

PHARMACOLOGICAL INVESTIGATIONS ON KHELLA- (*AMMI VISNAGA* L.)**Shah Alam^{*1}, Nighat Anjum², Jamal Akhtar² and Fouzia Bashir¹**¹Research Associate (Unani), Central Council for Research in Unani Medicine, New Delhi.²Research Officer (Unani), S-III, Central Council for Research in Unani Medicine, New Delhi.Article Received on
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Central Council for Research
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Delhi.**ABSTRACT**

Khella is one of the oldest medicinal plant documented in Eber's papyrus of Egypt. The plant is an annual or biennial herb, fruits of which are used for medicinal purpose. Traditionally khella (*Ammi visnaga* L.) has been described as emetic, purgative, antispasmodic and diuretic which is used to treat renal colic, bronchial asthma and whooping cough by the Unani physicians. It has also been used for the treatment of psoriasis, vitiligo and ureteric stones. Phytochemical analysis of *A. visnaga* has identified several important active constituents, such as khellin, visnagin and visnadin having cardioprotective, neuroprotective, antimicrobial, antispasmodic etc.

properties. Plant is grown commercially in several countries and is an industrial source for production of herbal medicines. 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.

KEYWORDS: *Ammi visnaga*, Khella, Khellin, Unani, Visnagin.**INTRODUCTION**

Ammi visnaga, known as Khella is one of the oldest herbs. It originates from the Nile delta and was used therapeutically by the ancient Egyptians, as is documented in Eber's papyrus (1546–1526 BC). The plant was used in Egyptian folk medicine to treat urinary calculi and bladder stones which was common as a result of widespread bilharzia. It was also used in the Middle Ages as a diuretic.^[28]

The name Ammi was used for the plant early by Dioscorides. The word comes from the Greek term ammos meaning “sand” and refers to the plant’s habitat. The term “visnaga” comes from “bis acutum” meaning “two pointed” referring to the seed heads. The Arabic name, khella is used throughout the Middle East and often in Europe. The plant’s dried umbels are still sold today in markets in the Middle East and the Far East, where the stems are used as toothpicks.^[25,28]

Lonicerus wrote that Ammi was a hot, dry plant and could therefore be used for “cold afflictions”, claiming its effectiveness: “against irritable stomachs and for the womb; it stimulates diuresis and women’s cycles (menstruation).” The seeds of this plant were at one time used to treat sterility, convulsions in the urethra and kidney stones; such conditions were regarded as “cold afflictions”.^[25,26,27,28]

Nomenclature

Ammi visnaga is commonly known as Ammi, Tooth-pick Ammi, Green Mist, Laceflower, False Queen Anne's Lace, Honey plant, Tooth-pick fruit, Spanish carrot and Khella / Khillah Sufli. The Arabic name 'khella' is popularly used throughout the Middle East and occasionally in Europe. The plant has several other Arabic names viz. Khilla or Khilla Baladi; Bizr, Al-khilla, Kulla, Khilal, Sowak, Alnabi, Saq, Alaroos. Old Latin names for the plant are Cuminum alexandrium, C. aethiopicum and C. regium - referring to Alexandrine, Ethiopian and Royal Cumin respectively.^[25,26,27,28]

Synonyms

Daucus visnaga Linn.; *Visnaga daucoides* Gaertn. It is sometimes confused with the wildflower *Daucus carota* which was originally called 'Queen Anne's Lace'.^[55]

Taxonomy

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Apiales
Genus	<i>Ammi</i> L. – ammi
Family	Apiaceae / Umbelliferae – Carrot family
Species	<i>Ammi visnaga</i> (L.) Lam. – tooth-pick plant

Habitat

Khella is native to Mediterranean region and is cultivated in Egypt, Morocco and Tunisia. There are now also large plantations in Europe, Argentina, Chile, Mexico, the southern States of the USA and the former Soviet Union. In India, the plants are cultivated in Kashmir, Jammu, Punjab, Dehradun and now in hot climate of North India also.^[55]

Plant description

Khella (*A. visnaga*) is an annual or biennial herb growing from a taproot erect to a maximum height of about 1.5 m. The root is fattened like the root of the carrot. Leaves are deltoid, 5-20 cm long, decompound, pinnately divided, ultimate divisions linear to filiform. Stems are solid, 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 mericarps and around 3 mm in length, somewhat resembling caraway. These fruits are used for medicinal purpose.^[44,50,55]

Temperament

Hot and Dry in second degree.^[11]

Ethnobotanical uses

In traditional system of medicine khella (*A. visnaga*) has been described as Muqi (emetic), Mus'hil (purgative) and Daf-e-Tashannuj (antispasmodic) which is used to treat renal colic, bronchial asthma and whooping cough by the Unani physicians.^[11,34] Waja' al-kuliya which includes renal colic and all types of loin pain, is relieved by a decoction of bizr al-khilla (*A. visnaga*) taken orally; this is believed to expel stones from the urinary passages. Seeds are used in treating burning micturition, loin pain, as diuretic, and ureteric muscle relaxant. Moreover, used for the treatment of peptic ulcer, gastrointestinal cramps, and painful menstruation.^[5,8] Also, it is used in the treatment of mild angina and various respiratory conditions such as asthma, bronchitis, and whooping cough.^[6,18] Khella is also used in cardiovascular disorders like hypertension, cardiac arrhythmias, congestive heart failure, atherosclerosis, angina pectoris / coronary insufficiency and hypercholesterolemia.^[11,46,47] Moreover, it is used as diuretic and for relieving liver and gall bladder disorders.^[6] When applied topically, Khella has been found useful in the recovery from vitiligo, psoriasis, wound healing, inflammatory conditions, and poisonous bites.^[2,51]

Phytochemistry

Phytochemistry of *A. visnaga* has revealed the presence of diverse groups of chemical constituents such as pyrones, saponins, flavonoids and essential oils.^[6,17,40] 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 furanocoumarins (xanthotoxin and ammoidin) only in trace amount.^[4,17,40,56] Two flavonols (quercetin and kaempferol) were identified in *A. visnaga* growing in Iraq.^[3] Eleven flavonols were isolated from the aerial parts of *A. visnaga*.^[7] 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.^[7] Very few studies have reported the analysis of the essential oils of *A. visnaga*.^[22,31,32] The main compounds reported in the essential oils of *A. visnaga* from Marocco 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 the North of Tunisia.^[31] The essential oils 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 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.^[31] The GC/MS analysis of essential oils of fresh aerial parts of *A. visnaga* from Constantine, Algeria revealed the presence of twenty one compounds.^[32] These compounds were characterized representing 97.3% of the essential oil with 2,2-dimethylbutanoic acid (30.1%), isobutyrate (14.0%), coveacin (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^[37], mainly containing Linalool (70.1%) and pentylmethylbutanoate (4.3%). The essential oil from Turkish origin.^[22] 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.^[47]

Biological activities of extracts and constituents of *A. visnaga*

Centuries ago, Arabs discovered that the seeds of khella (*A. Visnaga*) could relieve a number of ailments, such as the acute pain caused by a reduction in the flow of blood to the heart. Further, *A. visnaga* is considered anti-asthmatic, diuretic, vasodilator and an effective muscle relaxant. From ancient times, it has been used to alleviate the severe pain of kidney stones.^[13] The seeds 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.^[10] Essential oil of *A. visnaga* is well-known for its efficacy against coronary diseases and bronchial asthma.^[46,47]

Cardioprotective activity

The seeds of khella (*A. Visnaga*) are known to relieve the severe pain caused by a reduction in the blood flow to the 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.^[19,45] Visnadin was found the most active when used in In-vitro experiments.^[41] It inhibited vascular smooth muscle contraction and caused the dilatation of peripheral and coronary vessels and an increase in coronary circulation.^[14] 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.^[15] Further, visnagin has negative inotropic and chronotropic effects and helps in reduction of peripheral vascular resistance.^[14,48] Khellin and visnagin both are capable of inhibiting the spasms, indicating an involvement of a calcium channel blocking mode of action.^[45] Further khellin increased HDL-cholesterol in normo-lipidaemic subjects.^[23] Therefore, khellin also acts as a vasodilator and has bronchodilatory and spasmolytic activity.^[16]

Antimicrobial activities

Khella (*A. Visnaga*) is considered to have antimicrobial activities. Generally the antimicrobial activities were associated with khellin and visnagin. Both these constituents were considered to have antifungal, antibacterial, and antiviral activities.^[24] Because of the antimicrobial activities, *A. visnaga* could also be used for curing psoriasis, probably because

of the structural similarity between khellin and psoralen. *A. visnaga* has photo-sensitizing ability and it was considered useful as a photo-sensitizer in patients with psoriasis.^[2] 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).^[21] Similarly, 50% acetone, 50% aqueous or 95% ethanol extract of *A. visnaga* inhibited fungal growth (*Neurospora crassa*) *In-vitro*.^[35] Again, the aqueous extract of its fruits (in a concentration range of 210 mg/ml inhibited the growth and aflatoxin production by *Aspergillus flavus*.^[39] 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 MIC value (5 mg/ml) against *Enterococcus faecalis*. Though, a high concentration of extract was required to cause inhibition in yeast.^[20] When the essential oil of *A. visnaga* was tested against *E. coli* and different other bacteria, it showed the best antibacterial activity against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.^[32] 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. sanguis*.^[49]

Antispasmodic activity

Khella 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.^[23] Visnadin also caused nonspecific inhibition of vascular smooth muscles and selectively inhibited the contractile response in the rat aortic ring and portal vein segment.^[15,16] Similarly, aqueous extract of *A. visnaga* seeds induced relaxant result on contractibility of small intestine of rabbit.^[29] *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.

Melanoprotective activity

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.^[38] 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.^[1] In a study of 28 patients with vitiligo, a new photo-chemotherapeutic 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.^[43] 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.^[42] 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. 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.^[51] 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.^[38] Around 75% of the patients were satisfied with the cosmetic results and more than 75% repigmentation of the vitiligo areas was noted in 47% of the patients. The above mentioned research studies do emphasize further research in this area.

Anti-urolithiatic activity

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.^[33] 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.^[33] 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.^[52] The effect of oral administration of an aqueous seeds extract of *A. visnaga* was studied on urolithiasis in rats.^[54]

Hyperoxaluria was induced in male Sprague-Dawley rats by giving 0.75% ethylene glycol and 1% NH₄Cl via 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.^[54] 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 rats.^[53] 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.^[53] The inhibitory effect of *A. visnaga* extract (aqueous extract of whole plant and its seeds) was studied on the oxalo-calcic 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.^[12]

Hypoglycaemic activity

The effect of the aqueous extract of *A. visnaga* on blood glucose levels was investigated in fasting normal and streptozotocin (STZ) induced diabetic rats after single and repeated oral administration. The aqueous extract of *A. visnaga* at a dose of 20 mg/kg significantly reduced blood glucose in normal rats six hours after a single oral administration ($P < 0.005$) and nine days after repeated oral administration ($P < 0.05$). This hypoglycaemic effect is more pronounced in STZ diabetic rats ($P < 0.001$). These findings suggest that the aqueous extract of *A. visnaga* possess significant hypoglycaemic effect in both normal and STZ diabetic rats.^[30]

Neuroprotective activity

Visnagin which is an active principle of was investigated for neuroprotective effect against kainic acid (KA) -induced neuronal cell death. Visnagin administration (100 mg/kg, p.o. or i.p.) not only inhibited microglial and astroglial activation but also attenuated the inflammatory marker expressions concomitantly, suggesting that visnagin exerts its neuroprotective effects via an anti-inflammatory mechanism in KA model.^[36]

Adverse effects

Traditionally, *A. visnaga* is used as an emmenagogue and its fruits or extracts should be avoided during pregnancy. Intake of *A. visnaga* is not recommended at all along with blood thinners such as coumadin, anti-hypertensive drugs like calcium channel blockers or other drugs that lower blood pressure. 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* can lead to queasiness, dizziness, loss of appetite, headache, sleep disorders.^[9] 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.^[9]

Substitute(s)

Aatrilal (*Ammi majus* L.).^[11]

CONCLUSION

Khella (*A. Visnaga*) has been used in the Arab folk medicine for a number of ailments such as coronary insufficiency, angina pectoris, bronchial asthma etc. It has also been used for the treatment of psoriasis and vitiligo, as a diuretic for renal colic and ureteric stones. Phytochemical analysis of *A. visnaga* has identified several important active constituents, such as 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.

Competing interests: The authors declare no conflict of interest.

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