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PHYTOCHEMISTRY AND MEDICINAL PROPERTIES OF *AMMI VISNAGA* (APIACAE)

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Abstract

Ammi visnaga (bisnaga, toothpick weed or khella) belongs to the family *Apiaceae* and it is a herbaceous medicinal plant. It is found mainly in the Mediterranean regions and also distributed abundantly throughout the world as introduced species. Many times, *A. visnaga* is weed as well as used in many countries as herbal medicine for different purposes. Ancient records reveal various medicinal properties of *A. visnaga* as a popular source to cure variety of different ailments. The plant is used directly as a herb or as a component for production of a number of herbal medicines used in the cure of renal colic, ureteric stones, angina pectoris, the coronary vessels, cardiovascular disorders and asthma. Also it is used as a folk medicine for vitiligo and psoriasis. This review highlights the commonly recognized medicinal uses of *A. visnaga*, its chemistry and ethnobotanical uses and will also serve as ready reference for future research.

Introduction

There is great reliance on the curative properties of plants, besides great progress in synthetic drug production. Herbal medicines are obtained from different plants including weeds and used since ancient times for general health as well as specific diseases (Ali *et al*., 2012; Hashim *et al*., 2014). In developing countries, a large number of people depends on the traditional system of medicine and according to World Health Organization, 80% of the people living in rural areas depend on medicinal herbs as primary healthcare system. An estimated 35,000 to 70,000 plant species are used for medicinal purposes in the world (Akerele, 1992; Padulosi *et al*., 2002). Birds and animals are no exception (Khan, 2009; Javed *et al*., 2012; Khushdil *et al*., 2012). However, care should be taken as the products derived from herbs (herbal medicines) do not differ greatly from conventional drugs in terms of their mechanism and they are as effective as conventional medicines; this gives them the same potential to cause harmful side effects (Lai & Roy, 2004; Tapsell *et al*., 2006).

Ammi visnaga L. is known by many common names, including bisnaga, toothpick weed, and khella. It is a member of the *Apiaceae* (*Umbelliferae*) family; widely distributed in Europe, Asia, and North Africa and also throughout the world as introduced specie. The *Apiaceae* family (also commonly called parsley family) comprises about 300 to 455 genera and around 3000 to 3750 species (Rechinger, 1972; Heywood, 1999). Economically important vegetables (for example, carrot, parsnip, celery) and condiments (for example, coriander, anise, caraway, cumin, parsley and dill) are members of this family. Accordingly, species of *Apiaceae* family are well known for their economic importance and diversity of essential oils (Hegnauer, 1971, 1973).

The genus *Ammi* contains 6 accepted species according to The Plant List (Anon., 2010). The fruit extract of *A. visnaga* is a known herbal medicine and used in the treatment of coronary diseases and bronchial asthma, therefore, also an important raw material for pharmaceutical industry (Sitting, 1988; Kleeman *et al*., 1999). The medicinal proprieties of *A. visnaga* against coronary diseases and bronchial asthma are attributed to its essential oils (Rose & Hulburd, 1992; Satrani *et al*., 2004).

Hence, *A. visnaga* is commercially cultivated in many countries for its remedial properties (diaphoretic, carminative, antispasmodic and antiseptic). As, it contains a number of important chemical constituents, it is of immense importance to review the uses of *A. visnaga* for different purposes taking into account recent scientific findings.

Habitat: *A. visnaga* has its origins in the warm climate of the Mediterranean. It is also an indigenous plant of North Africa, West Asia, and a great part of the European Mediterranean (Tutin, 1968, Pignatti, 1982, Batanouny *et al*., 1999, Chevallier, 1996, Bueno *et al*., 2006). As naturalized specie, *A. visnaga* appears in North America, Argentina, Chile, Mexico, South-West Asia and some Atlantic islands (Kenner & Requena, 2001). Further, it is cultivated in North America, Mexico, Argentina, Chile, Egypt, Islamic Republic of Iran, Tunisia and Russian Federation (Zargari, 1989; Bisset, 1994). In Pakistan, *A. visnaga* was introduced as a medicinal plant (Fig. 1), which has become an invasive weed of cultivated fields (Marwat *et al*., 2010).

Botanical description: *A. visnaga* is an annual or biennial herb growing from a taproot erect to a maximum height of about 1.0 m. The root is fattened and looks like the root of the carrot. Leaves are up to 20 cm long and generally oval to triangular in shape but dissected into many small linear to lance-shaped segments. Stems are erect and highly branched. The inflorescence is a compound umbel of white flowers and highly swollen at the base, later on it becomes woody and used as toothpicks. Flowers are pentamerous, tetracyclic with radial symmetry, bearing five stamens and inferior ovary composed from two united carpels. The fruit is a compressed oval-shaped structure consisting of two mericarpes and around 3 mm in length (Tutin, 1968; Pignatti, 1982).

Fig. 1. *A. visnaga* infesting a sugarcane field. Inset shows the inflorescence of *A. visnaga* .

Ethnobotany of *A. visnaga*: *A. visnaga* has been commonly used for colic and gastrointestinal cramps, kidney stones and painful menstruation (Bisset, 1994). Also, *A. visnaga* is used in the treatment of mild angina. Further, it is used as a supportive treatment in the respiratory conditions such as asthma, bronchitis, cough and whooping cough (Farnsworth, 2001; Anon., 2007). *A. visnaga* is also used in the cardiovascular disorders for example hypertension, cardiac arrhythmias, congestive heart failure, atherosclerosis and hypercholesterolemia (Rose & Hulburd, 1992; Satrani *et al*., 2004). Moreover, it is used as diuretic and for relieving liver and gall bladder disorders (Anon., 2007). When applied topically, *A. visnaga* has been found useful in the recovery of vitiligo, psoriasis, wound healing, inflammation conditions, and poisonous bites (Abdelfattah *et al*., 1983; Valkova *et al*., 2004).

Phytochemistry: Studies on the phytochemistry of *A. visnaga* has revealed the presence of diverse groups of chemical constituents such as pyrones, saponins, flavonoids and essential oils (Martelli *et al*., 1984; Eldomiaty, 1992, Anon., 2007). The quantities and presence of these important metabolites depend on the parts of the plant analyzed. Further, growing conditions and the application of different bio-regulators also affect the profile of metabolites in *A. visnaga* (Talaat *et al*., 2013; Sellami *et al*., 2013). The major constituents of *A. visnaga* are γ-pyrones (furanochromone derivatives), which are up to 4%. Among the γ-pyrones, khellin (0.3-1.2%) and visnagin (0.05-0.30%) are the major ones. Khellinol, ammiol, visammiol, khellol, khellinin, khellinone, visnaginone are other important γ-pyrones. Coumarins (0.2-0.5%) is another important group of major constituents, the main one being the pyranocoumarins/visnagans (0.2-0.5%) comprising mainly of visnadin, samidin and dihydrosamidin. Coumarins also include furanocomarines (xanthotoxin and ammoidin) only in trace amount (Abou-Mustafa, 1990; Martelli *et al*., 1984; Eldomiaty 1992; Zgorka *et al*., 1998).

Two flavonols (quercetin and kaempferol) were identified in *A. visnaga* growing in Iraq (Abdul-Jalil *et al*., 2010). Eleven flavonols were isolated from the aerial parts of *A. visnaga* (Bencheraiet *et al*., 2011). There were four aglycones, four monoglycosides, two diglycosides and one triglycoside. Among the aglycones flavonoids, one was hydroxylated (quercetin) and three methoxylated (rhamnetin, isorhamnetin and rhamnazin). The monoglycosides were actually modified rhamnetin, isorhamnetin and rhamnazin with 3-O-glucosides and one 7-O-glucoside of isorhamnetin. The two diglycosides were 3-O-rutin of quercetin and isorhamnetin while the single trioside was quercetin 7,3,3'-O-triglucoside (Bencheraiet *et al*., 2011).

Very few studies have reported the analysis of the essential oils of *A. visnaga* (Günaydin & Neslihan, 2004; Khadhri *et al*., 2001; Khalfallah *et al*., 2011). The main compounds reported in the essential oils of *A. visnaga* from Marocoo were amyl isobutyrate (16%), linalool

(22.7%), methyl-2-isoamyl butyrate (27.7%) and amyl valerate (~10%). Forty-one constituents were identified in the essential oils of *A. visnaga* fruits, collected from Ichkeul and Djebba, the North of Tunisia (Khadhri *et al*., 2011). The essential oils from both samples were having high percentages of non-terpene esters (43.3 to 49.1%) and oxygenated monoterpenes (38.5 to 39.1%). The major constituents found in both samples were linalool (23.6 and 32%), isoamyl 2-methyl butyrate (24.2 and 36%) and isopentyl isovalerate (10 and 14.8%). Nonterpene esters were also present in a relative high abundance in both sample oils (Khadhri *et al*., 2011).

The GC/MS analysis of essential oils of fresh aerial parts of *A. visnaga* from Constantine, Algeria revealed the presence of twenty one compounds (Khalfallah *et al*., 2011). These compounds were characterized representing 97.3% of the essential oil with 2,2-dimethylbutaoic acid (30.1%), isobutyrate (14.0%), croweacin (12.2%), linalool (12.1%), bornyl acetate (7.3%) and thymol (6.0%). The composition of essential oil from Constantine sample was different compared to the essential oil isolated from the seeds of *A. visnaga* growing in Morocco (Lamiri *et al*., 2001), mainly containing Linalool (70.1%) and pentylmethylbutanoate (4.3%). The essential oil from Turkish origin (Günaydin and Neslihan, 2004) was characterized by high content of nerol (29.98%) and bisabolol (20.86%).

Differences in the chemical constituents of essential oils of *A. visnaga* and their respective percent amounts can possibly be explained by the differences in biotypes and geographic origins. Different constituent compositions are also attributed to variations in environment such as soil type, solar radiations and insect pest. These factors might lead to the activation/inactivation of certain enzymatic groups resulting in the up-regulation/down-regulation of certain biosynthetic pathways (Satrani *et al*., 2004).

Biological activities of extracts and constituents of *A. visnaga*: Centuries ago, Arabs discovered that the seeds of *A. visnaga* could relieve a number of ailments, such as the acute pain caused by a reduction in the flow of blood to heart. Further, *A. visnaga* is considered antiasthmatic, diuretic, vasodilator and an effective muscle relaxant. From ancient times, it has been used to alleviate the severe pain of kidney stones (Chevallier, 1996). The seeds of *A. visnaga* contain khellin, the chemical constituent considered as a selective coronary vasodilator and also used in the treatment of asthma. Further, both the extract and constituents of *A. visnaga* have antispasmodic action and also dilate bronchial, urinary and blood vessels without affecting blood pressure (Bown, 1995). Essential oil of *A. visnaga* is well-known for its efficacy against coronary diseases and bronchial asthma (Rose & Hulburd, 1992; Satrani *et al*., 2004).

Cardiovascular diseases: The seeds of *A. visnaga* are known to relieve the severe pain caused by a reduction in the flow of blood to heart. These properties of *A. visnaga* are attributed to its γ-pyrone constituents. The three constituents, visnadin, visnagin, and khellin, all are considered to have cardiovascular effects mainly because of their calcium channel blocking activities (Rauwald *et al*., 1994; Fetrow & Avila, 1999). Visnadin was found the most active when used in *In vitro* experiments (Martindale, 1999). It inhibited vascular smooth muscle contraction and caused the dilatation of peripheral and coronary vessels and an increase in coronary circulation (Duarte, 2000).

Visnagin, also exhibited peripheral and coronary vasodilator activities and has been used for the treatment of angina pectoris as it caused non-specific inhibition of vascular smooth muscle contractility (Durate *et al*., 1995). Further, visnagin has negative inotropic and chronotropic effects and helps in reduction of peripheral vascular resistance (Schindler, 1953; Duarte *et al*., 2000).

Khellin and visnagin both are capable of inhibiting the spasms, indicating an involvement of a calcium channel-blocking mode of action (Rauwald *et al*., 1994). Further khellin increased HDL-cholesterol in normolipaemic subjects (Harvengt *et al*., l983). Therefore, khellin also acts as a vasodilator and has bronchodilatory and spasmolytic activity (Duarte *et al*., 1997). Chemical structures of the major compounds of *A. visnaga* are shown in Fig. 2.

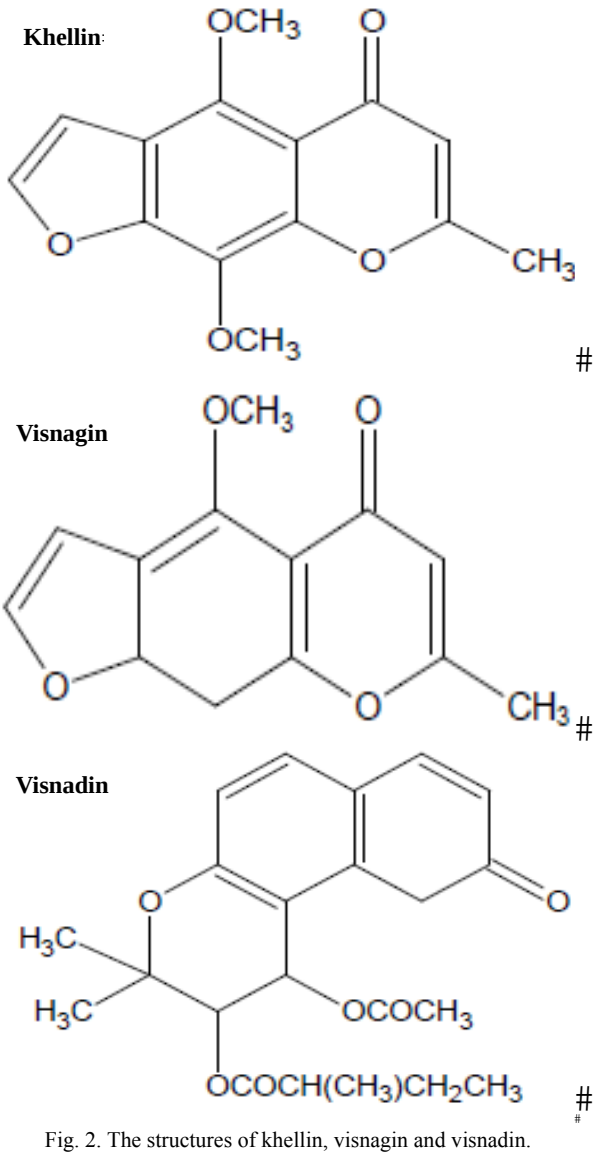

Fig. 2. The structures of khellin, visnagin and visnadin.

Antimicrobial activities: *A. visnaga* is considered to have antimicrobial activities. Generally the antimicrobial activities of *A. visnaga* were associated with khellin and visnagin. Both these constituents were considered to have antifungal, antibacterial, and antiviral activities (Hudson, 1999). Because of the antimicrobial activities, *A. visnaga* could also be used for curing psoriasis. Most probably because of the structural similarity between khellin and psoralen, *A. visnaga* has photosensitizing ability and it was considered useful as a photosensitizer in patients with psoriasis (Abdelfattah *et al*., 1983). The following examples do authenticate the antimicrobial potential of *A. visnaga*. Its fruit extract in 95% ethanol exhibited antibacterial activity, inhibiting the growth of *Mycobacterium tuberculosis* H37RVTMC 102 even in a very low concentration (dilution of 1:40) (Grange & Davey, 1990). Similarly, 50% acetone, 50% aqueous or 95% ethanol extract of *A. visnaga* inhibited fungal growth (*Neurospora crassa*) *In vitro* (Kubas, 1974). Again, the aqueous extract of its fruits (in a concentration range of 2-10 mg/ml inhibited the growth and aflatoxin production by *Aspergillus flavus* (Mahmoud, 1999). Ethanolic and aqueous extract of the *A. visnaga* were tested against eight pathogenic microorganisms. The most active extract against Gram-positive bacteria was ethanol extract with minimal inhibitory concentration value (5 mg/ml) against *Enterococcus faecalis.* Though, a high concentration of extract was required to cause inhibition in yeast (Ghareeb *et al*., 2011). When the essential oils of *A. visnaga* was tested against *E. coli* and different other bacteria, it showed the best antibacterial activity against *E. coli*, *Staphylococcus aureus* and *Psedumonas aeruginosa* (Khalfallah *et al*., 2011). Similarly the aqueous and hydro alcoholic extract of seed and stem of *A. visnaga* had a remarkable antibacterial activity against *S. mutans*, *S. salivarius* and *S. sangguis* (Semyaril *et al*., 2011).

Antispasmodic activity: *A. visnaga* is known to support body to combat spasms in smooth muscles and dilate blood vessels and therefore, its antispasmodic properties are very much valuable to treat asthma attacks. Now it is known that khellin and visnagin mitigate spasms in the bronchial passages (Harvengt & Desager, 1983). Visnadin also caused nonspecific inhibition of vascular smooth muscles and selectively inhibited the contractile response in the rat aortic ring and portal vein segment (Durate *et al*., 1995; 1997). Similarly, aqueous extract of *A. visnaga* seeds induced relaxant result on contractibility of small intestine of rabbit (Jawad *et al*., 2006). *A. visnaga* could induce relaxation of smooth muscles, including that of the ureter and coronary arteries, in a variety of animal species. A very slight amount of *A. visnaga*'s seeds could relieve the throbbing through its antispasmodic effects on the urinary tract muscles. For similar reasons, a number of asthma medications were formulated using *A. visnaga* in 1950s.

Against vitiligo disease: Vitiligo, also called leukoderma or white skin, is a skin disease, wherein there is a steady loss of the melanin pigment from the skin layers often with a progressive course causing destruction of melanocytes. As mentioned, topically *A. visnaga* is applied for curing vitiligo and psoriasis. The reason is that A. *visnaga* possesses phototherapeutic properties similar to those of the psoralens, however with significantly lesser phototoxic and DNA mutation effects (Leeuw *et al*., 2011). For example, a study of 60 people revealed that the combination of *A. visnaga* and natural sun exposure caused re-pigmentation in 76.6% of the treatment receiving group (Abdelfattah *et al*., 1982). In a study of 28 patients with vitiligo, a new photochemotherapeutic course of therapy using *A. visnaga*, a furanochromone (as photosensitizer) and ultraviolet A (UVA) irradiation was used. More than 70% re-pigmentation was achieved in 41% of the patients who received 100 to 200 treatments (Ortel *et al*., 1988). Similarly, a subsequent placebo-controlled study of 36 patients of vitiligo revealed that a topical *A. visnaga* gel plus UVA caused re-pigmentation in 86.1% of the treated cases compared to 66.6% in the placebo group (Orecchia *et al*., 1998).

Another pilot study was conducted on 33 patients to evaluate the effectiveness of local khellin and UVA (KUVA) and systemic psoralens and UVA (PUVA) therapy for vitiligo and to compare them in terms of the degree of re-pigmentation, duration of treatment, number of procedures, total UVA dose and side effects (Valkova *et al*., 2004). The results revealed that local KUVA effectively induced re-pigmentation of vitiligo-affected skin areas to an extent comparable to systemic PUVA, provided that treatment duration is long enough (Valkova *et al*., 2004). In a recent study, 19 patients with vitiligo disease, who did not respond to khellin in liposomes and ultraviolet light (KLUV) treatment for no less than a year were treated with Blister Roof Transplantation (BRT) followed by KLUV (Leeuw *et al*., 2011). Around 75% of the patients were satisfied with the cosmetic results and more than 75% re-pigmentation of the vitiligo areas was noted in 47% of the patients. The above mentioned research studies do emphasize further research in this area.

Against urolithiasis: Urolithiasis is a clinical condition referred to as kidney stone disease. These stones are usually composed mainly of calcium oxalate. Several factors are involved in the formation of kidney stones such as dehydration, consumption of certain foods containing high amount of calcium, oxalate or uric acid and some infectious diseases. In traditional Arab folk medicine, Egyptians have been using *A. visnaga* as tea preparation for the treatment and prevention of kidney stones. Therefore, the effect of *A. visnaga* seeds was investigated in animal experiment for urolithiasis (Khan *et al*., 2001). When oxalate nephrolithiasis was induced by 3% glycolic acid given for 4 weeks, it was found that daily oral treatment with *A. visnaga* (500 mg/kg) could inhibit the formation of kidney stones by lowering the deposition of calculi in kidney. The prophylactic effect of *A. visnaga* was attributed to its diuretic activity (Khan *et al*., 2001). Another study evaluated the effect of *A. visnaga* and its two major constituents (khellin and visnagin) on renal epithelial injury using LLC-PK1 and Madin-Darby-canine kidney cells. It was found that *A. visnaga* and its two major constituents could play a strong role in the prevention of stone formation due to hyperoxaluria (Vanachayangkul *et al*., 2010).

The effect of oral administration of an aqueous seeds extract of *A. visnaga* was studied on urolithiasis in stone-forming rats (Vanachayangkul *et al*., 2008). Hyperoxaluria was induced in male Sprague-Dawley rats by giving 0.75% ethylene glycol and 1% NH_4Cl via the drinking water. The *A. visnaga extract* (125, 250 or 500 mg/kg) was orally administered for 14 days. Rationally, a good correlation was obtained between the incidence of crystal deposition and the increase in urine pH. This study also demonstrated that *A. visnaga* could be used as a possible therapeutic approach for the prevention of kidney stones due to hyperoxaluria (Vanachayangkul *et al*., 2008). In another similar research study, the effect of aqueous seeds extracts and its two constituents khellin and visnagin was observed on the crystal deposition in stone forming rates (Vanachayangkul *et al*., 2011). The histopathological examination of the rat kidneys showed that *A. visnaga* significantly reduced the incidence of calcium oxalate crystal deposition. Similar to the aqueous seed extract, khellin and visnagin also reduced significantly the incidence of deposition in the kidneys. But both the constituents did not affect urinary citrate or oxalate excretion, unlike aqueous seed extract, signifying a mechanism of action different from the aqueous seed extract (Vanachayangkul *et al*., 2011). The inhibitory effect of *A. visnaga* extract (aqueous extract of whole plant and its seeds) was studied on the oxalocalcic crystallization in human urine. Even this study revealed the efficacy of extracts of the *A. visnaga* seeds in inhibiting the crystallization of calcium oxalate. Further, it was found that the extracts reduced oxalate calcium crystallization and specially monohydrate oxalate calcium (Charafi *et al*., 2012).

Adverse Indications and precautions: A word of caution that the herbs or herbal medicine do not differ greatly from conventional drugs in terms of their mechanism; this gives them the same potential to cause harmful side effects (Lai & Roy, 2004; Tapsell *et al*., 2006). Similarly herbs with certain drugs in combination may alter their action or produce unwanted side effects. Intake of *A. visnaga* is not recommended at all along with blood thinners such as coumadin, heart drugs called calcium channel blockers or other drugs that lower blood pressure. And probably the reason is that the aqueous extract of *A. visnaga* has significant hypoglycemic effects (Jouad *et al*., 2002). During treatment with *A. visnaga* and its constituents, the exposure to sun or other sources of ultraviolet light should be avoided, in order to minimize photosensitivity. Overdose or longer use of the *A. visnaga*'s drug can lead to queasiness, dizziness, loss of appetite, headache, sleep disorders (Blumenthal *et al*., 1998). Similarly, side effects like pseudoallergic reactions, reversible cholestatic jaundice and elevated activities of liver transaminases and γ-glutamyltransferase have been observed with the use of *A. visnaga* or its constituents ((Blumenthal *et al*., 1998). Traditionally, *A. visnaga* is used an emmenagogue and its fruits or extracts should be avoided during pregnancy.

Conclusion

The herb *A. visnaga* has been used in the Arab folk medicine for a number of health problems such as coronary insufficiency, angina pectoris, bronchial asthma. It has also been used for the treatment of psoriasis, as a diuretic for renal colic and ureteric stones. Phytochemical analysis of *A. visnaga* has identified several important active constituents, such khellin, visnagin and visnadin. The plant *A. visnaga* is grown commercially in Arab and several other countries and is an industrial source for production of herbal medicines. A number of products are already in the market for treating different ailments. However, *A. visnaga* still remains a plant for further research studies. Studies on the biological activities of extracts and individual components constitute a core area of research. The biodiversity of *A. visnaga* on molecular basis is still untapped and further research studies will reveal its unmasked potential. Little information is available on the molecular markers for *A. visnaga*. Similarly, there is little genomics work done on this important plant and EST data base is not available. Development of analytical tools for determining different chemical constituents and their precise functions, and characterization and exploitation of *A. visnaga* at molecular and biotechnological levels are areas for future research.

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