

Ammi visnaga L. and Nanocarrier Approaches in the Treatment of Skin Diseases

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ABSTRACT: *Ammi visnaga* L. is a plant that grows naturally in Europe and is very common in Türkiye. It has various pharmacological effects due to its γ -pyrones, coumarins, flavonoids and essential oils content. And its major phytoconstituent khellin, which has a furanochrome structure obtained by extraction from the seeds of the *Ammi visnaga* L. is effective on the photochemotherapy of skin diseases. In this context, researches on topical delivery of khellin has been increased and the use of nanocarriers has gain attention to achieve optimised efficacy and stability related khellin, for the treatment of some skin diseases especially psoriasis and vitiligo. In this review, studies on the efficacy of khellin, which is the major phytoconstituent of *Ammi visnaga* L. in the treatment of skin diseases and topical delivery of it via nanocarrier systems has been overviewed to draw attention to the potential of herbal sources accompanied modern carrier systems in the treatment.

KEYWORDS: *Ammi visnaga* L.; herbal medicine; psoriasis; vitiligo; topical; nanocarrier systems.

1. INTRODUCTION

Ammi visnaga L. (Apiaceae, toothpick-plant, toothpickweed, hiltan) is a plant that grows naturally in North Africa, East Asia and Europe with a Mediterranean climate and is widely grown in Türkiye mainly on the coasts and very common in the parts of Thrace, Southeastern Anatolia and the Islands [1,2]. *Ammi visnaga* L. has various pharmacological effects on the treatment of some kidney diseases, vitiligo, hair loss and it has antispasmodic, antidiabetic, anti-inflammatory, antimutagenic, antimicrobial, antioxidant, vasodilator, cardiovascular and immunostimulatory activities. *Ammi visnaga* L. contains γ -pyrones, coumarins, flavonoids and essential oils. Phytoconstituents that can be obtained from *Ammi visnaga* L. are listed as follows [3-5]:

- γ -pyrones [furanochromone up to 4%; khellin (0.3-1.2%), visnagin (0.05-0.30%), khellinol, ammiol, khellol, coumarins (0.2-0.5%), visnadin (0.3%)],
- Fixed oils (up to 18%),
- Essential oil [2,2-dimethylbutanoic acid (30.1%), isobutyl isobutyrate (14.0%), croweacin (12.2%), linalool (12.1%), bornyl acetate (7.3%), thymol (6.0%), α -thujene (1.5%), 3-methylpentenol (2.5%), β -myrcene (0.1%), methylbutyl 2-methylbutanoate (1.2%), α -isophorone (3.8%), 2-nonyne (1.2%), hexenyl isobutanoate (1.6%), endo-fenchyl acetate (0.2%), geranyl acetate (1.2%), lavandulyl acetate (1.2%), citronellyl propionate (0.6%) neryl isobutanoate (0.1%), lavandulyl 2-methylbutanoate (0.1%), and α -damascone (0.1%)].

Khellin, which is in the structure of γ -pyrones, is one of the major components of the plant [6] has been extracted from *Ammi visnaga* L. seeds and it is active in photochemotherapy of skin diseases. The amounts of khellin and visnagin are 11.0 ± 0.03 mg/g and 5.8 ± 0.01 mg/g respectively in seeds of *Ammi visnaga* L. Also, there have been found to be 22.6 ± 0.06 mg/g and 18.5 ± 0.06 mg/g and 6.8 ± 0.14 mg/g and 13.2 ± 0.27 mg/g khellin and visnagin respectively in flowers and leaves of *Ammi visnaga* L. [4].

Khellin shows its activity via exposure to UV radiation (365 nm) by forming a molecular complex with DNA [7]. Khellin is used for the treatment of vitiligo, psoriasis, alopecia areata, and dyshidrotic eczema [8-9]. Psoralen-ultraviolet A (PUVA) photochemotherapy is used as the first-line treatment of these diseases [10].

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Although psoralen containing injectable solution and psoralen containing liquid filled oral capsule form are authorised and commercially available, khellin is considered to be a useful alternative in the treatment of these diseases, since it is similar in structure to psoralen, but has less phototoxicity and less effect on DNA mutation [8,11]. In this context, many studies have been carried out on the effectiveness of khellin as an alternative to psoralen (8-methoxy psoralen) in the treatment of skin diseases such as psoriasis, eczema, alopecia areata and vitiligo. Studies that directly evaluate *Ammi visnaga* L. extract in vitiligo treatment are limited, but owing to *Ammi visnaga* L. flowers contains 22.6 ± 0.06 mg/g of khellin, its extract could be assumed as a good alternative to 8-methoxy psoralen treatment in vitiligo.

Between the application routes to overcome first-pass metabolism, the topical application is preferable also to get rid off the side effects such as nausea and hepatotoxicity, which can be seen in systemic application. In this direction, many studies have been carried out to improve topical application of active substances to treat skin diseases [12]. For this aim drug delivery systems accompanied topical dosage forms has gain attention in recent years and various researches have been accomplished to reduce the side effects of active substances either originated from chemical or natural sources, increase the stability, safety and bioavailability [13,14]. In case of phytoconstituent delivery liposomes, herbosomes, phytosomes, transferosomes, ethosomes, micro and nano emulsions/particles/capsules have been used to increase absorption and stability [15,16]. Since nanocarriers are too small to be detected by an immune system, they can deliver a powerful weapon system and drug against many diseases to the target organ. Therefore, drug doses and side effects are greatly reduced in nanocarriers. Many studies have been carried out to improve the topical effects of khellin by using different drug carrier systems such as nanosomes, liposomes, nano-vesicles, nano-structured lipid carriers, halloysite nanotubes, hydrogels, gel emulsions, and microemulsions [17-22].

In this review, the studies on the delivery of khellin the major phytoactive constituent of *Ammi visnaga* L. via nanocarrier systems are summarized and presented in order to draw attention to the studies on the treatment of various skin diseases.

2. KHELLIN AND SKIN DISEASES

Most of the studies on khellin in the literature are exist for the treatment of vitiligo. Vitiligo is a very common dermatological disease that is seen in 0.5-2% of the world population and can cause serious psychological problems [12,23]. Vitiligo results from a dynamic interaction between genetic and environmental risks that initiates an autoimmune attack on melanocytes in the skin. Melasma is a general condition of hyperpigmentation and specifically affects the face. Vitiligo (hypopigmentation or depigmentation) and melasma (hypermelanosis) are very common among pigmentation disorders [24]. Current vitiligo treatments used off-label are non-targeted immunosuppressants with only moderate efficacy. Developing safe and effective treatments requires a better understanding of disease pathogenesis to identify new therapeutic targets. In the pathogenesis of the disease, autoreactive cytotoxic T cells cause melanocyte loss and thus skin depigmentation [25]. Khellin shows its activity on vitiligo by acting on melanocytes in the bulge region of hair follicles [26]. In a study carried out in 2002, it was found that these melanocytes differentiate and migrate to the epidermis, forming the main source of melanocytes in the epidermis [27]. Khellin not only stimulates melanogenesis, but also allows melanocytes which are unaffected by vitiligo to migrate to the epidermis and proliferate. Therefore, studies have shown that khellin is very effective in the treatment of vitiligo by repairing the loss of melanocytes and melanin underlying the disease [26,28]. In the 1980s, the effect of khellin in the oral treatment of vitiligo was demonstrated and very successful results were obtained [29,30]. Thereupon, topical conventional formulations of khellin were prepared in the 1990s and its effectiveness in the treatment of vitiligo was investigated. However, positive results could not be obtained with khellin formulations applied in the forms of solution and cream [31,32].

Dyshidrotic eczema is a skin disease characterized by itchy, tight, deeply located vesicles on the palms and lateral surfaces of the fingers [33]. PUVA therapy is widely used against this disease. As an alternative to PUVA therapy, the effectiveness of topical application of khellin in dyshidrotic eczema was investigated in 2005. In this study, remarkable results were obtained and no side effects were observed [8].

Alopecia areata is an autoimmune disease characterized by the loss of scalp and body hair [34]. In 1993, a study was conducted against this disease using the combination of khellin and UVA for the first time. 10 patients were treated with a topical khellin solution applied to the area 1 hour before UV radiation. While complete hair growth was achieved in 50% of the patients, mild hair growth occurred in 30% and no response was obtained in 20% of the patients [9]. In another study, the effectiveness of narrowband UVB therapy and khellin-UVA (KUVA) therapy against alopecia areata was compared. After a 24-week treatment period, 57.89% positive response was obtained with KUVA treatment, while 10.52% positive response was obtained with narrowband UVB treatment. This shows that KUVA treatment responds better [34]. In 2017, the efficacy

and safety of topical khellin in combination with 308-nm excimer lamp (EL) in the treatment of alopecia areata was evaluated in a 5-year-old boy. Hair regrowth was observed after 1 year without recurrence. No significant side effects were detected except for a transient erythema [17]. This study showed that topical KUVA therapy may be safe for use in children.

Psoriasis is a common, chronic and non-contagious skin disease of unknown cause and treatment, which negatively affects quality of life. According to the report of the World Health Organization published in 2016, the prevalence of psoriasis varies between 0.09% and 11.43%, depending on the country, and it is stated that more than 100 million people worldwide are affected by psoriasis [35]. This disease is characterized by the formation of red, scaly, raised plaques as a result of epidermal hyperproliferation and abnormal keratinocyte differentiation [36,37]. Due to the unpleasant appearance of these plaques on the skin, depression and anxiety disorders are frequently encountered in psoriasis patients [38]. There are some studies in the literature about the effectiveness of khellin in the treatment of psoriasis.

In a study carried out in 1983, the effect of orally administered khellin on psoriasis was investigated in 10 patients. Positive results were observed in a total of 8 patients after 4 months of oral khellin and sunlight application [29]. On the other hand, oral administration of khellin has systemic side effects such as nausea and elevated liver enzymes.

70-80% of psoriasis patients have limited/localized disease and the disease is only treated topically. Therefore, topical therapy is the most common way of treatment used in the vast majority of patients and effective topical application is of great importance. In addition, it can be used in combination in resistant lesions in patients receiving topical therapy, phototherapy or systemic treatment. Especially in patients with skin lesions located on less than 5% of the body surface area, treatment with effective and safe topical drugs that directly target the lesion comes into prominence [39]. In the psoriatic stratum corneum, the lipid structure of the skin is disrupted and an abnormal increase in epidermal proliferation is observed. It has been reported that an increase in cholesterol levels and a decrease in ceramide levels cause skin thickening. This causes a significant decrease in the barrier function of the skin in psoriasis [12,40]. After years of use of coal tar, dithranol and salicylic acid, the current first-line drugs in the topical treatment of psoriasis are corticosteroids, vitamin D analogues, corticosteroid/vitamin D analogue combined preparation, tazarotene, tacrolimus and pimecrolimus [39]. In a study, gel formulations of khellin were prepared to investigate the effectiveness of topical khellin in psoriasis and it was observed that the release of the active substance from the gel carrier system and its penetration into the skin were not sufficient [11]. Also, khellin has a low solubility in water (0.025% w/v) and has a Log P \approx 3. Therefore, there is a need to develop nanocarrier systems in which drug release properties and drug penetration into the skin are improved so that therapeutic concentrations of khellin can reach the deep layers [41].

3. KHELLIN AND NANOCARRIER SYSTEMS

There are studies on traditional medicinal uses, homeopathic preparations and modern delivery systems of *Ammi visnaga* L. for the treatment of psoriasis and vitiligo [42,43]. In recent years especially the studies on the modern topical nano drug delivery systems of khellin have been increased in the treatment of some skin diseases [44]. Khellin included nanocarrier systems treatment for skin diseases are given in Table 1.

A phytosome approach to *Ammi visnaga* L. extract has shown increase in the bioavailability and stability of the herbal sources in case of topical delivery [16,45]. Nanosomes, also known as nanoscale liposomes, are microscopic vesicles composed of a single or multiple lipid bilayer capable of entrapping active pharmaceutical substances. These phospholipid nanosomes have the advantages of being similar in structure to the cell membrane, being non-toxic, selectively delivering drugs to their target sites, and being biodegradable [46]. Fennische *et al.* [17] evaluated the safety and effectiveness of a 1-year treatment of vitiligo with the combination of a 308-nm EL and khellin in nanosomes. The results show that this treatment improves the clinical outcomes of patients with resistant vitiligo.

Studies with liposomes, which are a type of nanosomes, have also been carried out with khellin. Phosphatidylcholine-based liposomes are thought to have a high potential to selectively target hair follicles. In 2003, the efficacy of khellin encapsulated in phosphatidylcholine liposomes and a UVA/UVB combination (KLUV combination) was investigated in an open-label study. After a mean treatment period of 12 months, 50% to 100% repigmentation was observed in 72% of the treated areas. The control group treated with only UVA/UVB showed almost no repigmentation [47]. These results demonstrate that khellin liposomes may be effective in the treatment of vitiligo. As a follow-up to this study, a new treatment combination was investigated in 2010 in 19 patients who did not respond to KLUV treatment. In this study, KLUV treatment was applied after transplantation treatment. 75% of patients stated that they were satisfied with the results of this treatment combination [18].

Nanostructured lipid carriers (NLC) is a carrier system in which partially crystalline lipid particles with an average diameter of ≤ 100 nm are dispersed in an aqueous phase containing an emulsifier [48]. NLCs are considered as potential drug carriers due to their biocompatibility, high drug loading capacity, fast drug release and superior formulation properties [49]. In a study in 2021 [50], it was shown that khellin-loaded NLCs have good biopharmaceutical properties for oral administration and increase the bioavailability and therapeutic effects of khellin.

Halloysite is a natural, nano-sized, tubular, porous clay mineral. It has advantages such as high biocompatibility, low cytotoxicity and low cost, which promise its safe use in various fields [51]. It also has the ability to encapsulate active substances within the core lumen and within any space found in the multi-layered walls of the cylinder. In a study in 2001 [52], the release properties of tetracycline HCl, khellin and nicotinamide adenine dinucleotide substances from halloysite were investigated *in vitro*. In this study, the release rate of khellin was found to be very slow. Too slow release rate of khellin from halloysite is a result of both its low water solubility and the fact that it remains as a hydrophobic solid under experimental conditions. It can be understood from this study that khellin loaded halloysites are not suitable for the treatment of vitiligo. On the other hand, in the study of Lisuzzo *et al.* [20], it was shown that halloysite nanotubes covered with chitosan layers could be effective as drug delivery systems. Electrostatic interactions occur between the outer surface of the halloysite and the cationic chitosan and an adjustable drug release system can be obtained due to these electrostatic interactions. As a result by loading khellin in this system, a controlled release system could be prepared.

Nanovesicles are advantageous carriers for drug solubility, skin penetration and release control. In another study, khellin was loaded into phosphatidylcholine-based ascorbic acid nanovesicles called ascosomes. This prepared formulation showed narrow size distribution, sufficient encapsulation efficiency and long-term stability. Therefore, it is suggested a suitable formulation for dermatological use [41]. In recent years, Risaliti *et al.* [19] carried out another study to improve the formulation properties of ascosomes. Since the ascosome formulation is liquid, its residence time in the skin is short and it carries the risk of forming aggregation. To overcome this, hydroxyethyl cellulose hydrogel was used as a carrier of ascosome vesicles. Although it is an advantage that the hydrogel is easily controllable, it is not a suitable carrier system for hydrophobic substances such as khellin due to its hydrophilic character [53]. However, due to the ascosome-hydrogel combination, khellin could be kept homogeneously in the hydrogels, the residence time of the ascosome in the skin was extended, the absorption of khellin from the skin could be improved, and sustained drug release and optimized bioavailability could be achieved [19].

In another study in 2020 [21], gel/oil emulsions of khellin were prepared and their *in vitro* drug release and skin penetration properties were investigated. Gel emulsion can be defined as an emulsion with gel-like network structure and mechanical properties similar to viscoelastic solid [54]. *In vitro* skin penetration studies of gel/oil emulsions of khellin showed that approximately 1% of khellin permeated through stratum corneum. Considering this value, it was suggested that gel/oil emulsion of khellin could be suitable for preclinical and clinical studies.

Microemulgel is a topical drug delivery system that incorporates the properties of both gel and microemulsion and displays a dual controlled release system. These systems have several advantages such as allowing hydrophobic drugs to be dispersed in the gel, having a large loading capacity, and controlled release [55,56]. In 2022, a khellin-loaded microemulgel formulation was developed for dermatological applications. Khellin was loaded into the oil phase of the microemulsion which was stabilized in the hydrogel. Improved skin penetration and prolonged controlled release were achieved with this combination [22].

In addition to these studies, in future studies, khellin can also be loaded into other nanocarrier systems such as nanoparticles, nanogels and nanofibers. Nanoparticles are stable nanocarriers that can be modified for targeting. Since they have a large surface area, their drug carrying capacity is quite high. Due to their ability to improve solubility, nanoparticles are ideal carrier systems for both hydrophobic and hydrophilic drugs. Particularly targeted smart nanoparticles can reach and accumulate high amounts deep in the hair follicle [57]. According to a study, the residence time of nanoparticles in the hair follicle is 10 times longer than the residence time in the stratum corneum [58]. Nanoparticles have the ability to reach deep areas of the hair follicle and accumulate there, collect in the follicular opening for a long time and penetrate through the follicular canal [57,59-61]. This shows that nanoparticles can be ideal carrier systems to deliver the khellin to its target area in the skin.

Nanogels are nano drug carrier systems consisting of cross-linked polymer networks and hydrogels. They are very advantageous due to being flexible, biocompatible and stable, having a long residence time in the skin, being able to easily penetrate tissues and having a high drug carrying capacity. They can significantly reduce the side effects of drugs and increase their therapeutic efficacy due to their controlled and targeted

release of drugs [62,63]. Thus, it could be expected that nanogel formulations may release the khellin over time, reach the lower layers of the skin and have a therapeutic effect.

Another innovative nano drug delivery system is nanofibers. Nanofibers are drug delivery systems that are nanosized, have a high surface area/volume ratio and porosity, and mimic the extracellular matrix structure of the skin [64]. Nanofibers, which have been widely used in topical/transdermal drug delivery and tissue engineering in recent years, have many advantages. It can be used for the controlled release of both hydrophilic and lipophilic drugs, and drug release rate can be controlled by nanofiber morphology, porosity and composition. Other advantages of nanofibers are that they have a high surface area/volume ratio and porosity, have a structure similar to the extracellular matrix of the skin, and their production methods are less costly and simpler compared to many nano-structured drug delivery systems. The morphology of nanofibers prepared from biocompatible natural (collagen, gelatin, alginate, chitosan, etc.) or synthetic (polyglycolide, polycaprolactone, polyvinyl alcohol, etc.) polymers or their mixtures can be changed depending on the parameters of the polymer solution and the electrospinning process. In addition, nanofibers are very advantageous because they are flexible, biocompatible and stable, have a long residence time in the skin, easily penetrate tissues and have a high drug loading capacity. They can significantly reduce the side effects of drugs and increase their therapeutic efficacy due to their controlled and targeted release of drugs [64-69]. By formulating khellin, which has a highly hydrophobic structure, into nanofiber carrier systems, its effectiveness on the skin will increase. Considering the advantages of these systems and the positive results obtained from innovative drug delivery systems in other studies, promising results of *Ammi visnaga* L. plant in the treatment of skin diseases can be predicted.

Table 1. Khellin included nanocarrier systems treatment for skin diseases.

Disease	Nanocarrier System	Reference
vitiligo	nanosome	[17]
vitiligo	liposome	[47]
vitiligo	halloysite	[52]
vitiligo	gel in oil emulsion	[21]
skin diseases	microemulgel	[22]
skin diseases	ascosome-hydrogel	[19]

4. CONCLUSION

Ammi visnaga L. is a plant widely grown in Europe and Turkey. Khellin, one of the major compounds contained in this plant, is used in many skin diseases. These diseases include psoriasis, eczema, alopecia areata and vitiligo. KUVA (khellin + UVA) therapy can be used as an alternative to PUVA (psoralen + UVA) therapy, which is used in these diseases and has many side effects. Oral and topical formulations of khellin have been developed since the 1980s. Topical application is mostly preferred due to systemic side effects and toxicities that may occur in oral administration. Innovative drug systems have been developed and improved effects have been achieved, as inadequate results were achieved with conventional topical formulations such as solutions and creams. Nanosomes, liposomes, nano-vesicles, nano-structured lipid carriers, halloysite nanotubes, hydrogels, gel emulsions and microemulsions can be given as examples of the systems developed. In addition, it is predicted that other nanocarrier systems such as nanoparticles, nanogels and nanofibers will be promising systems for future studies.

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