Review of Phytochemicals and Plant Extracts With Activity against Viruses and Protozoal Keratitis: *In Vitro* and *In Vivo*.

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Abstract:
Now a days Infectious keratitis is a leading cause of vision loss and total blindness world- wide. Proper diagnosis of causative organism is critical and although culture remains the prevailing diagnostic tool, newer techniques such as *in vivo* and *in vitro* plant extract are helpful for diagnosing Viral and Acanthamoeba. Next generation sequencing holds the potential for early and accurate diagnosis even for organisms that are difficult to culture by conventional method. In this review we covered types & various etiological agent that basically protozoa, viruses. The epidemiology & risk factor like contact lenses use, eye trauma, surface eye diseases, immunosuppression, agricultural work, and also emphasizing on the current treatment strategies and their limitations by using antiviral and antiprotozoal phytochemicals, also consist of need for novel therapeutic agents. We also covered types of phytochemicals and their classes like flavonoid, polyphenols, terpenoids etc. along with their sources, and extraction, and their families. Rationale for using phytochemicals in prevention of viral and acanthamoeba keratitis as antiviral and antiprotozoal agents include anti- inflammatory, antimicrobial, antioxidant, and immunomodulatory properties, also phytochemicals are plant derived products, so they have natural body acceptance. We also focused on mechanism of action and structural activity relationship of phytochemicals, their safety, efficacy and cytotoxicity to corneal cell. We also focused on how nanotechnology is useful for increment in efficacy and bioavailability at ocular surface. Methodologies such as MTT, PCR are also useful for this purpose. The combination therapies and also identification of gaps in current research and need for well designed in vitro and in vivo ,importance of standardized extracts and purified compounds ,development of suitable formulations for ocular delivery plays key role in keratitis prevention.

(Key words: keratitis, antiviral, antiprotozoal, phytochemical)

Introduction

Keratitis Definition and history Keratitis is an inflammation of the cornea, the transparent tissue in front of the eye. One of the leading causes of blindness worldwide is keratitis, an eye emergency. Keratitis is a clinical condition in which inflammatory cells invade various corneal layers in reaction to harmful stimuli, such as self-antigens or infectious foreign substances. Ulcers (keratitis) may develop as a result of the inflammatory response melting the corneal stroma and epithelium supportively. In addition to impairing corneal clarity, this also jeopardises the globe's integrity. Corneal blindness may arise from such situations. It is linked to both infectious and non-infectious conditions, which can affect the entire body or only the surface of the eye. Out of all the keratitis kinds covered above, "microbial keratitis" makes up the majority and is a serious concern in developing nations. It is not appropriate to disregard non-infectious keratitis, particularly in developed countries. Keratitis is primarily divided into two categories based on its etiological factors, infectious and non-infectious keratitis. Infectious keratitis is an infection of the cornea, also referred to as infectious corneal ulcer or corneal opacity. However, especially in affluent countries, non-infectious keratitis cannot be denigrated. Based on their separate etiological causes, keratitis is

primarily divided into two

types: infectious and non-infectious. A corneal infection called infectious keratitis is also referred to as an infectious corneal ulcer or corneal opacity.

The most prevalent types of infectious keratitis are bacterial and fungal. Although these statistics were underreported, the number of instances of infectious keratitis- related corneal blindness has dropped from roughly
1.6 million in 1990 to 1.3 million in 2015, and the number of cases of vision impairment has dropped from 3.3 million to 2.9 million in the same time frame. Up to 2015, infectious keratitis accounted for 3.5% (36 million) of all blind people, making it the fifth most prevalent cause of blindness overall and the most common cause of non-trachomatous corneal opacification. Since most epidemiological data for infectious keratitis are reported under "corneal blindness," which includes traumatic, infectious, inflammatory, and hereditary illnesses, it is challenging to get epidemiological data for infectious keratitis. The incidence of microbial keratitis varies globally. According to reports from industrialised nations, the frequency was
27.6 per 100,000 years in the US in 1999, 40.3 per 100,000 in England in 2006, and 6.6 per 100,000 in Australia in 2015. On the other hand, contagious keratitis poses a hazard to public health in developing Asian nations. Access to healthcare is difficult in these nations, and there are greater rates of workers in farming and agriculture (113 per 100,000 in Madurai, Tamil Nadu, India; 339 per 100,000 in Bhutan; 710 in Burma; and 799 in Nepal).

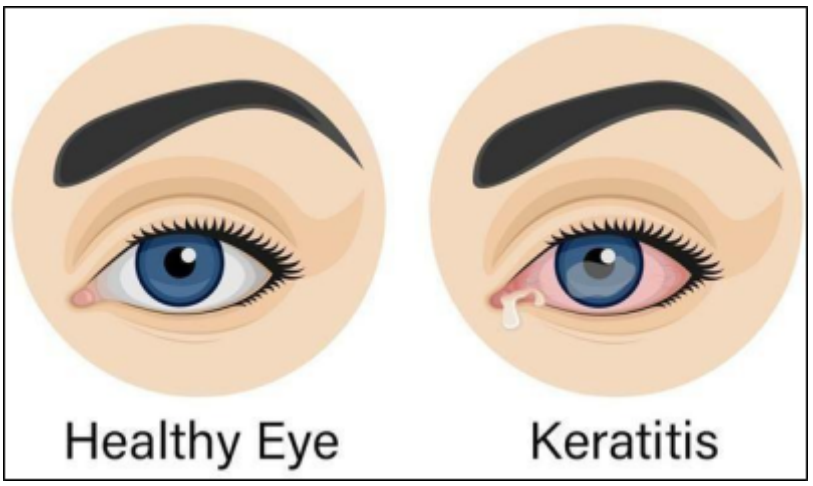

Figure 1: Comparision of infected eye in keratitis with normal eye

1.1. Types of keratitis (viral, protozoal)

Microbial keratitis (caused by the bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus pneumoniae, Pseudomonas aeruginosa*, and species of the *Enterobacteriaceae* family), fungal keratitis (caused by filamentous fungi like A*spergillus* and *Fusarium*), and yeasts *like Candida* are the different types of infectious keratitis. Other parasites (Acanthamoeba Keratitis), fungi (*Uvularia, Paecilomyces, Cladosporium*, and different phaeohyphomycotic), or viral keratitis (herpes viruses, Epstein - Barr virus (EBV), varicella-zoster virus, cytomegalovirus, Adenovirus, etc.) can also cause keratitis. Once more, disorders with different aetiologies and clinical presentations but comparable patient symptoms are used to further categorise non-infectious diseases. Thygeson superficial punctate keratitis is a self-limiting illness that flares up and goes away. Bilateral, central, raised intraepithelial white-grey lesions and neurotrophic keratitis, or corneal epithelial ulceration, which is linked to diminished or nonexistent corneal feeling, are its defining features. Numerous aetiologies can result in nerve dysfunction in NK, which impairs recovery. Epithelial filaments made of mucus and degraded epithelial cells are a hallmark of filamentary keratitis. Keratopathy in vortices Verticillate, another name for vortex keratopathy, is a corneal condition characterised by whorl-like brown-grey opacities in the basal epithelium.

1.2. Pathogenesis

Keratitis is an inflammatory disease of the cornea that can lead to blindness and vision loss if not treated. The disease is caused by various infections, including bacteria *(Staphylococcus aureus, Pseudomonas aeruginosa,*

Staphylococcus epidermidis, Streptococcus pneumoniae) viral (Herpes Simplex Virus (HSV) Varicella-Zoster Virus (VZV) Epstein-Barr Virus (EBV), fungal (*Fusarium species, Aspergillus species*) or parasitic ones[1, 2]. The first line of defence against keratitis is the corneal epithelium, which acts as a physical barrier. Tears contain enzymes that break down bacterial cell walls and inhibit microbial growth[3, 4]. The keratitis inflammatory response is triggered by an initial insult, activating Pathogen- Associated Molecular Patterns (PAMPs), which triggers the activation of immune cells[5].

This inflammatory response involves a complex interplay of cellular and molecular mechanisms, with cytokines and cytokine roles playing crucial roles in maintaining tissue homeostasis and preventing tissue damage[6, 7]. Inflammation in the eye can lead to corneal damage, ulceration, and infiltration. An imbalance between pro-inflammatory and anti- inflammatory responses can result in chronic inflammation, scarring, neovascularization, and permanent vision loss[8-10]. Factors contributing to imbalances include pathogen virulence, host factors, and environmental factors. In keratitis, the inflammatory response can lead to tissue destruction, including collagen degradation and neovascularization. Collagen degradation is the breakdown of collagen fibres in corneal tissue, causing tissue damage and vision loss[11]. Neovascularization, involving new blood vessels growing into the cornea, leads to vision loss. Angiogenic stimuli, such as inflammation and hypoxia, can activate angiogenic pathways, leading to endothelial cell proliferation and the formation of new blood vessels[12]. Corneal scarring, caused by inflammation, infection, or trauma, is a stage in keratitis where the cornea becomes opaque due to fibrosis and scarring. This can reduce transparency and affect vision, leading to distorted vision and blurred vision. Untreated corneal scaring can cause blindness and loss of corneal transparency, affecting the ability of rods and cones to process light[13].

1.1. Viral: Herpes simplex virus (HSV), Varicella-zoster virus (VZV), Adenovirus

Viral keratitis is caused by most commonly by HSV, then VZV, and followed by adenovirus due to contact lens warring.

1.2. Protozoal: Acanthamoeba species

Acanthamoeba keratitis is an uncommon infection that can cause blindness. frequently brought on by free-living protozoa called Acanthamoeba species, which are found in dust, soil, air, drinking water, and seawater. Although contact lens wearers are the main victims of AK, non-lens wearers may also get it, especially if they have weakened immune systems or have been exposed to tainted water. Both an infectious trophozoite form and a dormant resilient cyst are present. Instead of using genetic resistance, Acanthamoeba uses dormancy to survive extreme environments, which makes treatment more difficult. As a result, existing treatment plans frequently have drawbacks, underscoring the pressing need to find safe and efficient therapeutic agents to combat Acanthamoeba species while maximising benefits and reducing side effects. The Mannose-binding Protein allows acanthamoeba to adhere to ocular epithelial cells in the event of a corneal infection. This interaction promotes metalloproteinase, serin-, and cysteine proteinase release through acanthamoeba, which has lethal effects on human keratocytes and corneal epithelial cells and facilitates acanthamoeba's deeper corneal penetration.

1.3. Epidemiology and risk factors

Keratitis, an inflammation of the cornea, can be caused by infections, injuries, or underlying conditions. Key risk factors include contact lens use, poor hygiene, eye trauma, chemical exposure, ocular surface disease, dry eye, dysfunction, immune system issues, previous eye surgery, infectious exposure, socioeconomic and environmental factors, and prolonged corticosteroid use[14]. Key risk factors include contact lens use, eye trauma, chemical exposure, ocular surface disease, dry eye, immunosuppression, autoimmune diseases, previous eye surgery, infectious exposure, socioeconomic and environmental factors, and prolonged corticosteroid use[15, 16]. Keratitis is prevalent in India, with higher risk in South Indian states like Tamil Nadu, Andhra Pradesh, Telangana, Karnataka, and Kerala. Factors contributing to this include agricultural workforce, frequent eye trauma, tropical climate, high humidity, and high microbial load[17]. Middle and North Indian states like Uttar Pradesh,

Bihar, Madhya Pradesh, Chhattisgarh, Maharashtra, and Rajasthan face challenges such as lack of healthcare access, dust and dry conditions, traditional medicine use, and increasing contact lens misuse in urban centres [18, 19].

1.4. Current treatment strategies and their limitations (antiviral drugs, antifungals, etc.)

Keratitis, an inflammation of the cornea, can be caused by viral, bacterial, fungal, or parasitic infections, as well as non-infectious factors like injury or contact lens overwear. Treatment strategies are tailored to the specific cause, with antiviral medications like Trifluridine, ganciclovir gel, and acyclovir ointment being commonly used[20, 21]. Oral antivirals like acyclovir, valacyclovir, and famciclovir are prescribed for deeper infections or difficult topical administration. These may have absorption issues in lactose intolerance or gastrointestinal surgeries. Topical corticosteroids can reduce inflammation and scarring in stromal keratitis, but must be used cautiously[20].

Artificial tears can provide symptomatic relief for herpes simplex keratitis[22]. Debridement may involve removing infected cells. Antibiotic eye drops like fluoroquinolones and fortified antibiotics (e.g., tobramycin, cefazolin, vancomycin) are commonly used, with frequency depending on infection severity. Oral antibiotics may be used as adjunctive therapy in severe cases[23, 24]. Cycloplegic agents can alleviate ciliary spasm pain, while topical corticosteroids may alleviate inflammation and scarring post-infection control[25]. Natamycin is the FDA- approved topical antifungal for fungal keratitis, often used as the first- line treatment. Amphotericin B and voriconazole are also commonly used, with voriconazole having better ocular penetration[26]. Oral antifungal medications like voriconazole, itraconazole, fluconazole, and ketoconazole may be used in severe cases[27].

Intrastromal or intracameral injections of antifungal agents into the cornea or anterior chamber may be considered for deep or recalcitrant infections. Therapeutic keratoplasty may be necessary for severe cases or corneal perforation[28, 29]. Acanthamoeba Keratitis can be treated with antiparasitic eye drops like Propamidine isethionate and polyhexamethylene biguanide (PHMB), oral medications, or therapeutic keratoplasty in severe cases or if medical treatment fails, resulting in prolonged, long-lasting treatment[29]. Non-infectious keratitis can be treated with lubricating eye drops for mild cases, topical corticosteroids for severe cases, NSAIDs for pain management, and bandage contact lenses for cornea protection and healing[30].

1.7. Limitations of Current Treatment Strategies

Current treatment strategies for herpes simplex keratitis (HSK) have limitations due to their inability to eliminate the latent virus in corneal nerves, leading to cumulative damage and vision loss[31]. Prophylactic oral antivirals can reduce recurrence frequency but do not completely prevent them. Topical antivirals can cause epithelial toxicity[32, 33]. The corneal drug concentrations are difficult to achieve due to poor permeability and rapid clearance, with oral medications posing systemic side effects. There is no treatment for the neurotrophic effects of HSV, leading to complications like neurotrophic keratopathy. Drug resistance can still develop[34]. Antifungal drugs face limited availability and spectrum in some regions, with Natamycin effective against filamentous fungi but poor against yeasts. Amphotericin B has a broader spectrum but poor penetration, requiring compounding[35]. Large molecular sizes limit their ability to penetrate the corneal stroma effectively.

Fungal keratitis is a challenging condition that requires delayed diagnosis, prolonged treatment, potential drug toxicity, drug resistance, and variable efficacy[27]. It can lead to ocular surface toxicity, drug resistance, and a significant proportion of patients may require surgical intervention like corneal transplantation. Treatment duration can be weeks to months, increasing the risk of non-compliance[36, 37]. There is no definitive evidence that one antifungal drug or combination is superior for all fungal keratitis types. Current antiviral and antifungal drugs have limitations due to viral latency, drug toxicity, poor penetration, emerging resistance, and corneal challenges. Research is focused on developing novel drug delivery systems, improved efficacy and safety profiles, and alternative treatment approaches like corneal cross-linking to improve outcomes[38].

1.8. The need for novel therapeutic agents

In light of these drawbacks, creating new treatment options for viral and protozoal keratitis is essential to enhancing patient outcomes and averting vision loss. The best new treatments would be Target Viral Latency: In order to avoid recurrences and permanent corneal damage, medicines that can efficiently eradicate or suppress the latent virus are required for viral keratitis. New drug delivery methods such in situ gels, drug-eluting contact lenses, and nanoparticles may improve drug penetration, extend the duration of the drug's residence on the ocular surface, and lessen the need for frequent dosage, all of which could increase patient compliance and efficacy. New antiviral and antiparasitic agents are needed for long- term treatment of viral keratitis, aiming to reduce ocular toxicity and improve corneal nerve regeneration. These therapies can also combat drug resistance by developing new drugs with novel mechanisms of action. Additionally, new agents are needed to enhance efficacy against Acanthamoeba cysts, reducing treatment duration and improving cure rates, thus enhancing the overall effectiveness of these treatments. Advancements in diagnostic tools for early detection of viral and protozoal keratitis are crucial for timely intervention and better outcomes. Research is exploring new approaches, including antiviral molecules, immunomodulatory agents, gene therapies, and innovative drug delivery systems. The development of potent, less toxic antiparasitic drugs holds promise for improving management and reducing corneal blindness burden.

2. Phytochemicals and Plant Extracts

Phytochemicals, plant-derived compounds with antimicrobial, anti-inflammatory, and antioxidant properties, have potential in treating keratitis but face limitations due to poor bioavailability, stability, and corneal penetration. Advanced drug delivery systems and nanoparticles/nanoemulsions can improve solubility and cellular uptake, such as curcumin- loaded nanoparticles for HSV or fungal keratitis. Curcumin, a hydrophobic polyphenol, has numerous pharmacological activities.

2.1. Classes of phytochemicals (alkaloids, flavonoids, terpenoids, polyphenols, etc.) and their general properties

Alkaloids are crucial for biomedical science due to their unique ability to work as hydrogen- acceptor or hydrogen- donor for hydrogen bonding. They are found in about 20% of plant species and are major areas of research and development. Alkaloids can be classified into three types: true alkaloids (heterocyclics), protoalkaloids (non- heterocyclics), and pseudoal. True alkaloids are chemically complex compounds and derivatives of cyclic amino acids. Pyrrole alkaloids are the most important group, found in various natural and unnatural compounds with pharmacological properties. Quinoline alkaloids are found in *Cinchona ledgeriana* and quinidine alkaloids are found in *C. officinalis*. Tropane alkaloids are found in the *Solanaceae* family. Indole alkaloids have a bicyclic structure and are highly reactive with potent biological activities. Flavonoids are a class of phenolic compounds produced by plants, found in non-glycosylated or glycosidic forms. They consist of four types: flavones, flavanols, flavanones, isoflavonoids, flavanols, and anthocyanins. Flavones are found in celery, parsley, red pepper, chamomile, mint, and ginkgo. Flavanols, including quercetin, galanin, kaempferol, and myricetin, are mainly found in vegetables and fruits. Flavanones, also known as catechins or flavan-3-ols, are found in citrus fruits. Anthocyanins, glycosylated polyphenolic compounds, are soluble vacuolar pigments with a range of colours. Flavonoids have several properties, including antioxidant properties, anti-inflammatory effects, cardio- protective effects, and interference with lipid bilayers. Terpenoids are modified classes of terpenes with different functional groups and oxidized methyl groups. They are found in various plant and animal tissues, including oil glands, resin canals, and secretory tissues. Monoterpenes are found in secretory tissues, while sesquiterpenes occur naturally in hydrocarbons or oxygenated forms. Diterpenes can be linear, bicyclic, or macrocyclic. Sesterterpenes are found in fungi, marine organisms, insects, and lichens. Polyterpenoids are polymeric isoprenoid hydrocarbons.

2.2. Sources of plant extracts with reported activity (specific plants, families) Table 1.

Plant name	Family and botanical name	Parts used	Active constituents	Class	Type of study	Pharmacological target	Observed Effects/Mechanisms	Keratitis Type Targeted
Eucalyptus globulus	Myrtaceae	Leaves	1,8-cineole (eucalyptol), alpha-pinene	Terpenoid	*In vitro*	Acanthamoea	Significant reduction in amoebic viability	Protozoal
Camellia sinensis (Tea Plant)	Theaceae	Leaves	Catechins (e.g., EGCG), Caffeine	Polyphenol, Alkaloid	*In vitro*	Acanthamoeba	Lethal effect on cysts	Protozoal
Cuminum cyminum (Cumin)	Apiaceae	Seeds	EHP (1-(2-Ethyl, 6-Heptyl) Phenol)	Phenol	In vitro	HSV-1	Antiviral activity	Viral
Azadirachta indica (Neem)	Meliaceae	Leaves, seeds	Azadirachtin, Nimbin	Terpenoid	In vitro, In vivo	Candida albicans, Staphyloco ccus aureus	Antibacterial, Antifungal activity	Bacterial, Fungal
Curcumalonga (Turmeric)	Zingiberaceae	Rhizome	Curcumin	Polyphenol	In vitro, In vivo	Fusarium solani, Staphyloco ccus aureus	Antibacterial, Antifungal, Anti-inflammatory activity	Bacteria, Fungi
Allium sativum (Garlic)	Amaryllidaceae	Bulb	Allicin	Organosulfur	In vitro	Pseudomonasaeruginosa, Fusarium solani	Antibacterial, Antifungal, Anti-protozoal activity	Bacterial, Fungal, Protozoal
Melaleuca alternifolia (TeaTree)	Myrtaceae	Leaves	Terpinen- 4-ol	Terpenoid	In vitro	Candida albicans,E coli	Antibacterial, Antifungal activity	Bacterial, Fungal
Ocimum sanctum (Holy Basil)	Lamiaceae	Leaves	Eugenol, Ursolic acid	Phenolic compound, Triterpenoid	In vitro	Bacillus pumilus, Alternaria	Antibacterial, Antifungal activity	Bacterial, Fungal
Berberis vulgaris (Barberry)	Berberidaceae	Root, bark	Berberine	Alkaloid	In vitro	Acanthamoeba	Antibacterial, Antifungal, Anti-protozoal activity	Bacterial, Fungal, Protozoal
Glycyrrhiza glabra (Licorice)	Fabaceae	Root	Glycyrrhizin	Saponin	In vitro	HSV-1	Antiviral activity	Viral
Aloe vera	Asphodelaceae	Leaf gel	Acemannan	Polysaccharide	In vitro	Pseudomonas aeruginosa	Antibacterial, Antifungal activity	Bacterial, Fungal
Calendula officinalis (Marigold)	Asteraceae	Flower	Lutein, Zeaxanthin	Carotenoid	In vitro	Aspergillus niger	Antibacterial, Antifungal activity	Bacterial, Fungal
Echinacea purpurea	Asteraceae	Root, aerial parts	Echinacoside	Glycoside	*In vitro*	SARS- CoV-2	Antibacterial, Antiviral activity	Bacterial, Viral

Hypericumperfo ratum (St. John'sWor)	Hypericacea e	Flower,leave s	Hypericin, Hyperforin	Naphthodia nthrone, Phlorogluci nol	*In vitro*	Influenza	Antibacteril, Antiviral activity	Bacterial, Viral
Punica granatum (Pomegranate)	Lythraceae	Fruit peel, seeds	Punicalagin, Ellagic acid	Polyphenol	In vitro	Candida albicans	Antibacterial ,Antifungal activity	Bacterial, Fung al
Rosma rinus officin alis (Rosemary)	Lamiaceae	Leaves	Rosmarinic acid, Carnosol	Phenolic compound, Diterpene	In vitro	Streptococ cus mutans	Antibacterial , Antifungal activity	Bacterial, Fung al
Salviaofficin alis (Sage)	Lamiaceae	Leaves	Carnosol, Rosmarini c Acid	Diterpen e, Phenolic Compound	*In vitro*	*Penicillium aurantiogr iseum*	Antibacterial ,Antifungal	Bacterial, Fungal
Thymus vulgari s (Thyme)	Lamiaceae	leaves, flowers	Thymol, Carvacrol	Phenolic monoter pene	*I n v i t r o*	*Cladospori um spp.*	Antibacteria, Antifungal	Bacterial, Fungal
Zingiber officinale (Ginger)	Zingibera ceae	Rhizome	Gingerol, Shogaol	Phenolic compound	In vitro	Fusarium oxysporum	Antibacterial, Antifungal	Bacteri al, Fungal
Cinnamomu m zeylani cum (Cinnamon)	Lauraceae	Bark	Cinnamal dehyde	Aldehyde	In vitro	Staphyloco ccus aureus	Antibacterial, Antifungal	Bacte rial, Fung al
Syzygi um aromat icum (Clove)	Myrtaceae	Flower bud	Eugenol	Phenol	In vitro	E.Coli	Antibacterial, Antifungal	Bacte rial, Fung al
Propolis	Apidae	Resin	Flavonoid s, Phenolic acids	Flavonoi d, Phenolic compound	In vitro	Trichophyt ons	Antibacterial, Antifungal	Bacteri al, Fung al

1.1. Methods of extraction and characterization of phytochemicals

Plant extraction methods include traditional and modern techniques. Traditional methods include maceration, percolation, decoction, and infusion. Modern techniques include Microwave-Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE), Ultrasound- Assisted Extraction (UAE), Pressurized Liquid Extraction (PLE), Enzyme-Assisted Extraction (EAE), Solid-phase microextraction (SPME), membrane extraction, accelerated solvent extraction (ASE), and pulse electric field extraction (PEF). Maceration is a simple method with long extraction times and low efficiency, but is useful for thermolabile components. Percolation is a common method in traditional Chinese medicine, with simple equipment and easy operations. Decoction is an extract made using boiling water from plant materials like stems, bark, needles, roots, and seeds, with a small amount of lipophilic essential oils extracted.

Microwave-Assisted Extraction (MAE) is a conventional technique for extracting active components from medicinal plants using microwave energy to heat solvents. Supercritical Fluid Extraction (SFE) uses solid samples, with columns filled with pre-treated samples, pressurized supercritical solvents dissolved extractable

compounds. The dissolved compounds are transported to a separator for separation. Ultrasound-assisted extraction offers more effective extraction, energy savings, and moderate temperatures, especially for heat-sensitive compounds. Both methods utilize acoustic or ultrasonic cavitation for efficient extraction.

2. *In vitro* Studies

2.1. in vitro studies evaluating the activity of phytochemicals and plant extracts against:

Propolis, a natural substance produced by bees, has been studied for its antiviral and antiprotozoal properties, particularly in treating viral keratitis and Acanthamoeba keratitis. Propolis contains bioactive compounds such as flavonoids, phenolic acids, and a compound that inhibits NF-κB, a key factor in viral replication and inflammation[39]. It has demonstrated antiviral activity against various viruses, including HSV-1, VZV, and SARS- CoV-2. Propolis also has immunomodulatory properties, enhancing the host's immune response and reducing oxidative stress and inflammation in infected cells. It has shown potential as an antiviral agent in treating viral keratitis caused by HSV-1 and HSV-2, VZV, and adenovirus[40]. Propolis' broad-spectrum antiviral activity may be beneficial in treating adenoviral keratitis, as it may help reduce viral replication and inflammation. Further research is needed to fully understand the potential of propolis in treating viral keratitis and protozoal infections. Propolis, an antiprotozoal agent, has shown promise in treating protozoal infections, including Acanthamoeba, by inhibiting the growth and viability of trophozoites and cysts, disrupting their cell membrane, and causing apoptosis. Infection treatment is challenging due to resistance, limited options, and potential toxicity{Colorant, 2023 #347}. Phytochemicals, such as betulinic acid and betulin, have shown potential in managing viral and Acanthamoeba keratitis{Rocha, 2022 #346}. Betulinic acid, extracted from birch tree bark, has antiviral and anti- inflammatory properties, including against HSV-1, a leading cause of viral keratitis. It also inhibits protozoal pathogens by disrupting mitochondrial function and causing apoptosis-like death. Xanthones from mangosteen, particularly alpha- and gamma-mangostin, have significant antimicrobial, antioxidant, and anti-inflammatory properties, inhibiting viral replication and protozoa viability{Al- Massarani, 2013 #349}.

Caryophyllene-β-Caryophyllene, a natural sesquiterpene found in essential oils of cloves, black pepper, and cannabis, has anti-inflammatory, antiviral, and antimicrobial properties. It modulates immune response and reduces inflammation, which is crucial in keratitis treatment{Porto, 2024 #350}. Saponins, abundant in medicinal plants, have potent surfactant properties and can disrupt virus and protozoa lipid membranes, leading to cell lysis and immunostimulatory properties{Simões, 1999 #351}. Peppermint essential oil, with its components, has antiviral, antimicrobial, and analgesic properties, inhibiting growth of Acanthamoeba castellanii and HSV- 1{Camele, 2021 #352}. Ellagic acid, a polyphenol found in berries, pomegranates, and nuts, has antiviral activity and antioxidant and anti- inflammatory effects, making it a potential adjunct treatment for keratitis{AbouAitah, 2021 #353}. Lignans, phytoestrogens found in flaxseed and other plants, have antiviral, antiparasitic, and antioxidant properties. They have been found to inhibit HSV replication and target Acanthamoeba by interfering with energy metabolism and cell cycle processes{Xu, 2022 #354}. Several medicinal plants have shown promising in-vitro activity against Acanthamoeba species, targeting both trophozoite and cyst stages with low or no cytotoxicity to human cells. Propolis extracts were found to be cysticidal at concentrations above 15.62 mg/mL, while ethanolic extracts showed activity against both trophozoites and cysts{Topalkara, 2007 #355}. *Thymus sipyleus subsp. sipyleus var. sipyleus* showed effective activity against Acanthamoeba trophozoites at a concentration of 32 mg/mL. Further bio- guided fractionation may help identify the specific compounds responsible for its anti- amoebic effects{Polat, 2007 #357}.

Allium sativum (garlic) showed anti-Acanthamoeba effects with methanolic extracts, showing both amoebicidal and cysticidal activity. The extract was non-toxic at concentrations up to 3.9 mg/mL, suggesting potential therapeutic compounds{Polat, 2008 #358}. Aqueous extracts of *Ziziphus vulgaris* and *Trigonella foenum graecum* were effective against both trophozoites and cysts of Acanthamoeba. The cysticidal activity of *Arachis hypogaea L., Curcuma longa L., and Pancratium maritimum L*. was evaluated, with ethanol extracts showing minimal inhibitory concentrations (MICs) of 100 mg/mL across all tested durations{Niyyati, 2016 #359}. *Origanum syriacum* and *Origanum laevigatum* exhibited potent in-vitro amoebicidal effects, with *O. syriacum* eliminating trophozoites within 3 hours and cysts within 24 hours at a concentration of 32 mg/mL{Degerli, 2012 #360}. Among Peucedanum species, methanolic extracts of *Peucedanum caucasicum, P. palimbioides, P.* chryseum, and

P. longibracteolatum showed the strongest effect, eliminating both trophozoites and cysts within 24 to 72 hours

at a concentration of 32 mg/mL{Malatyali, 2012 #361}. *Salvia staminea* and *Salvia caespitosa* methanolic extracts demonstrated significant anti-Acanthamoeba activity, with no toxicity to human cells even at 16 mg/mL{Goze, 2009 #362}. Other medicinal plants, such as *Satureja cuneifolia* and *Melissa officinalis*, showed the highest amoebicidal and cysticidal activity. Extracts from *Ipomoea sp., Kaempferia galanga, and Cananga odorata* showed activity against multiple Acanthamoeba species. *Gastrochilus panduratum* showed lytic activity against

A. polyphaga and amoebistatic effects against *A. castellanii and A. culbertsoni*. Extracts *from Rubus chamaemorus, Pueraria lobata*, *Solidago virgaurea, and Solidago graminifolia* were effective in both in-vitro and in- vivo, with no observed toxicity{Malatyali, 2012 #364}. Extracts from *Olea europaea* were also effective in inhibiting *Acanthamoeba castellanii* trophozoites, with IC50 ranging from
8.234 µg/mL to 33.661 ± 1.398 µg/mL{Sifaoui, 2013 #365}.

2.2. Viruses (HSV, VZV, Adenovirus)

Viral plaque reduction assays are a classic virological technique used to evaluate antiviral activity against viruses, including in *in vitro* studies of viral keratitis. They quantify infectious virus particles, evaluate the effectiveness of antiviral drugs, and study viral replication kinetics in cell cultures. The process involves selecting susceptible cells, infecting them with a known virus titer, applying antiviral treatment, adding a semi-solid medium, incubating for 2-5 days, staining and counting viral plaques, and analyzing data to determine the IC50{Yin, 2023 #366}. These assays are used in viral keratitis research, resistance studies, and understanding host-virus interactions in ocular cell culture. Additionally, phytochemicals like propolis can be used to inhibit cytotoxic effect (CPE) in infected cells{Sohn, 2019 #367}{Guda, 2019 #368}. Quantitative PCR (qPCR) is a method used to measure viral DNA/RNA levels to assess antiviral activity. It has clinical applications in detecting HSV-1, HSV- 2, and VZV DNA in corneal samples, monitoring acyclovir-resistant HSV keratitis, and quantifying HSV-1 Latency-Associated Transcript (LAT). In a study in India, qPCR demonstrated higher sensitivity than immunofluorescence and conventional PCR, aiding in accurate diagnosis. It also showed a correlation between LAT presence and disease recurrence, indicating its potential in assessing viral reactivation and long-term management. Its diagnostic performance in tear samples was also evaluated[41-43]. Caffeic acid phenethyl ester (CAPE), quercetin, kaempferol, pinocembrin, and Brazilian green propolis extract are all known to have antiviral properties against HSV and other viruses, including VZV, HSV, and other bacterial infections[44].

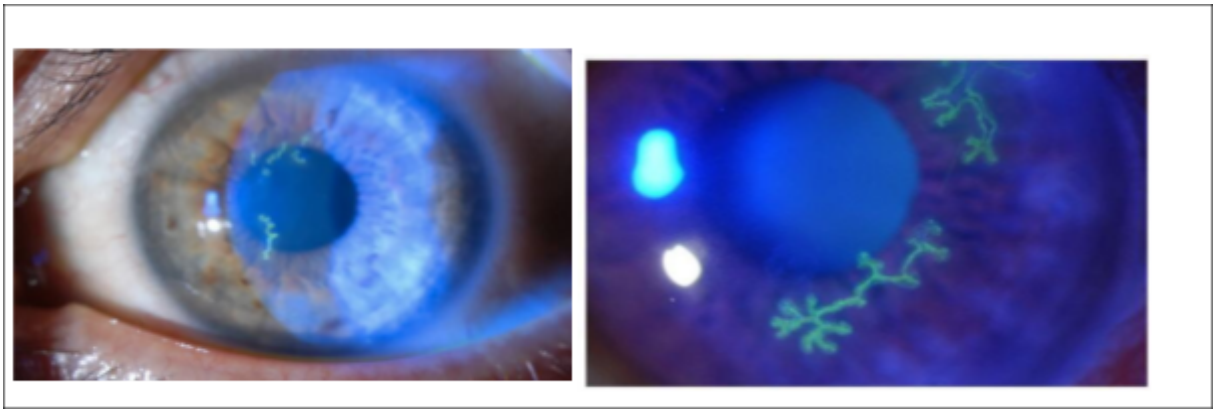

Fig . Eye infected by HSV-1

1.1. Acanthamoeba species (trophozoites and cysts)

Amoebicidal assays are used to measure the killing effect on Acanthamoeba trophozoites and cysts. These assays use methods such as Trypan Blue Exclusion Assay, Alamar Blue Assay, CellTiter-Glo Luminescence Assay, Complete-Kill Assay, and Excystation Assay. Trypan Blue Exclusion Assay measures the viability of viable cells by excluding the dye, while Alamar Blue Assay provides a quantitative measure of trophozoite metabolic activity. CellTiter-Glo Luminescence Assay measures cellular ATP levels, providing high sensitivity

in detecting viability loss in Acanthamoeba and other protozoa. Complete-Kill Assay assesses if a compound can completely eliminate all viable trophozoites and cysts, including regrowth potential over time. Excystation Assay evaluates the ability of cysts to transform into trophozoites under conducive conditions after treatment. Examples of amoebicidal compounds include hexamidine diisethionate, polyhexamethylene biguanide (PHMB), and chlorhexidine digluconate[45].

A study evaluated the adhesion of Acanthamoeba lugdunensis L3a trophozoites to silicone hydrogel contact lenses (SHCLs) with and without multipurpose contact lens care solutions (MPS). The lenses were tested with first-

generation Air Optix, second-generation Acuvue Oasys, and third-generation Biofinity. Discs were inoculated with trophozoites and incubated for 18 hours. The effects of MPS treatment were assessed by soaking lenses in Opti-Free Express, ReNu Fresh, or Biotrue solutions. Results showed significantly higher adhesion of trophozoites to Air Optix lenses compared to Acuvue Oasys and Biofinity. Treatment with Opti-Free Express reduced Acanthamoeba adhesion to both lenses, while ReNu Fresh and Biotrue did not. Biofinity lenses showed no Acanthamoeba adhesion. Surface morphology, particularly ridges, played a significant role in promoting adhesion. MPS containing myristamidopropyl dimethylamine (Opti-Free Express) was effective in reducing Acanthamoeba adherence to susceptible lens materials. Cell culture models like corneal epithelial and viral-infected cell cultures assess antiviral activity of propolis. Analytical techniques like HPLC and LC-MS help researchers understand its antiviral and antiprotozoal activities, indicating potential therapeutic applications. Phenolic compounds, flavonoids, CAPE, and Artepillin C show amoebicidal and antiprotozoal activity against Acanthamoeba trophozoites and cysts. Brazilian green propolis extract, red propolis extract, and poplar-type propolis extract show promising results against viral and Acanthamoeba keratitis[46].

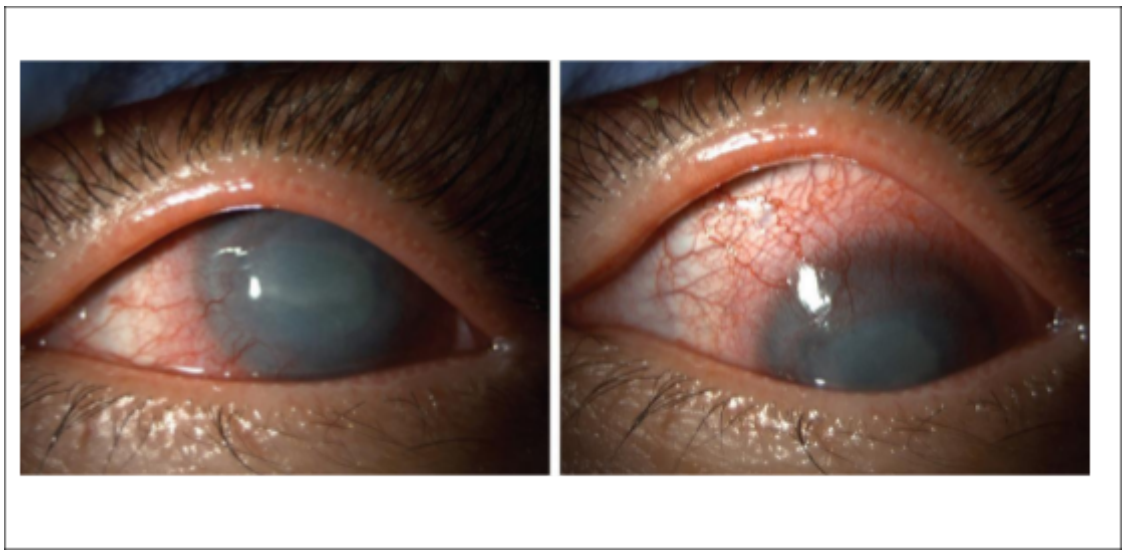

Fig . Eye infected by Acanthamoeba keratitis

1.2. Structure-activity relationships of effective compounds

Propolis, a resinous mixture produced by honeybees, is rich in bioactive phytochemicals with potent antimicrobial, antiviral, and antiparasitic properties. Its efficacy in managing ocular infections like viral keratitis and Acanthamoeba keratitis has gained attention. This review explores the structure-activity relationships (SARs) of major propolis constituents, highlighting how specific molecular features drive their therapeutic effect. Propolis offers a natural alternative rich in flavonoids, phenolic acids, and other bioactive compounds. Caffeic acid phenethyl ester (CAPE), quercetin, and curcumin are some of the SARs of propolis compounds in treating viral keratitis. CAPE is an ester of caffeic acid and phenethyl alcohol, with a catechol moiety responsible for antioxidant activity and NF-κB inhibition. Its SAR is related to its mechanism of action to viral keratitis, which may inhibit HSV replication by interfering with viral DNA synthesis or modulating host signalling pathways.

Quercetin, a flavonoid found in fruits and vegetables, has been identified as a bioactive compound with potential benefits in ocular viral infections. Its structure-activity relationship (SAR) reveals that its flavonoid backbone

plays a crucial role in its ability to interact with viral enzymes and nucleic acids, enhancing its ability to inhibit viral replication. Hydroxyl groups, such as 3-OH, 5-OH, 7-OH, and 3'-OH, play a significant role in its antiviral activity. Quercetin's glycosylated form, however, reduces cell membrane permeability and antiviral potency. The non-glycosylated form exhibits superior bioavailability and intracellular activity, making it more effective against ocular viral infections. Structural modifications like methylation or acylation can improve the lipophilicity of quercetin, enhancing corneal absorption. Quercetin's 3-OH and 4-carbonyl groups form a potent metal chelating site, binding divalent metal ions like Fe^{2+} and Cu^{2+} , which can inhibit key enzymatic steps in viral replication. Its antioxidant properties protect corneal epithelial cells from oxidative stress-induced apoptosis during viral infection.

Curcumin, a polyphenolic compound from the Curcuma longa plant, has shown potential as a therapeutic agent for treating viral keratitis, particularly Herpes Simplex Virus 1 (HSV-1) and Acanthamoeba keratitis (AK). Its multifaceted biological activities, including anti - inflammatory, antiviral, and antimicrobial properties, make it a promising candidate for treating these ocular infections. Curcumin's Structure-Activity Relationship (SAR) involves its distinctive chemical structure, consisting of two phenolic groups connected by a diene conjugated system. Its antiviral activity is well-documented, with key structural features including a diene conjugated system, phenolic hydroxyl groups, hydrophobic nature, and anti-inflammatory activity. Curcumin's ability to modulate pro-inflammatory cytokines, such as TNF-α and IL-6, is crucial in viral keratitis, as viral infections often induce excessive inflammation in the cornea, leading to tissue damage.

1.3. Cytotoxicity of phytochemicals to corneal cells

Cytotoxicity in natural compounds like propolis extracts is dose-dependent, with higher concentrations potentially damaging human corneal epithelial cells or fibroblasts. Factors influencing cytotoxicity include extract type, solvent residue, compound concentration, and duration of exposure. Most natural compounds show promising antimicrobial activity, but their cytotoxicity to corneal cells varies based on concentration, solubility, and cellular interaction mechanisms. Betulin and Betulinic Acid are generally considered safe with low cytotoxicity, sparing normal cells, including corneal epithelium, at therapeutic doses. EGCG and curcumin have shown potential therapeutic benefits in various studies, but their specific cytotoxicity in viral keratitis and Acanthamoeba keratitis requires further exploration. Curcumin's antagonistic effects against heavy metals like cadmium, arsenic, and nickel are dose-dependent and have potential therapeutic applications in treating inflammatory ocular conditions. Curcumin-loaded nanospheres have shown cytotoxic effects on certain cell lines, indicating potential applications in targeted therapy. In summary, while many compounds show therapeutic potential for keratitis, careful concentration adjustment and formulation are essential to ensure corneal cell safety.

2. *In Vivo* Studies

In vivo studies using animal models of viral and protozoal keratitis provide insights into host pathogen interactions, immune responses, and therapeutic efficacy. The Herpes Simplex Virus (HSV-1) and Feline Herpesvirus (FHV- 1) are commonly used models for studying these diseases. Recent advancements include gene therapy, siRNA, and nanoparticle-based drug delivery, while TLR agonist-containing nanoparticle eye drops have shown promising results in immunomodulatory therapy[4].

2.1. *in vivo* studies using animal models of viral and protozoal keratitis

In Acanthamoeba Keratitis (AK), Animal models are essential for understanding the pathophysiology of viral and protozoal keratitis, particularly Acanthamoeba Keratitis (AK). Rat models assess genotype-specific virulence and immune response, rabbit models evaluate drug efficacy and corneal healing, and ex-vivo porcine models study strain-specific invasion patterns. Recent innovations in immunotherapy and photodynamic treatment show promise in improving outcomes. Continued development of representative and translational animal models will enhance therapeutic development and clinical relevance{Dwia Pertiwi, 2021 #314}{de Souza Fernandes, 2023 #315}.

Viral keratitis study on total flavonoids from Ixeris sonchifolia (ISH) found that they significantly reduced corneal inflammation and viral replication in a mouse model of HSV keratitis. The treatment also enhanced corneal epithelium healing and downregulated pro- inflammatory cytokines[47]. The study was conducted using a topical ophthalmic solution, and found comparable efficacy to standard antiviral treatments like acyclovir in

some parameters. The findings suggest the potential of flavonoid compounds in antiviral therapy{Wang, 2020 #316}. Acyclovir-loaded nanofibers have been found to enhance the immune response against viral keratitis. The phytoconstituents in these nanofibers are total flavonoids from Ixeris sonchifolia (ISH), which modulate cytokine profiles, increasing levels of IL-2 and INF-γ, crucial for cellular immunity, and decreasing IL-4, a Th2 cytokine associated with allergic responses. Oral administration of ISH flavonoids led to decreased corneal edema and inflammatory cell infiltration in HSV-infected mice. Histopathological examination revealed improved corneal architecture and reduced damage in treated groups. The study found that treatment with ISH flavonoids resulted in dose-dependent reduction of corneal lesions, with 60% of mice exhibiting complete recovery at the highest dose (200 mg/kg). The treatment also led to a shift towards a Th1-dominant immune response{Guo, 2019 #317}.

Acanthamoeba keratitis is a rare eye infection that can lead to irreversible vision loss if not treated promptly. Researchers developed a mouse model to detect Acanthamoeba antigens in tears and eyeball lysates. They used two specific antibodies, one targeting the adenylyl cyclase associated protein (ACAP) and the other targeting the periplasmic binding protein (PBP) of Acanthamoeba castellanii [48]. The antibodies were confirmed through immunocytochemistry and successfully detected in the mice's tears and eyeball lysates. The study found that antigen detection levels for AK remained consistent regardless of infection progression, and IgM levels were the most elevated, while IgA was the least responsive [49]. These findings suggest ACAP and PBP antibodies hold promise as diagnostic tools for AK, potentially improving outcomes for patients at risk. The study explores the use of Nigella sativa Aqueous Extract and Chitosan Nanoparticles (NS-CNPs) in treating Acanthamoeba Keratitis (AK) using topical application. The treatment is primarily administered through eye drops or ocular gels, maximizing local therapeutic effects and minimizing systemic exposure. The study also uses rodent models, typically mice or rats, to induce AK, evaluate treatment efficacy through clinical examinations, corneal opacity grading, histopathological analysis, and microbiological assessments{Kim, 2022 #318}.

The study found that Nigella sativa aqueous extract encapsulated in chitosan nanoparticles (NS-CNPs) showed potent antimicrobial activity against Acanthamoeba cysts and trophozoites, reducing corneal infection severity in treated animals. The encapsulation of the active compounds enhanced their bioavailability and stability, leading to improved therapeutic outcomes. NS-CNPs achieved 100% cure rates in corneal opacity grades 1 and 2, compared to chlorhexidine's 25% cure rate. The combination therapy (Nigella sativa extract with chitosan nanoparticles) was the most effective, with 100% cure rates across all infection stages. NS-CNPs also exhibited anti-inflammatory properties, reducing the immune response and promoting faster healing. They were non-toxic to ocular tissue, making them a safe alternative to traditional treatments.

Recent studies have shown the potential of propolis extract, including its flavonoid components, in combating Acanthamoeba keratitis (AK). Propolis extract is typically administered topically in the form of eye drops or ocular gels to target the infection site. *In vitro* studies have assessed the effects of propolis extract on Acanthamoeba trophozoites and cysts, while *in vivo* models, particularly rats, have been used to evaluate its therapeutic efficacy in treating experimental AK{Elkadery, 2019 #319}. The extract demonstrated minimal inhibitory concentrations against Acanthamoeba trophozoites, inhibited encystation, and inhibited excystation and adhesion, potentially preventing infection recurrence.

Molecular docking studies revealed that propolis extract forms hydrogen bonds with the AcSir2 protein, a key regulator of encystation in Acanthamoeba{Sama-ae, 2023 #320}{Yang, 1997 #321}.

2.2. Efficacy of phytochemicals in reducing disease severity, inflammation, and tissue damage

Phytochemicals play a crucial role in mitigating inflammation and reducing disease severity in viral and Acanthamoeba keratitis, corneal inflammation caused by viral infections like HSV-1 and protozoan infections like Acanthamoeba. These diseases are often exacerbated by the pathogen and host's inflammatory response. Phytochemical efficiency refers to a compound's ability to exert therapeutic effects at low, non-toxic concentrations while achieving high biological activity. Efficient phytochemicals can mitigate these effects by scavenging reactive oxygen species, inhibiting inflammatory signaling pathways, and modulating immune responses{Caruso, 2022 #323}. Quercetin and resveratrol have shown efficacy in reducing pro-inflammatory cytokine levels and oxidative stress markers in HSV- 1-infected corneal cells, while propolis-derived flavonoids show anti-amoebic activity while preserving host tissue integrity. Phytochemicals have been optimized through various delivery and formulation strategies, including nanoencapsulation, surface modification, combination therapy, and hydrogel-based delivery. Nanoencapsulation enhances stability, bioavailability, and corneal

penetration, while surface modification increases adhesion. Combination therapy pairs phytochemicals with conventional agents, reducing drug concentrations. Hydrogel-based delivery provides prolonged contact time with the cornea, enhancing drug absorption. Photochemotherapy for Acanthamoeba keratitis uses photosensitizers activated by light to target Acanthamoeba trophozoites. Enhanced phytochemical delivery reduces corneal cytotoxicity and lowers dosing requirements, reducing the risk of side effects{Zangooie, 2024 #324}{Kim, 2020 #325}.

2.3. Strategies to Enhance Phytochemical Efficiency

Phytochemicals have been optimized through various delivery and formulation strategies, including nanoencapsulation, surface modification, combination therapy, and hydrogel-based delivery. Nanoencapsulation enhances stability, bioavailability, and corneal penetration, while surface modification increases adhesion. Combination therapy pairs phytochemicals with conventional agents, reducing drug concentrations{Qu, 2025 #326}. Hydrogel-based delivery provides prolonged contact time with the cornea, enhancing drug absorption. Photochemotherapy for Acanthamoeba keratitis uses photosensitizers activated by light to

target Acanthamoeba trophozoites. Enhanced phytochemical delivery reduces corneal cytotoxicity and lowers dosing requirements, reducing the risk of side effects{Gorantla, 2020 #327}.

2.4. Therapeutic Outcomes and Benefits Improving the efficiency of phytochemicals leads to several clinical benefits

The efficacy of phytochemicals in managing viral and Acanthamoeba keratitis offers numerous therapeutic benefits, including reduced corneal scarring, preserved vision, accelerated healing, lower cytotoxicity, and reduced recurrence due to sustained anti- inflammatory effects. Future research should focus on optimizing delivery systems and conducting large-scale clinical trials to establish standardized treatment protocols.

2.5. Bioavailability and ocular penetration of phytochemicals

Managing infections in the eye is challenging due to protective barriers and limited drug penetration. Phytochemicals, with their antimicrobial and anti-inflammatory properties, have emerged as potential therapeutic agents. However, their clinical application is hindered by poor bioavailability and limited ocular penetration{Onugwu, 2023 #328}. Nanotechnology advancements offer promising solutions to these challenges. Coumarin-rich extracts from Pterocaulon balansae show anti-Acanthamoeba activity, and incorporating them into nanoemulsions enhances solubility and ocular penetration. Berberine, an isoquinoline alkaloid with antimicrobial properties, is being investigated for its stability and delivery to ocular tissues. Nanotechnology has developed various nanocarrier systems to improve the delivery of phytochemicals to ocular tissues{Panatieri, 2017 #329}{Duong, 2021 #330}. Chitosan-based nanoparticles enhance mucoadhesion and facilitate drug penetration, while liposomes encapsulate both hydrophilic and lipophilic compounds{da Silva, 2016 #331}. Nanoemulsions enhance hydrophobic phytochemicals' solubility and penetration into ocular tissues, such as coumarin-rich extracts in Acanthamoeba keratitis treatment. Phytochemicals have antimicrobial and anti-inflammatory properties, but their clinical application is limited by challenges in ocular delivery. Advancements in nanotechnology offer innovative solutions to enhance the bioavailability and penetration of these compounds, paving the way for more effective and targeted ocular therapies{Gu, 2023 #332}{Panatieri, 2017 #333}.

2.6. Toxicity and safety evaluation in animal models

Animal models play a crucial role in understanding the pathophysiology and therapeutic windows of treatments for viral and Acanthamoeba keratitis. These preclinical models provide insights into disease mechanisms and enable the testing of novel therapeutics for ocular safety and efficacy{Zhang, 2021 #334}. Studies have shown that CMV can infect anterior segment tissues, leading to increased intraocular pressure and inflammation in the trabecular meshwork and Schlemm's canal. In a separate model, inflammatory infiltration and corneal edema were observed in mice infected with herpes simplex virus, but no systemic toxicity was reported. Acyclovir (ACV), a widely used antiviral for HSV keratitis, has undergone rigorous safety evaluations, demonstrating its safety for ophthalmic applications{Neelam, 2017 #335}{Tucker Jr, 1983 #336}. Acanthamoeba keratitis (AK) is a challenging infection, especially for contact lens wearers. Animal models have been used to study the disease's pathology and evaluate the safety of emerging therapies. A rabbit model showed deep stromal infiltration by

Acanthamoeba trophozoites and cysts, with no systemic toxicity{Sharma, 2021 #337}{Matoba, 2021 #338}. Miltefosine, an anti-leishmanial agent repurposed for AK, has raised safety concerns due to its ability to disrupt lipid rafts and cause cholesterol efflux in corneal tissues. Voriconazole, a broad- spectrum antifungal, has been explored for its amoebicidal properties, with *in vitro* studies showing minimal toxicity. An experimental cat model successfully inducing keratitis without systemic side effects{Rodrigues, 2023 #339}{Ledbetter, 2023 #340}.

The MTT assay was used to evaluate the cytotoxicity of various therapeutic drugs on the Human Corneal Epithelial Cell (HCEC) line. This assay relies on the ability of mitochondrial dehydrogenase enzymes in viable cells to reduce MTT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyl tetrazolium bromide) into an insoluble blue formazan product. A stock solution of MTT was prepared by dissolving it in growth medium at a concentration of 5 mg/ml. HCEC cells were seeded in 96-well microplates and incubated overnight at 37°C in a humidified atmosphere containing 5% CO. After 24 hours, freshly prepared dilutions of the therapeutic drugs were added to the wells. The study involved *in vitro* cytotoxicity assays using corneal cell lines to assess the toxicity of compounds used in ophthalmic therapeutics{Secchi, 2006 #341}. The cells were treated for various time intervals, washed with PBS, and then added to a freshly prepared MTT working solution. The cells were incubated for an additional 3 hours to allow for formazan formation. The MTT solution was discarded, and 100 µl of dimethyl sulfoxide (DMSO) was added to dissolve the formed formazan crystals. The optical density (OD) of each well was measured spectrophotometrically at a test wavelength of 596 nm. The

percentage of cell cytotoxicity was calculated using a formula: \% \text{Cytotoxicity at concentration} = \left(\frac{\text{OD of control} - \text{OD of treated wells}}{\text{OD of control}}\right) \times 100{Khan, 2019 #343}. The Draize eye test, a controversial ethical test, was used to assess ocular irritation in rabbits. Modern research increasingly relies on *in vitro* cytotoxicity assays using corneal cell lines, which offer a more ethical and reproducible measure of toxicity before moving to animal or human trials{Shareef, 2023 #344}{Dwia Pertiwi, 2021 #345}.

3. Clinical Studies

Phytochemicals, or plant-derived compounds, exhibit promise antibacterial, anti- inflammatory, and antioxidant characteristics; nevertheless, their therapeutic effectiveness in treating keratitis may be constrained by issues such as inadequate bioavailability, stability, and corneal penetration.

Advanced drug delivery systems enhance corneal retention time, penetration, and stability of phytochemicals, promising improved control over conventional formulations. Nanoparticles and nanoemulsions improve solubility and cellular uptake, such as curcumin-loaded nanoparticles for HSV or fungal keratitis. Curcumin, a hydrophobic polyphenol derived from Curcuma longa, has numerous pharmacological activities including anticancer, antimicrobial, anti-inflammatory, anti-amyloid, antioxidant, and neuroprotective effects. Cur-SLN has enhanced oral bioavailability. Natural products have potential treatments for ocular diseases due to their anti-oxidative, antiangiogenic, and anti-inflammatory effects. However, most beneficial compounds have low solubility, requiring frequent administration or high doses. The chemical structures of natural compounds play a crucial role in their therapeutic effects and biological properties.

Phytochemicals can be structurally modified to improve pharmacokinetics or target specificity. Combination therapy can enhance antiviral activity by combining EGCG with acyclovir. Permeation enhancers can facilitate corneal absorption by modifying tear film, mucous layer, and ocular membranes. Targeted delivery uses ligands that target infected corneal cells or immune pathways, improving specificity and minimizing toxicity. Examples include mannose-targeted systems for Acanthamoeba keratitis.

3.1. Efficacy in treating viral or protozoal keratitis in patients

Phytochemicals, plant-derived compounds, have potential antimicrobial, anti-inflammatory, and antioxidant properties but face limitations in treating keratitis due to poor bioavailability, stability, and corneal penetration. Advanced drug delivery systems enhance these properties, offering superior control over conventional formulations. Nanoparticles and nanoemulsions, like curcumin-loaded nanoparticles, improve solubility and cellular uptake. Curcumin, a hydrophobic polyphenol, has anticancer, antimicrobial, anti- inflammatory, anti-amyloid, antioxidant, and neuroprotective effects. Sandhir et al. developed a formulation with enhanced oral bioavailability of curcumin (Cur-SLN). Ocular disorders, including anterior and posterior segment diseases, are the main causes of blindness. Natural products have potential treatments due to their anti-oxidative, antiangiogenic, and anti-inflammatory effects. However, these compounds are often low solubilities, requiring

frequent administration or high doses. The chemical structures of these compounds play a crucial role in their therapeutic effects and biological properties. Lutein, for example, can effectively scavenge free radicals and prevent oxidation. Phytochemicals can be structurally modified to improve pharmacokinetics or target specificity. Combination therapy can enhance antiviral activity by combining EGCG with acyclovir. Encapsulation with permeation enhancers can facilitate corneal absorption. Targeted delivery uses ligands to target infected corneal cells or immune pathways, improving specificity and minimizing toxicity. Examples include mannose- targeted systems for Acanthamoeba keratitis.

3.2. Safety and tolerability in human eyes

The human eye's safety and tolerability refer to its ability to handle various exposures without causing damage or discomfort. It can tolerate natural daylight and indoor lighting, but can be harmed by UV light, blue light, and laser exposure. Chemical exposure is generally safe, but irritating substances like cleaning products, acids, and solvents can cause severe irritation or permanent damage. Mechanical tolerance is limited to foreign bodies, but injury risk can arise from blunt trauma or sharp objects. Radiation exposure, such as ionizing and infrared radiation, can cause eye tissue damage. Contact lens use is safe with proper hygiene and fitting, but poor practices can lead to infections or reduced oxygen supply to the cornea. Protective eyewear is essential in high-risk environments.

4. Mechanisms of Action

Phytochemicals, found in plants and easily extracted, have safe and eco-friendly effects. They produce antiviral and antiprotozoal effects against viral and acantha amoeba keratitis through various mechanisms [50]. Antiviral effects prevent virus attachment to host cells and target enzymes causing viral activity. Amoebicidal and anti-adhesion activities are observed against

trophozoites and cysts of A. Triangularis species. These chemicals disrupt cyst walls, inhibit trophozoite adhesion, and cause structural and permeability alterations, ultimately leading to parasite cell death[51] . Antiprotozoal effects control protozoa proliferation and reduce inflammatory damage through the immune system. Flavonoids affect enzymatic activity in fatty acid synthesis and ATP production[50]. Phytochemicals, including Quercetin flavonoids and Betulinic acid, have shown potent anti-acantha amoebic activity against Acanthamoeba castellanii. Quercetin conjugated silver nanoparticles effectively inhibited both encystation and excystation processes, suggesting superior anti-acantha amoebic activities [50]. However, their effectiveness should be evaluated in an animal model of keratitis caused by *A. castellani* [52, 53]. These phytochemicals also produce potential synergistic effects with other conventional drugs, such as knema retusa a phytochemicals and chlorhexidine. Modern technologies can enhance the viral effect of phytochemicals, as demonstrated in curcumin- loaded nano lipid carriers with N-acetyl L-cysteine PEG[54, 55]. The sustained release effect from modified nano lipid carriers was more pronounced than unmodified nano lipids, allowing enhanced curcumin permeation relative to solution. Therefore, phytochemicals are the best medicinal source for keratitis, as suggested by the World Health Organization[51, 56].

5. Future Directions and Challenges

Plant metabolites are being used as antimicrobial agents against drug-resistant microbes, but the FDA has only authorized a few phytochemicals, such as capsaicin, codeine, paclitaxel, reserpine, and colchicine. A UK government review suggests that AMR could kill 10 million people per year by 2050, prompting the need for novel antimicrobials. A study on Artemisia annua L. crude extracts showed a 6-18-fold reduction in plasmodial IC50 compared to pure artemisinin due to interacting compounds. Further research is needed to understand the interaction of phytocompounds in mixtures to develop efficient antimicrobials from plant secondary metabolites[57]. No chemotherapeutic agent has been described as a single effective treatment against acute mycobacterial reticulum (AK), due to the wide range of virulence traits of different isolates and the difficulty in establishing the most effective treatment regimen due to the small number of reported cases, variable pathogenicity of different strains, and the fluctuating nature of the disease process.

Steroids in Acanthamoeba keratitis treatment should be used cautiously as they can increase treatment duration by promoting cyst transition to trophozoites and trophozoite proliferation. Misdiagnosis is common in early stages, with 75%-90% of patients misdiagnosed. The German Acanthamoeba Keratitis Registry shows that correct diagnosis is not made until 2.8 ±

4.0 months. Despite new diagnostic methods, diagnosis is often missed or delayed, affecting patient prognosis and quality of life. Phytochemicals like miltefosine require enhanced delivery systems for better absorption and stability.

The study of phytochemicals' entry into the body is crucial due to their poor oral bioavailability and inefficient intestinal absorption. There is a lack of clinical trials to validate the safety, efficacy, and optimized dosing of phytochemicals for treating keratitis in humans[58]. As protozoan parasites and viral species are more resistant to chemical compounds, advanced clinical trials are needed to design safer molecules. Proper extraction processes, such as using solvent or elevated temperature, are essential[53]. It is crucial to develop effective and selective conventional extraction methods based on the nature of the plant matrix to be treated. Non-conventional techniques can help reduce extraction time and degradation of phytochemicals[59, 60].

5.1. Identification of gaps in current research

Natural phytochemicals' properties, effects on the body, and their entry into the body are crucial. Poor oral bioavailability and inefficient intestinal absorption have hindered their development. *In vitro* culture systems simulating intestinal epithelium have been used to predict intestinal permeability and accelerate the development of natural phytochemical foods. However, there is a lack of clinical trials to validate the safety, efficacy, and optimized dosing of phytochemicals for treating keratitis in humans. Future research should assess the antimicrobial activities of these novel derivatives against protozoan parasites and develop strategies to design alternative and safer molecules. The correlation between co-cultured cells and *in vivo* data has improved, with 3D multi-cell models and fluid dynamics systems making progress in GIT research. Proper convectional extraction methods are essential for the development of natural phytochemicals.

The development of effective and selective conventional extraction methods is crucial for improving efficiency in practitioners and researchers. Techniques should be studied based on the plant matrix, such as leaves, fruit, berries, roots, and tubers. Non-conventional techniques can be used to reduce extraction time and reduce degradation of phytochemicals, especially when extracting thermolabile or easily oxidizable principles.

Strategies to improve bioavailability and corneal penetration

Microencapsulation of polar or apolar bioactive compounds is an effective solution to overcome properties such as log P, melting point, phytochemical structure, and molecular weight. This technique preserves and stabilizes phytochemicals from physical-chemical stresses, increasing their bioavailability and bioactivity. For example, nanoparticles can improve the bioactivity of EGCG, resveratrol, curcumin, and quercetin. Phenolic compounds can be enhanced through dietary modifications, encapsulation, or stabilizing matrices.

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