(C)	H-donor	3.51	−0.9
		O	13	O	GLY	280	(C)	H-donor	2.55	−0.4
18	−8.5535	O	26	OE2	GLU	223	(C)	H-donor	3.16	−0.7
23	−9.9181	O	10	O	GLY	280	(C)	H-donor	2.78	−2.7
		O	42	ND1	HIS	324	(C)	H-acceptor	2.67	−0.5
		C	31	5-ring	HIS	323	(C)	H-pi	3.08	−1.6

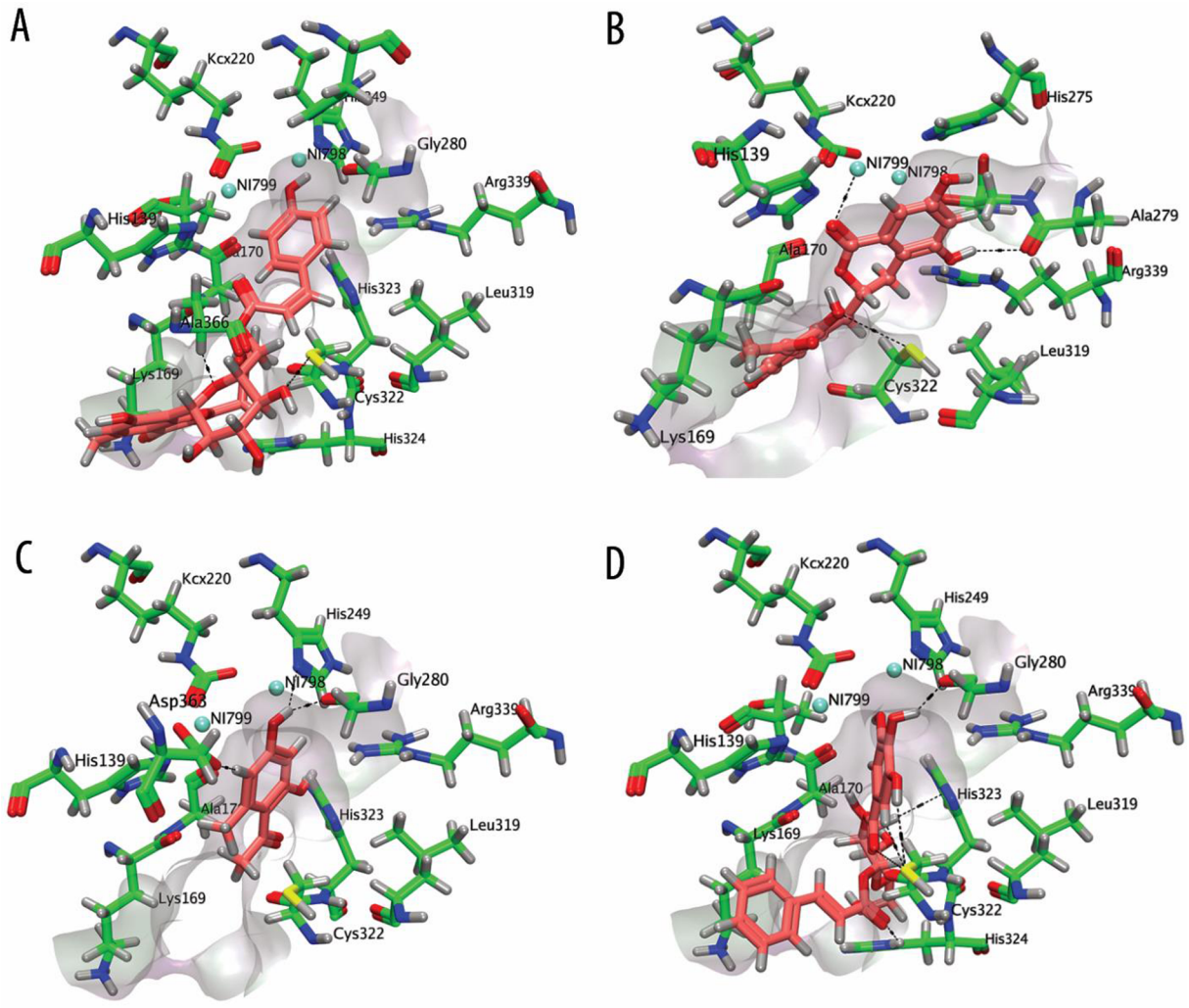

Fig. 3. Three dimensional interaction of compounds 4, 7, 11, 23 with active site residues of urease. Ligands are shown in pink color, key residues of the active site are shown in green stick mode, Hydrogen bonding and other interaction is shown in dark color dotted lines. (A) Hydrogen bonding and polar interaction of compound 4 with the active site residues (B) 3D interactions network of compound 7 (C) 3D interactions network of compound 11 (D) 3D interaction of compound 23.

each compound was further evaluated for binding mode. Detailed docking results are listed in Table 3.

The binding mode of the active compounds with the active site residues showed a healthy assignation with the backbone of the enzyme using hydrogen bonds, polar bonds, pi-pi and pi-H interactions. 3-D interaction of some favorable inhibitors is shown in Fig. 3. Fig. 3A presents the 3D binding mode of compound **4**. The Figure reflects that electron rich groups i.e., O, OH of the molecule by their electron donating inductive effect resulting in a good interaction pattern. The electronic cloud of OH moiety in compound interacts with the SH group of Cys322 and Ala366 is bonded to another electron rich O of the compound through an H-acceptor bonding. Compound **7, 11** and **23** are other active compounds that were found to be very good interactive. The electron rich species O, OH, in compound **7** were found interactive resulting in a good binding mode as shown in Fig. 3**B.** Polar hydrogen of OH group at one end of the compound formed H bonding with Ala279. The hydroxyl oxygen (OH) at the other end of the compound showed polar interaction with sulfur of SH group of Cys322 and carbonyl oxygen (C═O) at the mid part of the compound interacts with the Ni ion that has an important role in the active site of the enzyme. The docking mode of compound **11** depicted the inhibition of catalytic activities of the enzyme slightly in a different way by performing three H-donor interactions with His249, Gly280 and Asp363 (Fig. 3**C**). It was observed that electron rich OH group and aryl H which is induced polar are involved in interactions with the active site residues. The OH hydrogen electrostatically interacts with His249 and Gly280 by forming an H-donor bond. The induced polar hydrogen of aryl group of the compound is located at a distance of 3.36 Å to the carboxylate ion of Asp363 forming an H-donor contact.

Regarding binding mode, compound **23** offered better potential in this in-silico study in accordance to its biological activity. As illustrated in Fig. 3**D,** the electron rich species i.e., OH, O, NH in this compound are predominantly involved in bonding with the active site residues of the enzyme. Gly280 and Cys322 are observed to be involved in H-donor interactions with different OH groups of the compound. Similarly His324 and His323 showed H-acceptor and H-pi contacts with the compound respectively. Similarly moderately active compounds **5, 6** and **18** also presented better results as listed in Table 3.

The docking results complemented well the experimental results based on the multiple interactions of ligands with key residues of the urease enzyme and the docking scores calculated. The docking pose of almost all potent compounds computationally inhibited the catalytic activities of the urease by binding firmly through strong hydrogen bonding, hydrophobic, polar interactions with key residues.

4. Conclusion

Twenty three compounds were isolated from the methanol extracts of *A. vera* (**1–14**) and *L. shawii* (**15–23**) through bio-assay guided

fractionation. Among them, seven compounds (**4–7**, **11**, **18** and **23**) showed promising inhibition having IC_{50} value ranging from 14.0 to 39.3 μM. Two compounds **7** (14.5 μM) and **23** (14.0 μM) displayed the most potent activity when compared to the standard drug (Thiourea). This is the first report on the urease inhibition of these compounds except **18.** The molecular docking report revealed that all the active constituents were well accommodated in the active site of the enzyme. This study concludes that the most active urease inhibitors (**7** and **23**) may act as template drugs for diseases associated with hyperactivity of urease.

Conflict of interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.102955.

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