

Biological Efficacy of Ammi Majus (Apaceae) Extracts on Normal Human Lymphocytes

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Abstract

Ammi majus is a medicinal plant that contains bioactive compounds. Some of these active compounds were extracted using 80% ethanol and water by continuous extraction using the Soxhlet. The effective compounds in the extract were detected using qualitative disclosures. The effect of the active compounds of the extracts on the growth of normal human lymphocytes in vitro at five concentrations. 10, 5, 2.5, 1.25, 0.625 mg/ml were studied. The results showed that there were significant differences at the probability in $P < 0.05$ for the extracts, especially the concentrations of 10.5 mg/ml, where the percentage of cell viability for the ethanolic extract was 115.09% and 120.07%, respectively. As for the flavonoid extract, the percentage of cell viability was 106.74% and 157.19%, respectively.

Keywords: Ammi majus, normal human lymphocytes, plant extracts

1. Introduction

Plants are an important source for the manufacture of medicinal drugs because they contain compounds with biological efficacy, so they were adopted in the preparation of many medicines and medicinal drugs. Because of their pharmacological efficacy, the speed of their therapeutic effect, and the lack of negative side effects compared to chemically manufactured drugs, from here the importance of medicinal plants appears in the treatment of many diseases that affect humans).

researches have shown that plant extracts have multiple biological activities, including immune modulation activity [1, 2], there are many herbs that are immune modulators that modulate the immune response through stimulation or inhibition of that response, [3, 4]. Immune effect of garlic *Allium sativum* on cellular immunity, [4], and *Panax ginseng* is important in enhancing the immune response through stimulation of B and T lymphocytes [5].

Ammi majus is a medicinal plant belonging to the umbrella family Apiaceae, it has a straight stem and a thin branched in the upper part, the leaves are alternate on the stem lobed feathery, the flowers are small white in compound inflorescences mostly bisexual [6] spreads in different parts of the world, it grows in the Mediterranean basin, West Africa and some areas in Europe and Asia [7, 8]. In Iraq, it is usually found in fields, gardens, and the side of canals as weeds. Many uses in the medical field, including treating skin problems, psoriasis and vitiligo and others, and the aqueous extract of the fruits is used in the treatment of kidney stones, angina and asthma, [7].

A. majus contains many chemical compounds, the most important of which are coumarins, flavonoids, glucosides, tannins, [9]. The most important of these are antitumor and anti-inflammatory activity [10]. The aim of the research was to test the effect of the extract on human lymphocytes

2. Materials and Methods

A-Plant collection: The plant was collected during flowering from separate areas in Anbar Governorate and was classified in the herbarium of the College of Education for Girls / University of Anbar. Samples of the pneumatic parts of A.majus bricks were brought to the laboratory, where they were cleaned of the suspended dust well. After drying at room temperature, they were ground with an electric grinder, then filled in sealed bottles with a tight seal and kept away from direct light until use.

2-Prepare alcoholic extract: alcoholic extract of plant was prepared with a weight of 20g of plant powder, and put in a thimble in a Soxhlet device and used 200ml of 80% ethanol for a period of five hours, then concentrated the extract using a rotary evaporator and then dried the extract in an electric oven at a temperature of 40 ° C. And kept in opaque glass bottles in the refrigerator until use [11].

Extraction of flavonoids: 86 g of plant powder was weighed and 600 ml of distilled water was added to it 10% h/v of HCl, then Reflex extraction was carried out for 8 continuous hours to ensure that the glycosidic bond of flavonoids was broken to get the non-sugar fraction aglycan then filtered the extract The filtrate was cooled, and the non-sugar part, which is the active part of the flavonoids, was extracted by ethyl acetate, an organic solvent, by adding 50 ml of it for every 50ml extracted. Distilled to remove the acid residues Hcl used in the extraction, and finally the acetate layer is dried using a Rotary evaporator at a temperature of 45°C and save the product to complete the rest of the analyzes.

Chemical test: The qualitative statements were made to identify the active groups in the aqueous and alcoholic extracts of the plant as follows.

Phenols were detected by adding 2 ml of ferric chloride at a concentration of 1% to 3 ml of the crude extract, and a bluish-green precipitate appears indicating the presence of phenols [12].

Flavonoids were detected by mixing an amount of

sodium hydroxide solution with the crude extract of the plant and leaving it for a short period, so a bright yellow color appears indicating the presence of flavonoids [13].

Tannins test: Drops of 1% lead acetate solution were added, as the reagent was added to an equal amount of the plant extract, and when a white gelatinous precipitate was formed, it indicated the presence of tannins [14].

Saponins were detected in adding 2 milliliters of 1% of mercuric chloride to 2 ml of the crude extract of the plant, and when a white precipitate was observed, it indicated the presence of saponins [14].

Glycosides test: Put 2ml of the plant extract in a test tube, add to it 2ml of Benedict's reagent, then transfer to a boiling water bath for five minutes, after which the tube is cooled. If a red precipitate appears, this is evidence of the presence of clacosides.

Study of alcoholic extract (ethanol) and flavonoids extracted on cultures of normal human lymphocytes

The effect of the crude alcoholic extract (ethanol) and flavonoids extracted from the Ammi majus plant on normal human lymphocyte cultures was studied by a method.

(MTT) by treating lymphocytes cultured in a tissue culture dish

Micro Titer plate 96-well, u-Bottom, which contained 96 holes, and different concentrations of crude alcoholic extract and flavonoids extracted were added at 5 concentrations and three replicates for each concentration, then compared with the control model, which included 6 holes, represented by lymphocytes without treatment.

1- Preparing the culture media according to the method for tissue culture. Mix the above ingredients and then complete the volume to 1 liter by adding distilled water twice double distilled water (DDW), and then sterilize the resulting solution with a 0.22 µm liter Fitter filter. Lymphocytes are isolated and cultured according to the method [11], then every 100 microliters of suspended lymphocytes were transplanted into 96-hole plate titer micro-dissections, after which they were incubated in an incubator equipped with 5% CO₂ gas at a temperature of 37°C for a period of time. From 24-20 to stabilize lymphocytes so that the cells are not stressed and according to the Freshney protocol for live cell transplantation.

Preparation of solutions of crude alcoholic extract (ethanol) and flavonoids of different concentrations. A stock solution was prepared from the crude extract and flavonoids at a weight of 100 mg in 10 ml of distilled water so that the final concentration was 10 mg / ml, and 5 concentrations were prepared as follows: 0.625, 1.25, 2.5, 5, 10 mg / ml of the stock solution.

Treatment of transplanted lymphocytes

Lymphocytes cultured in a tissue culture dish were treated by adding 100µl of each prepared

concentration, starting from the stock solution 10 mg/ml, and by three replicates for each concentration with the addition of RPMI medium to 6 holes untreated with any solution of the prepared crude extract and flavonoid solutions, which contain Only on cells, lymph, and culture medium as a control model. After treatment, the dishes are incubated in a special incubator for culturing live cells at a temperature of 37 °C and in the presence of 5% CO₂ for 24 hours, and four hours before the incubation period, MTT dye is added to each hole of the tissue culture dish. Bile at a concentration of 5 mg/ml in an amount of 50µl and returned to the incubator again. After the end of the four-hour period of the second incubation, the living cells will have consumed the MTT dye without the dead cells, and transformed it into violet-colored crystals, called Forma zan crystals. Then all the pits were treated with 50 microliters of DMSO - Sulphoxide Dimethyl to dissolve the formed crystals, and then the violet color was read. At a wavelength of 620 nm to the dish by a reader Elisa.

The percentage of inhibition of lymphocytes was calculated to apply the equation $IR \% = (A - B) / a \times 100$

IR Retarding Ratio - A control - B read the form
The survival rate of lymphocytes is calculated by applying the following equation: Survival rate = (form reading rate) / (control reading rate) x 100

Statistical analysis: After collecting and tabulating the data related to the study, it was statistically analyzed: according to the simple experiment system applied using the Complete Randomized Design, each treatment was repeated three times. The least significant difference is L.S.D at the probability level of 0.05 [15].

3. Results and Discussion

Detection of the most important active compounds of the crude extract of the satanic ale plant A. majus, the results of the qualitative chemical analyzes and disclosures of the crude extract of the satanic plant showed that it contains the active substances, which are phenols, flavonoids, tannins, saponin, and glycosides, as shown in Table (1).

Results	Detector name	detection guide	Active Compound s
+	ferric chloride	bluish green precipitate	Phenols
+	SODUIM hydroxide	bright yellow color	Flavonoids
+	lead acetate	white gelatinous precipitate	Tannins
+	mercuric chloride	white precipitate	Saponins
+	Benedict detector	red precipitate	Glycosides
The (+) sign indicates positive detection			

Plant extracts contain a lot of active ingredients, and their concentrations differ from one plant to another, which made the tests related to the use of chemical reagents to investigate them necessary before starting to study their therapeutic efficacy [16]. The results of chemical detections of the crude extract

were similar to previous studies. The satanic trait contains these substances, including: [17], and the results obtained by [18] from his study of the chemical components of the plant, as he indicated that the extract contained flavonoids, tannins, glycosides, saponins, and others. And the results reached by [19] from their study of the flavonoid compounds of the plant, as they identified two types of flavonoids, kaempferol and quercetin, and [20] indicated that in another study.

The presence of these active compounds in the plant *A. majus* indicates its great importance and explains the reason for its use in medicine in ancient medicine as well as its use in modern medicine, as well as reveals its medicinal value, which calls for attention to this plant [17]. The detection, isolation and purification of these compounds has an effective effect in employing their results for the purpose of using them in controlling many diseases that afflict humans [21].

Effect of extracts of the satanic plant on cultures of normal human blood lymphocytes

The statistical results proven in Figures (1) and (2) showed that the ethanolic and flavonoid extract of *A. majus* had an effect on the growth of normal lymphocytes in general. The effect depended on the type and concentration of the extract with regard to the ethanolic extract. The increase in the number of lymphocytes had significant differences and at a level ($P < 0.05$) starting from a concentration of 1.25mg/ml and toward higher concentrations when compared with the control and the rest of the other concentrations, and the most effective was at concentration 5, 10 mg/ml with a cell survival rate of 115.09% and 120.07%, respectively, but the concentration of 2.5 mg/ml had a decrease in the number of lymphocytes compared with the control. Whereas, the results of the statistical analysis of the flavonoid extract of *A. majus* showed an increase in the vitality of human lymphocytes, starting at a concentration of 2.5 mg/ml, toward higher concentrations and with significant differences at the level ($P < 0.05$), when compared with the control and reading other concentrations. Effective at concentration 5 and 10 mg/ml, the percentage of cell viability was 106.74% and 157.19%, respectively.

L.S.D = 0.156

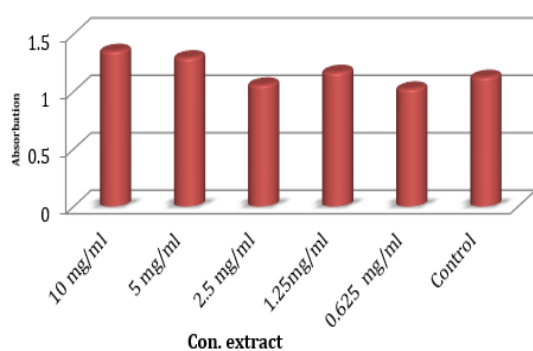


Figure (1): Effect of ethanolic extract concentrations on the growth of normal human lymphocyte cultures

L.S.D = 0.289

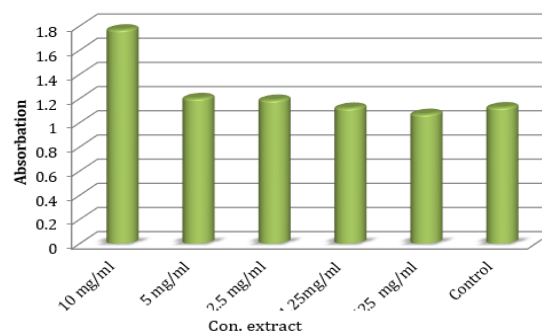


Figure (2) Effect of extracted flavonoids concentrations on the growth of normal human lymphocyte cultures

The discovery of new drugs of natural source that can enhance immune function has become an important goal of research in pharmacology and immunology, especially if they are from a natural source (vegetable) and have fewer side effects, and testing the inhibitory effect in normal cells is one of the main things that must be investigated About it when studying the effect of a substance for the purpose of using it as a treatment, and one of the most important of these cells are immune cells, especially lymphocytes that have the ability to divide outside the body by the effect of radiators [22] and since medicinal plants and herbs contain many compounds that have a stimulating effect or It inhibits the growth and division of some immune system cells such as lymphocytes [23]. Therefore, the effect of *A. majus* extracts on human lymphocytes was studied in vitro.

The results of the study also showed the positive effects of extracts (ethanol and flavonoids) of the plant *A. majus*, especially after noticing an increase in the number of lymphocytes when treated with these extracts, and this agrees with the study of Musini et al. [24] and they proved that phenols and flavonoids help increase the activity of human lymphocytes and protect them from Damage and oxidation, and in a study conducted in Iran on the effect of the ethanolic extract of the fruits of thistle plant on lymphocytes, the results of the study showed that the extract led to an increase in the content of DNA in cells [25, 26] indicated that the flavonoids present in *Al-Saad* herb has the ability to stimulate cellular immunity and produce an immune effect, knowing that the results obtained are identical to the above.

As for *A. majus* in particular, in a study conducted to evaluate some of the hematological and cytogenetic effects of the aqueous extract of the plant in male white mice, the results of the study showed that the extract had a significant and clear enhancement of the values of the studied treatments and that the total number of white blood cells showed a significant increase, as well as the study showed that the extract was Effective in raising the values of the split coefficient [27], the study was conducted to study the effect of anti-inflammatory activity of the alcoholic extract of satanic vinegar in rats, and it was found that it has an anti-inflammatory effect [28].

The biological activity of *A. majus* plant may be due

to the presence of active compounds such as phenols and flavonoids, which studies have proven biological and physiological activities that act as antioxidant [29]. The growth of lymphocytes and this is due to the chemical composition of these extracts and their containment of phenolic compounds, especially flavonoids, including rutin, quercetin, kaempferol, which are antioxidants and work to prevent oxidative damage to cells and DNA [16], as the secondary metabolites present in the extracts Plants can affect the median cellular immunity as well as reduce the inflammatory effect that occurs as a result of infection with various diseases, as plant secondary metabolites play an important role in activating this feature [30].

Reference

1. Yang X, Zhao Y, Yang Y, et al. Isolation and characterization of immunostimulatory polysaccharide from an herb tea, *Gynostemma pentaphyllum* Makino. *Journal of agricultural and food chemistry*. 2008;56(16):6905-9. <https://doi.org/10.1021/jf801101u><https://doi.org/10.1021/jf801101u>
2. Pandey RK, Shukla S, Hadi R, et al. Kirsten rat sarcoma virus protein overexpression in adenocarcinoma lung: Association with clinicopathological and histomorphological features. *J Carcinog*. 2020;19:9. <https://doi.org/10.4103/jcar.jcar.11.20>
3. Nagarathna P, Reena K, Reddy S, et al. Review on immunomodulation and immunomodulatory activity of some herbal plants. *Int J Pharm Sci Rev Res*. 2013;22(1):223-30.
4. Čolić M, Vučević D, Kilibarda V, et al. Modulatory effects of garlic extracts on proliferation of T-lymphocytes in vitro stimulated with concanavalin A. *Phytomedicine*. 2002;9(2):117-24. <https://doi.org/10.1078/0944-7113-00093>
5. Liou C-J, Li M-L, Tseng J. Intraperitoneal injection of ginseng extract enhances both immunoglobulin and cytokine production in mice. *The American journal of Chinese medicine*. 2004;32(01):75-88. <https://doi.org/10.1142/S0192415X04001771>
6. Bairwa R, Sodha R, Rajawat B. *Trachyspermum ammi*. *Pharmacognosy reviews*. 2012;6(11):56. <https://doi.org/10.4103%2F0973-7847.95871>
7. Al-Hadhrani RMS, Al Muniri RMS, Hossain MA. Evaluation of antimicrobial and cytotoxic activities of polar solvent extracts from leaves of *Ammi majus* used by the omanis. *Pacific Science Review A: Natural Science and Engineering*. 2016;18(1):62-5. <https://doi.org/10.1016/j.psr.a.2016.08.002>
8. Dodderi T, Puthiry M, Thomas S. How much is too much? Effect of volume on water-swallowing test. *J Nat Sci Biol Med*. 2020;11(2):169-75. <https://doi.org/10.4103/jnsbm.JNSBM.63.20>
9. Bartnik M, Sławińska-Brych A, Żurek A, et al. 8-methoxypsoralen reduces AKT phosphorylation, induces intrinsic and extrinsic apoptotic pathways, and suppresses cell growth of SK-N-AS neuroblastoma and SW620 metastatic colon cancer cells. *Journal of ethnopharmacology*. 2017;207:19-29.
10. Gledhill JR, Montgomery MG, Leslie AG, et al. Mechanism of inhibition of bovine F1-ATPase by resveratrol and related polyphenols. *Proceedings of the National Academy of Sciences*. 2007;104(34):13632-7. <https://doi.org/10.1073/pnas.0706290104>
11. Fernandez-Botran R, Vetvicka V. *Advanced methods in cellular immunology*. CRC Press, 2000. <https://doi.org/10.1201/9781420039238>
12. Cannell RJP. *Natural Products Isolation*. Humana Press, 1998. Available from: <https://books.google.com.pk/books?id=8Ye3uuy3fJUC>
13. Yadav R, Agarwala M. Phytochemical analysis of some medicinal plants. *Journal of phytology*. 2011;3(12).
14. Anbarasi A, Vidhya R. Evaluation of in vitro anti-inflammatory activity of *Tephrosia purpurea* (Seed). *Asian Journal of Pharmaceutical Research*. 2015;5(2):83-9. Available from: https://asianjpr.com/HTML_Papers/Asian%20Journal%20of%20Pharmaceutical%20Research_PID_2015-5-2-3.html
15. Al-Rawi KM, Allah AAMK. *Design of agricultural experiments*. Ministry of Higher Education and Scientific Research; 2000.
16. Boukhary R, Raafat K, Ghoneim AI, et al. Anti-inflammatory and antioxidant activities of *Salvia fruticosa*: An HPLC determination of phenolic contents. *Evidence-Based Complementary and Alternative Medicine*. 2016;2016. <https://doi.org/10.1155/2016/7178105>
17. Al-Snafi AE. Chemical constituents and pharmacological activities of *Ammi majus* and *Ammi visnaga*. A review. *International Journal of Pharmacy and Industrial Research*. 2013;3(3):257-65.
18. Adham AN. Comparative extraction methods, fluorescence, qualitative and quantitative evaluation of *Ammi majus* seed extracts. *Journal of Pharmacognosy and Phytochemistry*. 2015;4(1). Available from: <https://www.phytojournal.com/archives/2015/vol4issue1/PartA/31.1.pdf>
19. Abdul-Jalil TZ, Saour K, Nasser A. Phytochemical study of some flavonoids present in the fruits of two *Ammi* L. species wildy grown in Iraq. *Iraqi J Pharm Sci*. 2010;19(1):48-57. Available from: <https://www.iasj.net/iasj/download/6c8c97946b255d22>
20. Królicka A, Staniszevska I, Bielawski K, et al. Establishment of hairy root cultures of *Ammi majus*. *Plant Science*. 2001;160(2):259-64. [https://doi.org/10.1016/S0168-9452\(00\)00381-2](https://doi.org/10.1016/S0168-9452(00)00381-2)
21. Revathy J, Abdullah SS, Kumar PS. Antidiabetic effect of *Costus speciosus* rhizome extract in alloxan induced albino rats. *Journal of chemistry and biochemistry*. 2014;2(1):13-22. Available from: http://jcbnet.info/journals/jcb/Vol_2_No_1_March_2014/2.pdf
22. Araújo CRR, de Melo Silva T, Dos Santos MG, et al. Anti-inflammatory and cytotoxic activities of the extracts, fractions, and chemical constituents isolated from *Luehea ochrophylla* Mart. *BMC complementary and alternative medicine*. 2019;19(1):1-10. <https://doi.org/10.1186/s12906-019-2701-7>
23. Bhikha R, Glynn J. The pharmacological action of common herbal remedies. *American Journal of Internal*

- Medicine. 2018;6(5):99-107. Available from: <https://www.researchgate.net/publication/330287113>
24. Musini A, Gandhi S, Rao K, et al. HPLC polyphenolics profile and H₂O₂ induced cytoprotective effect of *Salacia oblonga* extracts on human lymphocytes. *Advances in Biological Chemistry*. 2016;6(1):19-27. <https://doi.org/10.4236/abc.2016.61003>
25. Amirghofran Z, Azadbakht M, Karimi MH. Evaluation of the immunomodulatory effects of five herbal plants. *Journal of Ethnopharmacology*. 2000;72(1-2):167-72. [https://doi.org/10.1016/S0378-8741\(00\)00234-8](https://doi.org/10.1016/S0378-8741(00)00234-8)
26. Chiang L-C, Chiang W, Chang M-Y, et al. In vitro cytotoxic, antiviral and immunomodulatory effects of *Plantago major* and *Plantago asiatica*. *The American journal of Chinese medicine*. 2003;31(02):225-34. <https://doi.org/10.1142/S0192415X03000874>
27. Al-Jumaily RMK, Ad'hiah AH, Halbosiy MMA, et al. Evaluation of Some Hematological and Cytogenetic Effects of *Ammi majus* Seed Aqueous Extract in Albino Male Mice. *Journal of Biotechnology Research Center*. 2010;4(1):104-10. <https://doi.org/10.24126/jobrc.2010.4.1.104>
28. Mutlag SH. Dose dependent anti-inflammatory effect of *Ammi majus* alcoholic extract in rat: chronic study. *Iraqi Journal of Pharmaceutical Sciences (P-ISSN: 1683-3597, E-ISSN: 2521-3512)*. 2012;21(1):82-6. <https://doi.org/10.31351/vol21iss1pp82-86>
29. Laura A, Moreno-Escamilla J, Rodrigo-García J, et al. Phenolic compounds. In *Postharvest physiology and biochemistry of fruits and vegetables 2019 Jan 1* (pp. 253-271). Woodhead Publishing.
30. Craig WJ. Health-promoting properties of common herbs. *The American journal of clinical nutrition*. 1999;70(3):491s-9s. <https://doi.org/10.1093/ajcn/70.3.491s>