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Antimicrobial and Immunostimulatory Effects of *Senna occidentalis* Ethanolic Extract against *Aspergillus flavus*-Induced Mycotic Keratitis: A Promising Therapeutic Approach

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ASEB, EEEM, MSES, SMA, and KMSA designed the research plan and wrote the manuscript. Authors ASEB and EEEM performed the experimental work. All authors read and approved the final manuscript.

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ABSTRACT

Background: Mycotic keratitis (MK) poses a significant risk as a severe and difficult-to-treat corneal infection with potentially life-threatening consequences. Fungi are commonly implicated as causative agents. Additionally, improper care of contact lenses increases the susceptibility to MK. **Aim:** This study aimed to investigate the phytochemical composition and therapeutic potential of *Senna occidentalis* ethanolic leaf extract in the treatment of *Aspergillus flavus*-induced mycotic keratitis.

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Methodology: The selected plant extract was tested for antimicrobial activity, analyzed for active ingredients and evaluated in vivo for physiological (Kidney and liver functions), histological (corneal tissue healing rates) and immunological responses (IgM levels).
Results: The GC/MS analysis revealed high levels of some active components, including o-methyl glucose, tetramethyl hexadecenol, hexadecenoic acid, and octadecatrienoic acid. The ethanolic extract of *S. occidentalis* demonstrated potent antimicrobial activity against *A. flavus* in an albino rabbit model. Treatment with the extract at a concentration of 12.5 mg/ml resulted in a significant healing rate without any side effects, as evidenced by reduced levels of blood urea, creatinine, AST, and ALT in infected rabbits. Moreover, *S. occidentalis* extract effectively stimulated the immune system, as indicated by increased levels of IgM in both infected and non-infected rabbits. Histopathological analysis of the eye cornea further confirmed the complete disappearance of inflammation and restoration of normal tissue appearance and layer arrangement.
Conclusions: These findings highlight the potential of *S. occidentalis* extract as a safe, affordable, and easily accessible treatment for *A. flavus*-induced mycotic keratitis, owing to its rich content of safe and bioactive phytochemicals.

Keywords: Aspergillus flavus; Senna occidentalis; mycotic keratitis; physiological parameters; corneal histology.

1. INTRODUCTION

Mycotic keratitis (MK) is the inflammation of corneal stroma due to the invasion of fungal elements, which is an exogenous infection, entering through injuries of the corneal epithelium [1]. Fungal keratitis is highly invasive into the layers of corneal stroma and antifungal agents are mostly static, leading to prolonged treatment and even surgical intervention [2,2a,2b]. The occurrence and type of filamentous fungal keratitis are significantly impacted by several environmental conditions, including humidity, rainfall, and wind, as well as seasonal fluctuations [3]. Less common predisposing variables that can contribute to mycotic keratitis include the use of antibiotics or corticosteroids, allergic conjunctivitis, immunological incompetence, and the use of hydrophilic contact lenses [4]. Furthermore, fungal keratitis is typically associated with pre-existing ocular conditions such as insufficient tear secretion or defective eyelid closure, as well as systemic conditions like diabetes mellitus or immunosuppression. It can also occur as a secondary infection on abrasions caused by contaminated contact lenses or on pre-existing epithelial defects resulting from herpes keratitis [5].

In two randomized controlled studies, it was found that many cases of mycotic keratitis were resistant to chlorhexidine when administered as a weak substitute for natamycin [6]. *Candida*, a prevalent yeast in ocular infections among immunosuppressed patients, was often managed using topical amphotericin B. However, this treatment had poor efficacy and resulted in calcification of the corneal tissues. Echinocandins (such as caspofungin and micafungin) and fluoroquinolones can be included as combination therapies to provide comprehensive treatment [6]. Corticosteroids are highly undesirable because they promote the growth of fungi by suppressing the immune mechanisms in the eye. This includes inhibiting the movement of immune cells toward the infection site, preventing the ingestion of pathogens by immune cells, reducing the number of immune cells, and blocking the release of certain substances. Therefore, it is not recommended to use topical steroids in the treatment of fungal keratitis [7]. Topical cyclosporine A was proposed as a complementary agent to antifungal treatment due to its ability to hinder the growth of filamentous fungi. Although therapeutic penetrating keratoplasty in certain cases demonstrated sustained graft survival, there is no clinical evidence suggesting that graft survival acts as a limiting factor for fungal keratitis [7].

Throughout human history, plants have been utilized as the foundation for numerous traditional remedies and continue to be utilized as supplies for various contemporary pharmaceuticals. According to the World Health Organisation (WHO), approximately 60% of populations in regions with limited resources are unable to acquire or purchase conventional medications. Consequently, almost three-quarters of these populations depend upon medicinal plants to meet their primary healthcare demands [8]. Due to their availability, affordability, safety, potential

efficacy, and environmental friendliness, traditional herbal medicines have recently attracted increased attention [9]. The development of Egyptian herbal medicine has a long and complex history, influenced by several cultures. The ancient pharaonic and more contemporary Arabic Unani medicine are the primary and most important origins of all formulations in today's herbal market [10]. Phytocompounds found in nature have been shown to have potential antifungal properties due to their chemical composition, which allows them to exhibit various biological activities [11]. These phytochemicals encompass a wide array of components such as phenols, flavonoids, tannins, alkaloids, essential oils, polysaccharides, terpenoids, lignans, glycosides, and more [9].

In their study, Essien et al. [12] reported that the leaf oil of *S. occidentalis* demonstrated antimicrobial effects against a range of bacteria including *P. aeruginosa*, *B. cereus*, *E. coli*, and *S. aureus*. However, studies on the antifungal activity of *S. occidentalis* are extremely rare. Generally speaking, the aerial portions of *S. occidentalis* were the only focus of the few research that was conducted on its antifungal activities [13]. Before contemplating the examination of natural plant extracts as viable antifungal alternatives for the management of fungal keratitis, deeper investigations must be performed to assess the safety of host tissues and functions. These studies are critical to making well-informed decisions about the acceptability and effectiveness of these extracts in the treatment of eye diseases. According to our knowledge and a deep literature survey, there have been no reported studies on the use of *S. occidentalis* leaf extract for treating mycotic keratitis. Accordingly, the primary aims of this work was to report on the chemical composition and antifungal characteristics of leaf extracts from *S. occidentalis*, with the aim of investigating their possible use in the treatment of fungal keratitis.

2. MATERIALS AND METHODS

2.1 Clinical Diagnosis of Different Corneal Ulcers

The study conducted a survey on patients diagnosed with different types of corneal ulcers, specifically focusing on mycotic keratitis. The patients were regularly visited at the Ocular Microbiology lab in the Ophthalmology Hospital of Tanta University twice a week from January to December 2022. The symptoms associated with corneal fungal infections include initial foreign body sensation, gradual increase in pain, the presence of a thick area of keratitis with a sticky hypopyon, stromal infiltrates with feathery edges and epithelial defects, as well as coagulative necrosis resulting from the secretion of various fungal enzymes. These enzymes lead to the destruction of keratocytes and collagen lamellae, causing puncture of Descemet's membrane and the passage of fungi into the anterior chamber. Additionally, the study observed various complications accompanying active mycotic keratitis, such as endophthalmitis, perforation, ring abscess, corneal melting, guttering, facet formation, thinning, and corneal opacity. Photographs and clinical descriptions were used to summarize the clinical complications associated with fungal keratitis in all infected eyes.

2.2 Collection, Purification, and Identification of Keratitis Fungi

Samples were collected from patients diagnosed with mycotic keratitis using appropriate tools and precautions to avoid perforation and contamination. A sterile and controlled environment was maintained in the hospital laboratory. Corneal scrapings were streaked on Petri dishes containing Sabouraud's dextrose agar (SDA) medium supplemented with chloramphenicol to prevent bacterial contamination. The plates were incubated at 27°C for up to 21 days. Purified fungal colonies were identified and photographed using established references. Identification of fungal genera and species was based on the works of Houbraken et al. [14]. The research protocol was approved by the Research Ethics Committee of Tanta University with an approval code of 34895/9/21.

2.3 Detection of Pathogenicity Tools of Isolated Fungi

The isolated fungi from corneal ulcers were tested for collagenase and protease activities, the main contributors to corneal ulcers. For the qualitative collagenase assay, fungal isolates were grown on a collagen-dependent liquid medium for one week. The supernatants from the cultures were tested for collagenase activity using a native bovine collagen substrate. The

formation of a purple color indicated the presence of collagenase activity [15]. To assess protease activity, a modified method using casein as a substrate was employed. Agar plates containing casein were inoculated with fungal isolates and incubated. The formation of cleared zones around the colonies indicated proteolysis, which was quantified as the percentage ratio of the diameter of the cleared zone to the diameter of the colony [16].

For the quantitative estimation of collagenase activity, a liquid medium containing gelatin, glucose, yeast extract, and native bovine collagen was prepared. Fungal isolates were incubated in the medium, and the supernatants were collected. The concentration of L-leucine produced from collagen degradation was measured, and the concentration of total proteins in the supernatants was determined. The collagenase activity was calculated by dividing the L-leucine concentration by the total protein concentration [17].

2.4 Collection, Identification, and Extraction of Wild Plants

A total of eight wild plants (*Cotula sericea* [L.f.], *Senna alexandrina* [Mill.], *Senna occidentalis* [L.], *Senna italica* [Mill.], *Heliotropium europaeum* [L.], *Pulicaria undulata* [L.], *Aerva javanica* [Burm.f.], and *Cleome droserifolia* [Forssk.]) were collected from Halayeb and Shalateen reserved region, Red sea cost, Egypt in the Spring season of 2022. The plants underwent meticulous air drying and were subsequently dried for further investigation. To prepare the different extracts from the dried plants, the method outlined by Kasim et al. [18] was followed.

2.5 Assessment of the Anti-fungal Characteristics of the Plant Extracts

A spore suspension of the collected fungi was mixed with sterile Sabouraud's dextrose medium and poured onto sterile Petri dishes. Wells were created in the agar plates, and each well was filled with 20 mg of the tested extract. Three replicates were prepared for each test, and all plates were incubated at 27°C for 3 days. The average diameters of the inhibition zones were then measured in millimeters and compared across all plates [19]. The MIC of the investigated plant extracts was performed, and the findings demonstrated that the ethanolic extract of *S. occidentalis* was the most potent plant extract against the predominant fungal keratitis-causing agent *A. flavus*, with MIC of 12.5 mg/ml.

2.6 Molecular Identification of *A. flavus* Strain

The predominant fungal keratitis-causing agent *A. flavus* isolate was cultivated on Sabouraud's dextrose medium and subjected to incubation at 28°C for 5 days, as described by [20]. DNA extraction was carried out utilizing the Patho-gene-spin DNA/RNA extraction kit manufactured by Intron Biotechnology, Korea. Polymerase chain reaction (PCR) and sequencing procedures were conducted in collaboration with SolGent, Daejeon, South Korea. The amplification of the ITS regions of the rRNA gene was accomplished using the universal primers ITS1 (forward) and ITS4 (reverse), which were incorporated into the reaction mixture. The primer compositions are as follows: ITS1 (5' - TCCGTAGGTGAACCTGCGG - 3') and ITS4 (5'- TCCTCCGCTTATTGATATGC -3').

Subsequently, the purified PCR product was subjected to sequencing using the same primers along with the incorporation of ddNTPs in the reaction mixture, following the approach outlined by [21]. The obtained sequences were then subjected to analysis using the Basic Local Alignment Search Tool (BLAST) available on the National Centre of Biotechnology Information (NCBI) website. Finally, the analysis of the sequences and the construction of phylogenetic trees were performed using MegAlign software version 5.05, developed by DNA Star.

The DNA sequence was aligned in the international GenBank database (http://www.ncbi.nlm.nih.gov/) using BLAST. Evolutionary history was inferred by the Maximum Likelihood method based on the Tamura-Nei model [22]. Evolutionary analysis was conducted by MEGA7 according to Kumar et al. [23] and the nucleotide sequences were deposited in the GenBank.

2.7 Phytochemical Investigation of the Ethanol Extract of *S. occidentalis* Using GC/MS

The phytochemical composition of *S. occidentalis* ethanolic extract was performed using Clarus 580/560S gas chromatography/mass spectrometry (GC/MS) (Perkin Elmer Inc., Waltham, MA, USA). An Elite-5 MS column (30 mm x 0.25 mm x 0.25 µm film thickness) was

utilized. The oven temperature was programmed to start at 80°C for 7 min., followed by an increase of 10°C/min. until 140°C with a 1 min. hold, then held at 200°C for 1 mi., and finally held at 280°C for 10 min. with a rate of 5°C per min. The input and transfer lines were maintained at 250°C. Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. A sample of 1 µL was automatically injected after a solvent delay of 5 min. by autosampler AS3000 and GC in split mode (1:20) at an ionization energy of 70 eV, EI mass spectra were obtained in full scan mode over the range of m/z 40–650. The ionization chamber temperature was set at 200°C. The identified components of the analyzed extract were determined by comparing their retention times and mass spectra to those of WILEY-09 and NIST 11 mass spectral databases.

2.8 Treatment of Experimental Animals with *S. occidentalis* Ethanolic Extract

As a safety confirmative test for the usage of *S. occidentalis* ethanolic extract to be applied as an antifungal agent in the treatment of corneal fungal infection of the experimental animals, the experimental design for the present investigation was performed according to El-Badry [24]. The research protocol, involving sampling, patient and animal model handling, was approved by the Research Ethics Committee of Tanta University (approval code: 34895/9/21). Twins male albino rabbits of the same weight (2 kg) were adapted to the lab conditions for 3 days before the start of work and divided into groups for different treatments as follows: albino rabbits were maintained under a 12 h. light-dark cycle at a temperature of 22±2°C and fed with standard diet (Soy protein, 18%; Vitamins mixture, 3.5%; Choline chloride, 0.2%; Soybean oil, 5.6%; Cellulose, 3.4%; L-lysine, 0.3% and Starch, 68.7%) and water. Each group of rabbits, with 3 individuals, was housed separately in special cages. The groups were as follows:

Group A: Healthy rabbits with no infection and no treatment, ordinary follow-up for normal growth, and normal incubation conditions.

Group B: The corneal surface of the right eyes of rabbits was infected with a spore suspension of *A. flavus* (10^6/ml) by scratching and swabbing with the spore suspension once daily for a week until noticeable fungal growth was established. The left eyes were left untreated to monitor the progression of the infection.

Group C: The right eyes of a healthy rabbit were treated twice daily for a week with a water suspension of S. occidentalis ethanolic extract that was redissolved in water.

Group D: Healthy rabbit right eyes were dropped with the standard concentration of the commercial antifungal drug twice daily for a week.

Group E: A. flavus infection of albino rabbits' right eyes, treated with the standard concentration of the selected commercial antifungal drug by dropping onto the infected eyes with one drop every 2 h till the end of treatment.

Group F: A. flavus infection of albino rabbits' right eyes, treated with a combination of the standard concentration of the selected commercial antifungal drug and the MIC of *S. occidentalis* extract by dropping onto the infected eyes every 2 h till the end of treatment.

Group G: A. flavus infection of albino rabbits' right eyes, treated with the MIC of *S. occidentalis* extract by dropping onto the infected eyes every 2 h till the end of treatment.

Group H: A. flavus infection of albino rabbits' right eyes, treated with the double MIC of *S. occidentalis* extract by dropping onto the infected eyes every 2 h till the end of treatment.

Following the completion of each treatment, rabbit eyes were photographed, and then rabbits were sacrificed after 2 h of the last dose (without using anesthesia). Blood samples were collected from each rabbit separately in sterile test tubes and kept for further assays and corneal samples were excised for further histological examinations.

2.9 Biochemical Parameters

2.9.1 Determination of serum AST and ALT levels

The enzymatic activity of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was determined using the method of Reitman and Frankel [25], employing the Diamond Diagnostic kit provided by Diamond Co. Egypt. The procedure involved adding 500 µl of 100 mM phosphate buffer with a pH of 7.2, together with 80 mM L-aspartate as a substrate for AST or 80 mM D-L-alanine as a

substrate for ALT, and 4 mM α-ketoglutarate to 100 µl of rabbit's serum. The mixture was subjected to incubation at a temperature of 37˚C for 30 min. Subsequently, the mixture was combined with 500 µl of the color developing reagent (4 mM 2, 4-dinitrophenylhydrazine), and then incubated at room temperature for 20 min. After that, 5 ml of 0.4 N NaOH was added, followed by thorough mixing, and then incubation at room temperature for 5 min. The absorbance was measured spectrophotometrically at 546 nm against the blank (assay combination without enzyme source). The AST and ALT activities were quantified as U/L AST and U/L ALT, respectively.

2.9.2 Determination of serum creatinine and urea levels

The quantification of creatinine level in the rabbit serum samples was performed following the manufacturer's instructions outlined in the Randox reagent kit manual (Crumlin, County Antrim, UK). The development of a yellow-orange colored complex with alkaline picrate, which could be measured at 492 nm, was the basis of creatinine determination [26]. The serum urea concentration was quantitatively determined using the Biolab Kit (Maizy, France) employing enzymatic reaction. The measurement was conducted at 578 nm, following the methodology established by Smith et al. [27].

2.9.3 Determination of serum IgM level

Serum immunoglobulin M (IgM) levels were quantitatively determined using an enzyme-linked immunosorbent assay (ELISA) method based on the protocol described by Takahashi et al. [28]. Various solutions and reagents were prepared, including coating buffer, wash solution, blocking solution, and sample/conjugate diluent. The step-by-step procedure involved coating the wells with a capture antibody, followed by the addition of an HRP detection antibody. The wells were then washed, and an enzyme substrate solution was added, allowing for a reaction to occur. The reaction was stopped using 2 M H_2SO_4, and the absorbance of the plate was measured at 450 nm. The results were calculated by determining the mean value of duplicate readings for each standard, control, and sample, and subtracting the blank reading. The concentration of IgM was calculated using a previously prepared standard curve.

2.10 Histological Examination of Corneal Tissues

The excision of rabbit right eye corneas in the experimental groups was performed according to the protocol outlined by Fischer et al. [29]. The corneas were fixed using alcoholic Bouin fixative, which involved injecting 0.1 ml of the fixative into the anterior chamber of the eye and dropping another 0.1 ml onto the eye surface. After excision, the corneal samples were immediately immersed in the fixative for 24 h. Subsequently, the samples underwent a series of steps, including washing with distilled water, dehydration in ethanol, soaking in a solution containing eosin dye, clarification in xylene, embedding in molten paraffin, and sectioning into 5 µm thickness using a microtome. The sections were then stained with hematoxylin and eosin, dehydrated, cleared with xylene, and mounted using a Hoyer mounting medium. The resulting slides were examined under a digital microscope, and images were captured at a magnification of 400X (Celestron Pentaview, USA).

3. RESULTS

3.1 Diagnostic Assessment of Corneal Ulcers

The survey conducted at the Ocular Microbiology lab, Ophthalmology Hospital, Tanta University, from January 2022 to December 2022, revealed that patients diagnosed with various corneal ulcers resulting from corneal fungal infections experienced initial sensations of a foreign object and subsequently experienced escalating pain. Observations revealed a dense region of keratitis accompanied by a viscous hypopyon, stromal infiltrates characterized by delicate borders, and an area of epithelial defect. The fungal enzymes, including phospholipase, collagenase, and proteases, induced coagulative necrosis, resulting in the depletion of keratocytes and the breakdown of collagen lamellae. Consequently, Descemet's membrane was punctured, enabling the fungus to infiltrate the anterior chamber. Mycotic keratitis that is active is frequently associated with complications such as endophthalmitis, perforation, ring abscess, corneal melting, gutter formation, facet formation, thinning, and corneal opacity.

3.2 Characterization of Keratitis-causing Fungi

According to our earlier investigation [30], we found that the fungal species responsible for

mycotic ulcers ranked in the following order: *Aspergillus flavus* > *Aspergillus niger* > *Candida albicans* > *Mucor fuscus*. We assessed the hydrolytic activity of the obtained isolates by conducting casienase and collagenase assays, as these fungi have aggressive capabilities that contribute to their spread in corneal diseases. Our previous investigation revealed significant levels of activity in both casienase and collagenase enzymes in *Aspergillus flavus*, suggesting its prominent function as a corneal invader.

3.3 The Anti-fungal Characteristics of the Investigated Plant Extracts

Our earlier study demonstrated that the ethanolic extract derived from *Senna occidentalis* exhibited the highest efficacy as an antifungal agent compared to other examined plants. Specifically, it displayed significant effectiveness against *Aspergillus flavus*, which is the most prevalent causal agent of fungal keratitis. Therefore, the ethanolic extract of *S. occidentalis* was selected to investigate its active components to determine its potential as a natural and safe treatment for fungal keratitis.

3.4 Molecular Identification of *A. flavus*

The utilization of 5.8S-ITS DNA sequences has been documented as a reliable method for the identification and classification of *A. flavus* [31]. Through phylogenetic analysis, it was verified that the *A. flavus* isolate PP756682 represents a mycelial fungus belonging to the Aspergillaceae family and Eurotiales order, characterized by the production of closed fruiting bodies (Fig. 1 and Table 1). The 5.8S-ITS DNA sequence of *A. flavus* PP756682 was deposited in the GenBank database with the accession number PP756682.1.

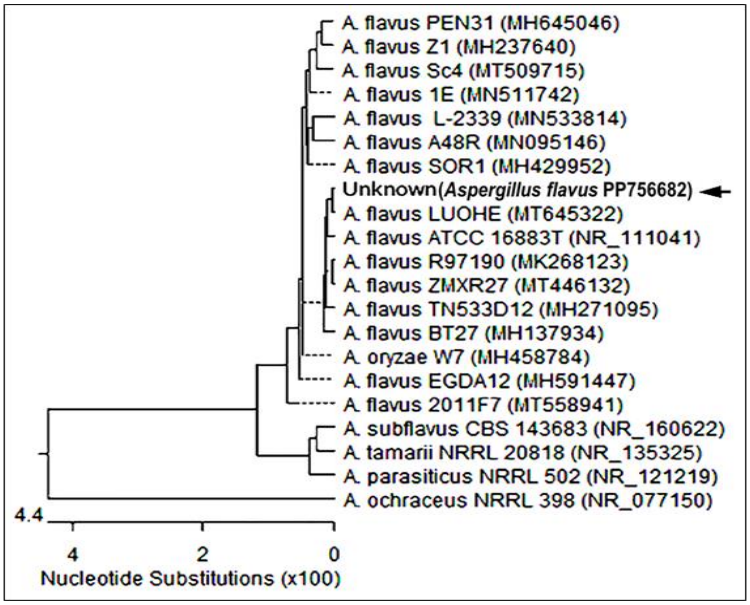

Fig. 1. Molecular phylogenetic analysis of the selected *Aspergillus flavus* PP756682 isolate

Table 1. Partial sequencing of 18s rRNA of *Aspergillus flavus* PP756682

No.	Sequence	No.
1	ctgcggaagg atcattaccg agtgtagggt tcctagcgag cccaacctcc cacccgtgtt	60
61	tactgtacct tagttgcttc ggcgggcccg ccattcatgg ccgccggggg ctctcagccc	120
121	cgggcccgcg cccgccggag acaccacgaa ctctgtctga tctagtgaag tctgagttga	180
181	ttgtatcgca atcagttaaa actttcaaca atggatctct tggttccggc atcgatgaag	240
241	aacgcagcga aatgcgataa ctagtgtgaa ttgcagaatt ccgtgaatca tcgagtcttt	300
301	gaacgcacat tgcgcccctt ggtattccgg ggggcatgcc tgtccgagcg tcattgctgc	360
361	ccatcaagca cggcttgtgt gttgggtcgt cgtcccctct ccgggggggga cgggccccaa	420
421	aggcagcggc ggcaccgcgt ccgatcctcg agcgtatggg gctttgtcac ccgctctgta	480
481	ggcccggccg gcgcttgccg aacgcaaatc aatcttttc caggttgacc tcggatcagg	540
541	tagggatacc cgctgaactt aagcatatca ataagcggag ga	582

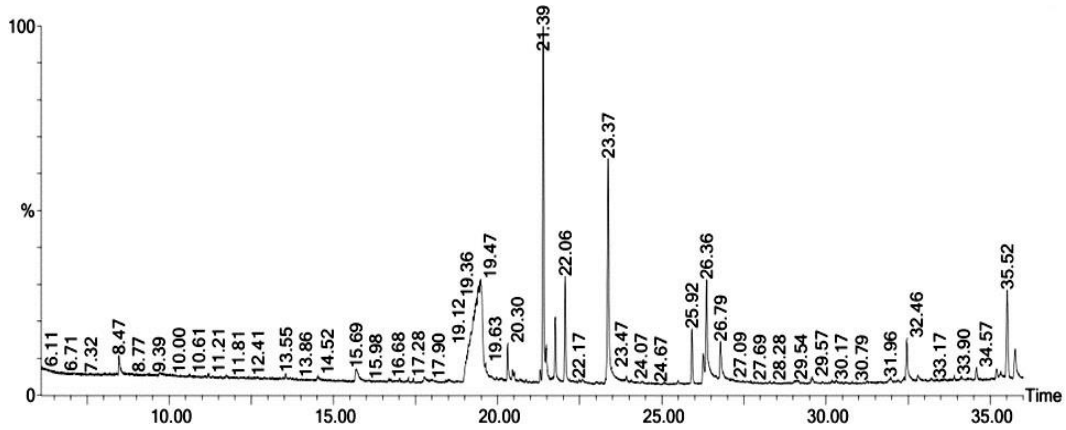

Fig. 2. GC/MS chromatogram of *Senna occidentalis* ethanolic extract analysis

Table 2. Chemical constituents and the biological activity of *Senna occidentalis* ethanolic extract

Peak No.	RT (min.)	Area (%)	Compound name	Chemical formula	Activity	Reference
1	8.469	0.768	9,12,15-Octadecatrienoic acid, 2- [(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)	$C_{27}H_{52}O_4Si_2$	Antimicrobial	[32]
2	8.534	0.**378**	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-,17-[O-(phenylmethyl)oxime], (3à,5à)	$C_{29}H_{43}NO_3Si$	Antimicrobial and antidiabetic	[33]
3	9.705	0.**736**	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{56}O_4Si_2$	Antimicrobial	[34]
4	13.551	**0.407**	9,12,15-Octadecatrienoic acid, 2- [(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)	$C_{27}H_{52}O_4Si_2$	Antimicrobial	[32]
5	15.692	1.090	d-Mannitol, 1-decylsulfonyl	$C_{16}H_{34}O_7S$	Antimicrobial and anti-cancer	[35]
6	17.763	0.**523**	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis	$C_{28}H_{44}O_4$	Antimicrobial and antiperspirant	[36]
7	19.484	24.581	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	Antimicrobial, immune activator, and signal regulator	[37]
8	19.634	0.981	l-Gala-l-ido-octose	$C_8H_{16}O_8$	Antibacterial, antiviral, antioxidant, and anti-coagulant	[38]
9	20.304	0.985	Myristic acid	$C_{14}H_{28}O_2$	Antifungal, antiviral, and anticancer	[39]
10	20.454	0.282	4-Cyclopentene-1,3-dione, 4,5-dimethyl-2-phenyl	$C_{13}H_{12}O_2$	Antimicrobial and disinfectant	[40]
11	20.509	0.235	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis	$C_{28}H_{46}O_4$	Antimicrobial, and antiperspirant	[36]
12	21.299	0.314	1-Dodecanol, 3,7,11-trimethyl	$C_{15}H_{32}O$	Antimicrobial, antioxidant, antitumor, antidiabetic, and antihypertensive	[41]
13	21.389	10.410	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Antimicrobial, antioxidant, anticancer, and	[42]

Peak No.	RT (min.)	Area (%)	Compound name	Chemical formula	Activity	Reference
					hepatoprotective	
14	21.479	1.361	2-Hexadecene, 2,6,10,14-tetramethyl	$C_{20}H_{40}$	Antimicrobial	[43]
15	21.760	2.190	1-Heptadecyne	$C_{17}H_{32}$	Antibacterial, anticancer, antioxidant, antidiabetic, antiemetic, hypoglycemic, antihypertensive, and hypolipidemic	[44]
16	22.060	3.397	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Antimicrobial, antioxidant, anticancer, and hepatoprotective	[42]
17	22.495	0.137	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{56}O_4Si_2$	Antimicrobial	[34]
18	22.580	0.246	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	Antimicrobial, antidiabetic, and cardiovascular-protective	[45]
19	23.370	10.154	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Antimicrobial, antioxidant, anti-inflammatory, anticancer, and hypocholesterolemic	[46]
20	23.840	0.165	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis	$C_{28}H_{44}O_4$	Antimicrobial, and antiperspirant	[36]
21	23.925	0.547	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)	$C_{27}H_{52}O_4Si_2$	Antimicrobial	[32]
22	25.916	1.715	2-Tridecen-1-ol (E)	$C_{13}H_{26}O$	Anticancer	[47]
23	26.261	1.196	2-Chloroethyl linoleate	$C_{20}H_{35}ClO_2$	Antibacterial, antifungal, and anti-inflammatory	[48]
24	26.361	5.378	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{19}H_{32}O_2$	Antimicrobial, antioxidant, anti-inflammatory, myorelaxant, anticancer, antigenotoxic, cardiovascular-protective, neuro-protective, and anti-osteoporotic	[49]
25	26.652	0.146	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	Antimicrobial, antidiabetic, and cardiovascular-protective	[45]
26	26.787	1.629	Stearic acid	$C_{18}H_{36}O_2$	Anticancer	[50]
27	29.108	0.281	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	Antimicrobial	[51]
28	29.578	0.620	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	Antidiabetic, anti-inflammatory, antioxidant, anti-ulcerogenic, hepato-protective, and antimicrobial	[52]
29	31.964	0.362	1-Heptatriacotanol	$C_{37}H_{76}O$	Antimicrobial, anti-	[53]

Peak No.	RT (min.)	Area (%)	Compound name	Chemical formula	Activity	Reference
					inflammatory, and antioxidant	
30	32.459	2.230	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	Antimicrobial, and antioxidant	[54]
31	32.799	0.309	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl	$C_9H_9F_3O_2$	Antimicrobial	[55]
32	34.590	0.850	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl	$C_{13}H_{22}OSi_2$	Antimicrobial	[56]
33	35.200	0.353	3,5,6-Trimethyl-p-quinone, 2-(2,5- dioxotetrahydrofuran-3-yl)thio	$C_{13}H_{12}O_5S$	Antimicrobial	[57]
34	35.515	3.648	Tricosane	$C_{23}H_{48}$	Ophthalmic and antimicrobial	[58]
35	35.760	1.673	Octadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	$C_{21}H_{42}O_4$	Antimicrobial, and antioxidant	[59]

3.5 Phytochemical Composition of *S. occidentalis* Ethanolic Extract Using GC/MS

The data reported in Fig. 2 and Table 2 depict the phytochemical constituents and the anticipated biological efficacy of the ethanolic extract of *S. occidentalis*, as assessed by the GC/MS technique. The data indicated the identification of 35 phytochemical components with varying prevalence ratios (expressed as area %) with their biological activity, based on the literature survey. The predominant constituents are o-methyl glucose (24.58%), tetramethyl hexadecenol (10.41%), hexadecenoic acid (10.15%), octadecatrienoic acid (5.38%), and tricosane (3.64%). The data additionally demonstrated the presence of various active phytochemicals in the ethanolic extract of *S. occidentalis*. These include d-mannitol, 1-decylsulfonyl, 9-octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis, l-gala-l-ido-octose, myristic acid, 2-hexadecene, 2,6,10,14-tetramethyl, 1-heptadecyne, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 2-tridecen-1-ol (E), 2-chloroethyl linoleate, stearic acid, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, and octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester. The components 1-monolinoleoylglycerol trimethylsilyl ether, 12-methyl-E,E-2,13-octadecadien-1-ol, and 9-octadecenoic acid, (2-phenyl-1,3-dioxolan-4- yl)methyl ester, cis were determined to be the least abundant in the ethanolic extract of *S. occidentalis*. The identified compounds exhibit many biological activities, including antioxidant, anti-inflammatory, anticancer, and antibacterial activities. Additionally, some of these have long-lasting analgesic properties, antigenotoxic effects, and the capacity to promote the human immune system, as detailed in Table 2.

3.6 The Visual Manifestation of Rabbit Eyes Afflicted with *Aspergillus flavus*

The histological implications of *Aspergillus flavus*, commercial antifungal agent (fluconazole 150 mg/ml), the ethanolic extract of *S. occidentalis*, and their combined effect on the external appearance of albino rabbit cornea are depicted in Fig. 3. Throughout the experimental stages, the eyes of albino rabbits exhibited a healthy and intact corneal structure, with normal eyeball and eyelid appearance in the non-infected, non-treated right eye (Fig. 3-a). Conversely, Fig. 3-b revealed complete damage to corneal tissues, fungal growth filling the anterior chamber and inner eyeball space, and pronounced inflammation in the *A. flavus*-infected, non-treated right eye of albino rabbits. Interestingly, the right eye of albino rabbits treated with *S. occidentalis* ethanolic extract at its MIC value (12.5 mg/ml) exhibited a normal appearance (Fig. 3-c). However, the commercial dose of fluconazole (150 mg/ml) caused observable inflammation and hypersensitivity in the treated right eye of albino rabbits (Fig. 3-d).

Treatment of *A. flavus*-infected eyes of albino rabbits with fluconazole (150 mg/ml) resulted in a slow response and low healing rate. Although fungal growth was inhibited within three weeks, inflammatory effects remained (Fig. 3-e). In contrast, the combination of *S. occidentalis* extract with fluconazole accelerated the visual manifestation of the healing rate and decreased inflammation symptoms during fungal infection treatment (Fig. 3-f).

Fig. 3-g illustrates the significant healing rate observed in *A. flavus* infection, with the complete disappearance of inflammation and the restoration of a normal appearance in the infected right eye of albino rabbits following treatment with the MIC dosage of *S. occidentalis* extract within only two weeks. Furthermore, a more definitive demonstration of the effect of *S. occidentalis* on fungal keratitis in vivo was achieved by treating the infected eye with double the MIC concentration (25 mg/ml) of the extract. This resulted in the highest healing rate of corneal tissues, complete inhibition of fungal growth, and the disappearance of inflammation in the treated eye within ten days (Fig. 3-h).

3.7 Biochemical Parameters of Treated Rabbits

To assess the safety parameters of synthetic and natural antifungal agents and evaluate the potential of *S. occidentalis* as an effective remedy against corneal fungal infection, various physiological and immunological responses were measured in blood samples collected from sacrificed rabbits *in vivo* (Table 3). Infection with *A. flavus* was found to significantly elevate the levels of liver function markers, with alanine aminotransferase (ALT) reaching 56 U/L and aspartate aminotransferase (AST) reaching 52 U/L in the infected, non-treated rabbit group (gp. b) compared to the healthy, non-treated rabbit group. However, treatment of infected rabbits with *S. occidentalis* extract demonstrated a positive impact on liver function, as indicated by ALT and AST levels of 39 and 36 U/L, respectively, in the infected and plant extract-treated with 12.5 mg/ml extract rabbit group (gp. g). These values were comparable to the ALT and AST levels of 44 and 43 U/L, respectively, in the non-infected, non-treated rabbit group (gp. a), and 38 and 34 U/L, respectively, in the non-infected, plant extract-treated rabbit group (gp. c). These findings were further supported by similar results obtained using double the MIC concentration of the plant extract (gp. h).

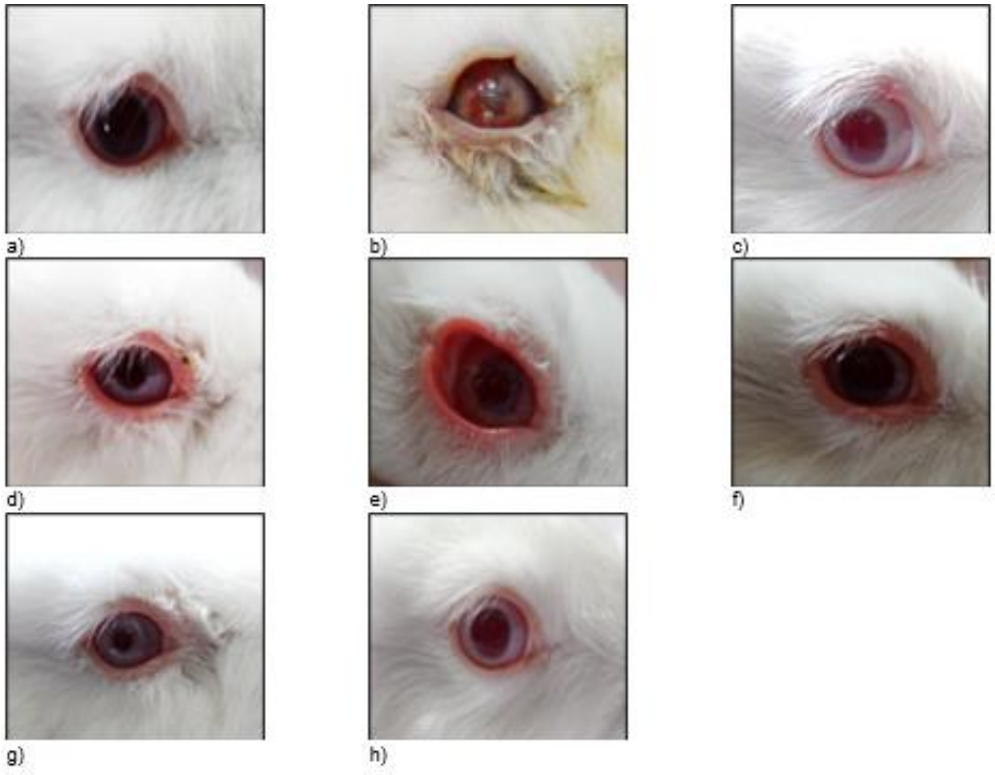

Fig. 3. Effect of *Senna occidentalis* ethanolic extract on the external appearance of the cornea of albino rabbits' eyes. a) non-infected untreated, b) *Aspergillus flavus*-infected untreated, c) *Senna occidentalis* MIC-treated without infection d) Fluconazole-treated without infection (150 mg/ml), e) *A. flavus*-infected treated with fluconazole, f) *A. flavus*-infected treated with combination of *S. occidentalis* MIC and fluconazole, g) *A. flavus*-infected treated with *S. occidentalis* MIC, and h) *A. flavus*-infected treated with *Senna occidentalis* double MIC

Table 3. Liver and kidney function parameters and immunoglobulin M (IgM) levels in response to the ethanolic extract of Senna occidentalis used in the treatment of in vivo-induced mycotic keratitis in rabbits

Rabbit groups	Liver function		Kidney function		Immune response
	ALT (U/L)	AST (U/L)	Urea (mmol/L)	Creatinine (mg/dL)	IgM (mg/dL)
a	44	43	45	1.0	18
b	56	52	58	1.2	24
	38	34	43	0.6	26
d	87	65	68	1.3	21
e	92	71	73	1.5	26
f	65	53	55	1.0	33
g	39	36	37	0.6	40
h	36	34	33	0.7	47

a) non-infected untreated, b) Aspergillus flavus-infected untreated, c) Senna occidentalis MIC-treated without infection d) Fluconazole-treated without infection (150 mg/ml), e) A. flavus-infected treated with fluconazole, f) A. flavus-infected treated with combination of S. occidentalis MIC and fluconazole, g) A. flavus-infected treated with S. occidentalis MIC, and h) A. flavus-infected treated with Senna occidentalis double MIC. ALT = alanine transaminase, AST = aspartate transaminase, and IgM = immunoglobulin M

Conversely, treatment with the commercial fluconazole solution resulted in a noticeable increase in liver function parameters, with ALT and AST reaching 87 and 56 U/L, respectively, in the non-infected, fluconazole-treated rabbit group (gp. d). These levels were even higher in the infected and fluconazole-treated rabbit group (gp. e), reaching 92 U/L for ALT and 71 U/L for AST. However, when fluconazole was combined with *S. occidentalis* extract to treat infected rabbits (gp. f), the levels were significantly reduced to 65 U/L for ALT and 53 U/L for AST, indicating a beneficial effect of the combined treatment. These results highlight the potential of *S. occidentalis* extract as a promising and safe alternative for the treatment of corneal fungal infections, as it exhibited positive effects on liver function markers, while the commercial fluconazole solution showed some hepatotoxicity. The combined treatment demonstrated a synergistic effect, effectively reducing liver function levels and suggesting enhanced efficacy in combating fungal infections.

The impact of experimental treatments on kidney function parameters, urea and creatinine levels, is presented in Table 3. The results revealed that fungal infection significantly elevated the levels of blood urea and creatinine in infected rabbits, reaching 58 mmol/L and 1.2 mg/dL, respectively, compared to the control group levels of 45 mmol/L and 1.0 mg/dL. However, S. *occidentalis* extract effectively reduced blood urea and creatinine levels to equal or even lower than those observed in healthy rabbits (43 mmol/L and 0.6 mg/dL, respectively).

Conversely, treatment with fluconazole resulted in a noticeable increase in blood urea and creatinine levels in the treated rabbits (68 mmol/L and 1.3 mg/dL, respectively). Moreover, in *A. flavus*-infected rabbits, fluconazole treatment further elevated these levels to 73 mmol/L and 1.5 mg/dL, respectively. However, the combination of *S. occidentalis* extract with fluconazole effectively lowered blood urea and creatinine levels in the infected rabbits (55 mmol/L and 1.0 mg/dL, respectively). Notably, a significant decrease in urea and creatinine levels was observed when the infected rabbits were treated with *S. occidentalis* extract at the MIC dosage, with levels of 37 mmol/L and 0.6 mg/dL, respectively. Furthermore, the enhancement of kidney function parameters in rabbit blood was remarkable when treated with a double MIC concentration of *S. occidentalis* extract, as it yielded levels even lower than those of the healthy control group (33 mmol/L and 0.7 mg/dL, respectively). These findings provide compelling evidence for the potential of *S. occidentalis* extract to restore kidney function parameters to normal or improved levels, surpassing the effects of fluconazole treatment. These results hold great promise for the development of novel therapeutic approaches for fungal infections of the cornea, emphasizing the remarkable potential of *S. occidentalis* extract.

The determination of serum immunoglobulin M (IgM) levels in the blood samples of experimental rabbits provides insights into the intrinsic immune response elicited by the various experimental treatments (Table 3). Notably, the levels of IgM in infected rabbits, fluconazole-treated rabbits, and

infected fluconazole-treated rabbits were found to exhibit a compromised immune response, with values of 24, 21, and 26 mg/dL, respectively, in comparison to the healthy control group's IgM level (18 mg/dL). In contrast, the administration of *S. occidentalis* extract demonstrated a beneficial effect on stimulating the immune system of both infected and non-infected rabbits. This fortuitous response played a vital role in expediting and facilitating the complete healing of the targeted infection. Notably, the treatment with *S. occidentalis* extract increased the IgM levels of healthy rabbits (26 mg/dL). Furthermore, the combination of *S. occidentalis* extract at the MIC dosage with fluconazole in infected rabbits led to a significant elevation of IgM level (33 mg/dL). Similarly, when *S. occidentalis* extract was administered at the MIC dosage in *A. flavus*-infected rabbits, a further increase in IgM levels was observed (40 mg/dL). The highest level of IgM was attained when *S. occidentalis* extract was applied at double the MIC concentration in *A. flavus*-infected rabbits, resulting in a remarkable level of 47 mg/dL.

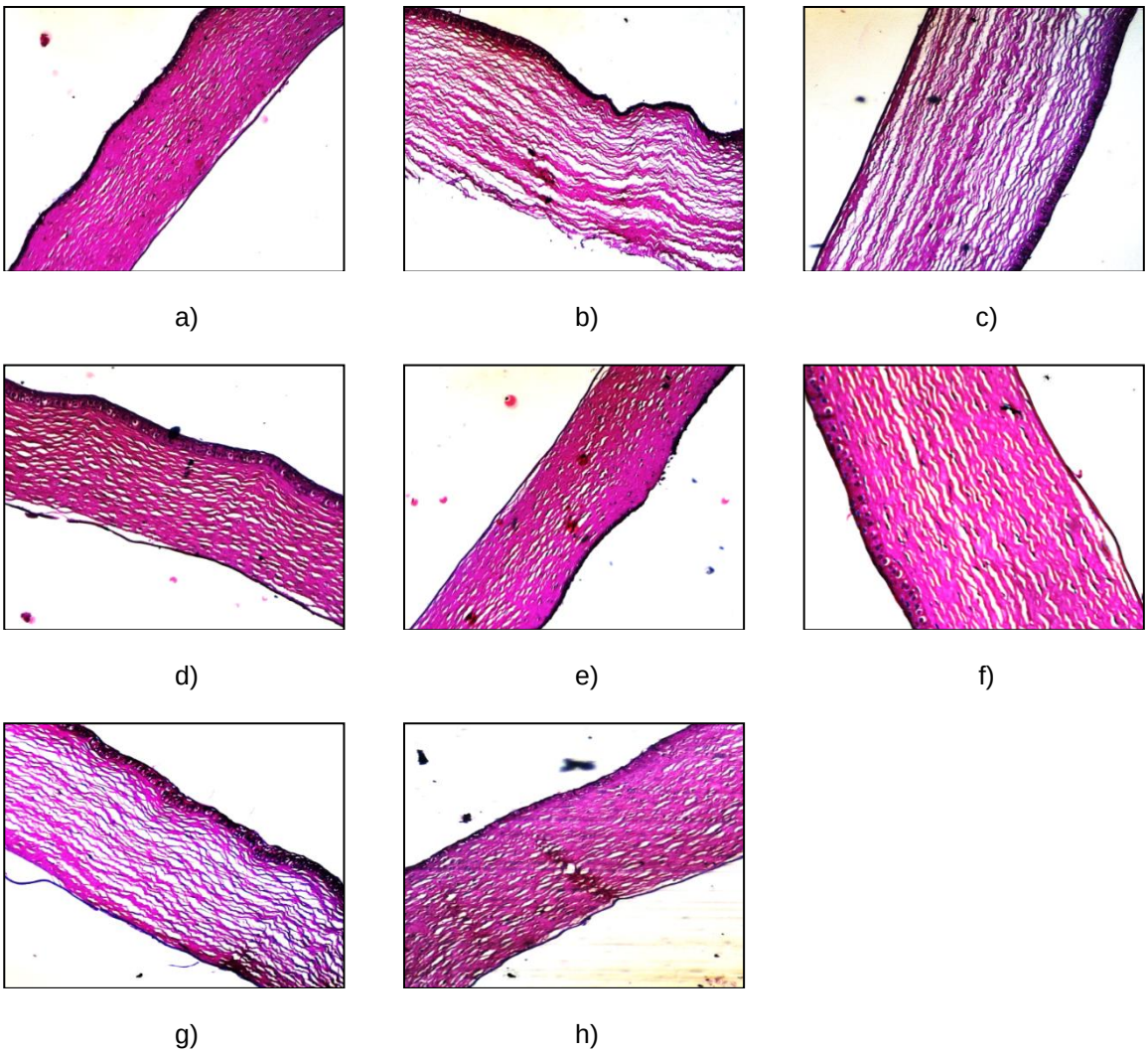

Fig. 4. Histopathological effects of *Senna occidentalis* ethanolic extract on the corneal tissues of albino rabbits. a) non-infected untreated, b) *Aspergillus flavus*-infected untreated, c) *Senna occidentalis* MIC-treated without infection d) Fluconazole-treated without infection (150 mg/ml), e) *A. flavus*-infected treated with fluconazole, f) *A. flavus*-infected treated with combination of *S. occidentalis* MIC and fluconazole, g) *A. flavus*-infected treated with *S. occidentalis* MIC, and h) *A. flavus*-infected treated with *Senna occidentalis* double MIC

3.8 Histopathological Effects of *Senna occidentalis* Extract on the Corneal Tissues

Following the *in vivo* investigation of the antifungal and histological effects of *S. occidentalis* extract on albino rabbit corneas, the corneas were excised meticulously and preserved in a 10% formalin solution. Subsequently, transverse sections of the corneas were stained, allowing for examination under a light microscope. Photographic documentation was conducted for all animal groups involved in the study (Fig. 4). Fig. 4-a illustrates the cornea of healthy rabbits, exhibiting an intact and regular layer with normal thickening and smooth epithelium and endothelium. In contrast, Fig. 4-b displays the cornea of *A. flavus*-infected rabbits, showing significant damage and distortion across all tissue layers.

The application of *S. occidentalis* extract to non-infected rabbit corneas, as depicted in Fig. 4-c, preserved the regular arrangement and thickness of the corneal layers. Conversely, treatment with a commercial fluconazole solution on non-infected rabbit corneas (Fig. 4-d) resulted in noticeable inflammation of the stroma, disintegration of the endothelium, and distortion of the epithelium.

Microscopic examination of infected rabbit corneas after three weeks of fluconazole treatment, as depicted in Fig. 4-e, revealed poor healing signs and persistent side effects of the drug. The corneal tissues exhibited stromal infiltration, thickening, incomplete endothelium regeneration, and irregular epithelium appearance. These complications manifested despite the inhibition of fungal growth. However, the combination of *S. occidentalis* extract at MIC dosage with fluconazole (Fig. 4-f) increased the likelihood of avoiding the side effects associated with the commercial drug. The corneas displayed an intact endothelium and relatively regular epithelium. Moreover, the use of the minimum inhibitory concentration (MIC) of *S. occidentalis* extract resulted in faster inhibition of fungal growth and stimulated the healing of corneal tissues after controlling the infection. This treatment led to complete regeneration of the endothelium, regular arrangement of the epithelium, and reduced stromal infiltration (Fig. 4-g). The fastest and most complete inhibition of fungal growth within corneal tissues was achieved by using double the MIC of *S. occidentalis* extract (Fig. 4-h). This treatment resulted in a healed cornea with a normal appearance, exhibiting regular arrangement and thickness of the epithelium, stroma, and endothelium.

4. DISCUSSION

Fungal keratitis poses a significant health concern in developing countries. The prevalence of this condition is notably higher in regions with limited access to quality healthcare, inadequate hygiene practices, and suboptimal agricultural and environmental conditions. Factors such as warm and humid climates, agricultural occupations, improper contact lens use, and ocular trauma contribute to the increased incidence of fungal keratitis in these settings [60]. Moreover, limited awareness about the disease, delayed diagnosis, and challenges in accessing appropriate antifungal medications further exacerbate the burden of fungal keratitis in developing countries. The lack of resources and infrastructure to support effective management and treatment strategies adds to the complexity of addressing this issue. Therefore, implementing comprehensive public health programs, improving healthcare infrastructure, promoting hygiene education, and ensuring the availability of affordable antifungal medications are crucial steps toward mitigating the impact of fungal keratitis in developing countries [61].

Despite chemical antifungal treatments being proven effective against fungal keratitis, their utilization involves potential dangers to patient well-being and health consequences. More precisely, these artificial pharmaceutical substances might lead to localized negative consequences such as irritation and allergic inflammation of eye tissues, as well as causing discomfort to the patient [62]. Administration of the drug throughout the body may also result in issues related to toxicity and possible interactions with other drugs, posing a risk to the health and safety of the patient [63]. Furthermore, an increasing issue in long-term treatment is the emergence of drug resistance among fungal infections.

In order to address these issues, the use of natural product extracts derived from plants has emerged as a possible alternative therapeutic strategy. In the current study, ethanolic extract of *S. occidentalis* was explored as a potential natural antifungal agent against *A. flavus* that causes fungal keratitis. The present work

investigated the potential of the ethanolic extract derived from *S. occidentalis* as a natural antifungal agent against *A. flavus*, the causative agent of fungal keratitis. The phytochemical analysis using GC/MS approach revealed the existence of several phytocompounds with a broad spectrum of biological activity. The prevalent compounds were o-methyl glucose, tetramethyl hexadecenol, hexadecenoic acid, octadecatrienoic acid, and tricosane. In addition, the ethanolic extract of *S. occidentalis* possessed other active phytochemicals, such as d-mannitol, 1-decylsulfonyl, 9-octadecenoic acid, l-gala-l-ido-octose, myristic acid, 2-chloroethyl linoleate, stearic acid, hexadecanoic acid, octadecanoic acid, and others. These compounds that were identified have been reported to provide health benefits to the human body, such as antioxidant, anti-inflammatory, anticancer, and antibacterial activity [64]. Additionally, they have long-lasting analgesic effects, antigenotoxic effects, and may stimulate the human immune system (Table 1).

The investigation conducted by Yadav et al. [65] revealed that the extract of *S. occidentalis* includes substantial quantities of saponins, flavonoids, sterols, triterpenes, and tannins. These phytochemicals are secondary metabolites that have been found to be highly effective against a variety of fungal infections, including *C. albicans*, *A. flavus* [9], *Trichophyton rubrum*, *T. mentagrophytes* [66], *A. niger* and *A. fumigatus* [67]. In addition, Yakubu et al. [68] conducted a GC/MS study on the ethanolic extract of *S. occidentalis* leaves and found a biologically active mixture. The extract exhibited noticeable antifungal activity. This finding was further supported by Abubakar and Umar [69], who validated the antifungal activity of the methanolic extract of *S. occidentalis* against *A. flavus, A. niger*, and *C. albicans*. Furthermore, according to Xu et al. [70], fatty acids, which are abundant in the ethanolic extract of *S. occidentalis*, could potentially contribute to the suggested therapeutic effect of antifungal activity. Therefore, *S. occidentalis* exhibited significant antifungal activity against the causative agent *A. flavus* of mycotic keratitis. The activity of this extract could be ascribed to bioactive secondary metabolites, including fatty acids, flavonoids, polyphenols, and other compounds. Additional investigation is necessary to investigate the therapeutic capacity of this plant in tailoring natural alternatives to commercially produced antifungals.

The results of our study revealed that the right eye of the albino rabbit treated with MIC of *S. occidentalis* extract seemed normal, but the commercial dose of fluconazole caused evident inflammation and hypersensitivity in the treated right albino rabbit eye. The extract of *S. occidentalis* maintained the normal architecture and thickness of the uninfected layers of the cornea. In addition, the utilization of MIC dosage of *S. occidentalis* extract resulted in a more rapid inhibition of fungal growth and promoted the recovery of corneal tissues following infection management. This was accompanied by the complete regeneration of the endothelium, the regular organization of the epithelium, and a reduction in the infiltration of the stroma. Our findings align with previous research, highlighting the significance of natural botanical extracts in the treatment of eye disorders. Ruszymah et al. [71] found that the aqueous extract of *Centella asiatica* may have the potential to enhance the healing of corneal epithelial wounds. Furthermore, Agarwal et al. [72] documented that the aqueous extract of *Curcuma longa* effectively inhibited endotoxin-induced uveal inflammations in rats by decreasing TNF-α activity.

An increase in liver function indicators, involving ALT and AST values, was found as a result of the *A. flavus* infection of albino rabbits under investigation. In contrast, the administration of *S. occidentalis* extract to infected rabbits had a positive impact on the liver function parameters. Conversely, the use of the commercial fluconazole solution in the treatment of the fungal infection resulted in a noticeable elevation of liver enzyme levels in the infected rabbits treated with fluconazole. The study indicated a promising potential for utilizing *S. occidentalis* extract as an antifungal treatment for mycotic keratitis. Additionally, it was found to effectively reduce the levels of blood urea and creatinine, which serve as indicators of kidney function, to a level that is equal to or lower than that of healthy rabbits. In their study, Roy et al. [73] concluded that oral administration of *Senna* sp. extract did not have any adverse effects on liver functions in animal models used for treatment. Furthermore, Yang et al. [74] validated the safety of *Senna* sp. extract on the various functions and tissues of the liver and kidney in albino mice while treating their dry eyes. Agbodjogbe et al. [75] examined the safety of *Senna* sp. leaf extract in rats by quantifying the toxicity of the extract to rats. The treatment of albino rats with *Senna* sp. leaf extract indicated that the extract can be classified as non-toxic to

rats. Nevertheless, the study on sub-acute toxicity revealed that the aqueous extract of *Senna* sp. had a negative impact on several biochemical markers associated with liver, kidney, and muscle function when administered at doses exceeding 2000 mg/kg. In addition, supporting to our data, experiments conducted on in vivo have demonstrated that the extracts obtained from the roots, leaves, and aerial parts of *S. occidentalis* can enhance liver function by reducing the activities of ALT, AST, alkaline phosphatase (ALP), and bilirubin in rats with chemically-induced liver damage [76,77]. The tissue protective action of *S. occidentalis* has been attributed to its distinct composition of secondary bioactive compounds.

The successful healing of cornea fungal infection in the treated rabbits depends on the combined effectiveness of the administered antifungal treatment and the internal immune response. The extract of *S. occidentalis* demonstrated a significant ability to enhance the immune system of rabbits in both infected and non-infected groups. This resulted in faster and more thorough healing of the targeted infection. The level of IgM, a key indicator of the immune response, reached its peak in infected rabbits treated with double the MIC of *S. occidentalis*. A recent study investigated the impact of *Senna* sp. leaf extract on dry eye in animal models. The study found that the extract promoted the regeneration of corneal tissues and did not negatively affect liver and kidney functions. Additionally, the extract enhanced the antifungal activity of macrophages, indicating an improved immune response against fungal eye infections [74]. Plant extracts, which are natural immunomodulators, enhance a compromised immune system and reduce an over-aggressive immunological response [78]. Therefore, it is crucial to evaluate the immunomodulatory activity, safety, and toxicity of local herbal formulations as they are reported to have these properties.

The administration of a novel antimicrobial product *in vivo* necessitates the conduction of safety approval tests on the host tissue and function, alongside the identification of discernible indicators implying the swift and complete healing of the host tissues after the effective inhibition of the microbial infection. Upon scrutinizing the corneal tissue layers of the treated groups, it was observed that the ethanolic extract of *S. occidentalis* exhibited a notable inhibition in *A. flavus* mycelial growth. This was accompanied by a positive rate of healing and regeneration of the corneal tissues, characterized by their normal arrangement, thickness, and texture on both the outer and inner surfaces. The positive outcome can be attributed to the potent antimicrobial activity of the phytochemical components present in the ethanolic extract of *S. occidentalis*, which effectively eradicated the *A. flavus* infection within the corneal stroma. Moreover, the rapid regeneration of the corneal stroma and collagen-bound layers can be ascribed to the invigorating effects of the *S. occidentalis* phytocomponents on the immune system and the production of IgM. These elements ensure an ample supply of blood and nutrients, expedite cell division in the corneal layers and contribute to the orderly arrangement, thickness, and texture of the regenerated layers. Furthermore, it was determined that the normal growth of corneal tissues relies on the proper functioning of other organs in the treated animal model, as evidenced by the normal levels of liver and kidney function parameters observed in the rabbits treated with the *S. occidentalis* ethanolic leaf extract in this investigation.

5. CONCLUSION

The phytochemical composition of *S. occidentalis* has shown potential as a safer alternative with reduced side effects and the ability to address anti-fungal resistance against fungal keratitis in rabbits caused by *A. flavus*. These findings highlight the efficacy of *S. occidentalis* extract in enhancing the immune response of rabbits, both in the presence and absence of infection, without adversely affecting liver and kidney function. The observed increase in IgM levels indicates the immunostimulatory properties of *S. occidentalis* extract, which contribute to a more robust and efficient healing process. Histopathological examination also revealed that *S. occidentalis* extract promotes the healing of affected corneal tissues in rabbits. These results suggest that *S. occidentalis* may be a promising adjunct treatment to enhance immune response and facilitate infection resolution. However, further clinical assessment is needed to thoroughly evaluate the effectiveness, toxicity profile, and optimal application of *S. occidentalis* extract for mycotic keratitis. Well-designed research studies are necessary to bridge the remaining knowl edge gaps and determine the effectiveness and utility of herbal medications for this specific purpose.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by the Research Ethics Committee of Tanta University with an approval code of 34895/9/21.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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