REVIEW ARTICLE
Eurasian J Bio Chem Sci, 7(2):178-185, 2024
https://doi.org/10.46239/ejbcs.1569164

Eurasian Journal of Biological and Chemical Sciences

Journal homepage: www.dergipark.org.tr/ejbcs

An overview of leech saliva and cosmetic potential

Fatma Çoruk[1], Sibel Kaymak[1*], Hüseyin Ayhan[1,2], Nilüfer Vural[1,3], Salih Mollahalioğlu[4]

*[*1]Department of Traditional, Complementary and Integrative Medicine, Biotherapeutic Products Research and Development Program, Ankara Yıldırım Beyazıt University, Ankara, Türkiye*

[2.] Department of Medical Laboratory Techniques Program, Health Services Vocational School, University of Ankara Yıldırım Beyazıt, 06760, Çubuk, Ankara, Türkiye

[3.] Department of Food Processing-Food Technology, Health Services Vocational School, University of Ankara Yıldırım Beyazıt, 06760, Çubuk, Ankara, Türkiye

[4.] Department of Public Health, Faculty of Medicine, Ankara Yıldırım Beyazıt University, Ankara, Türkiye

***Corresponding author :** sibelkaymak@aybu.edu.tr
Orcid No: https://orcid.org/0000-0002-6523-7637

Received : 18/10/2024
Accepted : 12/12/2024

To Cite / Atıf için: Çoruk F, Kaymak S, Ayhan H, Vural N, Mollahalioğlu S. 2024. An overview of leech saliva and cosmetic potential. Eurasian J Bio Chem Sci, 7(2):178-185 https://doi.org/10.46239/ejbcs.1569164

Abstract: The therapeutic benefits of leech secretion have been known for centuries, and the potential of this substance in modern cosmetics is now attracting increasing interest. Leech secretion contains various bioactive compounds, including enzymes, peptides, and proteins. The bioactive components found in leech saliva, including anticoagulants, growth factors, and antimicrobial peptides, are responsible for leech saliva's effectiveness in increasing skin hydration, reducing inflammation, and promoting wound healing. To utilize their potential, it is imperative to sift through the skin area. This study reviews the available literature and research studies on the chemical properties of leech saliva and its use in cosmetic formulations. It highlights the bioactive compounds, their potential mechanisms of action, and their efficacy in cosmetic applications. The sources consulted include peer-reviewed articles, clinical studies, and industry reports. Data from these studies are synthesized to provide a comprehensive overview of the current state of knowledge. The findings suggest that leech saliva has significant potential as an ingredient in cosmeceutical products and offers new benefits for skin rejuvenation and repair. The review highlights the need for further research to optimize extraction processes, ensure product safety, and investigate additional applications. By advancing our understanding of leech saliva's chemical properties and mechanisms, future studies may facilitate the development of innovative skincare solutions and increase the efficacy of cosmetic formulations.

Keywords: Leech, Leech saliva, Bioactive compounds, Cosmetic, Cosmeceutical

1. Introduction

The use of leeches in medical practice is a practice that has persisted for centuries and across various civilisations. The word 'leech' is etymologically derived from the old English word 'leace' (doctor). The first known use of leeches as medicine dates back to ancient Egypt when leech drawings from 1567–1308 BC adorned the walls of a pharaoh's tomb belonging to the 18th dynasty. Galen (130–201 AD) created the idea of humoral sickness and utilized leeches for bloodletting, which helped explain why leech therapy is so popular today. Medical records from the Anglo-Saxon, Chinese, Arabic, Ancient Greek, and Roman eras all make frequent mention of leech therapy (Mory et al. 2000; Whitaker et al. 2004). In the medieval period, Arab physicians employed the use of leeches in the treatment of alopecia, dermatological conditions, postoperative and pain relief, and joint diseases. Ibn Sina and Ibn Al-Quff provided a comprehensive account of medicinal leeches and were instrumental indistinguishing them from non-medical leeches (Alaama et al. 2024; Alharbi 2015). Furthermore, Ibn Sina (980-1037 AD) also documented the use of leeches for the treatment of certain dermatological conditions in his Canon of Medicine (Amani et al. 2020). For centuries, medicinal leeches have been employed in the treatment of a wide range of ailments, including eye diseases, malaria, typhoid, obesity, and skin disorders, among numerous other complications. It was also believed that leeches could be used to treat plague patients by eliminating the 'bad blood' in their bodies (Arabacı 2023; Sawyer 2013). In the late nineteenth century, Dr. John H. Haycraft's discovery of anticoagulants in leech head extract challenged the traditional belief that the benefits of leech therapy were

solely derived from bloodletting. Instead, he suggested that the real therapeutic benefit came from the substances injected into the patient's body by the leech (Haycraft 1883). Although the use of leeches for medical purposes declined in the early 20th century due to the development of precise theoretical bases for disease and treatment and the discovery of chemical drugs, leech therapy came back in the late 20th century, particularly in microsurgery and plastic surgery. The utilisation of leeches in medical applications has increased since the United States Food and Drug Administration (FDA) granted approval for the use of the medical leech *Hirudo medicinalis* as a medical device in plastic surgery in 2004 (Rados 2004).

Medical leeches (subclass Hirudinea) are hermaphroditic, ringed, amphibious animals belonging to the phylum Annelida. Some leeches are hematophagous, while others are carnivorous (Sawyer 1986). The bioactive substances present in the saliva of medicinal leech species, such as Hirudo sp., enable them to feed on and store blood, while also conferring therapeutic benefits to their hosts . Following the leech's bite, it must create a suction pathway (extracellular matrix degradation), inhibit adhesion and aggregation (antiplatelet activity), inhibit clotting (anticoagulant activity), increase blood flow, protect itself (antimicrobial activity) and avoid detection (analgesic and anti-inflammatory activity) (Sig et al. 2017).

The disturbance or absence of microcirculation represents a significant pathomechanism in numerous diseases, and as a result, leech therapy is employed in both traditional and complementary medicine for both therapeutic purposes and as an adjunct to treatment for a wide range of disorders. From a terminological perspective, hirudotherapy can be defined as an integrative treatment method in which medicinal leeches are applied to the body for therapeutic purposes. Currently, leeches have been used in the treatment of over twenty clinical conditions, including cardiovascular, musculoskeletal, plastic and reconstructive surgery, soft tissue injuries, and dental and skin diseases (Abdualkader et al. 2013; Ayhan and Mollahaliloğlu 2018). Of particular interest in recent years has been the investigation of the cosmetic applicability of hirudotherapy in dermatological diseases.

As the largest organ in the body, the skin serves as a physical barrier between the body and the external environment, and is involved in both physical and immunological processes. Furthermore, the skin, which is composed of hair and nails, performs additional functions such as the production of hormones and enzymes, maintenance of homeostasis, and regulation of body temperature (Xu et al. 2019). As the body's primary line of defense, the skin is frequently subjected to damage from external factors, which serves as the foundation for a multitude of related dermatological complications. As of today, over 3,000 dermatological diseases have been identified in the literature (Bickers et al. 2006). In comparison to conventional therapeutic and pharmacological treatments for dermatological conditions, hirudotherapy has a diverse range of applications across various fields, including cosmetology (Zabkowska and Piotrowska 2019). A cosmetic product is typically defined as any substance designed for application to the epidermis, nails, hair, lips, external genital organs, or the mucous membranes of the teeth and mouth. Its primary purpose is to cleanse, perfume, alter appearance, protect, maintain in good condition, or correct body odour, with an emphasis on aesthetic concerns (Anonym 2005). Cosmeceutics, a blend of "cosmetics" and "pharmaceuticals," refers to products that contain biologically active ingredients with medicinal or drug-like benefits. Unlike regular cosmetics, cosmeceutical products are designed not only to enhance appearance but also to improve skin health by addressing specific concerns such as aging, hyperpigmentation, and acne (Milam et al. 2021).

At present, with research and clinical applications reaching this point, leech extract, and particularly leech saliva extract, is being promoted as a potential therapeutic agent. Consequently, leech extract is being used and produced for medical purposes, including skin grafts, treatment of venous insufficiency, and other conditions, leading to the development of pharmacological, cosmeceutical, and cosmetic products (Zabkowska et al. 2022).

This study was designed to consolidate and elucidate the cosmetic processes associated with leech extracts and other purified leech products, and to shed light on the related therapeutic mechanisms. It aims to reveal the healing potential of leech saliva.

2. Leech Secretion Content

From a biochemical perspective, the secretions of most leech species consist of peptides, proteins, enzymes, and other small molecules. Therefore, although the content may vary, medicinal leeches generally contain these bioactive components. This composition includes approximately 100 bioactive chemicals, such as amino acids, peptides, proteins, enzymes, volatile compounds, phospholipids, and other components**.** Most of these components exhibit various biotherapeutic activities, and some may have cosmetic and dermatological potential. The biotherapeutic effects of the components in the cosmetic vision are presented in the chart given in Figure 1 (Zabkowska et al. 2022).

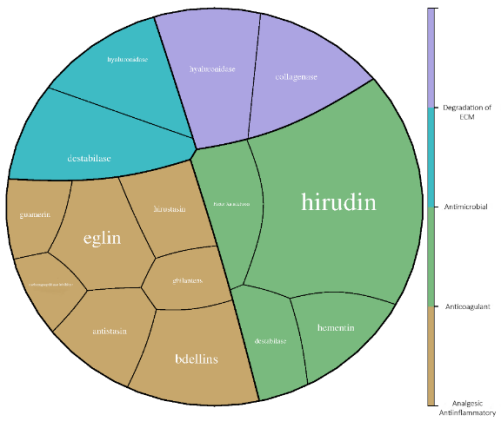

Figure 1. Voronoi diagram of the leech compounds and possible biotherapeutic activities

2.1. Proteins

2.1.1. Hirudin

Hirudin (PDB code: 5HIR and Uniprot code: P01050) is a protein consisting of 65 amino acids with a molecular weight of 6.97 kDa, represented by the sequence VVYTDCTESGQNLCLCEGSNVCGQGNKCILGSDGE KNQCVTGEGTPKPQSHNDGDFEEIPEEYLQ. It exhibits neutral properties and is stabilized by different secondary structural elements through disulfide bridges (Figure 2) (Folkers et al. 1989). Physicochemically, it has a hydrophilic structure, soluble in water and other polar solvents. Conformationally, its structural flexibility is limited due to the presence of disulfide bridges and beta-turns. Hirudin constitutes a significant portion (1-2%) of leech secretion and is an effective anticoagulant. It binds irreversibly to thrombin, inhibiting the activation of its active site and other bindings, thus preventing blood clotting processes. Hirudin also inhibits other thrombin-catalyzed hemostatic reactions such as the activation of clotting factors V, VIII, and XIII, as well as thrombin-induced platelet activation, exhibiting a synergistic anticoagulant behavior. Currently, hirudin is synthesized and produced recombinantly through biotechnological methods (Junren et al. 2021). As an anticoagulant, hirudin holds significant potential not only in medical applications but also in cosmetic fields. For instance, hirudin improves microcirculation by making the blood more fluid, which facilitates the delivery of more oxygen and other essential elements to the skin (Ren et al. 2021). This can be attributed to the increased brightness and vitality of the skin following leech applications on the facial area. In allergic skin disorders and other inflammatory skin issues, blood circulation and abnormal accumulation are among the most important symptoms. At this point, hirudin found in leech secretion has the potential to contribute to overall relaxation and support of blood flow processes (Peng et al. 2015). Additionally, the regulation and acceleration of blood flow contribute to cellular renewal, reducing wrinkle appearance and providing anti-aging effects. Therefore, hirudin-derived leech secretion has anti-aging potential. Furthermore, in terms of reducing skin blemishes, redness, increased capillary appearance, and other cosmetic and aesthetic concerns, hirudin could be a unique bioactive component and option.

2.1.2. Hirustasin

Hirustasin is a polypeptide composed of a 55 amino acid sequence, TQGNTCGGETCSAAQVCLKGKCVCNEVHCRIRCKY GLKKDENGCEYPCSCAKASQ, and has a molecular weight of 6.08 kDa. Structurally, it is a serine protease inhibitor that can also bind to kallikreins, a subgroup of serine proteases found in plasma and tissues, which cleave kininogens to form kinins (Liu et al. 2024). Additionally, being a definitive inhibitor of coagulation factor Xa further proves the synergistic anticoagulant effect of leech saliva (Zhao ey al. 2024). Besides other components, hirustasin emphasizes the importance of using leech saliva in regulating blood pressure and improving hypertension-related factors (Lemke 2020). Furthermore, studies in the literature have shown that hirustasin exhibits antimetastatic effects (Söllner et al. 1994). Cosmetically, hirustasin has the potential for treating bacterial infections on the skin, providing general moisture, and acting as an antioxidant.

2.1.3. Eglin

Eglin is a small protein consisting of 70 amino acid residues and weighing 8.1 kDa, inhibits chymotrypsin and subtilisin-like serine proteinases. Approximately 20 g of the dry weight of medicinal leeches has been determined to be eglin. Structurally, eglin inhibits alpha chymotrypsin, subtilisin, chymosin, granulocyte proteinases, elastase, and cathepsin G (Schnebli et al. 2021). Additionally, it suppresses neutrophil activity, thereby reducing inflammation, which is the main source of the anti-inflammatory activity in leech saliva (Suter et al. 1998). The mentioned anti-inflammatory effect indicates the potential of leech saliva containing eglin for cosmetic use in the treatment of acne, reduction of irritation, and treatment of eczema and dermatitis. Besides mediating the general anti-inflammatory effect of leech saliva, eglin highlights the broad range of cosmetic formulations in which leech saliva can be used, including improving skin elasticity and overall appearance.

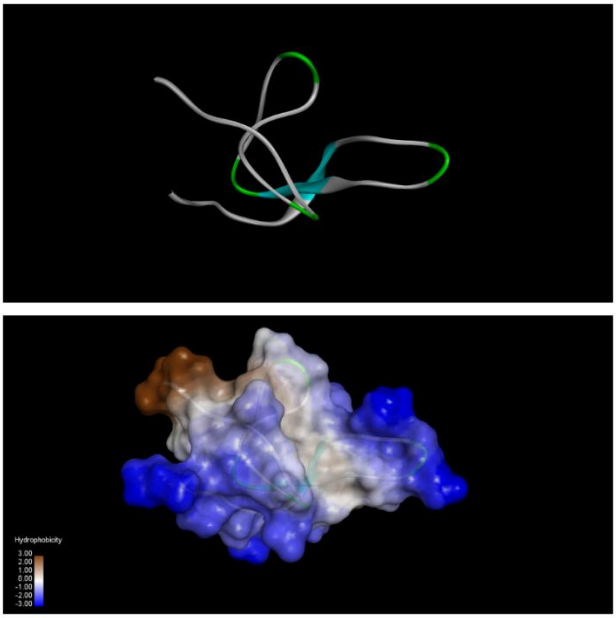

Figure 2. 3D structure of the Hirudin

2.2. Enzymes

2.2.1. Hyaluronidase

Hyaluronidase is a protein with a molecular weight of 40.9 kDa and consisting of 350 amino acids, containing one disulfide bridge. This enzyme is generally active under neutral or slightly acidic pH conditions, with an optimal pH range typically between 5.5 and 7.0 (Jung 2020) Hyaluronidase induces the breakdown of hyaluronan, the main glycosaminoglycan in connective tissue, and facilitates the diffusion of secretion components into tissues and the bloodstream, leading to systemic effects.

Bioterapeutically, hyaluronidase is used to enhance the spread of local anesthetics. By increasing tissue permeability at injection sites, it can help the drug spread over a larger area and assist in the resolution of fluid accumulation in tissues following trauma or surgery. Recently, in cosmetic applications, hyaluronidase can also be used to dissolve areas where hyaluronic acid has been improperly or excessively injected. Cosmetically, hyaluronidase increases the permeability of the skin barrier, potentially allowing bioactive substances commonly used in cosmetics (such as vitamins, peptides, antioxidants, etc.) to better reach the deeper layers of the skin (Weber et al. 2019). Furthermore, this enzyme found in leech secretion can break down accumulated hyaluronic acid in the skin, supporting the skin's natural renewal process. This can contribute to the smoothing and rejuvenation of the skin. In the context of hyaluronidase, leech secretion can also be used in eye creams aimed at reducing under-eye puffiness and bruising, as it may help break down the hyaluronic acid responsible for these conditions.

2.2.2. Destabilase

Destabilase (PDB code: 8BBW) is an enzyme with a molecular weight of 13.95 kDa, composed of 124 amino acid sequences, predominantly in an alpha-helix structure (Figure 3) (Marin et al. 2023). Its structure is relatively flexible, allowing binding adaptation during interactions with substrates. It is soluble in polar solvents like water and shows higher activity in saline environments. Due to its glycosidase activity, it exhibits fibrinolytic and antibacterial effects by targeting the β1-4 bonds in the peptidoglycan layer of the bacterial cell wall (Bobrovsky et al. 2021). It is structurally synthesized in different isoforms and derivatives for various leech species (Zavalova et al. 2000). The antibacterial property of Destabilase, by its nature, makes it a potent bioactive component against pimples and acne vulgaris. In addition to reducing swelling and redness on the skin, it can also act as a soothing agent.

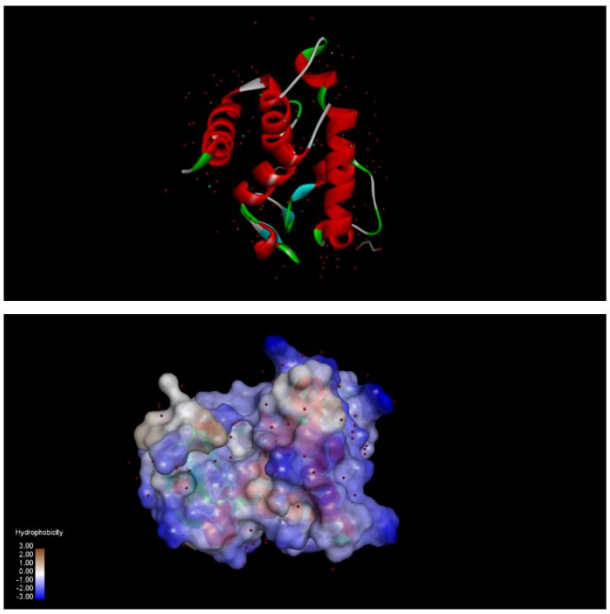

Figure 3. 3D structure of the Destabilase

2.2.3. Collagenase

Collagenase is an enzyme with a biotherapeutic effect that allows the breakdown of peptide bonds in collagen protein and is approved by the FDA. Collagenase is a Zn2+-dependent matrix metalloproteinase, and its active binding regions include Zn parts. Morphologically, it has a bent tertiary structure, and its side chains control the Zn part tetrahedrally (Brito et al. 2021). Collagenase is already used in medical applications such as breaking down burn scars instead of harsh surgical debridement, promoting wound healing, and removing necrotic tissue. In the United States, clostridial collagenase ointment is the only FDA-approved treatment for enzymatic wound debridement in severe burns, shortening wound healing time and reducing infection risk while supporting overall healing (Waycaster et al. 2018). Cosmetically, collagenase can be used in various areas such as skin renewal, post-acne scar treatment, cellulite and stretch mark treatment, and dissolving unwanted surgical fillers. This highlights the potential for leech saliva containing this enzyme to be used as a care cream or serum, especially in the treatment of cellulite and stretch marks.

2.3. Other Molecules

2.2.1. Histamine

Histamine, with the chemical formula $C_5H_9N_3$, is a biogenic amine containing an imidazole ring and is classified as an organic compound synthesized by the decarboxylation of the amino acid histidine (Moriguchi and Takai 2020). In humans, it interacts with different receptors and plays roles in various metabolic processes, including allergic reactions and inflammation (smooth muscle contraction, vasodilation), gastric acid secretion, and neurotransmitter release regulation (Lieberman 2011). Structurally, leeches also secrete histamine from their salivary glands during feeding, which causes partial relaxation of the blood vessels and smooth muscles at the bite site (Sandilands et al. 2013). Histamine is involved in vasodilation due to leech saliva and is studied as an important secretion component. Cosmetically, histamine has the potential to promote the use of leech saliva in anti-aging and anti-blemish products, as well as in products designed to reduce the appearance of capillaries, due to its effects on blood vessels.

2.2.2. Serotonin

Serotonin, with the chemical formula $C_{10}H_{12}N_2O$, is a compound belonging to the indolamine class, containing an indole ring and an amine group (Lent et al. 1991). It is formed by the hydroxylation and decarboxylation of the amino acid tryptophan. Serotonin is broken down into metabolites such as 5-hydroxyindoleacetic acid (5-HIAA) by the enzyme monoamine oxidase. This component is a neurotransmitter with various functions in the body. Serotonin has significant effects on mood, gastrointestinal function, and the circulatory system, influencing both the central nervous system and the peripheral system (Mohammad-Zadeh et al. 2008). In the cosmetic vision, serotonin has the potential to be one of the reasons for preferring leech saliva in managing stress-related skin

problems, as an anti-inflammatory agent, for skin rejuvenation, and for improving mood (as a fragrance agents) (Baskova et al. 2008).

3. Clinical Use

3.1 Wounds

A wound is defined as an interruption in the continuity of tissue structure. It occurs as a consequence of injury, surgical intervention, or accident, resulting in the destruction of tissue and blood vessels, extravasation of blood components, and hypoxia. The process of wound healing comprises three distinct stages: inflammation, proliferation, and tissue remodelling. The process of wound healing is the result of an intricate interplay between various biological factors, including cytokines, growth factors, blood, cellular elements, and the extracellular matrix (Waili et al. 2011). The anticoagulant compounds present in leech saliva have the effect of promoting continuous bleeding, which in turn results in a significant reduction in tissue congestion and relaxation of the capillary network. Furthermore, this has the additional effect of promoting haemorheology, increasing oxygen delivery, improving tissue metabolism, increasing lymphatic flow and eliminating tissue ischaemia. Furthermore, this results in favourable alterations to the local haemodynamic conditions. Consequently, the survival rate of the affected area is enhanced. Hirudotherapy represents a promising approach for the treatment of chronic non-healing wounds and post-traumatic wounds (Ünal et al. 2023). Amani et al. conducted a case study in which a 5% leech cream, prepared from lyophilised leeches and formulated in an oil/water pharmaceutical form, was applied to a patient with a grade 1 diabetic foot ulcer. The cream was applied twice daily, from the knee to the tip of the toes, for a period of one month. During this time, the patient did not receive any antibiotics. Two days after the commencement of treatment, a notable reduction in the intensity of the pain was observed. By the end of the third day, the patient reported that the pain had completely abated. One week later, new tissue was observed in the wound, and one month later, the wound had fully healed. The study reported that no recurrence was observed after two months of follow (Amani et al. 2020). Another study sought to develop a pharmaceutical cream comprising active enzymes and proteins derived from *lyophilised Hirudo orientalis* leech sources with wound healing and anticoagulant properties. In vivo results demonstrated that leech cream and phenytoin facilitated wound regeneration by accelerating re-epithelialisation and initial angiogenesis in all treatment groups (Amani et al. 2021).

3.2 Acne

Acne is a persistent inflammatory condition involving the sebaceous glands. Three factors play a role in its pathophysiology: Propionibacterium acnes growth in the pilosebaceous unit, aberrant follicular keratinisation, and hyperseborrhea. These factors interact to alter the cutaneous milieu and induce an inflammatory response in the host, which promotes the progression of the acne lesion (Taylor et al. 2011). A 23-year-old woman with active acne, acne scars on her forehead and both cheeks, and soreness in the area of her active acne was treated with leeches for a period of six months. Following a 15-day course of treatment, there was a notable reduction in the number of active acne lesions, acne scars and blemishes. In addition to a reduction in the number of active pimples, there was a notable decrease in their size, and the hyperpigmentation of both pimples and scars began to diminish (Kumari 2023). A case study reported that a patient who presented with acne, pruritus, and hyperpigmentation following four leech applications with an herbal mixture exhibited complete resolution of acne, complete resolution of pruritus, burning, and pain, and a significant improvement in facial discoloration from grade 3 to grade 1 (Jayant and Chandurkar 2018). In a case study reported by Habeshian et al., a 25-year-old woman with acne vulgaris was treated with hirudotherapy. Despite the use of pharmacological treatments, the recurrence of acne was not prevented, and the patient had been suffering from acne lesions for six months. The patient underwent four weekly sessions at regular intervals, during which four to five small leeches were placed at the site of the lesions. In addition to this treatment, the patient was advised to make lifestyle modifications, adopt a healthy diet and use an Ayurvedic preparation. Following the conclusion of the therapy, the patient exhibited complete disappearance of acne lesions, an absence of itching, burning and pain, and a notable reduction in facial discolouration (Habeshian et al. 2020).

3.3 Alopecia

Alopecia areata is a prevalent form of hair loss or baldness observed in humans. It is a variable autoimmune disease that can manifest in a number of ways, with the potential for remission or recurrence. When hair loss is extensive, the disease may become permanent (Pratt et al. 2017). A 28-year-old female patient with a six-year history of scalp hair loss, accompanied by dandruff, itching, and roughness, was treated with leech application. Following five sessions of leech application, a significant improvement in the patient's symptoms was reported, including hair growth in the affected area, hair thickness on the scalp, hair strand spores/hyphae, roughness, itching and dandruff (Yadav and Guguloth 2017). Waghmare treated a 29-year-old male patient diagnosed with alopecia with medicinal leeches in combination with herbal mixtures for a period of three months. The patient reported that the leech treatment relieved itching and dandruff complaints and resulted in significant hair regrowth (Waghmare 2019).

3.4 Eczema (Dermatitis)

The clinical manifestations of eczema, a non-infectious epidermodermitis, typically include pruritus, erythema, papules, seropapules, vesicles, scaling, crusting, lichenification. These findings are mainly attributed to hypersensitivity (Ring 1991).

The patient, who had been diagnosed with mild eczema on the flexor aspect of the right foot, was treated locally with leech therapy once a week for a period of six weeks. The efficacy of leech therapy was assessed at 15-day intervals using the EASI Score, and at the conclusion of the treatment

period, the leech application resulted in the complete cure of the eczema. No adverse effects were observed during the course of treatment, and no recurrence was reported during the post-treatment follow-up (Siddiqui et al., 2024).

3.4.1 Atopic Eczema

Atopic dermatitis is a type of eczema in which disruption of the skin barrier, IgE-mediated allergy and neural interaction represent the primary pathophysiological aspects (Ring 2015). In a study in which 27 patients diagnosed with eczema were treated with leech therapy at 7-day intervals, the efficacy of leech application in the treatment of eczema was evaluated using the standard clinical parameters Eczema Area and Severity Index (EASI) score, Atopic Dermatitis SCORing (SCORAD) index and Dermatology Life Quality Index (DLQI). The results demonstrated a significant improvement of 62.36% in the quality of life of the patients. The majority of patients exhibited no change in disease progression over the six-month follow-up period. The study demonstrated that leech application provided substantial relief from eczema symptoms, and no adverse effects were observed throughout the study period (Pratap 2014).

3.4.2 Stasis Eczema

Stasis eczema, also referred to as venous eczema, is a chronic form of endogenous dermatitis that is characterised by alterations in the skin as a consequence of deoxygenated blood failing to return to the heart with immediate efficacy. The hydrostatic pressure within the dermal capillaries is increased when there is a deficiency of deep perforating veins, resulting in the accumulation of extravascular blood along the capillary walls. This obstructs the diffusion of nutrients and oxygen. This ultimately results in dermatitis and oedema in malnourished skin. The condition is typically visible on the ankle, lower leg, and the back of the foot.[56] In a study conducted by Lalitha and colleagues, two to four leech applications were administered to ten patients presenting clinical symptoms of stasis eczema at one-week intervals, with a minimum of four sessions performed in each case. At the conclusion of the treatment period, the efficacy of the approach was evaluated using the EASI, with a 100% success rate reported (Lalitha et al. 2018).

3.5 Wrinkles

A request was made to experts in the field of cosmetology for their opinion on the use of hirudotherapy. The experts emphasised that they recommend the use of leeches to effectively and non-invasively reduce fine wrinkles, lighten and smooth the skin. They also noted that hirudotherapy has the potential to compete with other methods if the knowledge about the healing properties of hirudotherapy and enzymes from leech saliva is disseminated and the visual effect of treatments is noted (Zabkowska et al. 2022).

4. Advers Effects and Complications

Leeches carry several bacteria such as *Aeromonas spp*. in their bodies to aid digestion (Siddal et al. 2011). It is possible for these bacteria to be transmitted to humans when leeches suck blood. The presence of *Aeromonas spp.* and other gram-negative bacteria within the leech body can result in a range of bacterial infections in humans, including intestinal disorders, pneumonia, septicemia, necrosis, and valve failure. Infection occurs in 2-36% of leech treatments (Abduelkader et al. 2013). Furthermore, the potential for contamination with a range of blood-borne pathogens, including HIV and hepatitis viruses (hepatitis B, hepatitis C, etc.), has been documented and identified as a possible complication (Wollina et al. 2016). Allergic reactions and unstoppable bleeding may also occur after leech application (Pourrahimi et al. 2020). From a cosmetological perspective, one of the most significant adverse effects is the potential for an inverted Y-shaped scar, epidermal cyst, or scar formation to result from the procedure (Zabkowska et al. 2022).

5. Discussion

Immunosuppressive and anti-inflammatory drugs such as corticosteroids, together with antibiotics for secondary infections, are used in the treatment of eczema, acne, and alopecia. Sometimes these therapies only reduce the symptoms of the skin condition or are only partially successful (Amani et al. 2020) . The secretions of medicinal leeches contain a combination of compounds with medicinal properties that have the potential to exert beneficial effects on the skin. These include anti-inflammatory agents, such as antistasin, eglins and tryptase inhibitors; antibacterial agents, such as destabilase; and anticoagulant agents, such as hirudin, saratins and apyrase (Sig et al. 2017). For example, leeches act by inhibiting mast cell proteolytic enzymes with three isoforms of leech-derived tryptase inhibitor (LDTI) in secretion (Campos et al. 2004). Mast cell tryptases are serine proteases present within cell granules. Their release causes inflammatory reactions and is associated with allergic and inflammatory diseases, including anaphylaxis, asthma, and arthritis (Caughey 2016).

Nevertheless, the direct use of leeches or their secretions carries the inherent risks of adverse effects and complications, as previously mentioned. To avoid these complications, it is recommended that physicians applying leeches disinfect the leeches before use by following the appropriate procedures, use the leech once and then dispose of the leech (Ayhan 2020). .Due to the decline in medical leech populations, some species of the genus *Hirudo* are protected by international conventions (Utevsky et al. 2013). In this case, the disposal of leeches after a single use is highly controversial. The use of a live animal as a tool, disgust of the sight at the leech, and fear of blood may also explain the low interest in leech therapy compared to other natural therapies (Zabkowska et al. 2022).

Considering all of this information, research and development efforts in recent years have increased due to the significant potential and application reliability of cosmeceutical products that use biomaterials derived from leech saliva obtained under sterile conditions, rather than the direct use of leeches. These biomaterials offer great potential in terms of bioavailability, biocompatibility, and controlled active ingredient release. Through this study, we

aim to highlight the substantial product potential contained in leech saliva.

6. Conclusion

In conclusion, this review has illuminated the intricate chemical composition and diverse cosmetic applications of leech saliva. The rich array of bioactive compounds presents in leech saliva, including enzymes, peptides, and proteins, underscores its potential as a valuable ingredient in cosmetic/cosmeceutical formulations. These compounds contribute to various beneficial effects such as enhanced skin hydration, anti-inflammatory properties, and accelerated wound healing. A review of the literature reveals the existence of case studies and research on the effectiveness of leeches in dermatological applications. However, there is a paucity of reports on the efficacy of leeches for cosmetic indications, including the treatment of cellulite, wrinkles, and discolouration. Our review highlights that the unique bioactive profile of leech saliva not only supports its traditional use in therapeutic contexts but also offers promising advancements in cosmetic applications. The integration of leech saliva into cosmetic products could revolutionize the field by introducing innovative solutions for skin rejuvenation and repair. Future research should focus on optimizing extraction methods, ensuring product safety, and exploring the full spectrum of leech saliva's bioactive components. By advancing our understanding of its chemical properties and mechanisms of action, we can unlock new possibilities for its use in skincare and beyond.

Acknowledgements

The authors are very thankful to UCSF Chimera team for allowing using programs for molecular visualization.

Authors' contributions: The author contributed to each part of the study.

Conflict of interest disclosure:

The author(s) do not have any potential conflict of interest regarding the research, authorship and/or publication of this article

References

Abdualkader AM, Ghawi AM, Alaama M, Awang M, Merzouk A. 2013. Leech therapeutic applications. Indian J Pharm Sci. 75(2):127-37.

Alaama M, Kucuk O, Bilir B, Merzouk A, Ghawi AM, Yerer MB, Ahmado MA, Abdualkader AM, Helaluddin ABM. 2024. Development of leech extract as a therapeutic agent: A chronological review. Pharmacol Res - Mod Chin Med. 10:100355.

Alharbi MB. 2015. Different Types of Leeches for Medical Use in Surgery, Described by Ibn al Quff (630-685AH). Journal of Research on History of Medicine. 4(3):129-132.

Al-Waili N, Salom K, Al-Ghamdi A. 2011. Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice. Sci World J. 11(1):766-787.

Amani L, Motamed N, Mirabzadeh Ardakani M, Dehghan Shasaltaneh M, Malek M, Shamsa F, Fatemi E, Amin M. 2021. Semi-Solid Product of Medicinal Leech Enhances Wound Healing in Rats. Jundishapur J Nat Pharm Prod. 16(4):e113910.

Amani L, Fadaei F, Shams Ardakani M, Mirabzadeh Ardakani M, Sadati Lamardi SN, Shirbeigi L. 2020. Leech therapy in skin conditions from the viewpoints of Avicenna and modern medicine: Historical review, current applications, and future recommendations. Iran. J. Dermatol. 23(4): 168-175.

Amani L, Fadaei F, Shams Ardakani M, Mirabzadeh Ardakani M, Shirbeigi L. 2020 Treatment of Diabetic Foot Ulcer (DFU) with Pharmaceutical Product using Hirudo orientalis: A Case Report. J Adv Med Biomed Res. 28(129):225-229.

Anoym. 2005. Health Ministry, Republic of Turkey. Cosmetics regulation. Official Gazette, 2005, 23.

Arabacı B. 2023. 'Pearls' of the nineteenth-century: from therapeutic actors to global commodities medicinal leeches in the Ottoman Empire. Med. Hist. 67(2):128–47.

Ayhan H, Mollahaliloğlu S. 2018. Tıbbi Sülük Tedavisi: Hirudoterapi. Ankara Med J. 18(1):141-8

Ayhan H. 2020. Leech disinfection and leech saliva secretion (bioactive compounds). In: Parlakpınar H, editor. Hirudotherapy (medical leech application). Türkiye Klinikleri, Ankara, pp. 22-29.

Baskova IP, Ferner Z, Balkina AS, Kozin SA, Kharitonova OV, Zavalova LL, Zgoda VG. 2008. Steroids, histamine, and serotonin in the medicinal leech salivary gland secretion. Biochem. (Mosc.) Suppl. B: Biomed Chem. 2:215-25.

Bickers DR, Lim HW, Margolis D, Weinstock MA, Goodman C, Faulkner E, Gould C, Gemmen E, Dall T. 2006. The burden of skin diseases: 2004. J Am Acad Dermatol. 55(3):490–500.

Bobrovsky P, Manuvera V, Baskova I, Nemirova S., Medvedev A, Lazarev V. 2021. Recombinant destabilase from Hirudo medicinalis is able to dissolve human blood clots in vitro. Curr Issues Mol Biol. 43(3): 2068-2081.

Brito AMM, Oliveira V, Icimoto MY, Nantes-Cardoso IL. 2021. Collagenase Activity of Bromelain Immobilized at Gold Nanoparticle Interfaces for Therapeutic Applications. Pharmaceutics. 13(8):1143.

Campos ITN, Silva MM, Azzolini SS, Souza AF, Sampaio CAM, Fritz H, Tanaka AS. 2004. Evaluation of phage display system and leech-derived tryptase inhibitor as a tool for understanding the serine proteinase specificities. Arch Biochem Biophys. 425(1):87–94.

Caughey GH. 2016. Mast cell proteases as pharmacological targets. Eur J Pharmacol. 778:44-55.

Folkers PJ, Clore GM, Driscoll PC, Dodt J, Koehler S, Gronenborn AM. 1989. Solution structure of recombinant hirudin and the Lys-47. fwdarw. Glu mutants: a nuclear magnetic resonance and hybrid distance geometry-dynamical simulated annealing study. Biochem. 28(6): 2601-2617.

Habeshian KA, Cohen BA. 2020. Current Issues in the Treatment of Acne Vulgaris. Pediatrics. 145(2):225–230.

Haycraft JB. 1883. Iv. on the action of a secretion obtained from the medicinal leech on the coagulation of the blood. Proc. R. Soc. Lond. 36(228–231):478–87.

Jayant DS, Chandurkar SV. 2018. Role of Jalaukavacharan and Mahamanjisthadi kwath in the management of mukhadushika wsr to Acne Vulgaris-a case study. Int. J. Res.Granthaalayah. 6:30-31.

Jung H. 2020. Hyaluronidase: An overview of its properties, applications, and side effects. Arch Plast Surg. 47(04):297-300.

Junren C, Xiaofang X, Huiqiong Z, Gangmin L, Yanpeng Y, Xiaoyu C, Yuqing G, Yanan L, Yue Z, Fu P, Cheng P. 2021. Pharmacological activities and mechanisms of hirudin and its derivatives - A Review. Front Pharmacol. 12:660757.

Kumari P. 2023. Effect Of Jaloka (Leech Therapy) In Acne Vulgaris. World J Pharm Res. 12(9):158-1584.

Lalitha S, Anavarathan V, Mahalakshmi V, Periyasami D, Muthukumar NJ, Banumathi V. 2018. Effectiveness of Attai Vidal (leech therapy) on Naala Vibatha Karappan (stasis eczema). World J Pharm Sci. 6(12):129-34.

Lemke S, Vilcinskas A. 2020. European medicinal leeches—new roles in modern medicine. Biomedicines. 8(5):99.

Lent CM, Zundel D, Freedman E, Groome JR. 1991. Serotonin in the leech central nervous system: anatomical correlates and behavioral effects. J Comp Physiol A. 168(2):191-200.

Lieberman P. 2011. The basics of histamine biology. Ann Allergy Asthma Immunol. 106(2):S2-S5.

Liu Z, Zhao F, Huang Z, He B, Liu K, Shi F. Zhao Z, Lin G. 2024. A chromosome-level genome assembly of the non-hematophagous leech Whitmania pigra (Whitman 1884): identification and expression analysis of antithrombotic genes. Genes. 15(2):164.

Marin E, Kornilov DA, Bukhdruker SS, Aleksenko VA, Manuvera VA, Zinovev EV. Kovalev KV, Shevtsov MB, Talyzina AA, Bobrovsky PA, Kuzmichev PK, Mishin AV, Gushchin IY, Lazarev VN, Borshchevskiy VI. 2023. Structural insights into thrombolytic activity of destabilase from medicinal leech. Sci Rep. 13(1):6641.

Milam, EC, Rieder EA. 2021. An approach to cosmeceuticals. In Essential Psychiatry for the Aesthetic Practitioner; Wiley Online Library: Hoboken, NJ, USA. pp. 42–48.

Mohammad-Zadeh LF, Moses L, Gwaltney-Brant SM. 2008. Serotonin: A Review. J Vet Pharmacol The. 31(3):187–99.

Moriguchi T, Takai J. 2020. Histamine and histidine decarboxylase: Immunomodulatory functions and regulatory mechanisms. Genes Cells. 25(7):443-449.

Peng, Liu, Xinyuan Pan, and Guoqian Yin. 2015. Natural Hirudin Increases Rat Flap Viability by Anti-Inflammation via PARs/p38/NF-κB Pathway. Biomed Res Int. 1:597264.

Pourrahimi M, Abdi M, Ghods R. 2020. Complications of leech therapy. Avicenna J Phytomed. 10(3):222-234.

Pratap Shankar K, Rao Sd, Umar S, Gopalakrishnaiah V. 2014. A clinical trial for evaluation of leech application in the management of Vicarcikā (eczema). Ancient Science of Life. 33(4):236.

Pratt CH, King LE, Messenger AG, Christiano AM, Sundberg JP. 2017. Alopecia areata. Nat Rev Dis Primers. 3(1):1-17.

Rados. 2004. Beyond bloodletting: FDA gives leeches a medical makeover, FDA Consum. 38(5):9.

Ren K, Gong H, Huang J, Liu Y, Dong Q, He K. Tian L, Zhang F, Yu A, Wu C. 2021. Thrombolytic and anticoagulant effects of a recombinant staphylokinase-hirudin fusion protein. Thromb Res. 208:26-34.

Ring J. 1991. Angewandte Allergologie MMV. Medizin, München.

Ring J. 2015. Eczema–in the focus between dermatology and allergology. Allergo J Int. 24:129-142.

Sandilands EA, Crowe J, Cuthbert H, Jenkins PJ, Johnston NR, Eddleston M, Bateman DN, Webb DJ. 2013. Histamine-induced vasodilatation in the human forearm vasculature. Br J Clin Pharmacol. 76(5):699–707.

Sawyer RT. 1986. Leech Biology and behaviour. Clarendan, Oxford.

Sawyer RT. 2013. History of the Leech Trade in Ireland, 1750–1915: Microcosm of a Global Commodity. Med. Hist. 57(3):420–41.

Schnebli HP, Liersch MH. 2021. Properties and therapeutic potential of eglin c. Elastin and Elastases, Volume II. CRC Press. pp.137-143.

Siddall ME, Min GS, Fontanella FM, Phillips AJ, Watson SC. 2011. Bacterial symbiont and salivary peptide evolution in the context of leech phylogeny. Parasitology. 138(13):1815-27.

Siddiqui SA, Mohammad A, Shoaib M, Sultana A. 2024. Harnessing The Healing Power Of Leeches In Eczema Care: A Case Report. Eur J Pharm Med Res. 11(8):384-388

Sig AK, Guney M, Uskudar Guclu A, Ozmen E. 2017. Medicinal leech therapy—an overall perspective. Integr. Med. Res. 6(4):337–43.

Söllner C, Mentele R, Eckerskorn C, Fritz H, Sommerhoff C.P. 1994. Isolation and characterization of hirustasin, an antistasin-type serine-proteinase inhibitor from the medical leech Hirudo medicinalis. Eur J Biochem. 219(3):937-943.

Sundaresan S, Migden MR, Silapunt S. 2017. Stasis Dermatitis: Pathophysiology, Evaluation, and Management. Am J Clin Dermatol. 18(3):383-390.

Suter S, Chevallier I. 1998. The effect of Eglin C on the function of human neutrophils in vitro. Biol Chem. 369(2):573–8.

Taylor M, Gonzalez M, Porter R. 2011. Pathways to inflammation: acne pathophysiology. Eur J Dermatol. 21(3):323-33.

Ünal K, Erol ME, Ayhan H. 2023. Literature review on the effectiveness of medicinal leech therapy in the wound healing. Ank. Med. J. 23(1):151–64.

Utevsky S, Zagmajster M, Trontelj P. 2014. Hirudo medicinalis Linnaeus, 1758. The IUCN Red List of Threatened Species 2014: https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T10190A21415816.en

Waghmare G. 2019. Medicinal Leech Therapy in Alopecia areata patchy (Khalitya)-A Case Report. International Journal of AYUSH Case Reports. 23(3): 206-211.

Waycaster C, Carter MJ, Gilligan AM, Mearns ES, Fife CE, Milne CT. 2018. Comparative cost and clinical effectiveness of clostridial collagenase ointment for chronic dermal ulcers. J Comp Eff Res . 7(2):149–65.

Weber GC, Buhren BA, Schrumpf H, Wohlrab J, Gerber PA. 2019. Clinical applications of hyaluronidase. Adv Exp Med Biol. 255–77.

Whitaker IS, Izadi D, Oliver DW, Monteath G, Butler PE. 2004. Hirudo medicinalis and the plastic surgeon. Br J Plast Surg. 57(4):348–53.

Wollina U, Heinig B, Nowak A. 2016. Medical Leech Therapy (Hirudotherapy). Our Dermatol. Online. 7:91–96

Xu H, Timares L, Elmets CA. 2019. Host defenses in Skin. Clin. Immunol. 1: 273-283.

Yadav CR, Guguloth RA. 2017. Case Study of Leech Therapy (Jalaukavacharana) in Khalitya W.S.R. Alopecia. Int J Pharmacogn ChineseMed. 1(3): 000115.

Zabkowska E, Piotrowska A. 2019. Hirudotherapy in selected dermatological applications. Aesthetic Cosm. Med. 8:779–786.

Ząbkowska E, Czerwińska-Ledwig O, Bartnicka M, Piotrowska A. 2022. Case reports and experts opinions about current use of leech therapy in dermatology and cosmetology. Cosmetics. 9(6): 137.

Zavalova LL, Baskova IP, Lukyanov SA, Sass AV, Snezhkov EV, Akopov SB, Artamonova II, Archipova VS, Nesmeyanov VA, Kozlov DG, Benevolensky SV, Kiseleva VI, Poverenny AM, Sverdlov ED. 2000. Destabilase from the medicinal leech is a representative of a novel family of lysozymes. Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol. 1478(1):69–77.

Zhao F, Huang Z, He B, Liu K, Li J, Liu Z, Lin G. 2024. Comparative genomics of two Asian medicinal leeches Hirudo nipponia and Hirudo tianjinensis: With emphasis on antithrombotic genes and their corresponding proteins. Int J Biol Macromol. 270: 132278.