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ORIGINAL ARTICLE

Terfezia claveryi and Terfezia boudieri Extracts: An Antimicrobial and Molecular Assay on Clinical Isolates Associated with Eye Infections

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ABSTRACT

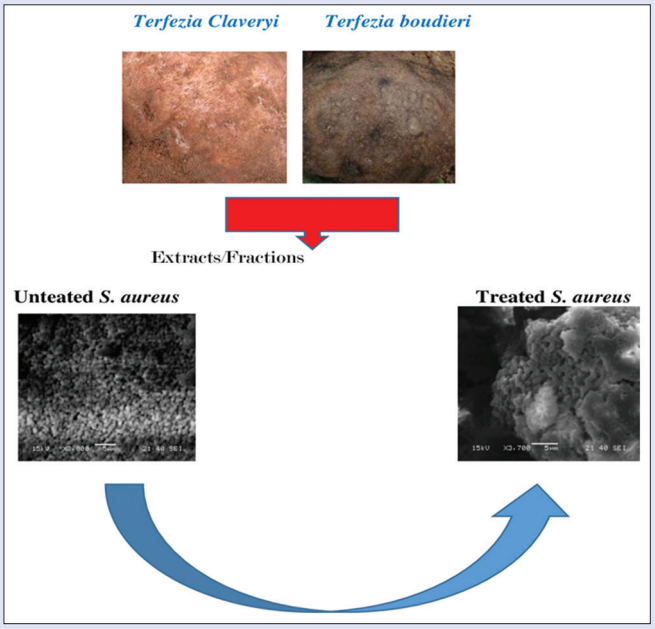
Background: Ocular infections are capable of spreading to different anatomical sites of the eyes and, if not appropriately treated, can lead to blindness. The emergence of difficult to treat microbial infections has led to the search of alternatives from natural sources. **Objectives:** The antimicrobial effects of *Terfezia claveryi* and *Terfezia boudieri* (*T. boudieri*) against bacteria isolates associated with eye infections and their molecular mechanism were investigated. **Materials and Methods:** Crude aqueous and methanolic extracts, including fractions of chloroform, petroleum, and ethyl acetate of *T. claveryi* and *T. boudieri*, were used for the investigation. Bacterial isolation and identification were carried out using basic microbiological and biochemical techniques. scanning electron microscopy. (SEM) and molecular docking were used to adduce possible antimicrobial mechanism of these extracts and their fractions. *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus lugdunensis, Serratia odorifera, Serratia liquefaciens, Pseudomonas stutzeri, Pseudomonas oryzihabitans, Proteus mirabilis, Kocuria kristinae, Kocuria rosea,* and *Micrococcus luteus* were isolated from patients with ocular infections. **Results:** Isolates were resistant to benzylpenicillin (78.0%), rifampicin (57.0%), tetracycline (56.0%), clindamycin (33.3%), and tigecycline (24.0%). Furthermore, the percentage resistance to gentamicin and ciprofloxacin was 13.0% each. All isolates were susceptible to extracts/fractions of *T. claveryi* and *T. boudieri*. Docking analysis showed binding with surface protein Sortase A of *Staphylococcus aureus,* indicating that stigmasterol, the active compound in both *Terfezia* species, interacted with valine amino acid 110. SEM imaging showed morphological alterations in treated isolated Staphylococcal species. **Conclusion:** Therefore, extracts of both *Terfezia* species have demonstrated the potential to possess antibacterial activity, which can be further exploited for clinical use.

Key words: Antimicrobials, extracts, isolates, ocular infections, *Terfezia boudieri*, *Terfezia claveryi*

SUMMARY

- Extracts and fractions of *Terfezia claveryi* and *Terfezia boudieri* exhibited significant antimicrobial activity against sensitive and resistant bacterial clinical eye isolates. Therefore is a potential source of future antibiotic.
- Molecular mechanism shows that it binds Sortase A protein on the surface of *Staphylococcus aureus*.

Abbreviations used: CoNS: Coagulase-negative staphylococci; SEM: Scanning electron microscope; ADT: Autodock tools; PBS: Phosphate-buffered saline; DMSO: Dimethyl sulfoxide; MICs: Minimum inhibitory concentrations; CAZ: Ceftazidime; FEB: Cefepime; AZM: Aztreonam; IMP: Imipenem; MINO: Minocycline; TGC: Tigecycline; PEN: Benzylpenicillin; ERY: Erythromycin; TET: Tetracycline; RIF: Rifampicin; CLI: Clindamycin; NLDO: Nasolacrimal duct obstruction; PUK: Peripheral ulcerative keratitis; WHO: World Health Organization Srt. A: Sortase A.



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INTRODUCTION

Ocular infections such as keratitis, conjunctivitis, orbital cellulitis, endophthalmitis, among a wide range of other eye infections, can be attributed to bacteria.[1-4] Particularly, 50%–70% of conjunctivitis cases are a result of bacterial infections.[5] Such infections might not remain localized but are capable of spreading to other anatomical sites of the eye[6] and consequently lead to corneal blindness or endophthalmitis if not timely treated.[7-9] Even when detected early, empirical management

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can either help in resolving the causative bacterial infection or failure. This is due to the global increase in difficult to treat bacterial infection, including those responsible for eye infections. Recent report[10] observed a growing resistance by Gram-negative bacteria associated with eye infections between 2013 and 2016 as compared to the observations for 2011 and 2013. These have been attributed to an increase in the use of antimicrobials like fluoroquinolones for both prevention and treatment.[11] Furthermore, earlier studies[12] have reported resistance to the fourth-generation fluoroquinolones; this growing resistance was particularly with concerns to moxifloxacin, gatifloxacin and imipenem, carbapenem. Therefore, there is a public health crisis arising as a result of difficult to treat bacterial infections to which the World Health Organization (WHO)[13] warned that the world could be entering an era like those preceding those of the discovery of antibiotics. The "golden era" of antibiotics resulted in the production and rise of antibiotics.[14] It is however of the view that the era ended due to misuse as well as the inability by researchers to keep up with the pace of the discovery of new drugs.[15] Antibiotics are not being produced as fast as they are needed, to meet with the rate at which antimicrobial resistance by bacterial isolates is evolving.

There is, therefore, a global urgency in the search for practicable alternative options. Thus, there is an increase in the search for, and the use of herbal medicinal remedies with about 25-50 % of current pharmaceuticals.[16] An increase in the discovery and use of herbal remedies could lead to interesting possibilities in combating antimicrobial resistance, as noted by researchers globally.[17] More so as these herbal remedies are less toxic when compared to conventional antibiotics.

Although the discovery of antibiotics was a defining moment in the medical management of microbial infections,[18] the advent globally of multi drug-resistant bacteria and the fact that the synthetic production of new antimicrobials has declined over the decades has led to a surge in the alternatives to antibiotics in herbal medicines. For ocular infections, there have been suggestions by researchers[19-21] for the use of dessert truffles as alternatives to currently used antibiotics.

Truffles are ectomycorrhizal fungi belonging to a family of complex hypogenous fungi containing species of which includes *Terfezia claveryi* and *Terfezia boudieri*.[22] Geographically, they are distributed in semi-arid and arid lands[23,24] and have been employed in traditional/folk medicine in Arab communities for over two millennia with no know toxicity to its users. The Bedouins recommend the use of its water extracts for the treatment of common eye infections.[25]

The antimicrobial effects of *Terfezia* species have been reported previously, particularly the aqueous extracts.[26] These reports show that *T. claveryi* extracts is effective against clinical isolates of methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[27,28] The documented efficacy of extracts of *T. claveryi* in curing trachoma disease and treatment of cornea infection in mice is based on its highlighted antibacterial bioactivity.[26,29] In line with the need to search for alternative medications in place of existing antibiotics, the present investigation seeks to look into the clinical bacteria isolates associated with ocular infections, their susceptibility to commonly used antimicrobial, as well as the effect of extracts and fractions of *T. claveryi and T. boudieri* and to elucidate molecularly the mechanism of these effects. This is with a view of providing further insight into the use of extracts and their fractions as alternatives in the prevention or treatment of ocular infections.

MATERIALS AND METHODS

Fungal material

Desert truffles species such as *T. claveryi* and *T. boudieri*, which are usually available in March and April, were procured from a weekly local market in Al-Ahasa located in the Eastern Region of Saudi Arabia. The fresh truffles were dried in the shadows for 21 days while avoiding direct sunlight until constant dried weights were obtained. The resultant dried truffles were grounded into fine powder and stored in a dark, dry place inside containers at ambient temperature until use. Voucher specimens were deposited at the College of Clinical Pharmacy in the Department of Pharmaceutical Sciences, King Faisal University, Al-Ahsa, Saudi Arabia.

Preparation of extracts of *Terfezia* species and fractionation

One hundred grams of fine powder of *T. claveryi* and *T. boudieri* were weighed out, soaked separately in distilled water in 1:3 ratio, for 48 h at temperatures of 4°C. The resultant solution was homogenized after 48 h, filtered through a double layer of cotton. The filtrate was centrifuged at 3000 rpm for 10 min at room temperature, with the resulting supernatants collected and labeled as crude sample of aqueous extracts. These crude samples were further dried under reduced pressure with a rotary evaporator to give 6.2 g for *T. claveryi* and 5.1 g *T. boudieri*. These were represented as total dried crude aqueous extracts.

For the preparation of methanolic extract, 400.0 g of dried powdered truffles extracts of *T. claveryi* and *T. boudieri* were exhaustively extracted three times with methanol (MeOH) for 7 days using 5.0 L of 70.0% MeOH under room temperature. The resulting extracts were then separately filtered using Whatman filter paper and evaporated to dryness. The concentration of the extracts was with rotary evaporator at reduced pressure to give a dark reddish-yellow extract weighing about 20.9 g for *T. claveryi* and 18.3 g for *T. boudieri* and labeled as total MeOH extracts.

Furthermore, 17.0 and 15.0 g of *T. claveryi and T. boudieri* were each suspended in 200.0 ml deionized water in separating funnels and partitioned with petroleum ether (5.0 × 500.0 ml). The resulting petroleum ether fractions were evaporated to dryness using rotary evaporator. These extractions were dried to give 5.1 and 4.6 g for *T. claveryi* and *T. boudieri*, respectively and then stored in closed containers. The rest of the mother liquor was mixed with chloroform (4.0 × 500.0 ml) and the chloroform fraction obtained from this mixture was also evaporated to dryness to concentrate it by the use of rotary evaporator, which was then freeze dried. A weight of 2.3 and 1.9 g was obtained, respectively, for *T. claveryi* and *T. boudieri*. The preparation of ethyl acetate extracts is the same as the above-mentioned protocol to give 4.9 and 3.7 g for *T. claveryi* and *T. boudieri,* respectively. The remaining mother liquor fractions were also dried to give 4.1 and 3.5 g for *T. claveryi* and *T. boudieri,* respectively, stored in an airtight container and kept in the freezer for further use.[30,31] From the ten extract fractions of crude aqueous extract, petroleum ether, chloroform fraction, ethyl acetate, MeOH extracts for *T. claveryi* and *T. boudieri* each, 20 mg/ml solutions were prepared and stored in the freezer for the evaluation of minimum inhibitory concentrations (MICs).

Minimum inhibitory concentrations determination

The MICs determination of *T. claveryi* and *T. boudieri* extracted fractions were done using the broth dilution method in accordance with a previously described.[32] All the extracted fractions were prepared to the highest possible concentration of 20.0 mg/ml (stock concentration) in 10.0% dimethyl sulfoxide (DMSO) solution. These were serially diluted to give concentrations of 15.0, 10.0, 5.0, and 2.5 mg/ml. A volume of 1.0 ml of the standardized microbial broth cultures were inoculated into the tubes containing the diluted extracts and labeled accordingly. The tubes were placed in CO_2 incubator at 37°C and were observed for 24 h. They were examined for the presence or absence of bacterial growth. The least concentrations of the extracts which inhibited the growths of inoculums were considered as the MICs. Therefore, MICs were found to

be 5.0 mg/ml for crude aqueous extract, MeOH extract, and chloroform fraction. However, ethyl acetate and petroleum ether fractions were 2.5 mg/ml, respectively.

Clinical ocular bacteria isolates

Specimens were from people with diabetes and hypertensive patients with glaucoma, bacterial and viral conjunctivitis, cataracts, which included those with mature and post-cataract surgical removal, nasolacrimal duct obstruction and peripheral ulcerative keratitis (PUK). Patient eye discharge was collected by the attending ophthalmologist using soft-tipped sterile cotton swabs under sterile conditions and was brought to the Microbiology department of the College of Medicine. Each swab was inoculated into the nutrient broth and incubated aerobically at 37°C for 24 hr. Overnight growth was plated on blood agar and MacConkey agar obtained from Oxoid, Hampshire, UK and was incubated aerobically for 24 h at 37°C.

Bacteria isolation and antimicrobial susceptibility test

Pure bacteria cultures were used for the identification using basic bacteriological and Biochemical techniques as recommended by Cheesbrough.[33] Confirmation of isolate identity was carried out using the VITEK 2 compact automated system (BioMerieux, Marcy L'Etoile, France) according to the manufacturer's guidelines, with GP and GN cards for Gram-positive and Gram-negative isolates, respectively. Susceptibility to antimicrobials and determination of MICs was carried out by the VITEK 2 compact automated system using antimicrobial susceptibility testing cards, against the following antibiotics: Ampicillin/Sulbactam (AMS); augmentin (20/10 μg); piperacillin/tazobactam (100/10 μg); ceftazidime (30 μg); cefepime (30 μg); aztreonam (AZM); ertapenem [10 μg]; imipenem (10 μg); meropenem (10 μg); amikacin (30 μg); gentamicin (10 μg); tobramycin (TOB); ciprofloxacin (30 μg); levofloxacin (5 μg); minocycline (30 μg); tigecycline (30 μg); moxifloxacin (MXF); oxacillin (OXA); trimethoprim/sulfamethoxazole (1.25/23.75 μg) benzylpenicillin (10.0 μg); erythromycin (15.0 μg), vancomycin (30 μg), and tetracycline (30 μg). Interpretation of results was according to the Clinical and Laboratory Standards Institute[34] recommendations.

Antimicrobial effect of *Terfezia claveryi* and *Terfezia boudieri*

Well diffusion antimicrobial susceptibility method[35,36] was used for the determination of the antimicrobial effect of the desert truffles against clinical ocular infection isolates. Each bacteria isolate was inoculated and spread on Muller Hilton agar. Using 0.8 mm cork borer, wells were cut into the agar with extracts of both desert truffles introduced into each well. All plates were incubated aerobically at 37°C overnight after which zones of inhibition were measured using mm ruler. Experiment was carried out in three replicates.

Molecular analysis

Scanning electron microscopy

The SEM was used to determine the effect of extracts fractions of *T. claveryi* and *T. boudieri* on the cells of species of *Staphylococcus* using the previous method[37] with modifications. Staphylococcal species were cultured in Muller Hilton broth at 37°C in a shaking incubator for 6 h. Final adjustment of turbidity was according to McFarland 0.5 standards with obtained bacteria cell suspension as described.[37]

Treated bacteria cultures were incubated in a shaker incubator at 37°C for 24 h while untreated bacteria cells were used as controls. The resulting growth was centrifuged, prefixed overnight in 2.5% glutaraldehyde solution at 4°C. All samples were then rinsed with phosphate-buffered saline and post fixed as described[37] with 100% acetone applied at the last stage. A 20 nm thick layer was obtained by sputtering of gold. The SEM micrographs were obtained with SEM (JSM 6390 LA, JOEL) at 15 KV accelerating voltage.

Molecular docking analysis

In other to further investigate the molecular mechanism of extracts of *T. claveryi* and *T. boudieri,* whose main active component is Stigmasterol, hence it was docked with *Staphylococcus aureus* surface protein. Molecular docking was done using Autodock tools V.1.5.4 and Autodock V.4.2 program. The aim was to perform *in-silico* analysis of the interactions between stigmasterol, a ligand candidate and Sortase A (Srt-A), a *Staphylococcus aureus* surface protein. The Srt-A (PDB ID: 1TD2P) three-dimensional (3D) chemical structure was retrieved from Protein Data Bank, while that of stigmasterol (CID_5280794) was obtained from PubChem compound database. Q-site finder was used for the identification of active sites of targeted protein with docked ligand reflected as rigid bodies to the receptors. Predicted binding energy was used for the evaluation and sorting of results. This described method was adopted from Hanieh *et al.*[38]

Statistical analysis

Data are presented as mean ± standard deviation and Graphpad Prism 8.2.3 (San Diego, USA) was used for statistical analysis. Two-way analysis of variance was used to compare the statistical difference between and within zones of inhibitions produced by different extract fractions of both plants under study. Furthermore, Paired *t*-test was used to compare zones of inhibitions produced by the same extract fractions from the two plants. The significant difference was taken as $P < 0.05$.

RESULTS

Patient demography

Collected specimens were from males (57%) and females (43%) with ages that ranged between 1 and 80 years. The isolated bacteria were *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus lugdunensis, Serratia odorifera, S. liquefaciens, Pseudomonas stutzeri, Pseudomonas oryzihabitans, P. mirabililis, Kocuria kristinae, Kocuria rosea, Micrococcus luteus,* as shown in Table 1.

Antimicrobial susceptibility

The results on the antimicrobial susceptibility of isolated Gram-negative and Gram-positive bacteria are presented in Tables 2 and 3, respectively. Table 2 shows that in addition to being intrinsically resistant to tigecycline, all isolated strains of *Proteus mirabilis* were resistant to minocycline, ceftazidime (CAZ), cefepime (FEP), and aztreonam (AZM). Two of the strains were also resistant to imipenem. For the Gram-positive Staphylococcal eye infection isolates, there was a 100% resistance to the penicillins, as shown in Table 3. Furthermore, there was high antimicrobial resistance against the following conventional antibiotics: erythromycin, clindamycin, tetracycline, and rifampicin. One strain of *S. epidermidis* isolates tested positive to cefoxitin screen, while another strain also showed high antimicrobial resistant to different groups of antibiotics [Table 3]. For all the isolates, according to the antibiotic classification, the β–lactams, there was a 78% resistance to benzylpenicillin, 50% resistance to cefepime, 30% to imipenem, and 22% ampicillin/sulbactam and ceftazidime each [Figure 1]. For other classes, Figure 2 shows antimicrobial resistance for the isolates as follows: rifampicin (57%), tetracycline (56%), clindamycin (33.3%), and tigecycline (24%). There was a 13% resistance to gentamicin and

ciprofloxacin, while 6% of the isolates were resistant to levofloxacin and trimethoprim/sulfamethoxazole.

Zones of inhibition against extracts and fractions

The results on growth inhibitory effects of fractions of *T. claveryi* and *T. boudieri* are shown in Table 4. Both Gram-positive and Gram-negative bacteria eye isolates were inhibited by crude aqueous and methanolic extracts of both plants to varying degrees, more so with fractions of *T. claveryi* extracts. Crude aqueous extract of *T. claveryi* showed better zones of inhibition than that of *T. boudieri* at a very statistically significant with *P* value of 0.0013. Furthermore, the chloroform fraction of *T. claveryi* showed better inhibition against all isolates as compared to those of the same fraction of *T. boudieri,* with the difference in the mean zone of inhibition being statistically significant ($P < 0.0001$), as shown in Table 4. A similar pattern was exhibited by ethyl acetate fractions of both plants, with *T. claveryi* having a better zone of inhibition, which was statistically significant ($P = 0.0001$). Results in Figure 3 showed that methanolic extracts observed zones of inhibition of bacteria growth was not statistically different when compared together. Of all plant fractions, those of petroleum ether were the least effective against the eye infection isolates, with observed differences between the two fractions being statistically not significant ($P = 0.077$).

Table 1: Patient demography, clinical presentations and associated bacterial infections

Lab ID	Patient demography			Bacteria isolates
	Age	Gender	Clinical presentation	
EY1	52	Female	Bacteria conjunctivitis	*M. luteus*
EY2	2	Female	NLDO	*S. liquefaciens*
EY3	55	Female	Cataract/dryness	*S. epidermidis*
EY4	36	Female	Bacterial conjunctivitis	*S. odorifera*
EY5	60	Male	Bacterial conjunctivitis	NG
EY6	1	Male	Viral conjunctivitis	NG
EY7	65	Male	Papillary conjunctivitis	*S. aureus*
EY8	55	Male	Glaucoma	*S. epidermidis*
EY9	32	Female	Bacterial conjunctivitis	*K. rosea*
EY10	40	Male	Bacterial conjunctivitis	*P. stutzeri*
EY11A	29	Female	Bacterial conjunctivitis	*P. oryzihabitans*
EY11B				*S. aureus*
EY12	NG		Chronic blepharitis	*S. hominis*
EY13	59	Male	Bacterial conjunctivitis	*S. epidermidis*
EY14	32	Male	PUK	*K. kristinae*
EY15A	23	Male	Bacterial conjunctivitis	*P. mirabilis 1*
EY15B				*P. mirabilis 2*
EY16	80	Male	Mature cataract	*P. mirabilis*
EY17	60	Female	Postcataract	NG
EY18	60	Female	Glaucoma	*S. epidermidis*
EY19	47	Male	Bacterial conjunctivitis	*S. lugdunensis*
EY20	23	Female	Bacterial conjunctivitis	*S. lugdunensis*

M. luteus: Micrococcus luteus; S. liquefaciens: Serratia liquefaciens; S. epidermidis: Staphylococcus epidermidis; S. odorifera: Serratia odorifera; S. aureus: Staphylococcus aureus; K. rosea: Kocuria rosea; P. stutzeri: Pseudomonas stutzeri; P. oryzihabitans: Pseudomonas oryzihabitans; S. hominis: Staphylococcus hominis; K. kristinae: Kocuria kristinae; P. mirabilis: Proteus mirabilis; S. lugdunensis: Staphylococcus lugdunensis; NLDO: Nasolacrimal duct obstruction; NG: Not Given

Comparison between antimicrobial susceptibility and fractions of extracts

The results in Table 2 are the antibiogram of the isolates to conventional antibiotics employed in the treatment of Gram-negative infections. It shows encountered strains of *P. mirabilis* to be resistant to ceftazidime (CAZ), cefepime (FEB), aztreonam (AZM), imipenem (IMP), minocycline (MINO), and Tigecycline (TGC). For the Gram-positive Staphylococcal species, antibiogram results presented in Table 3 showed resistance by different strains to benzylpenicillin (PEN), erythromycin (ERY), tetracycline (TET), rifampicin (RIF), and clindamycin (CLI). However, there was growth inhibition of this bacterium by extracts and

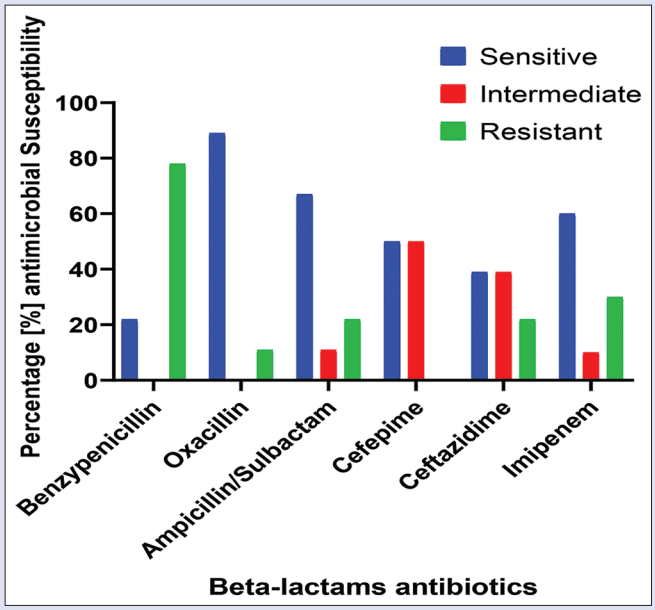

Figure 1: Antimicrobial susceptibility of isolates against β-lactam antibiotics

Table 2: Antimicrobial susceptibility of Gram-negative bacteria isolates

Bacteria isolate	Lab ID	Antibiotics																
		AMS	AUG	PTZ	CAZ	FEP	AZM	ETP	IMI	MEM	AMK	CN	TOB	CIP	LEV	MNO	TGC	SXT
P. mirabilis 1	EY15	S	N	S	I	I	I	S	S	S	S	S	S	S	S	R	R	S
P. mirabilis 2	EY15B	S	N	S	I	I	I	S	R	S	S	S	S	S	S	R	R	S
P. mirabilis 3	EY16	S	N	S	I	I	I	S	I	S	S	S	S	S	S	R	R	S
P. mirabilis 4	EY16B	S	N	S	I	I	I	S	S	S	S	S	S	S	S	R	R	S
S. odorifera	EY4	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. liquefaciens	EY2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
P. stutzeri	EY10	S	N	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
P. oryzihabitans	EY11	I	N	S	S	S	N	N	S	S	S	S	S	S	S	S	S	S

AMS: Ampicillin/sulbactam; PTZ: Piperacillin/tazobactam; CAZ: Ceftazidime; FEP: Cefepime; AZM: Aztreonam; ETP: Ertapenem; IMI: Imipenem; MEM: Meropenem; AMK: Amikacin; CN Gentamicin; TOB: Tobramycin; CIP: Ciprofloxacin; LEV: Levofloxacin; MNO: Minocycline; TGC: Tigecycline; SXT: Trimethoprim/sulfamethoxazole; AUG: Augmentin; *P. mirabilis: Proteus mirabilis; S. odorifera: Serratia odorifera; S. liquefaciens: Serratia liquefaciens; P. stutzeri: Pseudomonas stutzeri; P. oryzihabitans: Pseudomonas oryzihabitans*; R: Resistant; S: Sensitive; ERY: Erythromycin; N: Not detected; I: Intermediate

Table 3: Antimicrobial susceptibility of coagulase-positive and coagulase-negative *Staphylococcus* species

Bacteria isolate	Lab ID	Antibiotics																		
		PEN	OXA	AMS	CAZ	FUR	IMI	LEV	MXF	ERY	CN	CIP	CLI	LNZ	VAN	TET	TGC	F/M	RIF	SXT
S. aureus 1	EY11	R	S	N	N	N	N	S	S	S	S	S	S	S	S	S	S	S	I	S
S. aureus 2	EY7	R	S	N	N	N	N	S	S	R	S	S	S	S	S	R	S	S	I	S
S. epidermidis 1	EY8	I	N	R	R	R	R	R	R	S	N	N	S	S	S	R	S	S	N	S
*S. epidermidis 2**	EY3	R	R	N	N	N	N	I	S	R	S	R	R	S	S	S	S	S	I	R
S. epidermidis3	EY13	R	S	N	N	N	N	S	S	R	S	S	S	S	S	S	S	S	R	S
S. epidermidis 4	EY18	R	S	N	N	N	N	S	S	S	S	S	S	S	S	S	S	S	I	S
S. lugdunensis 1	EY19	R	S	N	N	N	N	S	S	S	S	S	S	S	S	R	S	S	S	S
S. lugdunensis 2	EY20	R	S	N	N	N	N	S	S	R	R	S	R	S	S	R	S	S	S	S
S. hominis	EY12	I	N	R	R	R	R	S	S	R	N	S	R	S	S	R	S	S	N	S

*Positive to cefoxitin screen. R: Resistant; S: Sensitive; PEN: Benzylpenicillin; OXA: Oxacillin; AMS: Ampicillin/sulbactam; CAZ: Ceftazidime; FUR: Cefuroxime axetil; IMI: Imipenem; LEV: Levofloxacin; MXF: Moxifloxacin; ERY: Erythromycin; CN: Gentamicin; CIP: Ciprofloxacin; CLI: Clindamycin; LNZ: Linezolid; VAN: Vancomycin; TET: Tetracycline; TGC: Tigecycline; F/M: Nitrofurantoin; RIF: Rifampicin; SXT: Trimethoprim/sulfamethoxazole; *S. aureus: Staphylococcus aureus*; *S. epidermidis: Staphylococcus epidermidis; S. lugdunensis: Staphylococcus lugdunensis; S. hominis: Staphylococcus hominis*

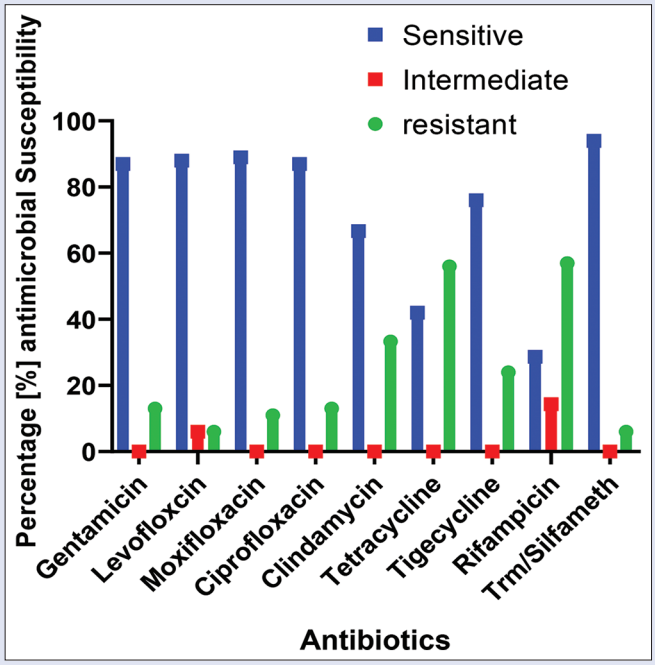

Figure 2: Antimicrobial susceptibility of the isolates against non-β lactams

extract fractions of both *T. boudieri* and *T. claveryi* as shown in Table 4. Extracts and fractions of *T. claveryi* showed better zones of inhibition than those of *T. boudieri* against the different isolated strains of *P. mirabilis*. The difference in growth inhibition by chloroform and ethyl acetate fractions were statistically significant ($P < 0.05$) as compared with other fractions of *T. claveryi*. Furthermore, with regard to Gram-positive Staphylococcal species, these isolates where found to be susceptible to extracts and fractions of *T. boudieri* and *T. claveryi* [Table 4], with *T. claveryi* exhibiting greater zones of inhibition. Results showed that both crude aqueous and methanol extracts displayed better antibacterial activity than other fractions, with methanol extract of *T. claveryi* exhibiting better inhibitory activity.

Results also showed that crude aqueous extract, methanol extract, and fractions of chloroform and ethyl acetate did significantly inhibit *S. epidermidis* more than other Staphylococcal species. *Staphylococcus aureus* was more sensitive to crude aqueous and methanol extracts, chloroform fraction, and ethyl acetate fraction isolated from *T. claveryi*.

Molecular analysis

The results of scanning electron microscopy and molecular docking used to determine the antimicrobial mechanism of action of *T. boudieri* and *T. claveryi* are presented in Figures 4 and 5, respectively. Morphological alterations in treated Staphylococcal species by fractions of *Terfezia* species are shown in Figure 4a-j. In Figure 4a, untreated *S. epidermidis* is seen with defined smooth clustered margins as compared with those treated with aqueous extract of *T. claveryi* [Figure 4b], which showed altered surface margins. A similar pattern is seen in the untreated [Figure 4c] and treated [Figure 4d] *S. epidermidis* with aqueous extract of *T. claveryi*. For the SEM micrograph of *S. epidermidis* untreated [Figure 4e] and treated [Figure 4f] with methanolic extract of *T. claveryi* shows the surface appearance of the bacteria was dramatically altered. This is similar to observations in the micrograph of *S. epidermis* with extracts of *T. boudieri* [Figure 4g and h]. While the micrographs of MeOH extract of *T. claveryi* showed complete distortion of treated *S. epidermidis* [Figure 4j] as against those of the untreated [Figure 4i].

Results in Figure 5 are those of 3D docking analysis of Srt-A surface transport protein of *S. epidermidis with* stigmasterol, the main constituent of *T. boudieri* and *T. claveryi*. Stigmasterol is the active compound of *T. boudieri* and *T. claveryi*. Docking analysis of stigmasterol with surface protein Srt-A of *Staphylococcus aureus* showed this active compound interacted with valine amino acid 110. This interaction with the Srt-A domain has a binding energy of-6.69, with a ligand efficacy of 0.22. The binding energy reflects a high binding efficacy to the bacteria, possibly depicts its antimicrobial activity.

DISCUSSION

The clinical features of ocular bacterial infections in the present study were diverse and so was their susceptibility against tested antibiotics. Bacteria conjunctivitis was the most prevalent of the encountered eye infections caused by both Gram-positive and Gram-negative bacteria. Researchers had previously reported similar findings in different regions of the world.[39-41] Causative bacteria included *Staphylococcus aureus*, co-agulase negative *Staphylococcus* [CoNS], Gram-negative bacteria with species of *Pseudomonas* and strains of *P. mirabilis* among others. The listed bacteria are similar to those reported by other researchers.[10,42] Differences in observed reports are in the antimicrobial susceptibility of the bacteria isolates against tested antibiotics.

In the present investigations, *Staphylococci* isolates were all (100%) resistant to Penicillins, observations that are similar to those of earlier researchers.[42,43] Furthermore, high resistance against erythromycin, clindamycin, tetracycline, and rifampicin, all of which are the drug of choice in the treatment of ocular eye infections, were noted. This is a

Table 4: Comparison in diameter mean±standard deviation zones of inhibition (mm) for extracts of *Terfezia boudieri* and *Terfezia claveryi*

Lab ID	Bacteria isolate	Crude aqueous extract		Petroleum ether fraction		Chloroform fraction		Ethyl acetate fraction		MeOH extract	
		1	2	1	2	1	2	1	2	1	2
EY1	*M. luteus*	20.22±0.26	38.93±0.40	23.97±0.56	15.09±0.11	15.07±0.12	10.5±0.36	15.07±0.21	20.03±0.06	32.1±0.1	40.33±0.58
EY2	*S. liquefaciens*	22.27±0.25	30.03±0.16	29.08±0.19	25.17±0.15	25.02±0.13	22.08±0.08	20.17±0.15	28.2±0.26	25.1±0.17	25.07±0.21
EY3	*S. epidermidis1*	21.27±.27	35.3±0.26	25.37±0.32	25.03±0.15	15.03±0.06	30.03±0.15	16.1±0.1	25.1±0.1	28.2±0.1	35.17±0.29
EY4	*S. odorifera*	25.23±0.25	21.5±0.5	25.18±0.18	12.1±0.17	22.03±0.06	20.03±0.06	20.1±0.1	28.13±0.06	20.07±0.6	19.3±0.61
EY5	*S. aureus 1*	25.13±0.15	20.17±0.15	23.07±0.21	15.08±0.08	26.13±0.15	22.1±0.1	0	28±0	22.07±0.12	20.03±0.06
EY7	*S. aureus 2*	21.97±0.15	22.13±0.15	23.12±0.10	0	0	22.1±0.1	0	21.07±0.12	23.07±0.12	18.27±0.25
EY8	*S. epidermidis2*	30.07±0.21	50.23±0.25	0.03±0.06	0	0	25.03±0.15	0	25.08±0.13	47.03±0.06	50.2±0.26
EY9	*K. rosea*	25.23±0.21	44.97±	25.06±0.22	27.27±0.25	20.03±0.15	30.8±0.44	20±0.1	25.03±0.06	50.33±0.6	35.1±0.1
EY10	*P. stutzeri*	20.1±0.1	30.07±0.21	21.13±0.32	20.19±0.35	20.03±0.06	25.1±0.1	20±0	21.2±0.26	26.02±0.08	30.1±0.1
EY11A	*P. oryzihabitans*	14.99±0.10	9.83±0.29	0	0	0	25.06±0.12	0	20±0	30.17±0.29	20±0
EY12	*S. hominis*	25.07±0.21	21.92±0.07	10.27±0.15	0	15.03±0.06	25.99±0.11	20.1±0.1	28.1±0.17	20±0	22.23±0.25
EY13	*S. epidermidis3*	20.2±0.26	20.13±0.15	0.23±0.25	0	16.13±0.12	30.03±0.06	12.03±0.06	30.13±0.12	15.17±0.29	26.07±0.12
EY14	*K. kristinae*	10.5±0.5	17.89±0.1	25.22±0.46	20.07±0.12	10.03±0.6	40.1±0.17	10.03±0.5	39.21±0.2	30.07±0.06	10.1±0.1
EY15A	*P. mirabilis 1*	20.27±0.25	0	0	0	0	20.1±0.1	0	25.17±0.21	20.03±0.06	20.17±0.21
EY15B	*P. mirabilis 2*	15.17±0.21	41.92±0.1	24.99±0.41	10.4±0.53	9.99±0.11	15.03±0.06	15.02±0.08	40.07±0.06	42.17±0.3	30.03±0.15
EY16	*P. mirabilis*	20.13±0.15	28.23±0.32	10.03±0.58	20.17±0.12	0	0.93±0.12	0	20.03±0.06	20.1±0.1	25.17±0.29
EY18	*S. epidermidis 4*	19.2±0.2	18.2±0.26	0	0	15.1±0.1	24.99±0.01	25±0.1	30.07±0.08	30.2±0.17	25.03±0.06
EY19	*S. lugdunensis*	20.13±0.23	24.03±0.15	0	10.07±0.21	0	19.97±0.06	0	25±0	24.93±0.12	30.1±0.1
EY20	*S. lugdunensis*	19.97±0.06	19.03±0.08	0	0.03±0.058	0	20.13±0.15	0	20.3±0.61	0	0.9±0.1

M. luteus: Micrococcus luteus; S. liquefaciens: Serratia liquefaciens; S. epidermidis: Staphylococcus epidermidis; S. odorifera: Serratia odorifera; S. aureus: Staphylococcus aureus; K. rosea: Kocuria rosea; P. stutzeri: Pseudomonas stutzeri; P. oryzihabitans: Pseudomonas oryzihabitans; S. hominis: Staphylococcus hominis; K. kristinae: Kocuria kristinae; P. mirabilis: Proteus mirabilis; S. lugdunensis: Staphylococcus lugdunensis; MeOH: Methanol

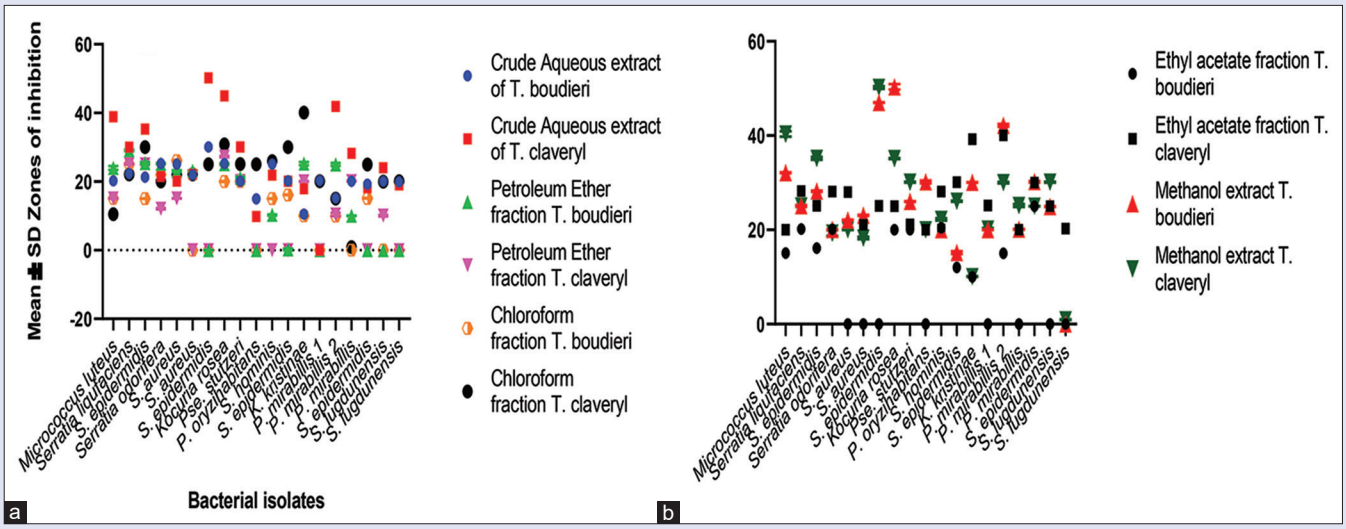

Figure 3: Mean ± standard deviation zones of inhibition by various fraction of *Terfezia boudieri* and *Terfezia claveryi*. (a) Represents zones of inhibition (mm) of crude aqueous extract, petroleum, ether and chloroform extracts for both *T. bouderi* and *T. claveryl*. (b) Zones of inhibition (mm) for ethyl acetate and methanol fractions for both *T. bouderi* and *T. claveryl*. Data represents Mean ± standard deviation zones

disturbing trend in antimicrobial susceptibility of bacteria associated with ocular infection but then reflects the global public health problem of the reduced susceptibility against antibiotic of choice by bacteria. Furthermore, troubling are the antimicrobial susceptibility of the various strains of *P. mirabilis* isolates with high resistance to minocycline, ceftazidime, cefepime, aztreonam, as well as some strains being resistant to imipenem. High antimicrobial resistance by bacteria associated with ocular infections, such as in this study, had also been reported by other researchers.[44,45] This trend in bacteria susceptibility to antimicrobials highlights the challenges faced globally in the general management of bacterial infections, inclusive of those involved in ocular infections, as shown in this report. It is, however, worthy of note that irrespective of antimicrobial susceptibility by the isolates in the present investigation, the extracts and or fractions of both desert truffles in the present investigation inhibited the growth of Gram-positive and Gram-negative bacteria isolated from patients with eye infections. There were, however, differences as seen in the study between *T. claveryi* and *T. boudieri* with regard to the zones of inhibition of the bacteria isolates as well as differences in the type of extracted component within a plant species. The statistically significant better zones of inhibition obtained from the crude aqueous extract, chloroform, and ethyl acetate fractions of *T. claveryi* than those of *T. boudieri* suggest variations between species of desert truffles that could be due to differences in their chemical compositions and content. A similar observation[46] pointed out differences in the antimicrobial properties of a number of desert truffles species. This they had attributed to possible differences in their chemical composition as well as the treated microbial strains. This might explain why methanolic extracts of *T. claveryi* and *T. boudieri* inhibited the growth of bacteria

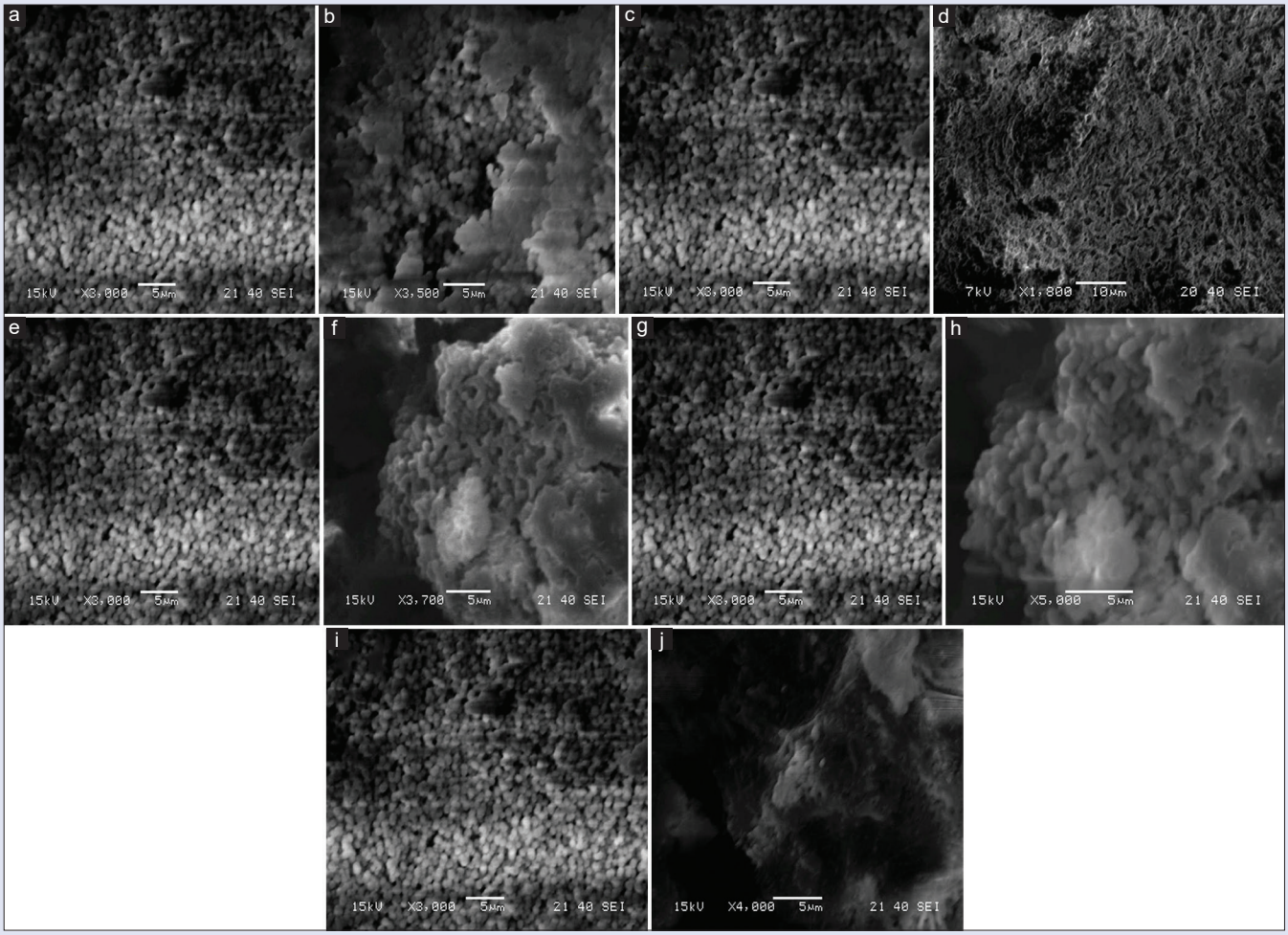

Figure 4: Micrograph of *Staphylococcus hominis* untreated (a) and treated (b) with aqueous extract of *Terfezia boudieri*. Micrograph of *Staphylococcus hominis* untreated (c) and treated (d) with MeOH of *Terfezia claveryi*. Micrograph of *Staphylococcus epidermidis* untreated (e) and treated (f) with aqueous extract *Terfezia claveryi*. Micrograph of *Staphylococcus epidermidis* untreated (g) and treated (h) with MeOH extract of *T. boudieri*. Micrograph of *Staphylococcus epidermidis* untreated (i) and treated (j) with MeOH extract of *T. calveryl*. Treatment shows complete distortion/killing of treated bacteria. The control bacteria were intact and round in structure

isolates in this study with the exception of *S. lugdunensis*. The bacteria strain could be contributory factor more so as the comparison in differences in zones of inhibition of the methanolic extracts of *T. claveryi* and *T. boudieri* were not statistically significant, an observation that is similar to those of other researchers.[47,48]

Besides the differences in plant species used in this study, there were observed variations in extract or fractions activity as encountered with both species of *Terfezia* in the present investigation. That petroleum ether fraction of both desert truffles was the least effective against the bacteria isolates suggest that this is might not be the most suitable for the inhibition of microbial growth, particularly in CoNS. These findings differ from earlier reports[29] where there was lack of growth inhibition by aqueous and petroleum extracts of *T. claveryi* against Gram-positive and Gram-negative bacteria. The report[29] suggested that this could be due to the chemical composition of the bacteria. Such differences could also be attributed to a number of other factors, such as of extraction methods that might affect the quantity and quality of antimicrobials present in them, a view that had also been expressed previously.[49]

Overall, it is, however, worth noting that there was growth inhibition of all the isolates in the present investigation by extracts and fractions of extracts of *T. claveryi* and *T. boudieri,* thus suggesting their suitability for the treatment of ocular infections. The molecular docking results and the SEM imaging gave an insight into the antimicrobial inhibitory mechanism of the desert truffles. The suggested mechanism is seen in the docking analysis by the interaction between stigmasterol that is one of the active compounds of *Terfezia* and Sortase A protein on the surface of *Staphylococcus aureus*.[50] This could be collaborated by the SEM analysis, where the bacterial cell wall showed partial or complete distortion when treated with *Terfezia* extracts.

CONCLUSION

Our study shows that *T. claveryi* and *T. boudieri* significantly inhibited the growth of clinical bacteria isolates associated with ocular infections, even for those that were resistant to conventional antibiotics. SEM and molecular docking analysis also confirm their antimicrobial activity. Therefore, the use of extracts of *T. claveryi* and *T. boudieri* as herbal remedy for eye infections could hereby be affirmed and justified.

Ethical consideration

Permission for the research was given by the Deanship of Scientific Research, King Faisal University [Research number 186176].

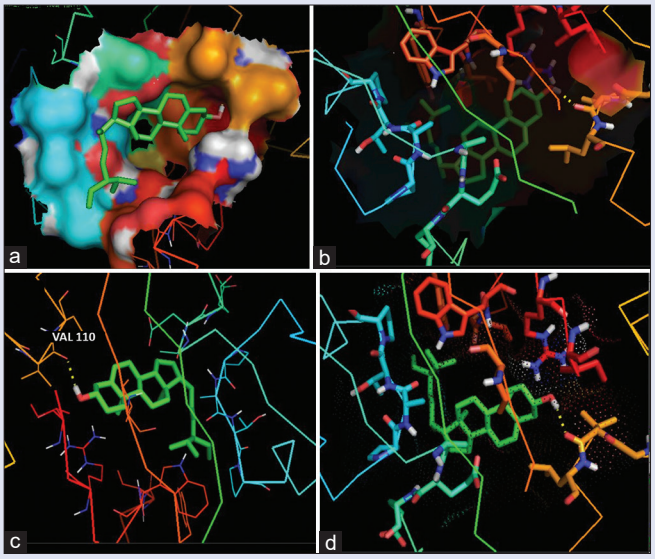

Figure 5: Molecular docking showing (a) electrostatic surface of the srt-A active binding site with Stigmasterol, (b) expanded view of the active hydrogen binding. Molecular docking showing (c) showing the putative Stigmasterol Srt-A binding with Valine 110. (d) Srt-A binding with Stigmasterol in similar orientation but a mirror three-dimensional image as in c

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Galvis V, Tello A, Guerra A, Acuña MF, Villarreal D. Antibiotic susceptibility patterns of bacteria isolated from keratitis and intraocular infections at fundación oftalmológica de santander (FOSCAL), floridablanca, colombia. Biomedica 2014;34 Suppl 1:23-33.
2. Iwalokun A, Oluwadun A, Akinsinde A. Niemogha MT, Nwaokorie FO. Bacteriologic and plasmid analysis of etiologic agents of conjunctivitis in Lagos, Nigeria J Ophthal Inflamm Infect 2011;1:95-103.
3. Choudhury R, Panda S, Sharma S, Singh DV. Staphylococcal infection, antibiotic resistance and therapeutics. In: Pana M, editor. Antibiotic Resistant Bacteria-A Continuous Challenge in the New Millennium. Chapter 10. Croatia: InTech Publication; 2012; 247-72. ISBN: 978-953-51-0472-8.
4. Bertino JS Jr. Impact of antibiotic resistance in the management of ocular infections: The role of current and future antibiotics. Clin Ophthalmol 2009;3:507-21.
5. Buznach N, Dagan R, Greenberg D. Clinical and bacterial characteristics of acute bacterial conjunctivitis in children in the antibiotic resistance era. Pediatr Infect Dis J 2005;24:823-8.
6. Ubani UA. Common bacterial isolates from infected eye. J Niger Optom Assoc 2009;15:40-7.
7. Willcox MD. *Pseudomonas aeruginosa* infection and in ammation during contact lens wear. Optom Vis Sci 2007;84:273-8.
8. Henry CR, Flynn W, Miller D, Forster RK, Alfonso EC. Infectious Keratitis progressing to Endophthalmitis: A 15-year-study of microbiology, associated factors and clinical outcomes. Ophthalmology 2012;119:2443-9.
9. Cao J, Yang Y, Yang W, Wu R, Xiao X, Yuan J, *et al.* Prevalence of infectious keratitis in central china. BMC Ophthalmol 2014;14:43.
10. Galvis V, Parra MM, Tello A, Castellanos YA, Camacho PA, Villarreal D, *et al.* Perfil de resistencia antibióticaen infecciones oculares en un centro de referencia en Floridablanca, Colombia. Arch Soc Esp Oftalmol 2019;94:4-11.
11. Yamada M, Hatou S, Yoshida J. *In vitro* susceptibilities of bacterial isolates from conjunctival flora to gatifloxacin, levofloxacin, tosufloxacin and moxifloxacin. Eye Contact Lens 2008;34:109-12.
12. Wong CA, Galvis V, Tello A, Villareal D, Rey JJ. *In vitro* antibiotic susceptibility to fluoroquinolones. Arch Soc Esp Oftalmol 2012;87:72-8.
13. Reardon S. WHO warns against "post-antibiotic" era. Agency recommends global system to monitor spread of resistant microbes. Nature news 2014; doi:10.1038/nature.2014.15135. © 2019 Macmillan Publishers Limited, part of Springer Nature. Available from: http://www.nature.com/news/who-warns-against-post-antibiotic-era-1.15135
14. Nathan C, Cars O. Antibiotic resistance-problems, progress and prospects. Engl J Med 2014;371:1761-3.
15. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, *et al.* Antibiotic resistance: A rundown of a global crisis. Infect Drug Resist 2018;11:1645-58.
16. Gupta PD, Birdi TJ. Development of botanicals to combat antibiotic resistance. J Ayurveda Integr Med 2017;8:266-75.
17. Narayanan S, Raja S, Ponmurugan K, Kandekar SC, Natarajaseenivasan K, Maripandi A. Antibacterial activity of selected medicinal plants against multiple antibiotic resistant uropathogens: A study from Kolli Hills, Tamil Nadu, India. Benef Microbes 2011;2:235-43.
18. Potroz M, Cho N. Natural products for the treatment of trachoma and Chlamydia trachomatis. Molecules 2015;20:4180-203.
19. Wang S, Marcone MF. The biochemistry and biological properties of the world's most expensive underground edible mushroom: Truffles. Food Res Int 2011;44:2567-81.
20. Patel S, Rauf A, Khan H, Khalid S, Mohammad S, Mubarak MS. Potential health benefits of natural products derived from truffles: A review. Trends Food Sci Technol 2017;70:1-8.
21. Schillaci D, Cusimano MG, Cascioferro SM, Di Stefano V, Arizza V, Chiaramonte M, *et al.* Antibacterial activity of desert truffles from Saudi Arabia against Staphylococcus aureus and Pseudomonas aeruginosa. Int J Med Mushrooms 2017;19:121-5.
22. Hamza A, Zouari N, Zouari S, Jdir H, Zaidi S, Gtari M, *et al.* Nutraceutical potential, antioxidant and antibacterial activities of Terfezia boudieri Chatin, a wild edible desert truffle from Tunisia arid zone. Arab J Chem 2016;9:383-9.
23. Norman JE, Egger KN. Molecular phylogenetic analysis of Peziza and related genera. Mycologia 1999;91:820-9.
24. Percudani R, Trevisi A, Zambonelli A, Ottonello S. Molecular phylogeny of truffles (Pezizales: Terfeziaceae, tuberaceae) derived from nuclear rDNA sequence analysis. Mol Phylogenet Evol 1999;13:169-80.
25. Neggaz S, Fortas Z, Chenni M, El Abed D, Ramli B, Kambouche N. *In vitro* Evaluation of Antioxidant, Antibacterial and Antifungal Activities of Terfezia claveryi Chatin. Phytothérapie. Available from: https://doi.org/10.1007/s10298-015-0993-4" https://doi.org/10.1007/s10298-015-0993-4.
26. Saddiq AA, Yousef JM, Mohamed AM. The potential antibacterial role of Terfezia claveryi extract against immune-inflammatory disorder and oxidative damage induced by Pseudomonas aeruginosa in rat corneas. Rom Biotechnol Lett 2016; 21:11781-801.
27. Dahhama SS, Al-Rawia SS, Ibrahima AH, Majidb AS, Majida AM, *et al.* Antioxidant, anticancer, apoptosis properties and chemical composition of black truffle *Terfezia claveryi*. Saudi J Biol Sci 2018;25:1524-34.
28. Malik HM, Gull M, Omar U, Kumosani TA, Al-Hejin AM. Evaluation of the antibacterial potential of desert truffles (Terfezia spp) extracts against methicillin resistant Staphylococcus aureus (MRSA) J Exp Biol Agric Sci 2018;6:652-60. Available from: http://dx.doi.org/10.18006/2018.6(4).652.660.
29. Alhussaini MS, Saadabi AM, Hashim K, Al-Ghanayem AA. Efficacy of the desert truffle *Terfezia claveryi* to cure trachoma disease with special emphasis on its antibacterial bioactivity. Trends Med Res 2016;11:28-34.
30. Mohammed MH, Hamed AN, Khalil HE, Kamel MS. Phytochemical and pharmacological studies of Citharexylum quadrangulare Jacq. Leaves. J Med Plants Res 2016;10:232-41.
31. Khalil HE, Aljeshi YM, Saleh FA. Authentication of Carissa macrocarpa cultivated in Saudi Arabia; botanical, phytochemical and genetic study. Int J Pharm Sci 2015;7:497.

32. Badger-Emeka LI, Hany EK, Emeka PM. Evaluation of different fractions of Garcinia kola extracts against multidrug resistant clinical bacterial and fungal isolates. Pharmacogn J 2018;10:1055-60.
33. Cheesbrough M. Biochemical tests to identify bacteria. In: District Laboratory Practice in Tropical Countries, Part 2. Cambridge: Cambridge University Press; 2000. p. 63-70.
34. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty Fifth Informational Supplement M100-S23. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2015.
35. Rios JL, Recio MC, Villar A. Screening methods for natural products with antibacterial activity. A review of literature. J Ethnopharmacol 1998;23:127-49.
36. Emeka LB, Emeka PM, Khan TM. Antimicrobial activity of nigella sativa L. Seed oil against multi-drug resistant *Staphylococcus aureus* isolated from diabetic wounds. Pak J Pharm Sci 2015;28:1985-90.
37. Sutthiwan T, Kusavadee S, Aphidech S. Time-kill profiles and cell-surface morphological effects of crude Polycephalomyces nipponicus Cod-MK1201 mycelial extract against antibiotic-sensitive and -resistant *Staphylococcus aureus*. Trop J Pharm Res 2017;16:407-12.
38. Hanieh H, Mohafez O, Hairul-Islam VI, Alzahrani A, Ismail MB, Thirugnanasambantham K. Novel aryl hydrocarbon receptor agonist suppresses migration and invasion of breast cancer cells. PLoS ONE 2016;11:E0167650.
39. Getahun E, Gelaw B, Assefa A, Assefa Y, Amsalu A. Bacterial pathogens associated with external ocular infections alongside eminent proportion of multidrug resistant isolates at the university of Gondar hospital, Northwest Ethiopia. BMC Ophthalmol 2017;17:151.
40. Shiferaw B, Gelaw B, Assefa A, Assefa Y, Addis Z. Bacterial isolates and their antimicrobial susceptibility pattern among patients with external ocular infections at Borumeda hospital, northeast Ethiopia. BMC Ophthalmol 2015;15:103.
41. Tesfaye T, Beyene G, Gelaw Y, Bekele S, Saravanan M. Bacterial profile and antimicrobial susceptibility pattern of external ocular infections in Jimma University specialized hospital, Southwest Ethiopia. Am J Inf Dis Microbiol 2013;1:13-20.
42. Belyhun Y, Moges F, Endris M, Asmare B, Amare B, Bekele D, *et al.* Ocular bacterial infections and antibiotic resistance patterns in patients attending Gondar Teaching Hospital, Northwest Ethiopia. BMC Res Notes 2018;11:597.
43. Teweldemedhin M, Gebreyesus H, Atsbaha AH, Asgedom SW, Saravanan M. Bacterial profile of ocular infections: A systematic review. BMC Ophthalmol 2017;17:212.
44. Anagaw B, Biadglegne F, Belyhun Y, Anagaw B, Mulu A. Bacteriology of ocular infections and antibiotic susceptibility pattern in Gondar University Hospital, Northwest Ethiopia. Ethiop Med J 2011;49:117-23.
45. Schimel AM, Miller D, Flynn HW Jr. Endophthalmitis isolates and antibiotic susceptibilities: A 10-year review of culture-proven cases. Am J Ophthalmol 2013;156:50-20.
46. Khalifa SA, Farag MA, Yosrie N, Sabir JS, Saeed A, Al-Mousawi SM, *et al.* Truffles: From Islamic culture to chemistry, pharmacology and food trends in recent times. Trends Food Sci Technol 2019;91:193-281.
47. Doğan HH, Aydın S. Determination of antimicrobial effect, antioxidant activity and phenolic contents of desert truffle in Turkey (Article). Afr J Tradit Compl Altern Med 2013;10:52-8.
48. Stojković D, Reis FS, Ferreira IC, Barros L, Glamočlija J, Ćirić A, *et al.* Tirmania pinoyi: Chemical composition, *in vitro* antioxidant and antibacterial activities and *in situ* control of *Staphylococcus aureus* in chicken soup. Food Res Int 2013;53:56-62.
49. Al-Qarawi AA, Mridha MA. Status and need of research on desert truffles in Saudi Arabia. J Pure Appl Microbiol 2012;6:1051-62.
50. Younis S, Taj S, Rashid S. Structural studies of *Staphylococcus aureus* Sortase inhibition via Conus venom peptides. Arch Biochem Biophys 2019;671:87-102.