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Investigating the Antimicrobial Potential of *in- vitro* Grown Microshoots and Callus Cultures of *Ammi visnaga* (L.) Lam.

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Abstract

Ammi visnaga (L.) Lam is a valuable herbal plant that is frequently collected for medicinal purposes. This study is conducted to evaluate the antimicrobial potential of the *in-vitro* grown microshoots and callus cultures of this plant against selected strains of bacteria and fungi. Shoot multiplication was obtained in MS medium containing 0.5 mg/L BA + 0.1 mg/L NAA, while callus multiplication was performed on MS medium containing 1.0 mg/L BA + 2.0 mg/L 2,4-D under light conditions. The aqueous and methanolic extracts were prepared from both culture types in addition to *in-vivo* grown plant material to experiment their antimicrobial activities. Generally, the *in-vitro* extracts of the microshoots and the callus cultures acted best against bacteria compared to the field plant extract. Moreover, the methanolic extracts were generally found to exhibit far better results and resistance against the tested microbes than the aqueous extracts. *C. albicans* was the most sensitive species to the microshoots extracts followed by gram-positive bacteria at MIC values of (3.125, 6.25) mg/mL. Meanwhile, *E. coli* bacteria were most sensitive to the microshoot extracts as they were completely inhibited at MIC value of (0.78 mg/mL). Moreover, the results of the callus extracts showed that *C. albicans* was the most sensitive at MIC values of (1.56 and 3.125) mg/mL, followed by gram-positive bacteria. On the other hand, gram-negative bacteria were the most resistant microbes to all experimented extract types in this study.

Keywords: *Ammi visnaga* , Antimicrobial activities, Callus, Microshoots

1. Introduction

Ammi visnaga (L.) Lam is a herbaceous medicinal plant which belongs to the family *Apiaceae*. It is found mainly in the Mediterranean regions, and is also distributed abundantly throughout the world as an introduced species. *A. visnaga* (L.) Lam is used frequently in many countries as a herbal medicine for different purposes. Ancient records revealed various medicinal properties of this plant as a popular source to cure a variety of different ailments (Hashim, *et al*., 2014).

Phytochemical studies on *Ammi visnaga* revealed the diversity of its chemical constituents, as they comprise several groups each with several compounds. The most important one includes Khellin (0.3-1.2 %) from the furanochromones group (2-4 %) (Hammouda *et al.,* 2005; WHO, 2007; Sellami *et al*., 2013; Hashim *et al*., 2014; Talaat, *et al*., 2014). It is worth mentioning that, furanochromones particularly visnagin, khellin, and khellol-glucoside are exclusively extracted from two species only, which are *A. visnaga* (L.) Lam (*Umbelliferae*) and *Eranthis hyemalis* L. (*Ranunculaceae*) (Kaul and Staba, 1967; El-Fiky *et al*., 1989).

Habitat destruction as a result of population growth, urbanization and uncontrolled collection caused overexploitation of wild plants particularly medicinal plants (Al-Quran, 2011). Moreover, about 90 % of the natural resources used by industries are collected from wild plants. This is due to the difference in price between the wild and cultivated plants, which could explain the lack of favorable plant materials, or to the high demands for the wild materials, which resulted, in turn, in the destruction of medicinal plants. Therefore, it has become an urgent matter to take legal measures and establish a compact program for the sake of saving all medicinal plants (Craker, 2007; Sharma *et al.,* 2010; Tahtamouni, *et al*., 2015).

The recent development in biotechnology particularly in the techniques of plant tissue culture have come as a boon to cope up with this alarming situation and to overcome these problems through the rapid micropropagation method in order to save the natural herbal wealth (Sridhar and Aswath, 2014).

Initially, plant tissue culture was exploited as a research tool and was focused on attempts to culture and study the development of small, isolated segments of plant tissues or isolated cells. Plant tissue culture is now a well-established

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technology. Like many other technologies, it has gone through different stages of evolution; scientific curiosity, research tool, novel applications and mass exploitation (Idowu *et al.,* 2009).

Although the discovery of antibiotics was a turning point that revolutionized medicine in human history (Davies and Davies, 2010), the uncontrolled use of antibiotics in addition to the their side effects on human health led to an imperative necessity to find therapeutic alternates such as semisynthetic antibiotics (Be´rdy, 2012), or plant-derived antimicrobials (Upadhyay *et al.,* 2014). More than a quarter of the modern medicines are derived from medicinal plants either directly or indirectly through the new technology applications of folk knowledge. This percentage can reach 60 % in the case of antitumoral and antimicrobial pharmaceuticals (WHO, 2011).

Several researches have been conducted recently for the extraction of vital substances and secondary metabolites from *A. visnaga* (L.) Lam which reveals the importance of this valuable plant (Al-Snafi, 2013; Hashim *et al*., 2014). In addition to its traditional importance in preventing or decreasing kidney stone formation in human patients, and its recent scientific importance against bronchial asthma and coronary diseases which are attributed to its essential oils (Rose and Hulburd, 1992; Satrani *et. al*., 2004), *Ammi visnaga* can be used as antitumoral (Beltagy and Beltagy, 2015) and is considered as a good antifungal and antimicrobial agent against both gram-positive and gram-negative bacteria (Rasooli *et al.,* 2007; Dababneh, 2008; Ghareeb, *et al*., 2011; Khalfallah *et al*., 2011; Mahmood, 2014; Sabry *et al*., 2014; Jaradat *et al*., 2015).

Accordingly, this study is conducted to investigate the antimicrobial potential of the *in- vitro* grown microshoots and callus cultures of *Ammi visnaga* as they contain a wide array of chemical constitutes reported to be active against some strains of bacteria and fungi

2. Material and Methods

2.1. Khellin Test

As mentioned earlier, khellin is a furanochromone and is exclusively extracted from two species which are *Ammi visnaga* (L.) Lam (*Apiaceae*) and *Eranthis hyemalis* L. (*Ranunculaceae*) (Kaul and Staba, 1967; El-Fiky, *et al*., 1989). Due to the high similarity between *Ammi visnaga* (L.) Lam and *Ammi majus* L seeds, the plant material was identified by the taxonomist, Professor Ahmad Aloqla from the Biology Department at Yarmouk University, Irbid, Jordan, using the Khellin test. This was very essential for the current research, as the tissue cultured plant material (microshoots and callus) used in our experiments were started initially *in-vitro* from the seeds and by applying the Khellin test this would make sure that the starting plant material (seeds) is true to its name.

The *Ammi visnaga* (L.) Lam seeds were bought from the market while the *Ammi majus* seeds were brought from the National Center for Agricultural Research and Extension (NCARE) in Amman, Jordan.

The seeds were dried, powdered, sieved and 2.0 g was weighed for extraction and added to a 20 ml of ethanol solvent for two days in an incubator under continuous shaking, where the shaker was adjusted to room temperature with 200 rpm. Then the alcoholic extract was filtered using vacuum buchner funnels, and was concentrated in a rotary thin-film evaporator and stored in a refrigerator in a stand manner to precipitate for a few days until the formation of three layers: an upper oily layer removed by filtration with a gentle suction and a middle cream, a fatty layer removed by the help of petroleum ether and a lower green crystalline layer from where pure khellin was obtained. The extracted khellin was checked based on the fact that mixing 5-8 mg of khellin with a small piece of solid NaOH will produce a distinct rose-red color (Barakat, And Badran, 1951; Hassan and Zubair, 1980; Kar, 2003).

2.2. In-Vitro Multiplication

2.2.1. In-vitro Multiplication of Microshoot and Callus Cultures

Preliminary experiments were conducted for the *in-vitro* establishment and multiplication of microshoots and callus cultures. The best multiplication rate for the microshoots was obtained in MS medium (Murashige and Skoog, 1962) containing 0.5 mg/L BA in combination with 0.1 mg/L NAA, while the callus multiplication was best in MS medium containing 1.0 mg/L BA in combination with 2.0 mg/L 2,4-D under light condition. To obtain the recommended mother stock for the present experiment, the microshoots (microshoot about 2.0 cm long and each flask contained four microshoots) and callus clumps (clump weight=0.5 g, with four clumps in each Petri dish) underwent five subculturing intervals (four weeks/interval).

2.3. Antimicrobial Effect

2.3.1. Crude Extraction

An aqueous extract of field and *in-vitro* grown plant material was prepared by stirring 4 g of previously dried plant powder with 40 ml of sterilized distilled water, while 1 g of the dried plant powder was added to 10 mL 100 % of a methanol solvent for the methanolic extract preparation. Both types of the extracts were left two days at 25°C with continuous shaking at the incubator shaker adjusted at 200 rpm. This was followed by centrifugation at 2000 g for ten minutes and the resulted supernatant was concentrated by complete solvent evaporation under vacuum using a rotary evaporator device, and was finally stored in sterile falcon tubes in a refrigerator at 4°C after being dissolved with dimethyl sulphoxide (DMSO) to reach the 100 mg/ml stock for each extract and was sterilized by a syringe filter with 0.45 µm pore size (Jaradat *et al*., 2015).

2.3.2. Antibacterial and Antifungal Activity of the Extracts.

Selected microbial strains were obtained from the Hamdi Mango Center for Scientific Researches (HMCSR). In this study, gram-negative bacteria *Escherichia coli* (American Type Culture Collection) ATCC 10536, *Klebsiella pneumonia* ATCC (31488) and gram-positive bacteria *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC (6633), and a species of fungi called *Candida albicans* ATCC (10231) were used to study the antimicrobial activity of these extracts.

Bacterial strains were cultured by streaking onto a solid nutrient agar (NA), while the fungi strain was cultured on a potato dextrose agar. The cultured Petri dishes were, then, kept in an incubator overnight at 37˚ C for twenty-four hours. For the antimicrobial microdilution assay, the Muller Hinton broth and potato dextrose broth were used for the bacteria and yeast respectively .

2.3.3. Microdilution Assay

The plant extract solutions were serially diluted (2-fold) ten times with Muller Hinton, potato dextrose broth. Well number eleven was considered negative control of bacterial growth, while well number twelve contained nutrient broth only and was used for positive control of bacterial growth. The achieved ten concentrations of the methanolic and aqueous plant extracts were from (50) to (0.098) mg/mL. A serial two-fold dilution of DMSO with Muller Hinton or potato dextrose broth was prepared to ensure that the antimicrobial activity was not from DMSO. Moreover, the blank or the back ground were two-fold dilution for each extract with broth. The final bacterial concentration in each well (except positive control) was adjusted to 0.75×10^6 CFU/mL. After the inoculation of bacteria, the plates were covered and incubated overnight at 37˚C for twenty-four hours. The plates were then scanned with an enzyme-linked immunosorbent assay (ELISA) reader at 600 Nano mole (nm) to examine the bacterial density (Karaman *et al.*, 2003) and at 405 nm to examine the yeast density (Scorzoni *et al*., 2007). The lowest concentration of the plant extract that did not allow any visible microbial growth in the test broth was considered the minimal inhibitory concentration (MIC) (Jorgensen and Ferraro, 2009; Jaradat *et al.,* 2015).

3. Results

3.1. Khellin Test

A red-rose color was obtained through the interaction between khellin and NaOH which revealed a positive result from the *A. visnaga* (L.) Lam seeds ethanolic extract, while negative results were obtained from the *A. majus* extracts as no changes in the NaOH color occurred (Figure 1). Hence, this proves that this species is *Ammi visnaga* (L.) Lam.

Figure 1. Khellin test experimented on *A. visnaga* (L.) Lam and *A. majus* seeds ethanolic extracts.

3.2. Antimicrobial Activity

3.2.1. Microshoots Extract

The results showed that both *A. visnaga* (L.) Lam *(in-vitro* microshoots and the field- collected plants) extracts possessed an antimicrobial activity against the tested bacterial and yeast strains; both types of the plant extract either aqueous or methanolic had a negative impact on the antimicrobial activities.

The aqueous extract of *A. visnaga* (L.) Lam at the level of 6.25 mg/mL was most effective against *E. coli*, while a concentration of 3.125 mg/mL was predominantly effective using the methanolic extracts (Figures 2 and 3). However the lowest MIC against *E. coli* were (0.78, 1.56 mg/mL) in the microshoots of methanolic and aqueous extracts respectively, while higher concentrations (12.50, 6.25 mg/mL) were needed to cause the inhibition of the microbes by the aqueous and methanolic extractions respectively in the field-collected shoot as shown in (Figures 2 and 3).

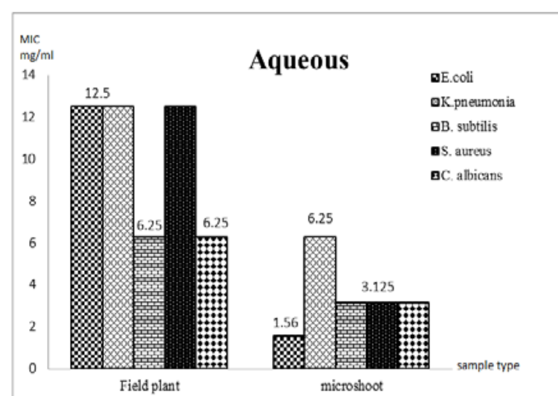

Figure 2. MIC values in mg/mL of aqueous *Ammi visnaga* extracts.

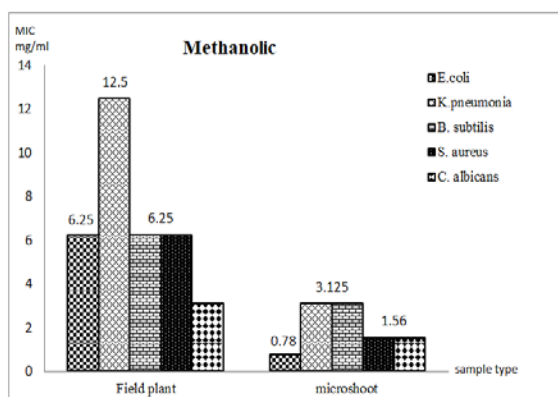

Figure 3. MIC values in mg/mL of methanolic *Ammi visnaga* microshoots extracts.

Similar finding were observed regarding the effect of different extracts against *K. pneumonia*, in which the predominantly effective concentrations of methanolic and aqueous extracts were 3.125, 6.25 mg/mL respectively, while the higher MIC (12.50 mg/mL) was obtained by both the methanolic and aqueous extracts of the *in-vivo* plant as shown in (Figures 2 and 3).

On the other hand, both methanolic and aqueous extracts have MIC of (3.125 mg/mL) against *B. subtilis,* and the *in- vivo* extract had the lowest efficacy (6.25

mg/mL) against this microbe as shown in (Figures 2 and 3). Interestingly, *S. aureus* was sensitive to the methanolic extracts even at low concentrations, in which the lowest activity was obtained by the field plant material extraction at a 6.25 mg/mL concentration, while *S. aureus* was less sensitive against the aqueous extracts in which the predominant effective concentration was (3.125 mg/mL) (Figures 2 and 3).

Regarding *C. albicans*, this could be the most sensitive microbial strain in this study because almost all inhibition concentrations ranged between 1.56 and 3.125 mg/mL, and only the aqueous field plant extract extract had 6.25 mg/mL MIC as shown in (Figures 2 and 3).

3.2.2. Callus Extracts

The current results showed that all *A. visnaga* (L.) Lam extracts regardless of their sources (*in vitro* calli and field-collected plants) whether (aqueous or methanolic) had an antimicrobial impact against the tested bacterial and yeast strains (Figures 4 and 5). As observed from the *A. visnaga* (L.) Lam aqueous extracts, a concentration of 6.25 mg/mL was the predominant effective concentration against *E. coli,* while a concentration of 3.125 mg/mL was the predominant effective using methanolic extracts (Figures 4 and 5). However, the lowest MIC against *E. coli* was (1.56 mg/mL) when using the methanolic control callus extract grown under light conditions, while higher concentrations (12.50, 6.25 mg/mL) were needed to cause inhibition by the aqueous and methanolic extractions respectively of the field-collected shoot as shown in (Figures 4 and 5). Similar findings were observed regarding the effect of different extracts against *K. pneumonia* in the current study, in which the predominant effective concentrations of methanolic and aqueous extracts were 3.125, 6.25 mg/mL respectively, Meanwhile, the higher MIC (12.50 mg/mL) obtained by both the methanolic and aqueous extracts of *in-vivo* plant as shown in (Figures 4 and 5).

Interestingly, *B. subtilis* was sensitive to the methanolic extracts even at low concentrations, in which the lowest activity was obtained by *in-vivo* plant material extraction at a 6.25 mg/mL concentration, while *B. subtilis* was less sensitive against the aqueous extracts in which the predominant effective concentration was (3.125 mg/mL) (Figures 4 and 5). On the other hand, both the methanolic and aqueous extracts have an MIC of (3.125, 6.25 mg/mL) respectively against *S. aureus*; only the *in-vivo* aqueous extract showed the lowest efficacy (12.5 mg/mL) against this microbe as shown in (Figures 4 and 5).

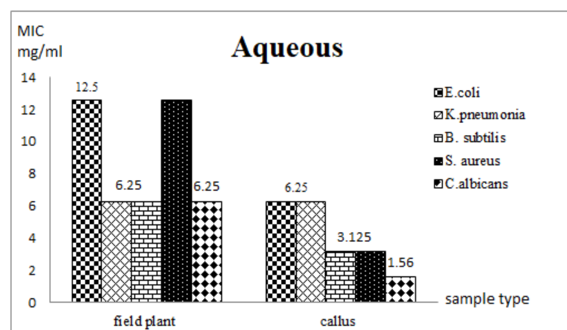

Figure 4. MIC values in mg/mL of *Ammi visnaga* (L.) Lam aqueous callus extracts**.**

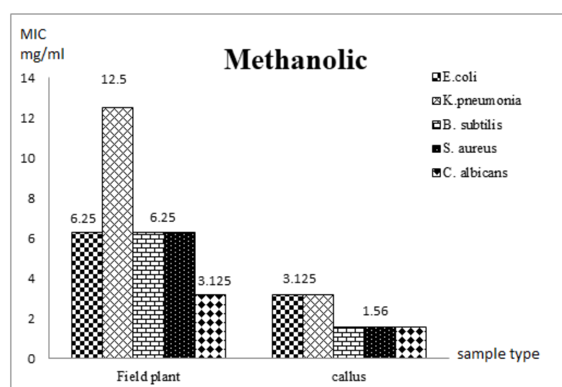

Figure 5. MIC values in mg/mL of *Ammi visnaga* (L.) Lam methanolic callus extracts

Regarding *C. albicans*, this could be the most sensitive microbial strain in this study where almost all inhibition concentrations ranged between 1.56 and 3.125 mg/mL; only the aqueous *in-vivo* extract had a 6.25 mg/mL MIC as shown in (Figures 4 and 5).

4. Discussion

It is evident from the aforementioned results that, the *in-vitro* extract concentrations of *A. visnaga* (L.) Lam exhibited far better results and resistance against both bacteria and fungi, hence it can be said that the *in-vitro* extracts possess greater antimicrobial activity compared to the field plant extracts. According to the Gobbo-Neto and Lopes (2007), the reason behind this may be that the plant secondary metabolites produced *in-vitro* are highly affected by the surrounding conditions including type and levels of growth regulators added to the media. Such findings were reported concerning many medicinally-important plants by several authors. For example, Barboza *et al.,* (2015) worked on *Anonna mucosa* (Jacq.), and Rasool *et al*., (2010) worked on *Prunella vulgaris* L., and both studies found out that biotechnological plant materials have the antibacterial properties similar to the *in-vivo* grown plants. Meanwhile, Kinyamasyo *et al*., 2014 and ElNour *et al*., (2015) revealed following a phytochemical screening of the seeds and callus extracts of *Nigella sativa* L and the plant material of *Aloe secundiflora* that the presence of secondary metabolites in such tested extracts may be responsible for their antimicrobial activity. Moreover, ElNour *et al.* (2015) results showed that the antimicrobial activities of the callus extracts of *Nigella sativa* L against *E. coli* were stronger than those of gentamicin and penicillin.

Moreover, the current study revealed that both the methanolic and aqueous extracts showed an antimicrobial activity against gram-positive and gram-negative bacteria as well as the studied fungi *C. albicans*. The methanolic extracts were as strong as or even more effective than the aqueous extracts, which may be related to the solubility of *A. visnaga* (L.) Lam active ingredients in organic solvents. This is similar to recent researches on *A. visnaga* (L.) Lam extracts conducted by (Ghareeb *et al*., 2011; Al-Snafi, 2015; Jaradat *et al.,* 2015) who proved the antimicrobial efficacy of this valuable plant against the studied microbes, and showed an increase in the antimicrobial activity in the methanolic extracts .

Also, it can be noted that gram-positive bacteria were affected by the *A. visnaga's* extracts more than gram-negative ones; this susceptibility differences may be due to cell membrane structural differences between these classes of bacteria. Particularly speaking, the outer membrane permeability barrier protects the gram-negative bacteria (Rasool *et al.,* 2010).

5. Conclusion

The results of the current study show that both extracts of the cultured tissue and the field grown *Ammi visnaga* (L.) Lam plant material could be useful in the development of new, alternative, and cheap antimicrobial drugs, particularly, against infections caused by these tested microbes. However, the obtained data revealed that the *in-vitro* extracts of the microshoots and the callus cultures gave the best antimicrobial results compared to those obtained by the field plant extract based on MIC values. Also, the present work clearly shows that the methanolic extracts exhibited far better results and resistance against the tested microbes. This finding may be attributed to the solubility of the active components of this plant in organic solvents.

References

Al-Quran S. 2011. Conservation of medicinal plants in Ajlun woodland -Jordan. *J Med Plants Res.*, **5 (24):** 5857-5862.

Al-Snafi A. 2013. Chemical constitute and pharmacological activities of *Ammi majus* and *Ammi visnaga*. A review. *IJPIR*, **3 (3):** 257-265.

Al-Snafi A.E. 2015. Therapeutic properties of medicinal plants: a review of their antibacterial activity (part 1). *IJPT*, **6 (3):** 137-158.

Barakat MZ and Badran N. 1951. New tests for the identification of khellin, visnagin and khellol-glucoside. *J Pharm Pharmacol*, **3 (1):** 576–580.

Barboza T J S, Ferreira A F, Ignacio ACPR and Albarello N. 2015. Antimicrobial activity of *Anonna mucosa* (Jacq.) grown *in vivo* and obtained by *in vitro* culture. *Braz J Microbiol*, **46 (3):** 785-789.

Be´rdy J. 2012. Thoughts and facts about antibiotics: Where we are now and where we are heading. *J Antibiot*, **65**: 385-395.

Beltagy A and Beltagy D. 2015. Chemical composition of *Ammi visnaga* l. and new cytotoxic activity of its constituents khellin and visnagin. *Int J Pharm Sci Res.*, **7 (6):** 285-291.

Craker L E. 2007. **Medicinal and Aromatic Plants—Future Opportunities**, *Issues in New Crops and New Uses*, 248-257.

Davies J and Davies D. 2010. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev.*, **74 (3):** 417-433.

Debnath M. 2008. Clonal propagation and antimicrobial activity of an endemic medicinal plant *Stevia rebaudiana. J. Med. Plants Res.*, **2 (2):** 045-051.

El-Fiky F K, Remmel R P and Staba E J. 1989. *Ammi visnaga*: Somatic embryo induction and furanochromone production in embryos, seedlings, and plants. *Planta Med.*, **55 (5)**: 446-451.

ElNour MEM, Ali AMA and Saeed BAT. 2015. Effect of different concentrations of auxins and combination with kinetin on callus initiation of *Trigonella foenum- graecum*.L. *Int J Eng Res Appl.*, **3 (2):** 117-122.

Ghareeb A, Zedan T and Gharb L. 2011. Antibacterial and antifungal activities of *Ammi visnaga* extracts against pathogenic microorganisms. *Iraqi J Sci.*, **52 (1):** 30-36.

Gobbo-Neto L and Lopes NP. 2007. Medicinal plants: factors of influence on the content of secondary metabolites. *Química Nova*, **30 (2):** 374-381.

Hammouda F M, Ismail S I, Abdel-Azim N S and Shams K A. 2005. *Ammi visnaga* (L.). In: Batanouny K H.(Ed), **A Guide to Medicinal Plants in North Africa, Malaga** : IUCN Center for Mediterranean Cooperation.

Hashim S, Jan A, Marwat K and Khan M. 2014. Phytochemistry and medicinal properties of *Ammi visnaga* (*apiacae*). *Pak J Bot.*, **46 (3):** 861-867.

Hassan MA and Zubair MU. 1980. **Analytical Profiles of Drug Substances**. (9th volume), United Kingdome, London: Academic press. 371-374.

Idowu A,Ibitoye D and Ademoyegun O. 2009. Tissue culture as a plant production technique for horticultural crops. *Afr. J. Biotechnol.*, **8 (16):** 3782-3788.

Jaradat N, Abualhasan M, Al-Masri M, Speih R, Johari M and Awad M. 2015. phytochemical screening and *in-vitro* evaluation of antioxidant and antimicrobial activities of the entire khella plant (*Ammi visnaga* L.) a member of Palestinian flora. *J Pharmacogn Phytochem.*, **7 (1):** 137-143.

Jorgensen J and Ferraro M. 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *J Med Microbiol.*, **49:** 1749-55.

Kar A. 2003. **Pharmacognosy and Pharmacobiotechnology,** New Delhi: New age international**.** 637-640.

Karamana I, Sahin F, Güllüce M, Ögütçü H, Sengül M and Adıgüzel A. 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *J Ethnopharmacol.*, **85:** 231-235.

Kau1 B and Staba E. J. 1967. *Ammi visnaga* (L) Lam. tissue cultures multi-liter suspension growth and examination for furanochromones. *Plant Tissue Cult.*, **15 (2):** 145-156.

Khalfallah A, Labed A, Semra Z, Kaki B, Kabouche A, Touzani R and Kabouche R. 2011. Antibacterial activity and chemical composition of the essential oil of *Ammi visnaga* (L.) (*Apiacae*). From Constantine, Algeria. *IJMAP*, **1 (3):** 302-305.

Kinyamasyo E M, Mwagandi CL, Kaingu FB and Gicharu GK. 2014. *In vitro* analysis of antibacterial and antifungal potency of tissue cultured and indigenous *Aloe secundiflora* plant extracts. *JAES*, **3 (2):** 53-64.

Kunduat S, Haque SK M and Ghosh B. 2015. Comparative analysis of bioactive compounds in different habitat of *Centella asiatica* (L.) Urban: Application for *in vitro* clonal propagation of elite ecotype. *J Appl Pharm Sci.*, **5 (2):** 030-036.

Mahmood N. 2014. Study the effect of the alcoholic and water extracts of *Ammi visnaga* (L.) and *Matrica chamomila* on different bacteria which isolates from diarrhea. *Int J Adv Res.***, 2 (2):** 139-142.

Murashige T and Skoog T. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures**.** *Plant Physiol.*, **15**: 473-497.

Rasool R, Ganai BA, Kamili A N, Akbar S and Masood A. 2010. Antioxidant and antibacterial activities of extracts from wild and *in vitro-* raised culture of *Prunella vulgaris* L. *Med Aromat Plant Sci Biotechnol.*, **4 (1):** 20-27.

Rasooli I, Taghizadeh M, Astaneh S, Rezaei M and Jaimand K. 2007. Phytobiological properties of *Ammi visnaga* L. and

Lavandula angustifolia Mill. Essential oils. *Int. J. Essen. Oil Ther.*, **1**: 1-7.

Rose J and Hulburd J. 1992. **The Aromatherapy Book Applications and Inhalations**. North Atlantic Books, Berkeley, California, 94712.

Sabry A, El-Said A, El-Zayat S, Abdel-Motaal F and Magraby T. 2014. Fungal contamination of *Ammi visnaga* seeds, antimicrobial activity of the plant seeds secondary metabolites and detection of alkaloids and non-alkaloids compounds. *Curr Microbiol.*, **3 (2):** 901-914.

Satrani B, Farah A, Fechtal M, Talbi M and Boumari, M.L. 2004. Chemical composition and antimicrobial and antifungal activities of the essential oil of *Ammi visnaga* (L.) Lam. *Acta Botanica Gallica*, **151:** 65-71.

Scorzoni L, Benaducci T, Almeida AMF, Silva DHS, Bolzani VDS and Gianinni MJSM. 2007. The use of standard methodology for determination of antifungal activity of natural products against medical yeasts *Candida* sp and *Cryptococcus* sp. *Braz J Microbiol.*, **38:** 391-397.

Sellami HK, Napolitano A, Masullo M, Smiti S, Piacente S and Pizza C. 2013. Influence of growing conditions on metabolite profile of *Ammi visnaga* umbels with special reference to bioactive furanochromones and pyranocoumarins. *Phytochem J.*, **95**: 197-206.

Sharma S, Rathi N, Kamal B, Pundir D, Kaur B and Arya S. 2010. Conservation of biodiversity of highly important medicinal plants of India through tissue culture technology- A review. *Agri Biol J North Amer.*, **1 (5):** 827-833.

Sridhar TM and Aswath CR. 2014. Review on medicinal plants propagation: A comprehensive study on role of natural organic extracts in tissue culture medium. *Am J Plant Sci.*, **5:** 3073-3088.

Tahtamouni RW, Shibli RA, Al- Abdallat AM, Al- Qudah TS, Younis L, Al-Baba H and Al- Ruwaiei H. 2015. *In vitro* conservation and cryopreservation of medicinal and aromatic plants: a review. *Jordan J Biol Sci.*, **11(1):** 147-167.

Talaat I M, Khattab H I and Ahmed A M. 2014. Changes in growth, hormones levels and essential oil content of *Ammi visnaga* L. plants treated with some bioregulators. *Saudi J Biol Sci.*, **21**: 355-365.

Upadhyay A, Upadhyaya I, Kollanoor-Johny A and Venkitanarayanan K. 2014. Combating pathogenic microorganisms using plant-derived antimicrobials: A mini review of the mechanistic basis. *Biomed Res Inter.*, 1-18.

World Health Organization, (2007), **WHO Monographs on Selected Medicinal Plants**. WHO, Geneva, Switzerland, p. 23.

World Health Organization, (2011), **Traditional Medicines: Global Situation**, **Issues and Challenges**. WHO, Geneva, Switzerland, p. 1.