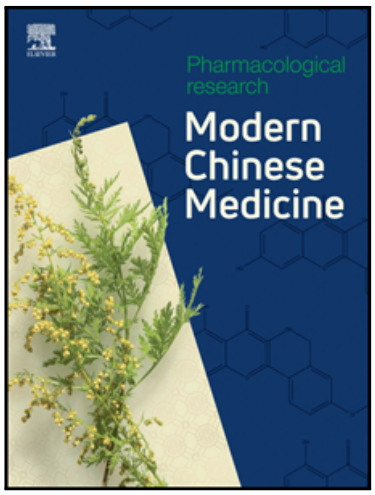

Development of Leech Extract as a Therapeutic Agent: A Chronological Review

MOHAMED ALAAMA , OMER KUCUK , BIRDAL BILIR , AHMED MERZOUK , ABBAS MOHAMMAD GHAWI , MUKERREM BETÜL YERER , MOHAMED ALAA AHMADO , ABDUALRAHMAN MOHAMMED ABDUALKADER , A.B.M. HELALUDDIN

PII: S2667-1425(23)00141-0
DOI: https://doi.org/10.1016/j.prmcm.2023.100355
Reference: PRMCM 100355

To appear in: *Pharmacological Research - Modern Chinese Medicine*

Received date: 25 September 2023
Revised date: 29 December 2023
Accepted date: 31 December 2023

Please cite this article as: MOHAMED ALAAMA , OMER KUCUK , BIRDAL BILIR , AHMED MERZOUK , ABBAS MOHAMMAD GHAWI , MUKERREM BETÜL YERER , MOHAMED ALAA AHMADO , ABDUALRAHMAN MOHAMMED ABDUALKADER , A.B.M. HELALUDDIN , Development of Leech Extract as a Therapeutic Agent: A Chronological Review, *Pharmacological Research - Modern Chinese Medicine* (2024), doi: https://doi.org/10.1016/j.prmcm.2023.100355

Title Page

Development of Leech Extract as a Therapeutic Agent: A Chronological Review

MOHAMED ALAAMA [a,b *], OMER KUCUK [c], BIRDAL BILIR [c], AHMED MERZOUK [d], ABBAS MOHAMMAD GHAWI [e], MUKERREM BETÜL YERER [f,b], MOHAMED ALAA AHMADO [g,b], ABDUALRAHMAN MOHAMMED ABDUALKADER [h], A. B. M. HELALUDDIN [i.]

[a] Biopep Medikal İlaç Sanayi ve Ticaret, Kayseri, Turkey.

[b] Drug Application and Research Center (ERFARMA), Erciyes University, Kayseri, Turkey.

[c] Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, Georgia, USA

[d] Biopep Solutions Inc., Vancouver, BC Canada,

[e] Department of Basic Medical Science, Kulliyyah of Pharmacy, International Islamic University Malaysia (IIUM), Kuantan, Malaysia.

[f] Department of Pharmacology, Faculty of Pharmacy, Erciyes University, Kayseri, Türkiye.

[g] Department of Medical Biology, School of Medicine, Erciyes University, Kayseri, Türkiye.

[h] Faculté de Pharmacie, Université de Montréal, Montréal, Québec, Canada,

[i] Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, International Islamic University Malaysia (IIUM), Kuantan, Malaysia.

[*] *Corresponding Authors: hass83pharm@gmail.com, +905525568757,*

https://orcid.org/0000-0002-9320-0583

Development of Leech Extract as a Therapeutic Agent: A Chronological Review

Abstract

Introduction

Leech extract contains many identified bioactive substances which have a variety of biological effects. Leech extract was discovered in the late 19th century and since then many pharmaceutical products have been produced using leech extract for different ailments, but many have been withdrawn. Ongoing studies focus on health authority compliant pharmaceuticals to be used as modern medicine.

Methodology

The online databases including Google Scholar, Scopus, PubMed, and Web of Science, were searched using different keywords: Leech, Leech extract, leech salivary gland extract and leech saliva extract. The purpose of this review was to discuss the development of leech extract as a therapeutic agent, including the evolution of extraction techniques, and the successful manufacturing of leech extract-based pharmaceuticals.

Results

Leech extract was successfully developed as a therapeutic agent. Some of the developed leech extract-based pharmaceutics were withdrawn and some are still in the market. The extraction methods played a vital role in the quality and efficacy of leech extract-based pharmaceutics and ultimately on their sustainability in the market. Also, the full characterization of leech extract components is a key factor in the development of leech extract as a therapeutic agent.

Discussion

This review provides a comprehensive historical perspective on the development of leech extract therapy, including its various stages of development and the key scientific and medical advances that have led to its current state and discusses potential future applications.

Conclusion

Leech extract is an invaluable source of bioactive substances that can be utilized for the treatment of mild and life-threatening medical disorders. This review will encourage other scientists to continue their research on leech extract, especially in the areas of formulation and marketing.

Key words: leech, leech extract, leech saliva extract, Hirudotherapy.

1. Introduction

Leeches are segmented worms belonging to the Phylum Annelida, with about 700-1000 species of leeches all over the world [1]. Leeches are found all over the earth, except terrestrial Antarctica, and their habitats include freshwater, estuarine, and marine aquatic ecosystems, as well as moist terrestrial ecosystems [2].

Some leeches are carnivorous; while some are sanguivorous and they feed on the blood of other animals including amphibians, birds, reptiles, fish and mammals. Blood feeding leeches, like other blood feeding animals, have active materials in their saliva to facilitate the feeding process. Because of these materials and their therapeutic effects, leeches have been used for medical purposes for centuries, mainly for bloodletting which was considered the key factor for its usefulness in medical treatment. Many leech species are considered medicinal and used for medical purposes such as *Hirudo medicinalis*, *Hirudo verbena* and *Hirudo orientalis* [3], figure 1:

The first documented evidence of the use of leeches in medicine goes back to Greek physicians who described leech therapy for the treatment of many diseases. They believed that most diseases could be treated by bloodletting, thus they have used many methods to achieve that, including leeching. It was reported that Greek physicians had used leeches for the treatment of arthritis pain, gout, ocular paralysis, acute liver disease, abdominal diseases associated with dyspepsia as well as for the treatment of satyriasis [4]. With the rise of Arabic medicine in the middle age centuries, leech therapy was well described and documented. Arab physicians used leeches for the treatment of skin diseases, alopecia, joint diseases, after surgery and for pain relief. The medicinal leeches were well described and differentiated from the non-medicinal leeches by Avicenna and Ibn Al-Quf. In Europe, during the 17th and 18th centuries, leech therapy was used for almost all diseases [5].

In the early 20th century, the use of leech for medicinal purposes has declined due to the discovery of chemical drugs and the development of specific theoretical bases of disease and treatment. However, in the late 20th century, leech therapy witnessed resurgence in popularity, particularly in plastic surgery and microsurgery. The United States Federal Drud Administration

(FDA) approved the use of the medicinal leech, *Hirudo medicinalis*, in plastic surgery in 2004 as a medical device, which led to an increased use of leech in medical practice [6].

Leech therapy, like any other therapy, may be associated with adverse effects and complications. Patient non-compliance, bleeding, the need for blood transfusion, local or systemic infection, allergic reaction and inflammation are the most reported complications of live leech therapy [7].

The theory of leech therapy underwent a significant change with the discovery of anticoagulants in the leech head extract by Dr. John H. Haycraft in the late 19th century. Haycraft's discovery challenged the traditional belief that the benefit of leech therapy was exclusively due to bloodletting. Haycraft suggested that the real therapeutic benefit was due to the substances injected into the patient's body by the leech [8]. This discovery led to a deeper understanding of the mechanism of action of leech therapy and paved the way for further research into therapeutic benefits of leech extract and its active compounds. Haycraft's contribution was an important turning point in the history of leech therapy and had a lasting impact on the field [9]. The discovery of anticoagulants in leech extract opened the door for a new era of leech therapy and encouraged researchers and physicians to explore the use of leech extract as a therapeutic agent. This helped to overcome some of the limitations and potential risks associated with live leech therapy, such as risk of infection, bleeding, and allergic reactions. Over the years, numerous studies have been conducted to identify the active compounds in leech extract and to develop safe and effective medicinal products based on leech extract [10-12].

Many terms were used to describe this extract such as leech extract [13], leech head extract [14], leech salivary glands extract [11], and leech saliva extract [15]. The use of leech extract as a therapeutic agent has a long history and has gone through several eras of development. The first era, which took place between 1884 and 1930, witnessed the discovery of leech extract and the manufacturing and marketing of commercial leech extract injections, which were primarily used to treat thrombosis and other circulatory disorders. However, the popularity of leech extract declined with the advent of modern pharmaceutical products, and it eventually disappeared from medical practice.

The second era, which lasted from 1930 to 1980, was characterized by a total lack of research and development in the field of leech extract therapy. During this period, leech extract was completely forgotten as a potential therapeutic agent.

The third era began in the 1980s with the reintroduction of leech extract, and specifically leech saliva extract, as a potential therapeutic agent. This period has been marked by a renewed interest in the use of leech extract for medical purposes, including the treatment of skin grafts, venous insufficiency, and other conditions.

In this review, the development of leech extract as a therapeutic agent, the evolution of extraction techniques, and the successfully manufactured leech extract-based pharmaceuticals are discussed.

2. Methodology

A comprehensive literature search was conducted using the keywords ”Leech extract”, ”Leech saliva extract”, ”Leech salivary gland extract” in the subject area of pharmacology and pharmaceutical technology. The data were collected from scientific publications and databases including Scopus, PubMed, Web of Sciences, and Google scholar. The English, German, and French literature between 1884 and 2022 were included in the search. The main criteria for including any article in this study was that it contained information about leech extract preparation, composition, development or formulation of it as a therapeutic agent.

A total of 316 articles were reviewed and only 106 articles were included in this review according to the main criteria.

3. Results

Leech extract had been discovered in 1883 by John Haycraft, and it was studied thoroughly in terms of pharmacological effects and chemical characteristics. In the meantime, many experiments were performed to prepare a leech extract-based pharmaceutics to replace the Hirudotherapy and utilize its therapeutic effect without exposing the patients to life leech therapy complications. The results showed that the journey of developing leech extract as a therapeutic agent has witnessed three major steps as follow:

3.1 The Discovery of "Leech Extract"

The old use of leeching and leech therapy depended on the theory of four humours when bloodletting was believed to help restoring the balance between body fluids which was ruined by the disease [16]. Haycraft had noticed that, unlike normal wounds, the bleeding from the wounds induced by leeches' bites continued for at least one hour after leech detached without coagulation. He reported that surgeons knew that the blood remained in a liquid form inside leech's body for a long time. His theory was that the leeches secrete ferment-containing juice which prevents coagulation of blood within its body and enough remaining around the edges of the wound to prevent, for some time, the outflowing blood from clotting. To prove his theory, he incubated rabbit blood with either 6% saline or a crude extract of leech intestines or leech heads in saline. The leech head extract prevented the blood from coagulation for 24 hours. In this way, he demonstrated that the leech head contains a substance that inhibited blood coagulation. For *in-vivo* studies, Haycraft developed an extraction method by soaking the anterior part of leeches in absolute alcohol for three days, then evaporating the alcohol off, grinding the leech with broken glass, extracting with water and filtering. Dogs and rabbits were injected with this newly obtained leech extract and the withdrawn blood showed prolonged coagulation time and the leech extract was proven to be eliminated by the kidney [8]. In the following years, researchers conducted many studies to understand the nature of leech extract and its action on blood [17].

To obtain better yield and higher efficacy, the extraction method was modified by Bock who cut the leeches' heads, rubbed them with dry sand, mixed them with sodium chloride 0.7 % and shook the mixture for 2 hours. Then centrifuged the mixture, collected the supernatant and labeled it as non-diluted leech extract. The remaining billets in centrifuge tubes were washed with sodium chloride and centrifuged again to obtain a diluted leech extract [18].

Later, Franz reported a more developed method for the extraction of leech extract. While conducting his study, he described for the first time the leech salivary glands and he believed that they were the source of the bioactive materials. He had also discovered and proved that the active material was in the head of a leech. The extraction method depended on Prof Jakobi's studies who named the full extract "Hirudin" for the first time [19]. The extraction

procedure was applied on the full leeches' heads or dissected leeches' heads where only the pharyngeal rings are taken and used for the extraction. The heads were rubbed with sand for 20 min then the thymolized saline was added and extracted for 1 hour at 37^0-38^0 C with stirring and then centrifuged to separate the non-dissolved parts. The extract was heated to 60^0 C for 2 hours and then centrifuged. After that it was exposed to chloroform vapors for 3 days, centrifuged again and finally dried in a desiccator over sulfuric acid. The yield was 8 -9 mg extract per head which corresponded to 12.5%. The study was extended to discover the time needed for leeches to recover their active materials after feeding and the time needed was 14 days. The study also reported a suitable method to stabilize the leech extract by drying the extract and keeping it away from moisture and air or by keeping it in thymol-sterile glass tubes. This product was then marketed as a medicine for the first time by (Sachsse and Co., Leipzig, Germany) [20]. There was likely growing interest in the therapeutic potential of leech extract during this period and companies were actively working to commercialize and market leech extract-based medicines. Overall, the development of this extraction method and the subsequent commercialization of leech extract-based medicines likely represented a significant milestone in the history of leech extract therapy.

The studies on the use of leech extract in the clinic had accelerated especially as an anticoagulant for blood transfusions. Later, as more was learned about the biological properties of leech extract, researchers began to explore its potential use in other areas, such as the treatment of eclampsia and as an anticancer agent. On the other hand, many studies were conducted to prove the safety or toxicity of leech extract.

The mechanism of anticoagulation activity of leech saliva extract was also studied and the antithrombin and antiplatelet aggregation effects of leech extract were described by [14]. In 1914 leech extract was used for blood transfusion in the dose of 7 mg/500 ml of blood and showed no toxic reaction except for some chills and febrile reactions [21]. However, other research showed toxicity of some hirudin preparations which was explained by the lack of hirudin standardization and the impossibility of sterilization of hirudin by heating which destroyed the anticoagulation effect of hirudin [22]. Leech extract was also used for the treatment of eclampsia, a condition characterized with seizures in pregnant women who

develop high blood pressure and damage to organs such as the liver and kidneys. The use of leech extract in the treatment of eclampsia was based on the belief that leech saliva could help to regulate blood flow and reduce the risk of blood clots, which could be a complication of preeclampsia [23].

The first report of the use of leech extract as anticancer agent goes back to 1913 [24]. While testing different materials on carcinoma in mice, they reported that leech extract not only inhibited the growth of tumor but also caused some regression of tumor. However, subsequent experiments showed that cancer cells eventually became resistant to the effects of hirudin, and the potential for long-term effectiveness as an anticancer agent was limited [25]. Additionally, there were concerns about the toxicity of hirudin, as seen in experiments with Guinea pigs [26]. Haas in 1924 reported the use of leech extract for hemodialysis and applied it on humans after a series of experiments on animals. The first experiment on human subjects aimed to confirm the non-toxicity of hirudin and the safety of the procedure. The hemodialysis lasted for 15 min with satisfactory results [27]. At the same time, Necheles used another commercial *hirudin* prepared by Passek and Wolf Hamburg for the dialysis in animals and he claimed that this preparation is less toxic [28]. Yet, the following experiments were not encouraging because of the toxicity of different hirudin preparation and at the same time the heparin was discovered and replaced hirudin in hemodialysis [29].

Along with the development of leech extract as a therapeutic agent, many scientists and physicians suggested the use of leech bite to deliver the active materials to the patients rather than using impure and non-standardized extract thereof. Termier was the first one to suggest and implement this method and called it “hirudinization of blood”. The method was applied successfully for the treatment of pulmonary embolism [30]. This method resembles the traditional hirudotherapy (leech therapy) in applying live leeches but differs in the mechanism (hirudinization of blood) which was completely understood at that time.

Unfortunately, this revival of medical leeches as therapeutic agents stopped completely when their supply from natural sources in Southeastern Europe was interrupted during World War II. Moreover, new powerful but much less expensive natural (heparin) and synthetic oral (coumarin) anticoagulants became available and soon proved to be useful in clinical settings.

Thus, the chance of developing hirudin as a therapeutic agent had declined, just because of the natural limitations in supply and the existence of competitor drugs which did not have these limitations [31].

To summarize the first era of developing leech extract as a therapeutic agent, it is obvious that researchers and physicians succeeded in producing an injectable leech extract which was used clinically. Commercial leech extract (hirudin) was produced and marketed by Sachsse and Co. Leipzig Germany, and Passek and Wolff of Hamburg. Regardless the reports about the toxicity and incapability of standardization of some hirudin preparations at that time, many papers stated that leech extract was used clinically for the treatment of some disease and ultimately injected into human patients. The reported toxicity of hirudin could have been due to allergy and shock which could also be partly explained by the presence of endotoxin in leech extract. The lack of standardiz ability can be overcome now by the utilization of suitable analytical methods for leech extract. The adverse event of bleeding could now be addressed by adjusting the dose and blood transfusion.

3.2 The Discovery of Leech Extract Constituents as Pure Compounds

The second era showed a decline in the use of leech extract as a therapeutic agent while the traditional use of live leeches or leech therapy continued without any support or approval from international health bodies. The studies on leech extract were limited to the isolation and purification of pure compounds and testing their activities on different diseases. The first isolated pure compound was the leech hyaluronidase [32], followed by pure compound “hirudin” [33], bdellins [34], eglins [35], decorsin [36], and many other active compounds. The biological activities of these isolated compounds were studied and they can be summarized in Table 1.

Recombinant hirudin was produced [73], which opened the door to produce injectable drug such as lepirudin, desirudin, and bivalirudin. Lepirudin was marketed under the brand name Refludan (Bayer) as anticoagulant in patients with heparin-induced thrombocytopenia (HIT) and thromboembolic disease to prevent further thromboembolic complication for the patients who are not able to use heparin [74]. Desirudin was approved by FDA under the brand name of

Iprivask as a direct thrombin inhibitor. Iprivask is indicated for the prophylaxis of deep vein thrombosis, which may lead to pulmonary embolism and in patients undergoing elective hip replacement surgery [75]. Bivalirudin was approved by FDA under brand name Angiomax is indicated for use as an anticoagulant in patients with unstable angina undergoing percutaneous transluminal coronary angioplasty (PTCA) [76].

3.3 The Return of Natural Whole Leech Extract as a Better Therapeutic Agent

Although leech therapy was not so popular during the first half of the 20th century, it started to have a great comeback beginning with the 1960s when physicians noticed its remarkable benefits in relieving venous congestion of skin transplants [77]. The use of leech therapy in plastic reconstructive surgery continued to gain popularity and was further optimized during the 1990s. This led to the U.S. Food and Drug Administration (FDA) recognizing and approving the use of medicinal leeches as a medical device for the treatment of venous congestion in graft tissue in plastic surgery [6]. Alongside with the comeback of leech therapy, leech salivary gland extract (SGE) had also gained more attention after the discovery of its unique composition and the possibility of employing the leech SGE for the treatment of complicated diseases such as lung tumor [11, 78]. This development helped leech extract to revive and find its way back to modern pharmacies as commercial products. Since 1983, many studies reported promising and successful leech extract-based pharmaceutical formulations especially in the Chinese market. The extraction method has also been modified and optimized to the level of obtaining the full leech saliva components without killing the leeches. Therefore, the following text will discuss the “leech extract in Chinese medicine”, and the “leech saliva extract” as different products according to their extraction methods and their application and their commercial products.

3.3.1 Leech extract in Chinese medicine

Leech therapy has been used in Chinese traditional medicine for thousands of years and the leech (Shuizhi) has been listed in Chinese pharmacopeia and identified as the full body dried powder of *Whitmania pigra*, *Hirudo nipponica* and *Whitmania acranulata* leech species. Leech extract can be found as oral, topical, or injected medicine in Chinese market.

3.3.1.1 Oral leech extract in Chinese traditional medicine

Oral leech extract is prepared as tablet or capsules, containing dried leech powder only or combined with other herbal or animal extracts. Many approved medicines can be found in Chinese pharmacopeia and Chinese market such as:

1. Zhixiong (capsules) has been used clinically for the treatment of acute and chronic cerebrovascular diseases due to its contents' abilities to improve the blood circulation, decrease blood coagulation, activate meridians and relieve stasi [79].
2. Shenyuandan (capsules) has been used for the treatment of coronary heart disease and angina pectoris. It protects against myocardial ischemia/reperfusion injury, ameliorates oxygen-free radical injury in ischemic myocardium, and improves the antioxidant ability of myocardial tissue. It attenuates atherosclerosis by promoting autophagy, probably through regulating genomic DNA methylation and Atg13 promoter demethylation [80]. Clinically, Shenyuandan's efficacy and safety in reducing the incidence of peri-procedural myocardial injury among patients with unstable angina who underwent elective percutaneous coronary intervention was tested using a prospective, randomized, double-blinded, placebo-controlled trial. The results showed a significant decrease in the number of peri-procedural myocardial injury in Shenyuandan group compared to placebo group [81].
3. Naoxintong (capsules) has been reported to have multiple protective effects on cardiovascular diseases including coronary artery disease, coronary microembolization, myocardial infarction, acute coronary syndrome, ischemic stroke, and ischemia-reperfusion injury [82].
4. Tongxinluo (capsules) has been used for coronary heart disease, unstable angina pectoris, and acute stroke in addition to its ability to improve serum lipid levels [83].
5. Dahuang Zhechong (pills) has been reported to improve pulmonary function, quality of life, and exercise capacity of silicosis patients [84], reduce serum biomarkers of liver fibrosis in patients with chronic hepatitis B [85] and suppress colorectal cancer liver metastasis via ameliorating exosomal CCL2 primed pre-metastatic niche [86].
6. Maixuekang (capsules) consists of leech *Whitmania pigra/Hirudo nipponica* extract only and has been prescribed for acute coronary syndrome [87].

3.3.1.2 Parenteral leech extract in Chinese medicine

The Chinese injectable leech extract Shuxuetong was prepared from the whole leech body and earthworm using different techniques. The leeches (*Hirudo nipponica Whitman*) and earthworms (*Pheretima* aspergillum) were cleaned and sterilized by soaking them in normal saline repeatedly. Then they were grinded and homogenized, and then exposed to freeze thaw cycles with a freezing temperature of -15 C. The mixture was centrifuged, and the supernatant was separated. The supernatant was ultrafiltered using a cut off membrane of 6000 Dalton and the filtrate was obtained, and the Shuxuetong was ready for medical use. Shuxuetong was prepared by mixing leech extract and earthworm extract in the percentage of 33%-84% and 16%-67%, respectively, as injectable solution 1-20ml, infusion 50-200ml/ bottle, or freeze-dried powder for injection [88].

Shuxuetong injection has been included in the Chinese Pharmacopoeia and had total sales of over $93 million in 2017 in China. Based on the source of the medicine, the main active ingredients were believed to be hirudin and lumbrokinase, so it is extensively prescribed as anticoagulant and fibrinolysis in acute cerebral infarction, acute phase of ischemic stroke and other coagulation related diseases [89]. Later, a study showed that hirudin is not detected in Shuxuetong, while the main ingredients are free amino acids, peptides, monosaccharides, polysaccharides, nucleic acids and other components. The extraction method could be the reason behind the absence of hirudin which is broken down into smaller polypeptides during the preparation of Shuxuetong [90]. Regardless of the presence or the absence of hirudin, Shuxuetong showed high anticoagulation effect making it a suitable candidate in the treatment of cerebral infarction. Animal studies have shown that Shuxuetong can effectively improve spinal cord injury due to its active materials ability to enhance spinal cord blood flow [91]. In addition to improving blood flow to the injured area, Shuxuetong facilitates angiogenesis during wound healing following traumatic brain injury in rats. The growth of new capillary blood vessels formation was enhanced after the treatment with Shuxuetong which promoted the endothelial cell proliferation via the vascular endothelial growth factor/vascular endothelial growth factor receptor-2 pathway [88].

Clinically, Shuxuetong injection was used in combination with other chemical drugs for the treatment of ischemic stroke [92].

3.3.2 Leech saliva extract

In leech farms, leeches are fed on blood using many techniques such as feeding on living frogs and fish, or on animals' blood through sieve, or artificial membranes. To study the effect of some chemicals found in blood on the feeding response of leeches, Galun and Kindler developed a method to feed leeches through artificial membrane consisting of silastic membrane [93]. This method was further developed and used for the extraction of diluted leech saliva extract [10]. Leeches were fed on a pre-heated phagostimulatory solution consisting of NaCl 0.015M and Arginine 0.001 M through a sausage membrane. While feeding, leeches secrete the saliva into the solution. Once the feeding is completed, the diluted saliva is obtained by squeezing leeches from posterior sucker towards the anterior sucker. This method was further optimized to ease the extraction process as leeches show some resistance against squeezing. Leeches can be stressed to regurgitate the saliva by adding salt or wood ash [94], ethanol [95] and ice [96]. The LSE extracted using this method showed remarkable biological activity such as anticoagulant [10], antidiabetic [97], antibacterial [98] and anticancer [99].

Many attempts have been reported to prepare a pharmaceutical formulation using whole leech saliva extract (LSE) which is extracted using this method [95]. Researchers claim that LSE is the suitable candidate as it contains the active materials only without any leech body components which were present in leech body extract and that could reduce the possibility of allergy and shock which may result from whole leech body extract injection [10]. LSE can be sterilized, depyrogenated, standardized and formulated as topical or parenteral drug. Up to date, there are no approved commercial products of LSE, but the literature contains many proven active products which could be marketed soon.

LSE was prepared as a topical liposomal gel for the treatment of knee osteoarthritis and the effect was assessed clinically [100]. A clinical trial was designed to evaluate the effect of adding LSE liposomal gel treatment to the physiotherapy of patients with knee osteoarthritis. The

results showed that the group receiving the combined treatment had significant pain reduction and quality of life improvement compared with the group receiving physiotherapy only [100].

Another LSE product produced using Asian leech (*Hirudinaria manillensis*) and prepared for the treatment of different types of cancers, especially prostate cancer. LSE effects were evaluated *in vitro* and *in vivo* models, but no clinical study has been performed yet [12]. LSE IC50 was determined *in vitro* in five prostate cancer cell lines using MTS cell viability assay. *In vivo* efficacy of LSE was determined in Lymph Node Carcinoma of the Prostate (LNCaP) and 22RV-1 in nude mice xenograft models. LSE was injected subcutaneously at a dose of 5mg/kg once a week for 4 weeks and docetaxel was used as positive control. The results showed that LSE has significantly reduced the tumor size and improved the PSA and other biological markers. LSE anticancer effect was similar to docetaxel effect with no side effects [101]. The studies revealed that LSE inhibited Proteinase Activated Receptor 1 (PAR-1) which is responsible for hormones like cellular signals in different cancer cells including those of the breast and prostate. Moreover, in LNCaP cells the Androgen Receptor (AR) activation and the Prostate Specific Antigen (PSA) expression were significantly reduced after treatment with LSE. Inhibition of phosphoinositide-3-kinase (PI3K) and Phospholipase C, Gamma 2 (PLCG2) were also observed. It has been found that LSE modulates a multitude of pathways which makes it a very promising candidate for treatment of cancer with an important advantage of low toxicity and reduced potential for development of drug resistance [102].

4. Discussion

Leech extract has been found to be effective for the treatment of many diseases due to its unique composition. Its extraction method has gone through huge development starting with the whole-body extract through the extraction of leeches' heads and salivary gland to the extraction of leech saliva without killing the leeches. Many leeches extract-based pharmaceuticals have been produced and marketed (Table 2)

The success of leech extract-based pharmaceuticals and the development of new extraction, filtration and formulation techniques along with the proven benefits of leech therapy enhance the scientific community's confidence in the possibility of obtaining a leech extract-based

product which is in compliance with modern health authorities' rules and regulations. However, apart from medicines marketed in China, there is no FDA approved leech extract-based pharmaceuticals yet. The published data shows promising products with enhanced pharmacological effects and wide safety margin comparing to existed chemical drugs.

A study was conducted using propensity score method to analyze the effect of Maixuekang capsule on the treatment outcome of coronary heart disease. The study team examined the electronic medical record database of 22 large-scale tertiary hospitals in China and reported the patients with coronary heart disease taking or not taking Maixuekang capsule in combination with other chemical drugs. After matching, there were 2464 cases in the Maixuekang and non-Maxiuekang groups. The results showed that Maixuekang group had better recovery and improvement compared to non-Maixuekang group [87].

Clinically, the effects of Shuxuetong (Leech extract injection) in adjuvant treatment of ischemic stroke on the degree of nerve injury, lipid metabolism and blood coagulation function were studied. The study included 74 patients who were divided into two groups, where the control group received regular chemical treatments, while Shuxuetong group have received Shuxuetong injection in addition to the regular treatments for three months. The chemical drugs included 100 mg aspirin enteric-coated tablets, 75 mg sulfate clopidogrel tablets, 20 mg atorvastatin and 30 mg edaravone injection with 250 mL normal saline. After one and three months of treatment, the degree of nerve injury, lipid metabolism and blood coagulation function indices were evaluated. The results showed that Shuxuetong had significantly alleviated the injury of nerve function, improved blood lipid metabolism and coagulation function in patients treated of ischemic stroke [92].

The wound healing properties of leech extract cream was compared to phenytoin cream, and the results showed that leech cream and phenytoin cream could promote wound regeneration by accelerating the re-epithelialization process and initial angiogenesis [106]. The antitumor properties of LSE and docetaxel in prostate cancer were assessed. The LSE has significant in vitro and in vivo anti-tumor activity with no apparent side effects compared to the chemical drug docetaxel [101].

5. Conclusion

The complexity of some diseases such as cancer and diabetes or the complications associated with aging which affect the whole body requires the patients to receive many medications at the same time increased the need for combined drugs. However, the increased number of medications taken by the patients at the same time led to severe and serious side effects which left some important organs at risk such as liver. Therefore, the health community confirms the need for a medication which can work on different sides with high efficacy and less side effects. Increasing number of scientific reports suggest health benefits of leech therapy. Centuries of use in diverse populations attesting to its relative safety warrant further investigation of leech saliva extract as a pharmaceutical agent. Leech saliva extract could be a treatment of choice for complex diseases due to the multitude of ingredients it contains which are combined naturally and demonstrate diverse biological activities. However, more research is needed to elucidate full chemical composition and mechanism of action of each compound that is present in leech saliva extract.

6. Financial support

This work received no financial support.

7. Acknowledgments

The authors expressed their gratitude to staff of Department of Pharmaceutical Chemistry, Faculty of Pharmacy, International Islamic University Malaysia and staff of the Drug Application and Research Center, Erciyes University for their technical assistance.

8. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] M.E. Siddall, Phylogeny of the leech family Erpobdellidae (Hirudinida: Oligochaeta), Invertebrate systematics 16(1) (2002) 1-6.

[2] A.J. Phillips, F.R. Govedich, W.E. Moser, Leeches in the extreme: Morphological, physiological, and behavioral adaptations to inhospitable habitats, International Journal for Parasitology: Parasites and Wildlife 12 (2020) 318-325.

[3] S. Utevsky, N. Kovalenko, K. Doroshenko, L. Petrauskienė, V. Klymenko, Chromosome numbers for three species of medicinal leeches (Hirudo spp.), Systematic parasitology 74 (2009) 95-102.

[4] M.R. Montinari, S. Minelli, From ancient leech to direct thrombin inhibitors and beyond: New from old, Biomedicine & Pharmacotherapy 149 (2022) 112878.

[5] I.A. Younis Munshi, H. Rafique, Z. Ahmad, Leeching in the history-a review, Pakistan journal of biological sciences 11(13) (2008) 1650-1653.

[6] C. Rados, Beyond bloodletting: FDA gives leeches a medical makeover, FDA Consum. 38(5) (2004) 9-9.

[7] M. Pourrahimi, M. Abdi, R. Ghods, Complications of leech therapy, Avicenna Journal of phytomedicine 10(3) (2020) 222.

[8] J.B. Haycraft, On the action of a secretion obtained from the medicinal leech on the coagulation of the blood, Proceedings of the Royal Society of London 36(228-231) (1883) 478-487.

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

[10] M. Rigbi, H. Levy, F. Iraqi, M. Teitelbaum, M. Orevi, A. Alajoutsijärvi, A. Horovitz, R. Galun, The saliva of the medicinal leech Hirudo medicinalis--I. Biochemical characterization of the high molecular weight fraction, Comparative biochemistry and physiology. B, Comparative biochemistry 87(3) (1987) 567-573.

[11] G.J. Gasic, A. Iwakawa, T.B. Gasic, E.D. Viner, L. Milas, Leech salivary gland extract from Haementeria officinalis, a potent inhibitor of cyclophosphamide-and radiation-induced artificial metastasis enhancement, Cancer Res. 44(12_Part_1) (1984) 5670-5676.

[12] M. Alaama, M. AlNajjar, A. Abdualkader, A. Mohammad, A.J.I.E.J. Merzouk, Isolation and analytical characterization of local Malaysian leech saliva, IIUM Engineering Journal 12(4) (2011).

[13] C.H. Wang, S. Pandey, K. Sivalingam, M.A. Shibu, W.-W. Kuo, V.P. Viswanadha, Y.-C. Lin, S.-C. Liao, C.-Y. Huang, Leech extract: A candidate cardioprotective against hypertension-induced cardiac hypertrophy and fibrosis, J. Ethnopharmacol. 264 (2021) 113346.

[14] R.I. Lee, B. Vincent, A Study of the Effect of Anaphylaxis and Leech extract on the Coagulation of the Blood, The Journal of Medical Research 32(3) (1915) 445.

[15] M. Alaama, A.B.M. Helaluddin, A. Mohammad, A. Merzouk, A.M. Abdualkader, M. Awang, Starvation time and successive collection effects on leeches saliva collection quantity and proteins quality and quantity in wet season, Sains Malaysiana 43(11) (2014) 1693-1697.
[16] B. Okka, Hirudotherapy from past to present, Eur J Basic Med Sci 3(3) (2013) 61-65.
[17] W.L. Dickinson, Note on “Leech-extract” and its Action on Blood, The Journal of Physiology 11(Suppl) (1890) 566.
[18] J. Bock, Untersuchungen über die Wirkung verschiedener Gifte auf das isolirte Sängethierherz, Archiv für experimentelle Pathologie und Pharmakologie 41(2) (1898) 158-178.
[19] C. Jacoby, Über Hirudin, Dtsch Med. Wochenschr 30 (1904) 786-787.
[20] F. Franz, Ueber den die Blutgerinnung auf hebenden Bestandtheil des medicinischen Blutegels, Archiv für experimentelle Pathologie und Pharmakologie 49(4) (1903) 342-366.
[21] H.S. Satterlee, R.S. Hooker, The use of herudin in the transfusion of blood, J. Am. Med. Assoc. 62(23) (1914) 1781-1783.
[22] E.K. Marshall, The toxicity of certain hirudin preparations, J. Pharmacol. Exp. Ther. 7(1) (1915) 157-168.
[23] A. Wallace, THE SUPPRESSION OF THE CONVULSION IN ECLAMPSIA, The Lancet 180 (1912) 1574-1576.
[24] L. Loeb, M.S. Fleisher, Intravenous injections of various substances in animal cancer, J. Am. Med. Assoc. 60(24) (1913) 1857-1858.
[25] M.S. Fleisher, M. Vera, L. Loeb, Immunization Against The Action of Substances Inhibiting Tumor Growth, The Journal of Experimental Medicine 20(5) (1914) 522-541.
[26] M.S. Fleisher, L. Loeb, The Effect of The Intravenous Injection of Substances Affecting Tumor Growth on The Cyclic Changes in The Ovaries and on Placentomata, The Journal of Experimental Medicine 20(2) (1914) 180-190.
[27] G. Haas, Versuche der blutauswaschung am lebenden mit hilfe der dialyse, Klin. Wochenschr. 4(1) (1925) 13-14.
[28] H. Necheles, Über dialysieren des strömenden Blutes am Lebenden, Klin. Wochenschr. 2(27) (1923) 1257-1257.
[29] G. Haas, über Blutwaschung, Klin. Wochenschr. 7(29) (1928) 1356-1362.
[30] A. Ochsner, H. Mahorner, The Use of Leeches in the Treatment of Phlebitis and the Prevention of Pulmonary Embolism, Ann. Surg. 98 (1933) 408.
[31] G. Nowak, K. Schrör, Hirudin–the long and stony way from an anticoagulant peptide in the saliva of medicinal leech to a recombinant drug and beyond, Thromb. Haemost. 98(07) (2007) 116-119.
[32] A. Claude, Spreading properties of leech extracts and the formation of lymph, The Journal of experimental medicine 66(3) (1937) 353.
[33] F. Markwardt, Hirudin, Blut 4(3) (1958) 161-170.

[34] H. Fritz, K. Krejci, Trypsin-plasmin inhibitors (Bdellins) from leeches, Methods Enzymol., Elsevier1976, pp. 797-806.

[35] U. SeemÜLler, M. Meier, K. Ohlsson, H.-P. MÜLler, H. Fritz, Isolation and characterisation of a low molecular weight inhibitor (of chymotrypsin and human granulocytic elastase and cathepsin G) from leeches, Hoppe Seylers Z Physiol Chem 358 (1977) 1105–1107.

[36] P.A. Orlandi, F.W. Klotz, J.D. Haynes, J. Cell, T.J. Hadley, Structure of the RGD Protein Decorsin: Conserved Motif and Distinct Function in Leech Proteins That, Chemistry (Easton) 17 (1978) 4756.

[37] I.P. Baskova, G.I. Nikonov, Destabilase: An enzyme of medicinal leech salivary gland secretion hydrolyzes the isopeptide bonds in stabilized fibrin, Biokhimiia (Moscow, Russia) 50(3) (1985) 424-431.

[38] E. Fink, H. Rehm, C. Gippner, W. Bode, M. Eulitz, W. Machleidt, H. Fritz, The primary structure of bdellin B-3 from the leech Hirudo medicinalis. Bdellin B-3 is a compact proteinase inhibitor of a “non-classical” Kazal type. It is present in the leech in a high molecular mass form, Biol Chem Hoppe Seyler 367 (1986) 1235–1242.

[39] R. Munro, C.P. Jones, R.T. Sawyer, Calin–a platelet adhesion inhibitor from the saliva of the medicinal leech, Blood Coagul Fibrinolysis 2 (1991) 179-184.

[40] M. Moser, E. Auerswald, R. Mentele, C. Eckerskorn, H. Fritz, E. Fink, Bdellastasin, a serine protease inhibitor of the antistasin family from the medical leech (Hirudo medicinalis), 253(1) (1998) 212-220.

[41] C.P. Sommerhoff, C. Söllner, R. Mentele, G.P. Piechottka, E.A. Auerswald, H. Fritz, A Kazal-type inhibitor of human mast cell tryptase: isolation from the medical leech Hirudo medicinalis, characterization, and sequence analysis, Biol. Chem. Hoppe Seyler 375(10) (1994) 685-694.

[42] D. Reverter, J. Vendrell, F. Canals, J. Horstmann, F.X. Avilés, H. Fritz, C.P. Sommerhoff, A carboxypeptidase inhibitor from the medical leech Hirudo medicinalis: Isolation, sequence analysis, cDNA cloning, recombinant expression, and characterization, J. Biol. Chem. 273(49) (1998) 32927-32933.

[43] I.P. Baskova, Z. Ferner, A.S. Balkina, S.A. Kozin, O.V. Kharitonova, L.L. Zavalova, V.G. Zgoda, Steroids, histamine, and serotonin in the medicinal leech salivary gland secretion, Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry 2(3) (2008) 215-225.

[44] A. Electricwala, R.T. Sawyer, C.P. Jones, T. Atkinson, Isolation of thrombin inhibitor from the leech Hirudinaria manillensis, Blood Coag Fibrinolysis, 2 (1991) 83-89

[45] E. Scacheri, G. Nitti, B. Valsasina, G. Orsini, C. Visco, M. Ferrera, R.T. Sawyer, P. Sarmientos, Novel hirudin variants from the leech Hirudinaria manillensis: amino acid sequence, cDNA cloning and genomic organization, Eur. J. Biochem. 214(1) (1993) 295-304.

[46] V. Steiner, R. Knecht, K.O. Börnsen, E. Gassmann, S.R. Stone, F. Raschdorf, J.M. Schlaeppi, R. Maschler, Primary structure and function of novel O-glycosylated hirudins from the leech Hirudinaria manillensis, Biochemistry 31(8) (1992) 2294-2298.

[47] M. Kordowicz, D. Gussow, U. Hofmann, T. Pacuszka, A. Gardas, Hyaluronidase from the Hirudinaria manillensis isolation, purification and recombinant method of production, Google Patents, 2006.

[48] A. Electricwala, N.A.E. Von Sicard, R.T. Sawyer, T. Atkinson, Biochemical Characterisation of A Pancreatic Elastase Inhibitor from the Leech Hirudin Aria Manillensis, J. Enzyme Inhib. 6(4) (1993) 293-302.

[49] S.J. Hong, K.W. Kang, Purification of granulin-like polypeptide from the blood-sucking leech, Hirudo nipponia, Protein Expr. Purif. 16(2) (1999) 340-346.

[50] H.I. Jung, S.I. Kim, K.-S. Ha, C.O. Joe, K.W. Kang, Isolation and Characterization of Guamerin, a New Human Leukocyte Elastase Inhibitor from Hirudo nipponia, J. Biol. Chem. 270(23) (1995) 13879-13884.

[51] D.R. Kim, K.W. Kang, Amino acid sequence of piguamerin, an antistasin-type protease inhibitor from the blood sucking leech Hirudo nipponia, Eur. J. Biochem. 254(3) (1998) 692-697.

[52] M. Salzet, V. Chopin, J.-l. Baert, I. Matias, J. Malecha, Theromin, a novel leech thrombin inhibitor, J. Biol. Chem. 275(40) (2000) 30774-30780.

[53] V. Chopin, M. Salzet, J.-l. Baert, F. Vandenbulcke, P.-E. Sautiere, J.-P. Kerckaert, J. Malecha, Therostasin, a novel clotting factor Xa inhibitor from the rhynchobdellid leech, Theromyzon tessulatum, J. Biol. Chem. 275(42) (2000) 32701-32707.

[54] V. Chopin, T.V. Bilfinger, G.B. Stefano, I. Matias, M. Salzet, Amino-acid-sequence Determination and Biological Activity of Cytin, a Naturally Occurring Specific Chymotrypsin Inhibitor from the Leech Theromyzon tessulatum, Eur. J. Biochem. 249(3) (1997) 733-738.

[55] V. Chopin, I. Matias, G.B. Stefano, M. Salzet, Amino acid sequence determination and biological activity of therin, a naturally occuring specific trypsin inhibitor from the leech Theromyzon tessulatum, Eur. J. Biochem. 254(3) (1998) 565-570.

[56] V. Chopin, G.B. Stefano, M. Salzet, Amino-acid-sequence determination and biological activity of tessulin, a naturally occurring trypsin-chymotrypsin inhibitor isolated from the leech Theromyzon tessulatum, Eur. J. Biochem. 258(2) (1998) 662-668.

[57] A. Tasiemski, F. Vandenbulcke, G. Mitta, J. Lemoine, C. Lefebvre, P.-E. Sautiere, M. Salzet, Molecular characterization of two novel antibacterial peptides inducible upon bacterial challenge in an annelid, the leech Theromyzon tessulatum, J. Biol. Chem. 279(30) (2004) 30973-30982.

[58] V. Laurent, M. Salzet, Isolation of a renin-like enzyme from the leech Theromyzon tessulatum, Peptides 16(8) (1995) 1351-1358.

[59] T.M. Connolly, J.W. Jacobs, C. Condra, An inhibitor of collagen-stimulated platelet activation from the salivary glands of the Haementeria officinalis leech. I. Identification, isolation, and characterization, J. Biol. Chem. 267(10) (1992) 6893-6898.

[60] G.P. Tuszynski, T.B. Gasic, G.J. Gasic, Isolation and characterization of antistasin. An inhibitor of metastasis and coagulation, J. Biol. Chem. 262(20) (1987) 9718-9723.

[61] S.M. Malinconico, J.B. Katz, A.Z. Budzynski, Fibrinogen degradation by hementin, a fibrinogenolytic anticoagulant from the salivary glands of the leech Haementeria ghilianii, The Journal of laboratory and clinical medicine 104(5) (1984) 842-854.

[62] S. Finney, L. Seale, R.T. Sawyer, R.B. Wallis, Tridegin, a new peptidic inhibitor of factor XIIIa, from the blood-sucking leech Haementeria ghilianii, Biochem. J. 324(3) (1997) 797-805.

[63] R. Brankamp, G. Manley, D. Biankenship, T. Bowlin, A.D. Cardin, Studies on the anticoagulant, antimetastatic and heparin-binding properties of ghilanten-related inhibitors, Blood Coagul Fibrinolysis, 2 (1991) 161-166.

[64] C.S. Barnes, B. Krafft, M. Frech, U.R. Hofmann, A. Papendieck, U. Dahlems, G. Gellissen, M.F. Hoylaerts, Production and characterization of saratin, an inhibitor of von Willebrand factor-dependent platelet adhesion to collagen, Semin. Thromb. Hemost. (2001) 337-348.

[65] A.M. Chudzinski-Tavassi, E.M.A. Kelen, A.P. de Paula Rosa, S. Loyau, C.A.M. Sampaio, C. Bon, E. Anglés-Cano, Fibrino (geno) lytic properties of purified hementerin, a metalloproteinase from the leech Haementeria depressa, Thromb. Haemost. 80(07) (1998) 155-160.

[66] F. Faria, E.M.A. Kelen, C.A.M. Sampaio, C. Bon, N. Duval, A.M. Chudzinski-Tavassi, A new factor Xa inhibitor (lefaxin) from the Haementeria depressa leech, Thromb. Haemost. 82(11) (1999) 1469-1473.

[67] E.M.A. Kelen, G. Rosenfeld, Fibrinogenolytic substance (Hementerin) of Brazilian blood-sucking leeches (Haementeria lutzi Pinto 1920), Pathophysiol. Haemost. Thromb. 4(1) (1975) 51-64.

[68] K.-H. Strube, B. Kröger, S. Bialojan, M. Otte, J. Dodt, Isolation, sequence analysis, and cloning of haemadin. An anticoagulant peptide from the Indian leech, J. Biol. Chem. 268(12) (1993) 8590-8595.

[69] J.L. Seymour, W.J. Henzel, B. Nevins, J.T. Stults, R.A. Lazarus, Decorsin. A potent glycoprotein IIb-IIIa antagonist and platelet aggregation inhibitor from the leech Macrobdella decora, J. Biol. Chem. 265(17) (1990) 10143-10147.

[70] P. Mazur, W.J. Henzel, J.L. Seymour, R.A. Lazarus, Ornatins: potent glycoprotein IIb-IIIa antagonists and platelet aggregation inhibitors from the leech Placobdella ornata, Eur. J. Biochem. 202(3) (1991) 1073-1082.

[71] V.V. Babenko, O.V. Podgorny, V.A. Manuvera, A.S. Kasianov, A.I. Manolov, E.N. Grafskaia, D.A. Shirokov, A.S. Kurdyumov, D.V. Vinogradov, A.S. Nikitina, S.I. Kovalchuk, N.A. Anikanov, I.O. Butenko, O.V. Pobeguts, D.S. Matyushkina, D.V. Rakitina, E.S. Kostryukova, V.G. Zgoda,

I.P. Baskova, V.M. Trukhan, M.S. Gelfand, V.M. Govorun, H.B. Schiöth, V.N. Lazarev, Draft genome sequences of Hirudo medicinalis and salivary transcriptome of three closely related medicinal leeches, BMC Genomics 21(1) (2020) 331.

[72] I.P. Baskova, E.S. Kostrjukova, M.A. Vlasova, O.V. Kharitonova, S.A. Levitskiy, L.L. Zavalova, S.A. Moshkovskii, V.N. Lazarev, Proteins and peptides of the salivary gland secretion of medicinal leeches Hirudo verbana, H. medicinalis, and H. orientalis, Biochemistry (Moscow) 73 (2008) 315-320.

[73] M. Courtney, G. Loison, Y. Lemoine, N. Riehl-Bellon, E. Degryse, S.W. Brown, J.P. Cazenave, G. Defreyn, D. Delebassee, A. Bernat, Production and evaluation of recombinant hirudin, Semin Thromb Hemost 15 (1989) 288-292.

[74] T.E. Warkentin, Management of heparin-induced thrombocytopenia: a critical comparison of lepirudin and argatroban, Thromb. Res. 110(2) (2003) 73-82.

[75] T.J. Graetz, B.R. Tellor, J.R. Smith, M.S. Avidan, Desirudin: a review of the pharmacology and clinical application for the prevention of deep vein thrombosis, Expert Rev. Cardiovasc. Ther. 9(9) (2011) 1101-1109.

[76] T.E. Warkentin, A. Greinacher, A. Koster, Bivalirudin, Thromb. Haemost. 99(11) (2008) 830-839.

[77] M. Deganc, F. Zdravic, Venous congestion of flaps treated by application of leeches, Br. J. Plast. Surg. 13 (1960) 187-192.

[78] G.J. Gasic, E.D. Viner, A.Z. Budzynski, G.P. Gasic, Inhibition of lung tumor colonization by leech salivary gland extracts from Haementeria ghilianii, Cancer Res. 43(4) (1983) 1633-1636.

[79] J. Zhou, Z. Song, M. Han, B. Yu, G. Lv, N. Han, Z. Liu, J. Yin, Evaluation of the antithrombotic activity of Zhi-Xiong Capsules, a Traditional Chinese Medicinal formula, via the pathway of anti-coagulation, anti-platelet activation and anti-fibrinolysis, Biomed. Pharmacother. 97 (2018) 1622-1631.

[80] M. Zhou, P. Ren, Y. Zhang, S. Li, M. Li, P. Li, J. Shang, W. Liu, H. Liu, Shen-Yuan-Dan capsule attenuates atherosclerosis and foam cell formation by enhancing autophagy and inhibiting the PI3K/Akt/mTORC1 signaling pathway, Front. Pharmacol. 10 (2019) 603.

[81] X. Li, Y. Zhang, H.-X. Liu, J.-J. Shang, Q. Zhou, A.-Y. Li, X.-L. Lai, W.-L. Xing, S.-H. Jia, A randomized, placebo-controlled, double-blind trial to evaluate efficacy and safety of Shen-Yuan-Dan capsules, a traditional Chinese medicine, for treatment of peri-procedure myocardial injury following percutaneous coronary intervention, Complement. Ther. Med. 69 (2022) 102841.

[82] J. Han, H. Tan, Y. Duan, Y. Chen, Y. Zhu, B. Zhao, Y. Wang, X. Yang, The cardioprotective properties and the involved mechanisms of NaoXinTong Capsule, Pharmacol. Res. 141 (2019) 409-417.

[83] J. Lv, S. Liu, S. Guo, J. Gao, Q. Song, X. Cui, Tongxinluo capsule as supplementation and cardiovascular endpoint events in patients with coronary heart disease: A systematic review and meta-analysis of randomized, double-blind, placebo-controlled trials, J. Ethnopharmacol. 289 (2022) 115033.

[84] W.-Y.-N. Tang, J.-T. Liang, J. Wu, L. Liu, M.-Z. Lu, X.-Y. He, L.-J. Wu, H.-Y. Jiang, F. Wang, X. Meng, S.-P. Li, Efficacy and Safety of Dahuang Zhechong Pill in Silicosis: A Randomized Controlled Trial, Evid. Based Complement. Alternat. Med. 2021 (2021) 4354054.

[85] F. Wei, Y. Lang, D. Gong, Y. Fan, Effect of Dahuang zhechong formula on liver fibrosis in patients with chronic hepatitis B: A meta-analysis, Complement. Ther. Med. 23(1) (2015) 129-138.

[86] C. Chen, X. Yao, Y. Xu, Q. Zhang, H. Wang, L. Zhao, G. Wen, Y. Liu, L. Jing, X. Sun, Dahuang Zhechong Pill suppresses colorectal cancer liver metastasis via ameliorating exosomal CCL2 primed pre-metastatic niche, J. Ethnopharmacol. 238 (2019) 111878.

[87] R. Chen, G. Gai, W. Zhang, Y. Zhuang, Y. Xie, Application of propensity score method to analyze the effect of Maixuekang capsule on the treatment outcome of coronary heart disease, Journal of Chinese Physician (2020) 365-368.

[88] X. Jin, G. Shen, F. Gao, X. Zheng, X. Xu, F. Shen, G. Li, J. Gong, L. Wen, X. Yang, X. Bie, Traditional Chinese drug ShuXueTong facilitates angiogenesis during wound healing following traumatic brain injury, J. Ethnopharmacol. 117(3) (2008) 473-477.

[89] Z.Y. Sun, F.J. Wang, H. Guo, L. Chen, L.J. Chai, R.L. Li, L.M. Hu, H. Wang, S.X. Wang, Shuxuetong injection protects cerebral microvascular endothelial cells against oxygen-glucose deprivation reperfusion, Neural Regen Res 14(5) (2019) 783-793.

[90] Z. Yu, X. Liu, Y. Xing, X. Wang, X. Wang, Y. Huang, L. Han, G. Pan, Identification and Quantification of Characteristic Peptides (Oligopeptides) in Shuxuetong (SXT) Injection by LC–MS/MS, Chromatographia 85(12) (2022) 1029-1039.

[91] L.-Y. Jia, A.-H. Yao, F. Kuang, Y.-K. Zhang, X.-F. Shen, G. Ju, Beneficial Effect of the Traditional Chinese Drug Shu-Xue-Tong on Recovery of Spinal Cord Injury in the Rat, Evid. Based Complement. Alternat. Med. 2011 (2011) 862197.

[92] W. Zhang, Effects of Shuxuetong injection applied in acute ischemic stroke, Journal of Acute Disease 5(6) (2016) 507-511.

[93] R. Galun, S.H. Kindler, Chemical specificity of the feeding response in Hirudo medicinalis (L.), Comp. Biochem. Physiol. 17(1) (1966) 69-74.

[94] B.R. West, L.S. Nichter, D.E. Halpern, Emergent reuse leech therapy: a better method, Plast. Reconstr. Surg. 93(5) (1994) 1095-1098.

[95] A. Shakouri, N. Adljouy, J. Abdolalizadeh, Anti-Cancer Activity of liposomal Medical leech saliva extract (LSE), Proceedings of the 3rd World Congress on Recent Advances in Nanotechnology (RAN'18), April, 2018 Budapest, Hungary.

[96] M. Alaama, A.M. Abdualkader, A.M. Ghawi, A. Merzouk, R.S. Khalid, A.B.M. Helaluddin, Assessment of Trace Heavy Metals Contamination in the Tissues and Saliva of the Medicinal Leech Hirudinaria manillensis, Turkish Journal of Fisheries and Aquatic Sciences 21(5) (2021) 225-231.

[97] A.A. Mohammed, G.A. Mohammad, A. Mohamed, A. Mohamed, M. Ahmed, In vivo anti-hyperglycemic activity of saliva extract from the tropical leech Hirudinaria manillensis, Chinese journal of natural medicines 11(5) (2013) 488-493.

[98] B. Malik, D.A. Astuti, D.J.F. Arief, M. Rahminiwati, A study on antioxidative and antimicrobial activities of saliva extract of Indonesian local leeches, IOP Publishing, 2019, p. 012061.

[99] A. Merzouk, A.M. Ghawi, A.M. Abdualkader, A. Abdullahi, M. Alaama, Anticancer effects of medical Malaysian leech saliva extract (LSE), Pharm Anal Acta S 15 (2012) 2-6.

[100] A. Shakouri, N. Adljouy, S. Balkani, M. Mohamadi, H. Hamishehkar, J. Abdolalizadeh, S. Kazem Shakouri, Effectiveness of topical gel of medical leech (Hirudo medicinalis) saliva extract on patients with knee osteoarthritis: A randomized clinical trial, Complement. Ther. Clin. Pract. 31 (2018) 352-359.

[101] A.E. Ammar, M.H. Hassona, G.R. Meckling, L.G. Chan, M.Y. Chin, A. Abdualkader, M. Alaama, A. Merzouk, A. Helaluddin, A. Ghawi, O. Kucuk, E.S. Guns, Assessment of the antitumor activity of leech (huridinaria manillensis) saliva extract in prostate cancer, Cancer Res. 75 (2015).

[102] A. Ammar, E. Guns, O. Kucuk, A. Abdualkader, M. Alaama, A.H. Uddin, A. Ghawi, M.J.C.R. Hassona, Mechanism of anticancer activity of BPS-001 (lyophilized leech saliva extract), 77(13_Supplement) (2017) 107-107.

[103] I.P. Baskova, O.M. Aguejouf, F. Azougagh-Oualane, L.L. Zavalova, A.V. Basanova, C. Doutremepuich, Arterial antithrombotic effect of piyavit, the novel pharmacological preparation from the medicinal leech, and of its components, prostanoids and enzyme destabilase, Thromb. Res. 77(6) (1995) 483-492.

[104] J. Qiu, W. Lingna, H. Jinghong, Z. Yongqing, Oral administration of leeches (Shuizhi): A review of the mechanisms of action on antiplatelet aggregation, J. Ethnopharmacol. 232 (2019) 103-109.

[105] Y. Su, A. Jing, Y. Wang, Clinical research of Huangqi Injection combined with Shuxuetong Injection therapy on aged acute cerebral infarction, Chinese Traditional Patent Medicine (1992).

[106] L. Amani, N. Motamed , M. Mirabzadeh Ardakani, M. Dehghan Shasaltaneh, M. Malek, F. Shamsa , E. Fatemi , M. Amin, Semi-Solid Product of Medicinal Leech Enhances Wound Healing in Rats, 16(4) (2021) e113910.

Figures:

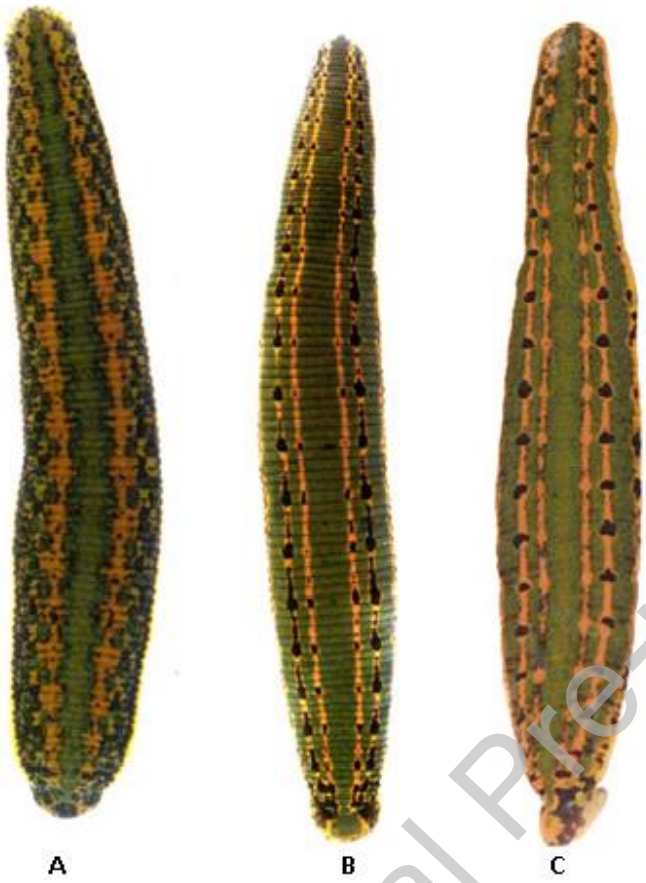

Figure 1: some medicinal leech species, A: *Hirudo verbena*, B: *Hirudo medicinalis*, C: *Hirudo orientalis*

Table 1: Isolated chemical compounds from different leech species

Leech species	**Isolated compounds**
Hirudo medicinalis	Hirudin[33], Destabilase[37], Bdellin B[38], Calin[39], Factor Xa inhibitor, Bdellastasin[40], Hirustatin, Leech-derived tryptase inhibitor (LDTI)[41], Hyaloronedase[32], Eglins[35], Apyrase[10], Collagenase[10], Leech carboxy peptidase inhibitor [42], Lipase (triglyceridase), Cholesterol-esterase[43], Steroids, Histamine, and Serotonin[43].
Hirudinaria manillensis	Bufrudin[44], HM1, HM2[45], Hirullin P6, Hirullin P18[46], Manillase[47], Gelin[48].
Hirudo nipponia	Leech granulin[49], Guamerin[50], Piguamerin[51]
Theromyzon tessulatum	Theromin[52], Therostasin[53], Cytin[54], Therin[55], Tessulin[56], Theromacin and Theromyzin[57], Renin-like enzyme[58]
Haementeria officinalis	Leech antiplatelet protein (LAPP) [59], Antistasin [60]
Haementaria ghilianii	Hementin[61], Tridegin[62], Ghilantin[63], Saratin[64],
Haementeria depressa	Hementerin[65], Lefaxin[66],
Haementarea lutzi pintu	Hementerin[67]
Haemadipsa sylvestris	Haemadin[68],
Macrobdella decora	Decorsin[69]
Placobdella ornate	Ornatins[70]
Leech in general	100++ unidentified proteins [71, 72]

Table 2: Leech extract-based pharmaceuticals

Leech extract-based product	**Route of administration**	**Claimed usage**	**Year of marketing**	**Used for**
Hirudin Sachsse and Co., Leipzig, Germany	Injection	Anticoagulant [20]	1904-1925	Blood transfusion [21], Eclampsia [23], hemodialysis [27]
Hirudin Passek and Wolf Hamburg	Injection	Anticoagulant [28]	1920-1925	hemodialysis [28]
Piyavit	Oral	Anticoagulant [103]	1993-1995	acquired heart valvular disease [103]
Shuizhi and other oral Chinese formulations	Oral	Anticoagulant, antiplatelet aggregation [104]	1960s-today	Acute and chronic cerebrovascular diseases [79], atherosclerosis [80], ischemic stroke [82], acute coronary syndrome [87].
Shuxuetong	Injection	Anticoagulant [105]	1990s-today	Ischemic stroke [92]
Recombinant hirudin derivatives				
Lepirudin	Injection	Anticoagulant [74]	1997	heparin-induced thrombocytopenia [74]
Desirudin	Injection	Thrombin inhibitor [75]	2003	Antithrombin in elective hip replacement surgery [75]
Bivalirudin	Injection	Anticoagulant [76]	2000	unstable angina [76]

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: