Biotechnology Reports 44 (2024) e00862

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Review

Bee products: An overview of sources, biological activities and advanced approaches used in apitherapy application

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ARTICLE INFO

Keywords:
Apitherapy
Bee products
Apis mellifera
Beehive air therapy
Apilarnil
Bee venom therapy

ABSTRACT

Background: Bee therapy (Apitherapy, Api-treatment, Bee treatment) is a type of biotherapy that uses bees and their products as medicinal or preventative measures to control progression of diseases. In many countries today, apitherapy is a section of complementary and integrative medicine. The aim of this review is to explore the different bee products and their therapeutic potentials.
Method: We searched the literature and then explored and evaluated evidence for bee products' composition, therapeutic abilities and novel techniques used to enhance their effectiveness.
Results: Data revealed that there are continuous advances in research and clinical trials of bee therapy. A better understanding of the composition of bee products generated great interest in their use for medical treatments. Bee products either collected or synthesized promote healing through reducing inflammation, enhancing circulation, and inducing a healthy immunological response, Furthermore, researchers have developed innovative approaches such as nanoparticles, scaffold, nanofibers, and others to increase the bioavailability of bee products and overcome problems with the traditional use of these products.
Conclusion: Bee therapy is a simple, accessible, and easy-to-use pharmaceutical that is used in conventional medicine and has the potential to treat a variety of diseases. However, further studies are needed to prove its efficacy, and safety. Lack of practice regulations is still an issue.

1. Introduction

The prevalence and incidence of life-threatening diseases such as cancer, autoimmune diseases, chronic diseases, bacterial, fungal, and viral infections, etc., are increasing worldwide. Additionally, owing to the adverse effects of synthetic medications on human health and the rising rate of drug resistance to these substances, current pharmaceutical drugs are now experiencing a serious crisis [1–3] It is critical to discover and create innovative, natural, conventional, and non-synthetic pharmaceuticals to treat these diseases. Folk medicine has always made use of natural ingredients. Apitherapy is one of the natural remedies [4]. Honeybees, Apis mellifera are the "Golden insects" that produce a large number of vital products that contain bioactive constituents that have been used by different civilizations for centuries to treat various diseases [5].

Apitherapy derives its name from the Latin word "*Apis*" (bee). It is based on the use of beehive products (honey, pollen, wax, royal jelly, propolis, and bee venom) in preventing and treating various diseases, as well as in increasing the resistance of the human body [6]. Nowadays, the definition of bee therapy has been broadened and developed to include the usage of bee products, bee acupuncture, and even the extensive natural fundamentals of bee activities and associated apiaries to treat humans. Apitherapy is defined as "the art and science of treatment and holistic healing through the honeybee and her products for the benefit of mankind and all of the animal kingdom" by Dr. Stefan Stangaciu, editor in chief of the International Federation of Beekeepers' Association [7].

The use of honey and other bee products in human treatments can be traced back to olden times. Therapeutic benefits of bee products are documented in various religious books, such as the Bible, Vedas, and Quran [8,9]. A whole Surah in the Holy Qur'an carries the name of bees (Al Nahl).

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https://doi.org/10.1016/j.btre.2024.e00862
Received 19 March 2024; Received in revised form 11 August 2024; Accepted 17 October 2024
Available online 18 October 2024

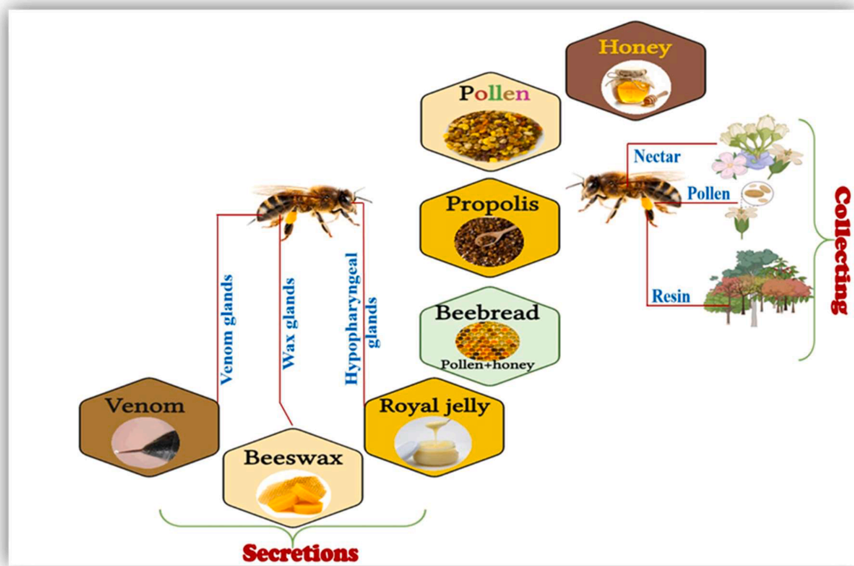

Fig. 1. The origin of honeybee products.

Although it is still impossible to determine the exact beginnings of apitherapy. Rose (**1994**) revealed that Apitherapy can be traced back to ancient Egypt and Greece. It has been practiced in China for 3–5 thousand years [10]. Ancient records from India and Egypt mention the use of honey to heal wounds. Honey is registered in 147 prescriptions for external applications on the Ebers papyrus (1550 BC). The Greeks and Romans also used bee products for medicinal purposes. Hippocrates, the ancient Greek physician and "father of medicine," listed the physical effects of honey: "It causes heat, cleans sores and ulcers, softens hard ulcers of the lips, and heals carbuncles and running sores" [11]. The medicinal value of bee products was described by Hippocrates (460–370 BC), Aristotle (384–332 BC) and Galen (130–200 AD), who prescribed the use of honey and bee venom as a cure for baldness. According to Aristotle, eating honey can help people maintain their health and vitality. The most beneficial ingredient in the ancient Roman pharmacopoeia was honey. Aristotle wrote in his Historia Animalium that "honey is good as a salve for sore eyes".

Other than honey, pollen has also been mentioned in the past, with a Chinese ruler named Shen Nung discussing its benefits as early as 2735 BC. Pollen is referred to as "life-giving dust" in Egyptian papyri. Hippocrates suggested pollen as a treatment for a number of ailments. Hindus claimed that pollen and honey may bring forth health, vitality, happiness, and wisdom. According to Wagner et al. (1970), bee pollen is nutrient-dense and contains up to a hundred vitamins, enzymes, minerals, and amino acids in addition to water, protein, carbohydrates, fatty acids, and antioxidants [12].

Also, beeswax is known for its medicinal use in ancient civilizations. Beeswax was the main ingredient in many recipes for ointments and creams used to treat burns, wounds, bruises, joint pain, and fractures as mentioned in the Ebers Papyrus (1550 BC.) and ancient Rome. Hippocrates recommended the use of beeswax in cases of purulent tonsillitis. Beeswax was one of the components of the first cosmetic cream, which was created by Galen, the great Greek physician, in 150 BC., composed of beeswax and olive oil, with an emulsion of rose water [13].

In Egypt, propolis was used specially in antiquity. Propolis was used effectively in wound treatment Throughout World War II and the Anglo-Boer War [14]. Also, the fact that propolis has Greek name suggests that the ancient Greeks were also familiar with it [15].

In addition, royal jelly has been used in traditional Chinese medicine for 70–80 years. Royal jelly was highly valued by Chinese monarchs as a way to increase longevity and sexual power. Japanese case study on royal jelly demonstrated its widespread use and effectiveness for fatigue [16,17]. Finally, bee venom is used dates to ancient times. Hippocrates used bee venom for therapeutic purposes [18]. Recent years have seen the development of bee products in pharmaceutical bee formulations. Honey is included in pharmaceutical dosage forms such as hydrogels and scaffolds for patients' convenience [19,20].

Bee pollen product is prepared in the form of candy, bars, granules, pills, oral liquids, and human tonics [21]. Also, propolis can be used as shampoos, skin creams, antiseptic solutions, and toothpastes in cosmetics as well as candies and chocolates in food technology [22]. Beeswax is an ideal ingredient for natural skincare products, because of its soothing and moisturizing properties. So that it is used in several cosmetics and personal care things, such as lip balms, cream and ointment moisturizers, and hair care items [23]. Furthermore, royal jelly is packed in gel capsules for health care application [24], as well as its use as vaginal cream in treatment of genitourinary syndrome [25]. In addition to using bee venom as vials for injection, bee venom can be used as body cream for skincare [26].

Consequently, with this background, we have endeavored in this review to categorize natural bee products based on their origin. In addition, we summarize the current knowledge regarding their phytochemical compositions, biological activities offered by each of these products as well as different techniques used to potentiate its pharmaceutical applications. Comparatively, some efforts have been made to introduce the biological activities of bee venom, hence it has attracted the interest of the scientific community worldwide. Thereupon, numerous studies have found beneficial effects exerted by this natural product on human health in the last few years. Finally, regulation and limitation of the currently practicing Apitherapy in addition to the potential directions of the newly discovered bee products in health aides were obviously revealed in this article.

2. Main bee products and different advanced techniques used in their applications

Before going into the advantages and applications of bee products, it is important to know the origin of each bee product. As shown in Fig. (1) bee products can be categorized into secreted products and collected ones. The bees collect plant-derived materials and modify them to produce propolis, honey, bee bread, and bee pollen. While bee venom, royal jelly, and beeswax are secreted from bees themselves through specialized glands [27].

2.1. Collected bee products

2.1.1. Honey

Honey is a liquid, viscous, light or dark amber in color and the natural sweet substance produced by honeybees from the floral nectar or secretions of living parts of plants, which are collected and transformed by bees and combined with specific substances of their own. It is deposited, dehydrated, stored, and left in the honeycomb to ripen and mature [28].

Honey has diverse compositions depending on its botanical source because bees use plants to manufacture it [29]. The main ingredients of honey include sugars (such as glucose, maltose, fructose, sucrose, isomaltose, maltotriose, maltulose, melezitose, and threalose), water, and enzymes such as (invertase, α- and β-amilase, glucose oxidase, and catalase). Low concentrations of proteins, amino acids, some vitamins (mostly B and C), minerals (Na, K, Ca, Mg, Fe, Cu, Mn, and Zn), pollen grains, and other phytochemicals are also present. Qualities of honey, including color, flavor, and scent, are influenced by the bee species that produce the honey as well as floral sources, geographical locations, and seasonal influences. Additional factors affecting honey include handling, storage, and climate [30,31].

Enzymes are a crucial part of honey. The existence and activities of these enzymes provide important data for the natural state and quality of the honey because their activity declines in old or heated honey [32].

Dark honeys typically feature significant levels of phenols in addition to high antioxidant capabilities and other biological properties [33]. A reliable indicator of honey's antioxidant capabilities is its total phenolic content. Antioxidant is the most explored characteristics of honey [34]. Also, Honey has anti-inflammatory and anticancer properties as a result of the activity of polyphenolic chemicals (phenolic acids and flavonoids), vitamins C and E, and enzymes (catalase, peroxidase) in it [35].

Honey has been utilized as a medicine to treat a number of diseases by people all over the world. Studies have shown that honey has nutritional benefits and healing effects, including its role as an antioxidant, antimicrobial, anti-parasitic, anti-inflammatory, anti-tumor, cardiovascular guardian [36].

2.1.1.1. Antibacterial activity of honey and wound healing. Honey acts as bacteriostatic and bactericidal with no antibiotic resistance reported. The antibacterial activity of honey is related to different factors. Honey dehydrates bacteria by withdrawing moisture from surrounding it. Its high sugar content and low pH inhibit microbial growth. Finally, and most important, the presence of hydrogen peroxide [37]. **Mama et al. (2019)** found that lower water content of honey and the presence of glucose oxidase in its components led to prevention of bacterial growth and destroying methicillin-resistant Staphylococcus aureus (MRSA) microorganisms that isolated from infected wounds [38].

Therefore, honey is considered an effective treatment for wounds.

Large burn wounds are more susceptible to infection and fluid loss for the patient. Therefore, inhibition of bacterial infections and excessive fluid loss is necessary for healing. There are numerous studies showing how well honey works to heal burn wounds [39–41]. Traditionally raw honey was applied topically to treat wounds and burns. However, the use of honey in raw form requires more time than the conventional medical approach, though produces positive outcomes. Therefore, recently some efforts have been made to develop it in pharmaceutical dosage forms. A scaffold strategy was explored to enhance the medical effects of bee products on skin injuries. Common design criteria include creating a framework that is simple to handle, suitable for commercialization, and secure for use with home medications. **Angioi et al. (2021)** reported that the honey incorporation within scaffolds provided an ideal atmosphere to promote thorough healing across all skin layers. Manuka honey-loaded hydrogels have been created using pectin, a naturally occurring polyuronide, to treat excisional wounds in rats. These hydrogels significantly accelerated wound contraction as compared to direct liquid honey administration [20]. In another study, **Al-Musawi et al.** developed a honey/tripolyphosphate (TPP)/chitosan (HTCs) nanofibers wound bandage which was loaded with capsaicin, gold nanoparticles or both. The antibacterial activity of the developed formulations was tested against different species, and it showed better inhibition of bacterial growth compared to antibiotics. Further, developed nanofibrous mat showed higher wound closure rate compared to control when tested on rabbits. Thus, nanofibrous mat proved to be a promising bandage material [42].

2.1.2. Propolis

Propolis, mostly identified as bee glue, is a viscous, sticky, dark yellow or brownish material that honeybees collect from plant buds and shoots in order to protect their colonies. Bees use propolis to repair the interior walls of the hive, close holes and honeycombs, and stop the deterioration of invaders who have perished inside the hive. It serves as a deterrent to intruders and is used to combat a variety of pathogens in the hive [43].

In various raw propolis samples, about 300 distinct compounds have been found, including phenolic compounds, flavonoids such as flavones, flavanones, and stilbenes, chalcone, steroids, and lignans [43,44]. Hence, researches have shown that bee glue has various biological actions such as antimicrobial, antioxidant, and anti-inflammatory effects. According to reports, Chinese propolis has effective cytotoxic activity against human breast cancer cells [45].

Raw propolis needs to be cleansed before it can be used as a feedstock. Because active compounds are more readily soluble in ethanol, ethanolic extraction is the most common method for producing raw propolis [46].

Typical ethanolic propolis extract has a limited range of applications, because it contains alcohol. New techniques have been developed to improve the bioavailability of bioactive propolis particles such as its combination with chemotherapeutic drugs, encapsulation and fabrication electrospun nanofibers [47].

For example, propolis combined with doxorubicin (DOX) enhanced its anticancer activity on breast cancer cell lines according to two *in vitro* studies [48,49]. **Rouibah et al. (2021)** investigated the anti-cancer activity of 70 % ethanolic propolis extract and various concentrations of DOX on MDA-MB-231 breast cancer cells. The study suggested that the combination exhibited greater cell apoptosis as evidenced by a 10-fold lower IC_{50} value compared to DOX alone [49].

Propolis was formulated as nanofiber for wound healing dressings by electrospinning technique [50,51]. **Alberti et al.** developed a nanofiber wound dressing of PVA and propolis [51]. The scaffold showed compatibility with fibroblast in vitro. Moreover, it showed higher wound closure rate than group treated with allantoin solution and untreated group [22].

To improve the usability of propolis, it can be encapsulated into consumable alcohol-free propolis beads. **Keskin et al. (2019)** encapsulated ethanolic propolis extract by sodium alginate and then dried them into alcohol free beads to enhance their bioavailability [52].

2.1.3. Bee pollen

A pellet known as bee pollen is gathered from flowers of plants by foraging bees then delivered to the hive in the form of pollen loads or granules. Lumpy particles are wet by a slight nectar or honey and joined with different enzymes formed through the bees, such as amylase and catalase [53].

Bee pollen contains a variety of bioactive substances, such as proteins, carbohydrates, fatty acids, polyphenols, vitamins, carotenoid pigments, phytosterols, enzymes, and co-enzymes. The composition of honey pollen depends on ecological factors, such as flora, habitat, and season [53]. Although bee pollen has been utilized as a nutrition since ancient times, it also contains bioactive compounds that have a variety of health benefits, including antioxidant, antimicrobial, anticancer, antiallergenic, anti-atherosclerotic, hepatoprotective, chemopreventive, and immunomodulatory properties [54]. The treatment of metabolic diseases like diabetes, obesity, hyperlipidemia, and associated cardiovascular problems has benefited from the bee pollen usage [21].

Experimental studies on animals have reported that bee pollen exhibit good hepatoprotective activity through reduction of liver enzymes such as alanine, aspartate transaminase, and acid phosphatase in rats [55]. For the first time, bee pollen extract-loaded nanoparticles were used to treat lung cancer cells by polymer nanoparticles technique. As a natural product, bee pollen extract has proven its potential ability to inhibit the proliferation of A549 lung cancer cells [56]. Also, the ant-inflammatory and anticancer activity of bee pollen were studied by **Alshehri and Abdella, 2023** as they encapsulated bee pollen and thymol oil extract into chitosan nanoparticle matrix. The nano-formulation showed significant inhibition of cytokines production in two different cell lines [57].

The addition of bee pollen to yoghurt improves its shelf life while enhancing its look, taste, odour, and stiffness [58]. Furthermore, bee pollen has the potential to be employed in cosmetics, protecting cells from unusual melanogenesis in skin illnesses and removing age spots, freckles, melasma, and malignant melanoma. [59].

2.1.4. Bee bread

Bee bread, known as perga or ambrosia, is produced by the lactic acid fermentation of bee pollen, probably performed by microorganisms that are associated with bees such as bacteria, yeasts, or both. Bee bread is the stored form of bee pollen in the colony, which is then eaten by adults the larvae of bees. Compared to fresh pollen, it is a more reliable and nourishing meal for honeybees [60,61]. Bee bread is very easy to use because it is highly soluble in water. Honeybee bread has a high probiotic value and a higher yield than normal bee pollen, and is another new health-oriented product [60]. There is not any proof that bee bread was overdosed; it is ingested as a food supplement, and an adult human's recommended daily intake is 20–40 g. Bee bread diet supplementation has been shown to have positive effects on both animals and people [62].

Bakour et al. studied the protective effect of bee bread against toxicity caused by titanium dioxide. It was found that the biochemical parameters (glucose level, lipid profile, liver enzymes and others) were changed upon administration of titanium dioxide nanoparticles. Co-treatment with bee bread resulted in restoring the normal levels of these biochemical parameters [63].

2.2. Secreted bee products

These products are synthesized through specialized glands of honeybees.

2.2.1. Beeswax

Beeswax is a complex compound secreted in liquid form by eight ceriferous glands (specialized wax glands) in the ventral part of young worker abdomen of (bees aged between 12–18 days). When it comes into contact with air, it hardens, and bees form the structure of a honeycomb with their jaws to store both honey and pollen. At the beginning of secretion, the pure beeswax is almost white; only after contact with honey and pollen does it assume a variably intense yellowish or golden color and turn dark brown after about four years, because it contains the cocoon [64,65].

The composition of the beeswax may vary among the different families and different breeds of bees, because it is probable that wax production is closely related to bee genetics and diet [66]. However, in general, beeswax is a mixture of more than 300 compounds, including complex wax esters (15–27 %), hydrocarbons (12–16 %), free fatty acids (12–14 %), hydroxyl-monoesters (35–45 %), fatty alcohol (~1 %), di-esters, and exogenous substances, such as residues of propolis, pollen, and small pieces of floral components [67].

Antiquity was aware of the therapeutic properties of beeswax. In his famed "Canon of Medicine," Avicenna mentions various medicine formulations that contain beeswax. Furthermore, archaeological indication of beeswax ointments dating back to the 16th century has been discovered [68].

Recently, Bees wax is used as natural lipid components to prepare nanostructured lipid carriers for different biological activities [69]. Also, beeswax has been widely used in cosmetic products. The durability of beeswax makes it a superior wax for skin care and cosmetic applications. Beeswax is also a fantastic product for finishes, polishes, and waxes that maintain, add sheen, and mostly improve objects covered with it due to its strength, flexibility, and water-proofing properties [70].

2.2.2. Royal jelly

Royal jelly is a viscous, milky-white or yellowish, creamy, acidic substance with a slightly pungent odor and taste. It is secreted from the hypo-pharyngeal and mandibular glands of young worker bees**.** Royal jelly is a superfood for the queen bee throughout her entire life cycle. Additionally, during the first two to three days of development, the young larvae, workers' brood, and drones are temporarily dependent on it for feeding [71].

Worker bees only survive for around 2 months, but life span of queens can reach up for over 4 years. It has been hypothesized that the nutritional supplements found in royal jelly is the reason for the difference between queens and worker bees life span. Although the exact mechanism is unknown, short-chain hydroxy fatty acids found in the proteins of royal jelly are also believed to show a significant role [71,72]. Royal jelly is believed to have an immunostimulatory effect, and therefore has been described as "a secret of life" or an elixir [73].

Royal jelly is used in two different forms, fresh or lyophilized. Many factors, including the method of production, seasonal variations, the way the bees are fed, and the type of bees, affect the composition of royal jelly [74]. The major components of royal jelly are water (60–70% w/w), proteins (9–18% w/w), sugars (7–18% w/w), and lipids (3–8% w/w). Also, there are other important components such as vitamins (A, B complex, C, and E), amino acids (Ile, Leu, Val, Thr, Phe, Met, Trp, and Lys), minerals (Ca, Fe, Mn, K, Na, Zn, Mg, and Cu), nucleotides, hormones, polyphenols, enzymes, and minor heterocyclic composites. The pH of royal jelly is 3.6 [75,76].

In addition to serving as a nutrient, the main royal jelly protein MRJP1 is a crucial component in bees' ability to learn, remember, and engage in social behaviours. Royal jelly contains fatty acids that rarely occur in other natural products. These fatty acids and proteins have wide biological properties such as antioxidants, neurodegenerative diseases and immunomodulatory effects [77–79]. Furthermore, the protein and lipid components of royal jelly have the ability to prolong the lives of a variety of organisms, including mice, honeybees, crickets, silkworms, and nematodes. They also have the ability to prevent the senescence of human tissues in cell cultures by up-regulating epidermal growth factor signaling and down-regulating of insulin-like growth factors [80].

Despite the wide range of biological activities of royal jell, it is particularly sensitive to light and temperature. Its color is changed and degradation of its component because of enzymatic, lipid-protein, and Maillard reactions. Its storage is a major problem as it oxidizes in contact with air. Royal jelly should be kept at a temperature of −20 °C [81].

To prevent its degradation, several techniques such as lyophilization, encapsulation and coating of nanoparticles are used [82]. For example, a new royal jelly delivery system was developed by **Spanidi et al., 2022.** The delicate components of royal jelly are shielded by the encapsulation. Royal jelly's stability was enhanced by the liposome/cyclodextrin suspension, which kept stable for 6 months. Additionally, the time-controlled release of 10-hydroxy-2-decenoic acid is made possible by the royal jelly encapsulating twofold in cyclodextrin/liposomes, which confirmed helpful for skin applications [83].

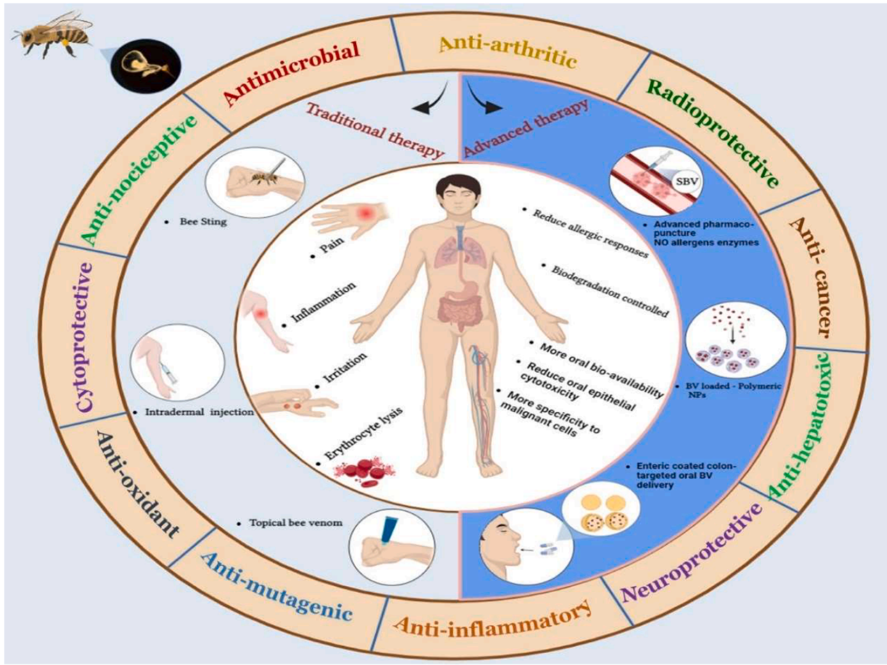

Fig. 2. Schematic representation of the biological activities of bee venom and its traditional and advanced application methods.

2.2.3. Bee venom

The bee venom medicinal effects were renewed by the Lubarski and Russians Lokumski, in 1868 under a book titled "Bee venom, a remedy" [18]. In recent years, honeybee venom is applied to treat patients with autoimmune and chronic disorders by physicians and certified api-therapists [84].

Bee venom (Apitoxin) is a complex, yellowish, bitter, pungent, and transparent acidic liquid secreted from two poisonous glands in the abdominal cavity and associated with the sting apparatus of bees. Its production increases during the first two weeks of their lives. It is a significant defensive tool used by honeybees. Through their stinger, only female bees are able to sting. Approximately 0.3 mg of bee venom is produced in the venom sac. The honeybee venom immediately crystallizes and dries when it comes into touch with the air. Several highly volatile chemicals included in bee venom are quickly lost during collection [85].

Bee venom comprises numerous active components, like peptides (including melittin, adolapin, and apamin) as well enzymes such as hyaluronidase and phospholipase A2, and a mast cell degranulating peptide in addition to non-peptide molecules, similar to histamine, norepinephrine and dopamine. Melittin, which represent about 50 % of the dry venom is main constituents followed by phospholipase A2 (PLA2), which makes up around 12 % [86,87].

2.2.3.1. Biological activity of bee venom. *In vitro* as well as *in vivo* studies have shown multiple diverse therapeutic effects of bee venom (Fig. 2) such as anti-arthritic [84], anti-mutagenic, anti-nociceptive, and radioprotective [88], anti-hepatotoxic, cytoprotective [89], anti-oxidant, anti-inflammatory [89,90], anti-microbial [90,91], anti-viral, neuroprotective [90], anti-metastatic, and anti-cancer effect [86,92].

2.2.3.2. Application therapy of bee venom

2.2.3.2.1. Traditional techniques in bee venom therapy.

i. **Bee sting therapy** Bee sting therapy (BST) or apipuncture is performed by licensed practitioners/ acupuncturists using a live bee. Practitioners hold a live bee with a tweezer and place it on affected area letting it to sting Allergy to bee venom should be tested before the procedure. Treatment should be gradual with 1–2 stings in the first session. Sting therapy is applied in direct and indirect ways.

In direct application, bee is held by head or thorax with tweezers and then put at the intended area. Stinger is removed within 1 min and these steps are repeated at different areas as needed. While, Indirect application involves removing the sting with forceps, and putting it on the body for few seconds. The same stinger can be used on 10 different spots.

ii. **Injectable bee venom therapy** Bee venom therapy (BVT) or use of Apis mellifera venom is a remedy applied intra-dermally. Subcutaneous injection of venom solution is allowed up to 2.0 mL even though intradermal injection is more efficient. Such injections must be regulated and monitored by authorized acupuncture physicians. Korean Food and Drug Administration approved first BVT in 2003 [93].

iii. **Topical bee venom therapy** BV is now used to heal a variety of skin diseases and used in cosmetic field as a topical preparation in ointment or cream forms. Due to its application on large skin areas, unfavorable effects can be observed in the form of skin reactions such as lesions and irritations. In some cases, these unfavorable effects may up to severe immunological responses, including anaphylaxis [94].

Toxic effects of traditional bee venom therapy

Safety of bee venom therapy remains a significant limiting issue [95]. The span of adverse effects is wide from mild skin irritation to life-threatening anaphylactic reaction. In 2015, a systematic review reported adverse events happened in 58 studies out of 145 studies including various skin irritation symptoms [96]. Other sever adverse reactions may occur (Fig. 2) [97].

This may be related to hypersensitivity [98], frequency and concentration of administered dose [95]. Pain and inflammation are common in sting and injection and this is due to melittin content [99]. Melittin produced pain in both animals and humans when injected subcutaneously [100]. Furthermore, lysis erythrocyte and human peripheral blood lymphocytes are also reported [101,102].

2.2.3.2.2. Advanced techniques in bee venom therapy. Bee venom can be beneficial in treating several diseases when used in appropriate doses.

However, honeybee stings, bee venom injections and topical bee venom therapy have many disadvantages. Therefore, the researchers turned to a solution that could overcome the disadvantages of bee venom use through the design of a safer and more appropriate sustainable launch system (Fig. 2).

i. **Advanced pharmaco-puncture method** Bee venom pharmacopuncture (BVP) is a novel treatment that combines the efficacy of acupuncture with the pharmacological actions of artificial venom. BVP has been applied in rheumatoid arthritis and spine disorders [103]. However, hypersensitivity is still a difficulty facing such treatment. Finally, the investigation revealed that a patient who received BV acupuncture experienced intravascular coagulation and died from hypovolemic shock, despite the fact that the patient had no history of medical illnesses, responses to bee stings, or allergies [104].

   Thus, another type of treatment was developed to reduce these allergic responses and solve this problem using sweet bee venom (SBV). Sweet bee venom is modified from bee venom via the removal of harmful substances like enzymes, known as allergens. The elimination of these allergens was done through a protein separation technique which resulted in melittin formation. Due to fewer allergic responses reported, SBV or melittin treatment is considered as effective as or better than BVP treatment [105]. In the same manner, **Ryu et al. (2022)** found that SBV has therapeutic activity against THP-1 monocytic leukemia cells in a concentration dependent mannar [106]. Interestingly, melittin that is extracted from BV significantly inhibited the proliferation of two types of cancer cells (Huh-7 and HCT 116). Nevertheless, melittin does not affect the viability of normal human lung Wi-38 cells [107].

ii. **Polymeric (NPs) bee venom therapy** Bee venom-loaded polymers have been suggested to improve its biological potentials and solve its problems. Biodegradable polymer nanoparticles such as chitosan, alginate, and poly-D, L-lactic-co-glycolic acid (PLGA) are effective carriers of bioactive composites. The delivery and release of bee venom can be enhanced by this way. Further, NPs degradation is controlled by modulating certain aspects to maintain sustained release, reduced frequency and improved patient compliance [108].

iii. **Oral enteric coated bee venom therapy** Oral route of administration can be good alternative to avoid injection pain (Fig. 3). However, bee venom is protein in nature, digested by gastrointestinal enzymes administration with subsequent very low oral bioavailability, where only 5.22 % of the bee venom is absorbed [90].

   For the first time in 2022, it has overcome this problem through the development of an optimized formulation from bee venom cross-linked chitosan enteric microspheres coated with Eudraget S 100 for cancer treatment. In this study, the coating protected bee venom from enzyme's action (Fig. 3). The authors concluded that the optimized microspheres formula compared to doxorubicin was more effective at inhibiting the proliferation of human prostate cancer (PC3) cells. Furthermore, microspheres protected normal oral epithelial cells against cytotoxic effect of bee venom revealing the specificity of microsphere to malignant cells. This indicated that targeted formulation could be promising option for cancer therapy [92].

   Bee venom has multiple therapeutic potentials. Some of these are discussed below.

2.2.3.3. *Therapeutic activity of bee venom.*

A. **Anticancer activity**

Many investigations have been conducted to evaluate the anticancer activity of bee venom. **Taher et al. (2017)** prepared a novel compound of chitosan nanoparticles loaded with bee venom for cancer treatment. They found that the loading of bee venom on chitosan NPs improved the efficacy against colon cancer cells proliferation indicated by lower IC_{50}. Also, data showed that chitosan nanoparticles recorded the lowest inhibitory effect on the viability of the normal Vero cell line compared to bee venom [109]. In the same regard, another study showed that chitosan NPs loaded with bee venom were more effective against hepatocellular carcinoma (HEPG2) with appreciable arrested cell cycles in the G2/M for HePG2 and PC3 [110]. In another study, **Alalawy et al. (2020)** observed that encapsulating bee venom in fungal-derived nano-chitosan improved anti-cancer activity against cervix carcinoma (HeLa) cells [111].

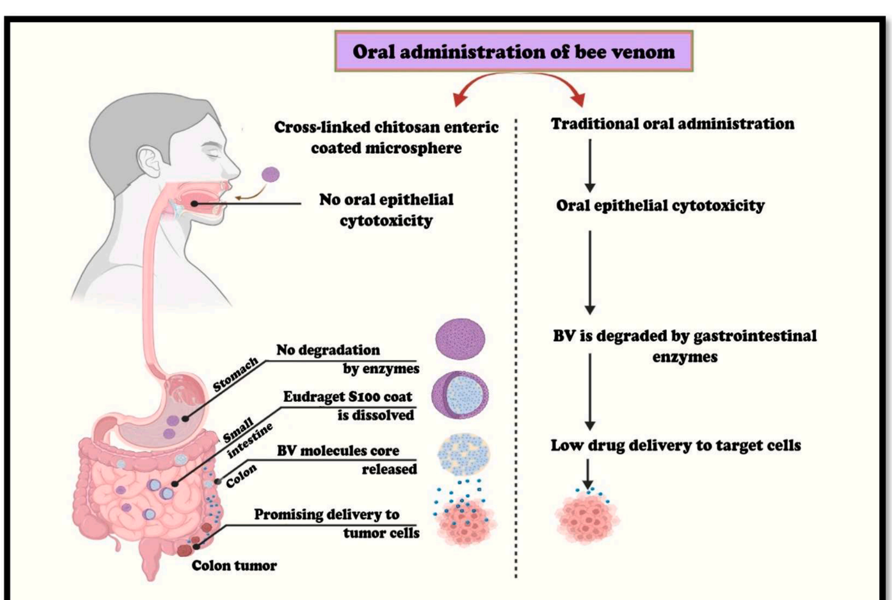

Fig. 3. Schematic representation of oral administration of bee venom before and after enteric coated technique.

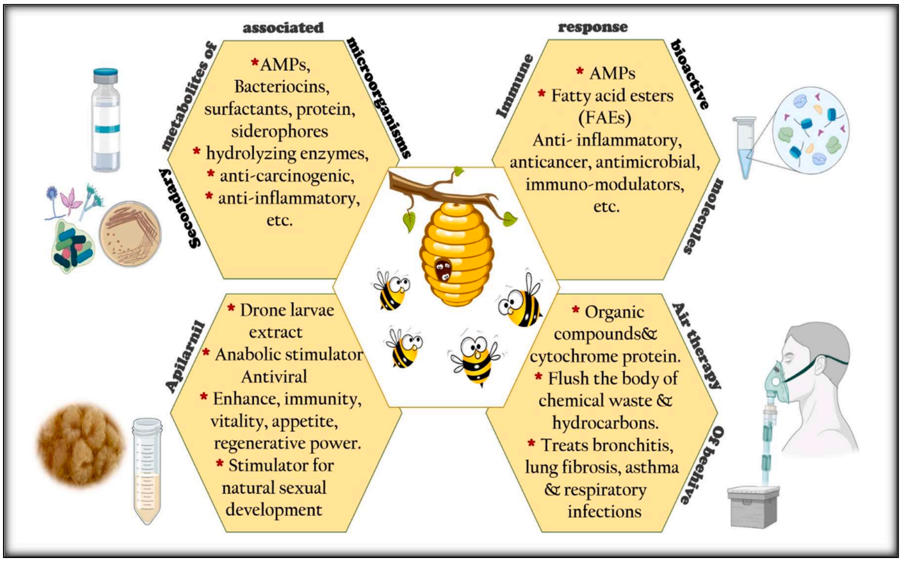

Fig. 4. Newly discovered honeybee products.

B. **Antiviral activity**

Based on research studies, bee venom destroys HIV, but the powerful toxin “melittin” of venom can cause considerable damage to normal cells or cell lysis [112]. The scientists showed that loading melittin on nanoparticles protect normal, healthy cells. Melittin kills HIV virus by attacking a vital part of its structure. It fuses with its envelope, which results in its rupture [113]. Thus, NPs loaded with bee venom have a destructive effect on HIV virus without harming surrounding cells [114].

Lee et al., investigated the antiviral activity of prepared bee venom loaded with chitosan/alginate NPs against virus of respiratory and porcine reproductive syndrome in pigs when administered via nasal route. The chitosan/alginate NPs with bee venom reduced viral burden significantly in sera and lung tissues. On both a microscopic and gross testing of the lungs, the interstitial pneumonia symptoms improved. Further, high PRRSV-specific IgG level was found. Accordingly, chitosan/alginate NPs loaded with bee venom could be a better solution than classical PRRSV vaccination as a defence mechanism against immune-suppressive viruses like PRRSV [115].

C. **Antifungal activity**

The antifungal activity of bee venom loaded on nanoparticles was developed in 2022, through the preparation of a novel agent, chitosan nanoparticles loaded with bee venom (BV-CNPs) to control human fungal pathogens. Kodamaea ohmeri, Cryptococcus neoformans, and Candida albicans were used to evaluate the antifungal activity of BV-CNPs against unicellular fungal pathogens. Loading bee venom onto chitosan nanoparticles led to an improvement in its activity against both C. neoformans and Candida albicans. It was found that it was more potent than fluconazole. Moreover, fluconazole had no inhibitory effect against fungus K. ohmeri. Further, BV-CNPs intimidated biofilm production and the yeast-to-hyphal transition formed by the tested unicellular fungal pathogens. This indicated that chitosan nanoparticles loaded with bee venom are an alternative antifungal agent designed for human fungal pathogen [91].

D. **Antibacterial activity**

Antibacterial action of bee venom-loaded nanoparticles is less studied. The antibacterial activity of bee venom is improved when loaded with nanoparticles. According to **Elnosary et al. (2023)**, when compared to Chitosan nanoparticles, free BV, and a conventional medication (tetracycline), synthesized bee venom loaded chitosan nanoparticles (BVCNPs) demonstrated better antibacterial efficacy against both Gram-positive and Gram-negative bacteria. The MIC were 250, 125, 500, & 500 μg/mL for BV, 62.5, 15.6, 500 & 500 μg/mL for ChNPs, 31.3, 15.6, 31.3 & 125 μg/mL for BV-ChNPs and 500 μg/mL for tetracycline against all tested strains, respectively [116].

3. Newly discovered bee products and approaches

Currently, bee therapy is being developed and is no longer limited to the use of bee products and bee acupuncture known from ancient times, but it now includes natural factors or substances related to beehives. Bees bring multiple products of nature to the hive, plus those that they produce from their own body. It is said that the hive is nature’s pharmacy, and many chemical processes take place inside it (Fig. 4).

3.1. *Apilarnil (Drone larvae)*

It was discovered by a very famous Romanian beekeeper, Mr. Nicolae Iliesiu, and the word "Apilarnil" comes from *Api*, the bee’s Latin name; Lar, larvae, nil comes from inventor’s name. Usually, drone larvae are not utilized in the beehive, and honeycombs, cut and discarded by beekeepers.

Apilarnil (Drone larvae) is a honeybee product that is not common in most countries. Apilarnil is a drone cell extract made from 3 to 7-day old drone larvae. Apilarnil extract is collected from drone larvae cells and then lyophilized [117].

Its biological value comes from beneficial ingredients that exist in the bodies of both eggs and larvae [118]. Apilarnil comprises 25–35 % dry matter, 9–12 % proteins, 6–10 % carbohydrates 5–8 % lipids, 2 % ash, and 3 % unknown compounds. [119] It has many biological functions, which include being anabolic stimulator, an antiviral, immune system enhancer, and enhancing appetite, vitality, energy of body, and regenerative power [120,121]. Moreover, apilarnil has high androgenic hormones content, and enhances spermatogenesis. Due to androgenic and anabolic effects, it is considered a natural alternative for sexual development stimulation [120]. **Bolatovna et al. (2015)** demonstrated that apilarnil increased seminal glands weight, germ cells density, and sperm motion when injected parenterally in young pigs [122]. Also, apilarnil induced the sexual maturation in male broilers at a young age [123].

Apilarnil has a catbolic action producing energy in the body [117]. Some studies revealed that apilarnil may prevent oxidative stress and hepatic injury in rats [124]. Also, apilarnil has antioxidant and

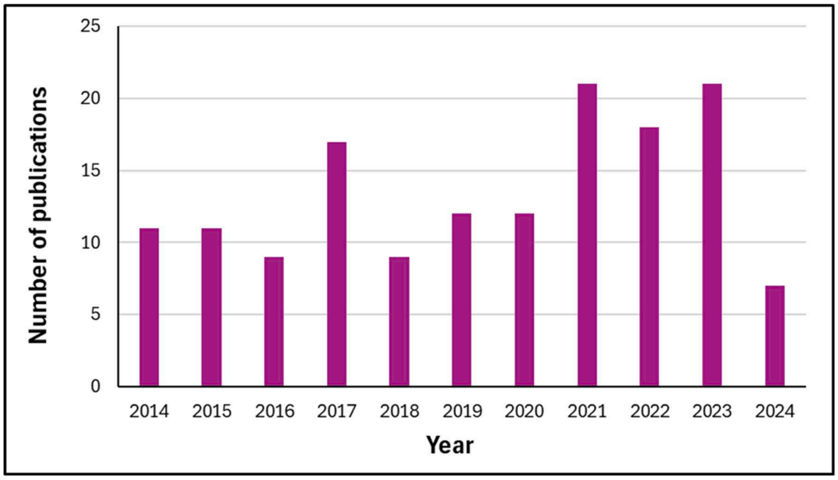

Fig. 5. Flowchart showing number of published articles on clinical studies of bee products for treatment of various diseases during the last 10 years.

antiradical activity due to its phenolic content [125]. Finally, complementary medicine uses Apilarnil for variety of reproduction diseases (ovarian dysfunction and male infertility), as well as other health problems (immune disorders, and children malnutrition [126].

3.2. Secondary metabolites of honeybees associated microorganisms

While outside foraging and returning with pollen and nectar to the colony, honeybee workers interact with ambient bacteria, leading in the development of *Apis mellifera*-related microorganisms [127]. A variety of helpful related fungi enable honeybees to make a number of biochemical contributions, including the enhancement, transformation, and storage of pollen in comb cells as bee bread [128]. These fungi produce short-chain fatty acids that provide bees with their main source of energy as well as neuroactive compounds for improved behavior and brain health [129]. Additionally, by degrading both pectin and lignin, the associated microbes produce a natural defense shield and could enhance the host honeybee's diet [130].

Bacteria (*Bacillus* sp. and *Enterobacteriaceae*), moulds (*Penicillium*, and various zygomycetes such as *Rhizopus* and *Mucor sp*.), and yeasts (*Torulopsis sp*.) belong to the microorganisms associated with honeybees [131,132]. Interestingly, Antimicrobial peptides, bacteriocins, surfactants, siderophores, protein hydrolyzing enzymes, and cell wall-destructing enzymes are examples of secondary metabolites produced by related microbes [133]. A number of chemical compounds with anti-carcinogenic and anti-inflammatory properties are also secreted by the related bacteria, and they are crucial for the function of immune system and the generation of a defense response [134]. Because of this, in recent years, scientists have tended to isolate these species, take advantage of their secondary metabolites, and use them as natural sources for the treatment of diseases.

In the study of **Kalaba et al.*,*** Mucor bainieri MK-Bee-2 was isolated from honeybees, Apis mellifera for the first time and identified morphologically and genetically. Secondary metabolites of isolated fungus showed antimicrobial activities against human pathogenic Gram-positive (S. aureus and B. cereus) and Gram-negative (E. coli, S. typhimurium and K. pneumonia) bacterial strains as well as Candida albican. The findings demonstrated that the extract possessed high antioxidant properties, as evidenced by robust suppression of both DPPH and ABTS free radicals. Additionally, it showed a remarkable potential anticancer activity through apoptosis against both human lung cancer cells (A549) with IC_{50}= 6.45 μg/ml, and human liver cancer (HepG2) cells with IC_{50}= 27.48 μg/ml. Furthermore, it showed less cytotoxic activity against normal cells, with higher IC_{50} values of 106.99 and 132.57 μg/ml against normal human lung fibroblasts (Wi-38) and oral epithelial cells (OEC), respectively. The *Mucor bainieri* MK-Bee-2 secondary metabolite was found to include bioactive chemicals that have the potential to be effective therapeutic candidates for the treatment of lung cancer, bacterial infections, and fungal infections in addition to being a robust antioxidant, according to these findings [135].

3.3. Beehive air therapy

Abd El-Wahed *et al.*, (2021) reported that the hive's air is rich in organic compounds and cytochrome protein, which can flush the body of chemical waste and hydrocarbons that have become trapped there. As a result, beehive air therapy is acknowledged as a viable treatment for respiratory conditions such bronchitis, lung fibrosis, asthma, and respiratory tract infections [136].

Currently, beehive air permitted in Germany, Austria, Slovenia, Hungary, and Slovenia. Both chemical and biological investigations are necessary as a proof of idea because there isn't enough scientific evidence to support its usefulness. Heinrich Huttner, an Austrian beekeeper, created a mechanism to breathe hive air for therapeutic purposes. The air current is separated from the bees in this method using a sieve, and the air is then extracted through a tube. [137,138]

The concept of using beehive air was inspired by research on a variety of volatile compounds released from apiaries as well as a Malaysian study that demonstrated beekeepers have a longer life expectancy than the general population.

Nasir et al. *(2015)* examined telomere length in 30 male beekeepers and 30 male non-beekeepers using Southern analysis of terminal restriction fragments (TRFs) produced by Hinf I/Rsa I digestion of human genomic DNA using the Telo TAGGG Telomere Length Assay and associated them with longevity of life. It's interesting to note that they discovered that the male beekeepers' telomere lengths were significantly longer than those of the male non-beekeepers' ($p < 0.05$), confirming that beekeepers live longer than non-beekeepers. Additionally, they discovered a correlation between telomere length and both frequent daily use of bee products and long-term consumption of bee products. Consuming bee products for an additional year is linked to a mean increase in telomere length. [139].

Through using solid phase micro-extraction (SPME) and gas chromatography-mass spectrometry (GC–MS), volatile compounds were extracted from the air of beehives. Six fatty acids, six alcohols, ten aldehydes, five esters, one ether, nine hydrocarbons, one phenol, seven ketones, one nitrogenous molecule, and ten terpenes have been discovered in the beehive air for a total of 56 volatile compounds. Short-chain fatty acids made up the majority of the contents (26.32 %), whereas nitrogenous compounds formed the least amount (0.82 %). Moreover, in an agar-well diffusion assay, beehive air and venom had the strongest antibacterial effects on multidrug-resistant *Staphylococcus* *aureus, Acinetobacter baumannii*, and *Klebsiella pneumoniae* [136]. However, there are many factors to consider before using beehive air for respiratory therapy.

3.4. Bioactive molecules secreted by honeybees as immune response

Honeybee's humoral and cellular immunity shape the innate immune system that together tackles many biotic and abiotic stressors [140]. Humoral responses indicate the secretion of different immune molecules such as antimicrobial peptides (AMPs) [141].

In the medical field, antimicrobial resistance is a rising crisis that needs critical solutions [142]. AMPs come to exhibit a solution for this crisis with advantages do not present with antibiotics therapy. AMPs have an immunomodulatory effect [143], and do not easily develop resistivity [144]. AMPs are indicated to have a special mode of action against a wide spectrum of infections and health-related challenges [145].

Four AMP species are detected in honeybees: apidaecin[146], abaecin[147], hymenoptaecin[148], and defensins. The defensins are represented by two peptides; defensin 1 and defensing 2 [149]. **Li et al. (2019)** confirmed a defensin-wide broad of antibacterial activity against Gram-positive and Gram-negative bacteria, and inhibiting MRSA with low cytotoxicity [150]. Also, defensins (α- and β-defensins) are included among the anti-HIV peptides (a subclass of anti-viral peptides) [151].

Other bioactive molecules that have been indicated in honeybees as a part of immunity defensive responses and in maintaining their homeostasis are free fatty acids (FFAs) [152]. Antimicrobial activities of methyl stearate and methyl palmitolate were detected [153]. Lauric acid was the most effective antibacterial saturated fatty acids. Recently, **Šamšulová et al. (2023)** confirmed the antibacterial of lauric acid against honeybee pathogen *Paenibacillus larvae*. [154] Also, **Elhoseny et al. (2024)** conducted a GC/MS to identify the bioactive components of honeybee larval hemolymph that render antimicrobial activities especially antifungal activity against tested *Candida albicans* and two other fungal honeybee pathogens. Data showed that antifungal activities were attributed to the presence of 9,12-Octadecadienoic acid, methyl ester (methyl linolelaidate), and ethyl oleate (methyl oleate) [155].

4. Clinical trials and the synergistic effect of bee products

Currently, apitherapy is used as complementary and integrative medicine in diverse regions of the world. Furthermore, the use of bee products as dietary supplements and nutraceuticals has grown because of their nutrients. People consume various quantities of bee products either occasionally or multiple times a day, and there are numerous recommendations for their use [156]. In the past few years, more research has been conducted on the pharmacological action of bee products, revealing a variety of biological activities. Studies conducted in *vivo* and in *vitro* have demonstrated that bee products are useful for treating a range of illnesses and maintaining homeostasis and balance in the body [27]. In addition, numerous studies and reports investigated the clinical trials of various bee products on human health. Honey has been utilized by people for wound healing all over the world. **Moghazy et al.,** carried out a study using an effective wound dressing containing honey for treatment of Thirty patients infected with diabetic foot ulcers. After 3 months a study confirmed that honey is a clinical and cost-effective dressing for diabetic injury [157]. In another study, oral application of honey 8 times per day reduced postoperative pain after tonsillectomy in 38 patients [158]. Honeybee bread is another new health-oriented product. Bee bread diet supplementation has been shown to have positive effects on people. A diet consisting of protein-filled pollen and bee bread is benefit to kids who deficient appetite and patients recovering from surgery, in whom malnutrition by strengthening their immune systems [60]. Also, royal jelly is useful in lowering premenstrual syndrome and one of the aims of royal jelly supplementation is to enhance the quality of lifespan in postmenopausal age. According to clinical research, taking women of reproductive age 1 g of royal jelly orally every day for two months can lessen the severity of premenstrual syndrome and enhance their quality of life[159]. Furthermore, sudden reduction of estrogen levels during menopause impacts the autonomic nerve system, resulting in the emergence of mood disorders (e.g., anxiety, sadness), headache, low back pain, or backache, as well as neurodegenerative diseases (e.g., Alzheimer disease). For three months, 42 postmenopausal women who reported experiencing anxiety, back discomfort, and low back pain were randomly assigned to receive 800 mg of dextrin or 800 mg of royal jelly treated with enzymes. Following this time, the royal jelly group's anxiety and backache scores were considerably lower than those of the placebo group. Also, no adverse reactions were noted when royal jelly was administered [160].

According to previous studies, special attention was given to clinical trials in the last few years. Consequently, the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) was used to search for the clinical studies of each bee product. The following keywords were evaluated: "honey clinical trials", "propolis clinical trials", "bee venom clinical trials", "bee pollen clinical trials", "bee bread clinical trials", "royal jelly clinical trials", "apilarnil clinical trials", "beeswax clinical trials" and "beehive air clinical trials". The criteria for selecting the published articles were only articles dealing with the clinical trials of each product were included. Other articles were discarded. The number of manuscripts published from 2014 until 2024 is shown in Fig. (5).

Many products derived from bees are well-known for their useful qualities, which include a wide range of biological activities such as antibacterial and antioxidant effects. However, investigation on these substances has until now tended to focus on them individually and independently of each other [88]. The combined use of several popular botanicals and honeybee products achieved much better pharmacological benefits than single uses [161,162].

Indeed, the biological efficacy of natural products is significantly improved by the synergistic interactions among their constituents. Therefore, in this study we focus on binary combination of honeybee products to investigate their synergistic biological activities. **Noori et al.,** reported that honey can be applied in combination with other bee products. Honey has synergistic effect against antibiotic resistant microorganisms (*E. coli, S. aureus*, and *C. albicans*) in single and polymicrobial culture when used with propolis [163]. In the same way, binary combination of honeybee products (honey, bee pollen, bee bread, bee wax, old wax comb, propolis, royal jelly, Drone brood homogenate, worker brood homogenate, queen brood homogenate, bee venom) were applied to test their antioxidant activity. Honeybee products exhibited more synergistic effects in most of the binary combinations [164]. Another study was carried out to assess the potential synergistic effect of three bee products (honey, propolis, and bee pollen) on the antibacterial activity against clinical strains of *Escherichia coli* and *Staphylococcus aureus* and the suppression of free radicals. It was shown that the biological activities were increased as a result of synergistic effect between these products [165]. Additionally, *Pseudomonas aeruginosa* was treated with honey and royal jelly. It was found that using of honey and royal in combination enhanced the effectiveness of their antibacterial potential [166]. On the other hand, no synergism was observed in the antibacterial and antioxidant actions when honey and propolis were applied against pathogenic bacteria (*Salmonella enterica, Yersinia enterocolitica, Staphylococcus aureus*, and *Listeria monocytogenes*) [167].

5. Currently practicing apitherapy (regulation & limitation)

Apitherapy has been used extensively throughout the world for millennia and is gaining more and more attention from bee scientists [168] and practitioners of complementary medicine [10] despite having its roots in the East, where it has been practiced since the beginning of time, its intellectual and scientific aspects didn't start to take hold in Europe until quite recently. The history of apitherapy in the USA dates back around 100 years, during which time it was used by many eminent medical professionals, including Dr. Bodog Beck, who began treating patients in New York City. Charles Marz, a beekeeper from Middlebury, Vermont, is the last surviving pupil of Dr. Beck. He was hailed as the "King of bee venom therapy" and is now the final living patient. Having been an apitherapist for more than 60 years, today, the application of venom via "bee venom therapy" is commonly used in the United States, and its development is promoted through research activities. Apitherapists believe that bee products can be utilized to treat a wide range of diseases. Although many people believe that natural goods are risk-free, using bee products in apitherapy can have unfavorable impacts. The negative effects of bee products range from minor reactions that go away to severe or catastrophic anaphylactic reactions when bee venom is involved. Therefore, this strategy requires professional skills. There is several organizations exist to regulate apitherapy on scientific outlines, as indicated by the Apimondia's proposed definition, International Federation of Beekeepers Associations. Bee therapy has recently been used in many nations., such as Brazil, the USA, China, Japan, Turkey, Switzerland, Croatia, and Bulgaria. Turkey has approved Apitherapy as a complementary/alternative medicine. Also, in Brazil, Apitherapy tends to be an expanding practice and has become part of the National Policy of Integrative and Complementary Practices (PNPIC– Ministry of Health, Regulation [156]. In Romania, medical doctors can get competences in Apitherapy following specialized training courses organized by the Ministry of Health. This is common for most of the Eastern European countries [169]. However, the use of bee products in traditional medicine is restricted to a few conditions where they have demonstrated outcomes that are comparable to or superior to those of normal therapies, such as in the treatment of burns and wounds and as an intriguing treatment for arthritis [4].

6. Conclusion and future perspective

Apitherapy dates to ancient times, but recently, it has received pronounced consideration from researchers and is carried out in several regions of the world. It is concluded that main products of bees including (Propolis, honey, bee pollen, bee bread, wax, bee venom and royal jelly) as well as their constituents have a diverse spectrum of biological and pharmaceutical actions. Despite documentation of the medicinal benefits of bee products, some products' safety is still a major challenge. In an attempt to improve the therapeutic effect of bee products, researchers developed optimized formulations to increase its effectiveness and reduce its systemic side effects. Several studies have found that combining bee products with conventional medications can result in achieving the same effectiveness with lower concentrations or doses of drugs and minimizing side effects as the drug alone. Additionally, bee products were formulated in different pharmaceutical formulations such as hydrogel, cream, nanofiber membranes and scaffolds to enhance product efficiency through its incorporation within these structures. Another consideration is the improvement and control release of bee venom and its component melittin through nano-formulations onto nanoparticle polymers. Finally, the utilization of beehive air for treating respiratory diseases and extraction of some important products such as apilarnil and secondary metabolites from bees associated microorganisms are novel emerging approaches in the field which represent a promising novel option for the treatment of diseases and enhanced healthcare. More study is needed to identify, find, and extract hidden bioactive compounds and their roles. More proof is also needed to support the efficacy, safety, precise quantity, and quality needed to deliver anticipated health benefits. Moreover, regulations are necessary for practicing such therapy to ensure patients' safety.

Ethics approval and consent to participate

There are no ethical issues with this paper

Consent for publication

Not applicable.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

The data presented in this study are available on request from the corresponding author

CRediT authorship contribution statement

Samia E. El-Didamony: Conceptualization, Visualization, Investigation, Writing – original draft, Writing – review & editing, Formal analysis, Data curation, Resources. **Hend I.A. Gouda:** Writing – review & editing, Software. **Mahmoud M.M. Zidan:** Writing – review & editing, Software. **Reham I. Amer:** Investigation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing 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.

Acknowledgments

Not applicable.

Data availability

Data will be made available on request.

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