

Conclusion

According to the data presented in [Table XX] and [Figure 42], it turns out that, among the different concentrations used in this assay 10.3125 $\mu\text{l/ml}$ of *O. basilicum* oil displayed the highest stimulation index of 1.585 which reflects a significant immunostimulant activity.

Regarding essential oil of *P. anisum* 20.3125 $\mu\text{l/ml}$ of the oil showed the highest stimulation index of 1.20 but it does not reflect a significant immunostimulant activity.

Conclusion and Recommendations

From the previous results, one can conclude that plant tissue culture offered a good source for production of secondary metabolites which are considered to be promising compounds of significant biological activities. So, we recommended further studies for optimizing tissue culture conditions in term of precursors, minerals, organic nitrogen (amino acids), vitamins, hormonal balance, environmental conditions, etc. aiming at establishing the most favourable culture conditions for getting overproducer cell lines that can supply a higher yield of volatile oil with biologically effective natural components quality and quantity wise.

Also, further investigations for a wider spectrum of bioactivities to include antiinflammatory, antiviral, antimicrobial and neural effects of the tested oils. We suggest pre-clinical and subsequent clinical trials of these compounds for the use of these compounds as therapeutic drugs.

General summary

GENERAL SUMMARY

The work presented in the thesis represents plant tissue culture of two medicinal aromatic plants (*Ocimum basilicum* L. and *Pimpinella anisum* L.) and biological screening of cytotoxicity, antioxidant, and immunostimulant activities of their extracted oils.

The thesis consists of three parts:

Part I

Plant tissue culture of *Ocimum basilicum* L. and *Pimpinella anisum* L. and examination of calli for cytodifferentiation and organogenesis.

Since plant tissue culture systems represent a potential renewable source of valuable medicinal compounds which cannot be produced by microbial cells or chemical synthesis. So, we applied this technology on two important aromatic plants which can be widely used for medicinal and non- medicinal purposes all over the world. The formed calli and tissues from culture system indicates the formation of different organs and xylem tissue which refers to a high degree of cytodifferentiation and these tissues were microscopically examined.

Part II

- A. Extraction of essential oil from *Ocimum basilicum* L. and *Pimpinella anisum* L. calli and assessment of the yield.
- B. Gas chromatography- Mass spectral analysis (GC/MS) of the extracted oils.

Since the main purpose of our tissue culture work was the production of secondary metabolites of interest. So, essential oils for both cultures were extracted and their yield of essential oil was obtained at different time intervals of the culture time. Gas chromatography- Mass spectral analysis was essential for identification of components in the extracted oils.

Part III

Chapter 1- Cytotoxic activity of essential oil of *Ocimum basilicum* L. and *Pimpinella anisum* L. on normal peripheral blood cells (PBMCs), hepatocellular carcinoma (HepG-2) and colorectal adenocarcinoma (Caco-2).

Cancer research is a compromising area for work. Discovery of new safe cytotoxic agent is paid a great effort. In this thesis, the extracted oils from cultures were tested for their cytotoxicity against both normal peripheral blood

cells (PBMCs) to determine the safe concentrations that can be used next in testing cytotoxicity against two different cancerous cell lines; hepatocellular carcinoma (HepG-2) and colorectal adenocarcinoma (Caco-2). Both of the tested oils showed no toxicity against normal cells and they showed significant anticancer activity against the tested cancer cell lines.

Chapter 2- Antioxidant activity of essential oil of *Ocimum basilicum* L. and *Pimpinella anisum* L. using DPPH assay.

Natural products from dietary components such as Indian spices and medicinal plants are known to possess antioxidant activity, there are epidemiological evidences correlating higher intake of foods with antioxidant abilities to lower incidence of various human mortalities. We performed, here screening of antioxidant activity for both of the extracted oils, both of them showed antioxidant activity *in vitro* by reducing absorbance of DPPH radical in a quite low doses.

Chapter 3- Immunostimulant activity of essential oil of *Ocimum basilicum* L. and *Pimpinella anisum* L.

Here, we performed a biological screening for both extracted oils to show if they have a significant immunostimulant activity or not. Only essential oil extracted from calli of *O. basilicum* showed a significant immunostimulant activity with a stimulation index value more than 1.5 at a certain concentration.

APPENDIX I

Preparation of growth regulator stock solutions

Benzyladenine (BA) 1mg/ml

25 mg BA suspended in 20 ml water in a 25 ml volumetric flask. Add drop-wise 1N HCl until BA dissolved and made up 25 ml volume with water.

2,4- Dichlorophenoxyacetic acid (2,4-D) 0.1mg/ml

Dissolve 25 mg 2, 4-D in 5 ml absolute alcohol in a 250ml volumetric flask and made up to 250 ml volume with water.

Kinetin 10 mM stock solution

Dissolve 21.5 mg of Kinetin in 1 ml of 1N NaOH . In a 10ml volumetric flask, bring the solution to 10 ml volume with water. Sterilize the solution by filtration and store at -20° C.

References

REFERENCES

1. Fowler, M. W., *Commercial application and economic aspects of mass cell cultures*, in *Plant biotechnology*, Mantel, and S.H. Smith, editors. 1983, Cambridge University Press. p. 3-37.
2. Arora, R., and Bhojwani, S.S., *In vitro propagation and low temperature storage of Saussurea lappa C.B. Clarke-an endangered, medicinal plant*. Plant Cell Rep, 1989, **8**: p. 44-47.
3. Michael, H., and Clive, K., *100 Families of flowering plants*, 2nd edn . 1988, Cambridge University Press .p. 391.
4. Bailey, L. H., *Manual of cultivated plants*, MacMillan, New York, 1924.
5. Darrah, H.H., *the cultivated basil*, Buckeye Printing, Independence MO, 1980.
6. Gamal El-Din, H. M., *Medicinal Plants*, 1st edn.1967, Anglo-Egyptian Bookshop. p. 407.
7. Constance, L., *History of the classification of Umbelliferae (Apiaceae)*, in *the biology and chemistry of the Umbelliferae*, Heywood, V.H., editor. 1971, Academic Press.p.1-8.
8. Deutschmann, F., Hohmann, B., and Stahl E., *Morphologie und Anatomie, Pharmazeutische Biologie. Drogenanalyse I* Fischer, Stuttgart New York. 1979.
9. Tas, A., *Analgesic effect of Pimpinella anisum L. essential oil extract in mice*. Indian Veterinary Journal, 2009. **86** (2): p.145–147.
10. Carson, C.F., Mee, B.J., and Riley, T.V., *Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage and salt tolerance assays and electronmicroscopy*. Antimicrob Agents Chemotherapy, 2002. **46**: p. 1914–1920.
11. Deshpande, R. S., Tipnis, H. P., *Insecticidal activity of ocimum basilicum L*. Pesticides I I, 1997. p. 1-12.
12. Chaterje, A., Sukul, N., C., Laskal, S., and Ghoshmajumdar, S., *Nematicidal principles from two species of Lamiaceae*. Journal of Nematology, 1982. **14**: p. 118-120.
13. Reuveni, R. A., Fleishcr, A., and Putievsky, E. , *Fungistatic activity of essential oils from Ocimum basilicum chemotypes*. Journal of Phytopathology, 1984. **110**: p. 20-22.
14. Simon, J. E., Moralis, M.R., Phippen, W., B., Vieira, R., F., and Hoa, Z., *Perspectives on new corps and new uses*, in Janick, J., editor. *A source of aroma compounds and apopular culinary and ornamental herb*, Alexandria, VA: ASHS Press, 1999. p. 499-505.
15. Wannissorn, B., Jarikasem, S., Siriwangchai, T., and Thubthimthed, S., *Antibacterial properties of essential oils from Thai medicinal plants*. Fitoterapia, 2005.**76**: p. 233-236.
16. Dachler, M., and Pelzman, H., *Arznei-und Gewurzpflanzen*, Agravelag Klosteneuburg, 1999. p. 141-143.
17. Hanus, H., Heyland, K.U., and Keller, E., *Faserpflanzen, Arzneipflanzen und Sonderkulturen*, Handbuch des Pflanzenbaues 4, Eugen Ulmer KG, 2006. p.346-349.
18. Marquard, R., and Kroth, E., Agrimedia GmbH, 2002. p. 26-33.
19. Ismail, M., *General properties and chemical composition of ocimum basilicum essential oil*. Pharmaceutical Biology, 2006. **44** (8): p.619-626.

20. Aoshima, H., and Hamamoto, K., *Potentiation of GABA A receptors expressed in Xenopus oocytes by perfume and phytoncid*. Bio Sci Biotech Bioch, 1999. **63**:p. 743-748.
21. Boissier, J.R., Simon, P., and Le Bourhis, B., *Experimental psychotropic effect of isomeric cis and trans-anetholes*. Therapie, 1967. **22**: p. 309-323.
22. Elisabetsky, E., Brunt, L., and Souza, D., *Anticonvulsant properties of linalool in glutamate-related seizure models*. Phytomedicine , 1995. **6**: p.107-113.
23. Librowski, T., Czarnecki, R., Mendyk, A., and Jastrzebska, M., *Influence of new monoterpene homologues of GABA on the central nervous system activity in mice*. Pol. J Pharmacol, 2000. **52**: p. 317-321.
24. MacDonald, R.L., and Kelly, K.M., *Antiepileptic drug mechanisms of action*. Epilepsia , 1995. **36**: p. 502-512.
25. Narusuye, K., Kawai, F., Matsuzaki, K., and Miyachi, E., *Linalool suppresses voltage-gated currents in sensory neurons and cerebellar Purkinje cells*. Neural Transm, 2005. **112**: p. 193-203.
26. Robbers, J.E., Speedie, M.K., and Tyler, V.E., *Terpenoids*, in *Pharmacognosy and Pharmacobiotechnology*, Santos, F.A., and Rao, V.S., editors. 1996, New York, Williams, Wilkins, **375**: p.79-104.
27. Santos, F.A., and Rao, V.S., *Anti-inflammatory and antinociceptive effects of 1,8-cineole, a terpenoid oxide present in many plant essential oils*. Phytother Res, 2000. **4**: p. 240-244.
28. Silva, L., Emanuelli, T., Souza, D., and Elisabetsky, E., *Effects of linalool on glutamate release and uptake in mouse cortical synaptosomes*. Neuro Chem Res, 2001. **26**: p.191-194.
29. Szabadiss, J., and Erdelyi, L., *Pre and postsynaptic effects of eugenol and related compounds on Helix pomatia L. neurons*. Acta Biol Hung , 2000. **51**: p. 265-273.
30. Wie, M.B., Won, M.H., Lee, K.H., Shin, J.H., Lee, J.C., Suh, H.W., Sond, D.K., and Kim, Y.H., *Eugenol protects neuronal cells from excitotoxic and oxidative injury in primary cortical cultures*. Neuro Sci Lett, 1997. **225**: p. 93-96.
31. Dallmerier, K., and Carlini, E.A., *Anesthetic, hypothermic, myorelaxant and anticonvulsant effects of synthetic eugenol derivatives and natural analogues*. Pharmacology, 1981. **22**: p. 582-593.
32. Hobbs, W.R., Rall, T.W., and Verdoorn, T.A., *Hypnotics and sedatives* in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 9th ed, Hardman J.G., et. al, editors. 1996, New York, McGraw-Hill, p. 361-396.
33. Trojakova, L., Reblova, Z., Nguyen, H. T.T., and Pokonry, J., *Antioxidant activity of rosemary and sage extracts in rapeseed oil*. Journal of food lipids, 2001. **8**: p.1-13.
34. Petersen, M., and Simmonds, M.S.J., *Rosmarinic acid*. Phytochemistry, 2003. **62**: p. 121-125.
35. Javanmardi, J., Khalighi, A., Kashi, A., Bias, H.P., and Vivanco, J.M., *Chemical characterization of basil (Ocimum basilicum L.) found in local accessions and used in traditional medicines in Iran*. Journal of Agricultural and Food Chemistry, 2002. **50**: pp. 5878-5883.
36. Lee, J., and Scagel, C.F., *Chicoric acid levels in commercial basil (Ocimum basilicum) and Echinacea purpurea products*. Journal of Functional Foods, 2010. **2**: p. 77-84.
37. Madsen, H.L., Nielsen, B., R., Bertelsen, L.H., and Skibsted, L.H., *Screening of antioxidative activity of spices. A comparison between assays based on ESR*

- spins trapping and electrochemical measurement of oxygen consumption*. Food Chemistry, 1996. **57**: p. 331–337.
38. Dube, S., Upadhyay, P.D., and Tripathi, S. C., *Antifungal, physicochemical, and insect-repelling activity of the essential oil of Ocimum basilicum*. Canadian Journal of Botany, 1989. **67**(7): p. 2085-2087.
 39. Ahmet, A., Medine, S., Weryem, O., Hatice, S., Fikrettin, S., and Isa, K., *Antimicrobial effects of Ocimum basilicum (labiatae) extract*. Turk J BIOL, 2005. **29**: p.155-160.
 40. [Opalchenova, G.](#), and [Obreshkova, D.](#), *Comparative studies on the activity of basil-an essential oil from Ocimum basilicum L -against multidrug resistant clinical isolates of the genera Staphylococcus, Enterococcus and Pseudomonas by using different test methods*. [Journal of Microbiological Methods](#), 2003. **54**(1): p. 105–110.
 41. Oxenham, S.K., *Classification of an Ocimum basilicum germplasm collection and examination of the antifungal effects of the essential oil of basil*. PhD thesis, Glasgow, UK, University of Glasgow, 2003.
 42. Pandey, R., Karla, A., Tandon, S., Mehrotra, N., Singh, H.N., and Kumar, S., *Essential Oils as Potent Source of Nematicidal Compounds*. Journal of Phytopathology, 2000. **148** (7-8): p. 501–502.
 43. Chinnasamy, S., *Potential Anti-inflammatory Properties of Crude Alcoholic Extract of Ocimum basilicum L. in Human Peripheral Blood Mononuclear Cells*. Journal of Health Science, 2007. **53**(4): p. 500-505.
 44. Arshad, K., Dar, A., Siddique, B., Kabir, N., Aslam, H., Ahmed, S.H., Erum, S.H., Habib, S.H., and Begun, S., *Anticancer Activity of Ocimum basilicum and the Effect of Ursolic Acid on the Cytoskeleton of MCF-7 Human Breast Cancer Cells*. [Letters in Drug Design & Discovery](#), 2010, **7** (10): p. 726-736.
 45. **Stajković, O., et al.**, *Antimutagenic Properties of Basil (Ocimum basilicum L.) in Salmonella typhimurium TA100* [Food Technology and Biotechnology](#). Lipan j, 2007. **45**(2).
 46. Tsetkov, R., *Study on the fruit quality of some umbelliferous essential-oil plants*. Planta Med, 1970. **18**:p. 350–353.
 47. Weiss, R.F., editor, *Lehrbuch der Phytotherapie*, in Hippokrates, Stuttgart, 1974.
 48. Wagner, H., editor, *Pharmazeutische Biologie*, in Drogen und ihre Inhaltsstoffe. Fischer, Stuttgart. 1980, New York.
 49. Zepernick, B., Langhammer, L., and Lüdcke, J.B.P. , editors, *Lexikon der offiziellen Arzneipflanzen*, in De Gruyter. 1984, Berlin New York.
 50. Gildemeister, E., Hoffmann, F.R., and Die ätherischen. Öle, vol 1. 1956, Akademie, Berlin.
 51. Özcan, M.M., Chalchat, J.C., *Chemical composition and antifungal effect of anise (Pimpinella anisum L.) fruit oil at ripening stage*. Annuals of Microbiology, 2006. **56**(4): p.353–358.
 52. Hossein, K., Mohsen, F., and Masoud, A., *Chemical composition of essential oils of Iranian Pimpinella anisum L. and Foeniculum Vulgare Miller and their antifungal activity against post harvest pathogens*. Journal of essential oil bearing plants, 2008. **11**(5): p. 514-522.

53. Akhtar, A., Dshmukh, A.A., and Bhonsle, A. V., *In vitro* Antibacterial activity of *Pimpinella anisum* fruit extracts against some pathogenic bacteria. *Veterinary World*, 2008. **1**(9): p.272–274.
54. Ates, D.A., Erdogru, Ö.T., *Antimicrobial activities of various medicinal and commercial plant extracts*. *Turkish Journal of Biology*, 2003. (27): p.157–162.
55. Shukla, H.S., Tripathi, S.C., *Antifungal substance in the essential oil of anise (Pimpinella anisum L.)*. *Ultral and Biological Chemistry*, 1987. **51**(7): p.1991–1993.
56. Kosalec, I., Pepeljnjak, S., and Kuatrak, D., *Antifungal activity of fluid extract and essential oil from anise fruits (Pimpinella anisum L., Apiaceae)*. *Acta Pharmaceutica*, 2005. **55**(4): p.377–385.
57. Tirapelli, C.R., Andrade, C.R., and Cassano, A.O., *Antispasmodic and relaxant effects of the hydroalcoholic extract of Pimpinella anisum (Apiaceae) on rat anococcygeus smooth muscle*. *Journal of Ethnopharmacology*, 2007. **110**(1): p.23–29.
58. Pourgholami, M.H., Majzob, S., Javandi, M., Kamalinijad, M., and Fanaee, GHR., *The fruit essential oil of Pimpinella anisum exerts anticonvulsant effects in mice*. *Journal of Ethnopharmacology*, 1999. **66**(2): p.211–215.
59. Al Mofleh, I.A, Alhalder, A.A, Mossa, J.S., Al-Sooalbani, M.O., and, Rafatullah, S., *Aqueous suspension of anise “Pimpinella anisum” protects rats against chemically induced gastric ulcers*. *World Journal of Gastroenterology*, 2007. **13**(7): p.1112–1118.
60. Picon, P.D., Picon, R.V., and Costa, A. F. , *Randomized clinical trial of a phytotherapeutic compound containing Pimpinella anisum, Foeniculum vulgare, Sambucus nigra, and Cassia augustifolia for chronic constipation*. *BMC Complementary and Alternative Medicine*, 2010. **10**, article 17.
61. Sahraei, H., Ghooshoni, H., and Hossein, S., *The effects of fruit essential oil of the Pimpinella anisum on acquisition and expression of morphine induced conditioned place preference in mice*. *Journal of Ethnopharmacology*, 2002. **80**(1): p.43–47.
62. Tas, A., *Analgesic effect of Pimpinella anisum essential oil extract in mice*. *Indian Veterinary Journal*, 2005. **86**(2): p. 145-147.
63. Tas, A., Ozbek, H., Atasoy, N., Altag, M.E., and Ceylan, E., *Evaluation of analgesic and antiinflammatory activity of Pimpinella anisum fixed oil extract*. *Indian Veterinary Journal*, 2006. **83**(8): p.840–843.
64. Nahidi, F., Tahetpoor, M., Majob, F., and Majd, H., *Effect of Anise extract on hot flush of menopause*. *Pajoohandeh*, 2008. **13**(3): p.167–173.
65. Khoda Karami, N., Moattar, F., and Ghahiri, A., *Comparison of effectiveness of an herbal drug (celery, saffron, Anise) and mephnamic acid capsule On primary dismenorrhea* . *Ofoghe Danesh*, 2008. **14**(1): p.11–19.
66. Rajeshwari, C.U., Abirami, M., and Andallu, B., *In vitro and in vivo antioxidant potential of aniseeds (Pimpinella anisum)*. *Asian Journal of Experimental Biological Sciences*, 2011. **2**(1): p.80–89.
67. Singh, G., Kapoor, IPS, Singh, P., de Heliani, C.S., and Catalan, CAN, *Chemical composition and antioxidant potential of essential oil and oleoresins from anise seeds (Pimpinella anisum L.)*. *International Journal of Essential Oil Therapeutics*, 2008. **2**(3): p.122–130.
68. Erler, F., Ulug, I., and Yalcinkaya, B., *Repellent activity of five essential oils against Culex pipiens*. *Fitoterapia*, 2006. **77**(7-8): p.491–494.

69. Tunç, I., Berger, B.M., Erler, F., and Dagli, F., *Ovicidal activity of essential oils from five plants against two stored-product insects*. Journal of Stored Products Research, 2000, **36**(2): p.161–168.
70. Lee, H.S., *p-anisaldehyde: acaricidal component of Pimpinella anisum seed oil against the house dust mites Dermatophagoides farinae and Dermatophagoides pteronyssinus*. Planta Medica, 2004.**70**(3): p.279–281.
71. Shukla, H.S., *Antiviral properties of essential oils of Foeniculum vulgare and Pimpinella anisum L*. Agronomie, 1989. **9**(3): p.277–279.
72. Lee, J.B., Yamagishi, C., Hayashi, K., and Hayashi, T., *Antiviral and immunostimulating effects of lignin-carbohydrate-protein complexes from Pimpinella anisum*. Bioscience, Biotechnology and Biochemistry, 2011. **75**(3): p.459–465.
73. Mulabagal, V., and Tsay, H., *Plant Cell Culture- An Alternative and efficient source for the production of biologically important secondary metabolite*. International Journal of Applied Science and Engineering, 2004. **2**(1): p. 29-48.
74. Pand, S. S., and Gupta, P., *Plant tissue culture of Stevia rebaudiana (Btoni)*. Journal of pharmaconosy and phytotherapy, 2013. **5**(1): p.26-33.
75. Dodds, J., and Roberts, L., *Experiments in Plant Tissue culture*, 2nd edn.1985, Cambridge university press.p.70.
76. Thomas, E., and Davey, M.R, *From single cells to plants*, 1975.
77. Thorpe, T.A., *Organogenesis in vitro: structural, physiological, and biochemical aspects*, in *Perspectives in plant cell and tissue culture*, I.K., Vasil, editor. 1980, New York Academic press.p.71-111.
78. Skooge, F., *Growth and organ formation in tobacco tissue cultures*. Am.J.Bot, 1944. **31**: p.19-24.
79. Tran Thanh Van, K., *Control of morphogenesis by inherent and exogenously applied factors in thin cell layers*, in *Perspectives in plant cell and tissue culture*, Vasil, I.K., editor. 1980a, New York Academic press. p.175-194.
80. Tran Thanh Van, K., and Trinh, H., *Morphogenesis in thin cell layers: concept, methodology and results*, in *Frontiers of plant tissue culture*, Thorpe, T.A., editor. 1978, International association for plant tissue culture. p. 37-48.
81. Tran Thanh Van, M., Chlyah, H., and Chlyah, A., *Regulation of organogenesis in thin layers of epidermal and sub-epidermal cells*, in *Tissue culture and plant science*, Street, H.E., editor.1974, London: Academic press.p.101-39.
82. Torrey, J.G., *The initiation of organized developement in plants*. Adv. Morphogenesis, 1966. **5**:p.39-91.
83. Ross, M.K., Thorpe, T.A., and Costreton, J.W., *Ultra structural aspects of shoot initiation in tobacco callus culture*. Am.J.Bot, 1973. **60**:p.788-795.
84. Street, H.E., *The anatomy and physiology of morphogenesis*, in *Studies involving tissue and cell culture*, R.J.Gautheret, editor. 1977, Paris: Masson. p. 20-33.
85. Gresshoff, P.M., *Phytohormones and growth and differentiation of cells and tissues cultured in vitro*, in *Phytohormones and related compounds a comprehensive treatise*, Letham, D.H., Goodwin, P.B., and Higeins, T.J.V., editors. 1978, Amesterdam: North- Holland. **2**:p.1-29.
86. Gauthert, R.J., *Investigations on root formation in tissues of Heliantbus tuberos cultured in vitro*. Am.J.Bot, 1969.56:p.702-717.
87. Thomas, E., and Street, H.E., *Organogenesis in cell suspension cultures of Atropa belladonna L., and Atropa belladonna cultivar lutea Doll*. Am.J.Bot, 1970. **34**:p.657 -669.

88. Gautheret, R.J., *Factors affecting differentiation of plant tissues in vitro*, in *cell differentiation and morphogenesis*, Beerman, W., editor. 1966, Amsterdam: North-Holland. p.55-95.
89. Meins, F., *Heritable variation in plant cell culture*. *Annu. Rev. plant physiol*, 1983. **34**:p.327-347.
90. Welander, M., and Huntrieser, I., *The rooting ability of shoots raised in vitro from the apple rootstock A2 in juvenile and in adult growth phase*. *Physiol. Plant*, 1981. **53**:p.301-306.
91. Gould, A.R., *Diverse pathways of morphogenesis in composite *Brachycome lineariloba* (2n=4)*. *Protoplasma*, 1978. **27**:p.125-135.
92. Murashige, T., and Nakano, R., *Morphogenic behavior of tobacco tissue cultures and implication of plant senescence*. *Am. J. Bot.*, 1965. **52**:p.819-827.
93. Skooge, F., and Tsui, C., *Chemical control of growth and bud formation in tobacco stem segments and callus cultured in vitro*. *Am. J. Bot.* 1984. **35**:p.782-787.
94. Sterling, C., *Origin of buds in tobacco stem segments cultured in vitro*. *Am. J. Bot.*, 1951. **38**:p.761-767.
95. Mada, E., and Thorpe, T.A., *Shoot hisogenesis in tobacco callus cultures*. *In Vitro*, 1979. **15**:p.415-424.
96. Huxter, T.A., Reid, D., and Thorpe, T.A., *Ethylene production by tobacco (*Nicotiana tabacum*) callus*. *Physiol. Plant*, 1979. **46**:p.374-380.
97. Thorpe, T.A., *Carbohydrate utilization and metabolism*, *In Tissue culture in forestry*, Bonga, J.M. and Durzan, D.J., editors. 1982b, The Hague: Martinus Nijhoff/Junk. p. 325-368.
98. Gross, P.R., *Biochemistry of differentiation*. *Annu. Rev. Biochem*, 1968. **37**:p.631-660.
99. Roberts, L.W., *Cytodifferentiation in plants*, in *Xylogenesis as a model system*. 1976, Cambridge University Press.
100. Phillips, R., and Arnott, S.M., *Studies on tracheary element differentiation in cultured tissues of tubers of the Jerusalem artichoke, *Helianthus tuberosus**. *Histochem. J*, 1983. **15**:p.427-436.
101. Roberts, L.W., and Baba, S., *Exogenous methionine as a nutrient supplement for the induction of xylogenesis in lettuce pith explants*. *Am. Bot. J.*, 1978. **42**:p.375-379.
102. Miller, A.R., and Roberts, L.W., *Ethylene biosynthesis and xylogenesis in *Lactuca* pith explants cultured in vitro in presence of auxin and cytokinin: The effect of ethylene precursors and inhibitors*. *Experimental Bot. J*, 1984. **35**:p.691-698.
103. Aloni, R., *Role of cytokinin in differentiation of secondary xylem fibers*. *Plant Physiol.*, 1982. **70**:p.1631-1633.
104. Roberts, L.W., and Fosket, D.E., *Further experiments on wound vessel formation in stem wounds of *Coleus**. *Bot. Gaz.*, 1962. **123**:p.247-254.
105. Gresshoff, P.M., *Phytohormones and growth and differentiation of cells and tissues cultured in vitro*, in *Phytohormones and related compounds a comprehensive treatise*, Letham, D.H., Goodwin, P.B., and Higeins, T.J.V., editors. 1978, New York: Elsevier. **2**:p.1-29.
106. Dalessandro, G., and Roberts, L.W., *Induction of xylogenesis in pith parenchyma explants of *Lactuca**. *Am. J. Bot.*, 1971. **58**:p.378-385.

107. Wetmore, R.H., and Rier, J.P., *Experimental induction of vascular tissues in callus of angiosperms*. Am. J. Bot., 1963. **50**:p.418-430.
108. Jeffs, R.A., and Northcote, D.H., *Experimental induction of vascular tissue in an undifferentiated plant callus*. Biochem.J., 1966.**101**:p.146-152.
109. Fadia, V.P., and Mehta, A.R., *Tissue culture studies on cucurbits: The effect of NAA, sucrose, and kinetin on tracheary differentiation in Cucumis tissues cultured in vitro*. Phytomorphology, 1973. **23**:p.212-215.
110. Haddon, L.E., and Northcote, D.H., *Quantitative measurement of the course of bean callus differentiation*. J. Cell. Sci., 1975. **17**:p.11-26.
111. Kintzios, S., and Makri., O., *In vitro rosmarenic acid accumulation in sweet basil (Ocimum basilicum L.)*. Biotechnology letters, 2003. **25**:p. 405-408.
112. Mandal, J., Patiniak, S., and Chand K., P., *Alginate encapsulation of axillary buds of ocimum americanum L.(horny basil), O.basilicum L. (sweet basil), O.gratissimum L.(shrubby basil), O. sanctum L.(sacred basil)*. In vitro cell. Dev. Biol. –Plant, 2000. **36**:p.287-292.
113. Siddique, and Anis, M., *Rapid micropropagation of Ocimum basilicum using shoot tip explants pre-cultured in thidiazuron supplemented liquid medium*. Biologia Plantrum, 2007. **51** (4): p. 787-790.
114. Winthrop, B., Phippen, and James, E., Simon, *Shoot regeneration of young explants from Basil. (Ocimum basilicum L.)*, In vitro cell. Dev. Biol. Plant, 2000. **36**:p.250-254.
115. Sahoo, Y., Pattnaik, S., K., and Chand, P., K., In vitro cell. Dev. Biol. Plant, 1997. **33**: p.293-296.
116. Itoh, K., Nakamura, K., Utsuki, T., Sakamaki, H., and Horiuchi, A., *Stereoselective oxidation of racemic 1- arylethanol by basil cultured cells of Ocimum basilicum cv. Purpurascens*. Biotechnol. Lett, 2008. **30**: p.951-954.
117. Gopi, C., Ponmurugan, P., [Somatic embryogenesis and plant regeneration from leaf callus of Ocimum basilicum L.](#) Journal of Biotechnology, 2006. **126**(2):p.260-264.
118. Bais, H., [Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of Ocimum basilicum](#). Plant Physiology and Biochemistry, 2002. **40** (1): p. 983-995..
119. [Livadariu, O.](#), *Experimental research on in vitro propagation through direct somatic embryogenesis of basil (Ocimum basilicum L.)*. Animal Science and Biotechnologies, 2011. **68** No. 1/2 p. 332-337.
120. [Saha, S., Ghosh, P.D., and Sengupta, C.](#), *An efficient method for micropropagation of Ocimum basilicum L.* [Indian Journal of Plant Physiology](#), 2010. **15** (2): p.168-172.
121. Gopi, C., Nataraja Sekhar, Y., and Ponmurugan, P., *In vitro multiplication of Ocimum gratissimum L.through direct regeneration*. African Journal of Biotechnology, 2006. **5** (9): p. 723-726.
122. Khan, F., and Saeed, A., *Evaluation of comparative scavenging efficiency of in vitro grown Ocimum tenuiflorum (TULSI) for copper and lead using atomic absorption spectroscopy*. Pakistan Journal of Science, 2012. **64** (2).
123. Saha, S., Dey, T., and Ghosh, P., *Micropropagation of Ocimum Kilmandscharicum Guerke (Labiatae)*. Acta biologica craciviensia series botanica, 2010 .52/2: p.50-58.
124. Lutzenberger, A., and Theime, R., R., *Fatty Acid B-Oxidation and Glyoxylate Cycle Enzyme Activities of Induced Glyoxysomes from Anise Suspension Cultures*. Plant Cell Report, 1983. **2**: p. 160-163.

125. Noma, M., Huber J., Ernst, D., and Pharis, R., *Quantitation of gibberellins and the metabolism of [³H] gibberellin A₁ during somatic embryogenesis in carrot and anise cell cultures*. *Planta*, 1982. **155**: p.369-376.
126. Ernst, D., Schafer, W., and Oesterhel, D., *Isolation and identification of a new, naturally occurring cytokinin (6-benzylaminopurineriboside) from an anise cell culture (Pimpinella anisum L.)*. *Planta*, 1983. **159**: p. 222-225.
127. Reichling, J., Martin, R., and Kemmerer, B., *Biosynthesis of pseudoisoeugenol-derivatives in liquid tissue cultures of Pimpinella anisum*. *Plant Cell, Tissue and Organ Culture*, 1995. **43**(2): p. 131-136.
128. Eguchi, Y., Bela, S.J., and Shetty, K., *Simulation of Somatic Embryogenesis in Anise (Pimpinella anisum) Using Fish Protein Hydrolysates and Proline*. *Journal of Herbs, Spices and Medicinal Plants*, 1998. **5**(3): p. 61-68.
129. Bela, J., Ueno, K., and Shetty, K., *Control of Hyperhydricity in Anise (Pimpinella anisum) Tissue Culture by Pseudomonas spp.* *Journal of Herbs, Spices and Medicinal Plants*, 1998. **6**(1): p. 57-67.
130. Andarwulan, N., and Shetty K., *Phenolic Content in Differentiated Tissue Cultures of Untransformed and Agrobacterium-Transformed Roots of Anise (Pimpinella anisum L.)*. *J. Agric. Food.*, **1999**. **47**(4): p. 1776–1780.
131. Radetzky, R., and Langheinrich, U., *Induction of accumulation and degradation of the 18.4-kDa oleoresin in atriacylglycerol –storing cell culture of anise (pimpinella anisum L.)*. *Planta*, 1994. **194**: p.1-8.
132. Ernst, D., and Oesterhel, D., *Changes of cytokinin nucleotides in an anise cell culture (pimpinella anisum L.) during growth and embryogenesis*. *Plant cell reports*, 1985. **4**: p.140-143.
133. Ernst, D., and Oesterhel, D., *Effect of exogenous cytokinins on growth and somatic embryogenesis in anise (pimpinella anisum L.)*. *Planta*, 1984. **161**: p. 246 -248.
134. Ernst, D., Schafer, W., and Oesterhel, D., *Isolation and quantitation of isopentyladenosine in an anise cell culture by single- ion monitoring, radioimmunoassay and bioassay*. *Planta*, 1983. **159**: p. 216 -221.
135. Salem, K.H., Charlwood, B., *Accumulation of essential oils by Agrobacterium tumefaciens-transformed shoot cultures of Pimpinella anisum*. *Plant Cell, Tissue and Organ Culture*, 1995. **40**(3): p. 209-215.
136. Chand, S., Ashok, S., and Prakash, D., *In vitro culture of Pimpinella anisum L.(anise)*. *Plant biochemistry and biotechnology*, 1997. **6**: pp.91-95.
137. Saxina, S., Khan, I., and Saxina, R., *Organogenesis in anise*, *Journal of spices and aromatic crops*, 2012. **21**(1): p. 59-63.
138. Shellie, R., Mondello, L., and Marriott, P., *Characterization of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography*. *Journal of Chromatography A*, 2002. **1** (2000).
139. Bicchi, C., Binello, A., D'Amato, A., and Rubiolo P., *reability of Van don Dool indices in the analysis of essential oils*. *Journal of Chromatographic science*, 1999. **37**:p. 288-294.
140. Kováts, E., *Gas-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retention sindices aliphatischer Halogenide, Alkohole,*

- Aldehyde und Ketone*. Helvetica Chimica Acta Volume, 1958. **41**(7): p.1915–1932.
141. Thiem, B., Kikowska, M., Kurowska, A., and Kalemba, D., *Essential oil composition of the different parts and in vitro shoot culture of Eryngium planum L.* *Molecules*, 2011. **16**: p.7115-7124.
 142. Anderson, N., H., Costin C.R., Kramer, C.M., Ohta, Y., and Huneck, S. *Phytochemistry*, 1973. **12**: p. 2709.
 143. Leffingwall, J., and Alford, E., D., *Volatile constituents of Perique tobacco*. *Electronic Journal of environmental, agricultural and food chemistry*, 2005. **4**(2): p.899-915.
 144. Swigar, A., A., and Silverstein, R., M., *Monoterpenes*, Aldrich, Milwaukee wi, 1981.
 145. Jennings, W., and Shibamoto, T., *Qualitative analysis of flavor and fragrance volatiles by glass capillary column GC*. Academic Press, New York, 1990.
 146. Politeo, O., M., Juki and Milo, M., *Chemical composition and antioxidant activity of essential oils of twelve spice plants*. *Croatica Chemical Acta.*, 2006. **79**(4):p.545-660.
 147. [Krkosova, Z.](#), [Kubinec, R.](#), [Addova, G.](#), [Jurdakova, H.](#), [Blasko, J.](#), [Ostrovsky, I.](#), and [Sojak, L.](#), *Gas chromatographic - mass spectrometric characterization of monomethylalkanes from fuel diesel*. *Petroleum & Coal*, 2007. **49**(3):p. 51-62.
 148. Amri, I., Mancini, E., De Martino, L., and Marandino, A., *Chemical composition and biological activities of the essential oil from three Melaleuca species grown in Tunisia*. *Int J Mol Sci.*, 2012. **13**(12): p.16580-16591.
 149. Maarse, H., *J. Chromatography*, **106**(1975): p.369.
 150. Adams, R.P., *Identification of essential oil components by gas chromatography/mass spectrometry*. Allured Publishing Corporation, Carol Stream, IL, 1995.
 151. Tepe, B., [Askin Akpulat, H.](#), [Sokmen, M.](#), [Daferera, D.](#), [Yumrutas, O.](#), [Aydin, E.](#), and [Polissiou, M.](#), *Screening of the antioxidative and antimicrobial properties of the essential oils of Pimpinella anisetum and Pimpinella flabellifolia from Turkey*, *Food Chem.*, 2006. **97**:p. 719-724.
 152. [Zeller, A.](#), and [Rychlik, M.](#), *Character impact odorants of fennel fruits and fennel tea*. *J. Agric. Food Chem.*, 2006. **54**:p. 3686-3692.
 153. Loloiu, T., and Radulescu, V., *Capillary GC-MS investigation of chemical composition of Hypericum perforatum*, 2000.
 154. [Tudor, E.](#), [Moldovan, D.](#), *Temperature Dependence of the Retention Index for Perfumery Compounds on a Se-30 Glass Capillary Column. II. The Hyperbolic Equation*. *J. Chromatogr.*, 1999. **848**: p.215-227.
 155. Barath M, et al., *Synthesis of a Precursor of a Lipid A Mimic*. *Chemical Papers (Slovak Acad. Sci.)*, 2003. **57**(2): p.125-130.
 156. Danaei, G., Vander Hoorn, S., Lopez, A.D., Murray, C.J., and Ezzati, M., *causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors*. *Lancet*, 2005. **366**(9499): p.1784-1793.
 157. Doll, R. and Peto, R., *The causes of cancer: quantitative estimates of avoidable risks of cancer in United States today*. *J Natl Cancer Inst*, 1981. **66**(6): p.1191-1308.
 158. *World health organization [homepage on the internet]. Country profiles*. Available from: <http://www.emro.who.int/emrinf/index.asp?Ctry=egy>. 2010.

159. Dey, S., Soliman, A., Hablas, A., Seifeldin, IA., Ismail, K., and Ramadan, M., *Urban-rural differences in breast cancer incidence by hormone receptor status across 6 years in Egypt*, *Breast Cancer Res Treat*, 2010. **120**(1): p.149-160.
160. Dey, S., Soliman, AS., Hablas, A., Seifeldein, IA., Ismail, K., Ramadan, M., El-Hamzawy, H., Wilson, ML., Banerjee, M., Boffetta, P., Harford, J., and Merajver, SD., *Urban-rural differences in breast cancer incidence in Egypt(1999-2006)*, *Breast*, 2010.**19** (5): p.417 -423.
161. Dey, S., Hablas, A., Seifeldin, IA., Ismail, K., Ramadan, M., El-Hamzawy, H., Wilson, ML., Banerjee, M., Boffetta, P., Harford, J., Merajver, SD., and Soliman, AS., *Urban-rural differences of gynaecological malignancies in Egypt (1999-2002)*. *Bjog*, 2010.**117** (3): p.348 -355.
162. Dey, S., Zhang, Z., Hablas, A., Seifeldein, IA., Ramadan, M., El-Hamzawy, H., Soliman, AS., *Geographic patterns of cancer in the population-based registry of Egypt : possible links to environmental exposure*. *Cancer Epidemiology*, 2011. **35** (3): p.254-264.
163. Reddy, L.B., Odhav, and Bhoola, *Natural products for cancer prevention: a global prespective*. *Pharmacology& amp; Therapeutics*, 2003. **99**(1): p.1-13.
164. Timbrell, J.A., *Toxic responses to foreign compounds, in principles of biochemical toxicology*. 2000, Taylor & Francis: London. p. 272-273.
165. Jemal, A., et al., *Global cancer statistics*. *A Cancer Journal for Clinicians*, 2011. **61**(2) p. 69-90.
166. Beasley, R., *Hepatitis B virus: the major etiology of hepatocellular carcinoma*. *Cancer*, 1988. **61**(10) p. 1942-1956.
167. Parkin, D.M., *The global health burden of infection-associated cancers in the year 2002*. *Int J Cancer*, 2006. **118**:p.3030-3044.
168. London, W.T., and McGlynn, K.A., *Liver cancer*. In *Cancer Epidemiology and Prevention* 3rd ed , Schottenfeld D., and Fraumeni FJJr, editors. 2006, New York: Oxford University Press. p. 763-786.
169. Ming, L., Bray, F., Melissa, M., Ferlay, J., Ward, E., and Forman, D., *Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China*. *Hepatology*, 2002.**36**:p. 1214-1220.
170. International Agency for Research on Cancer (IARC), *Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol **82**. Lyon, France: International Agency for Research on Cancer, 2002.
171. El-Serag, HB., *Epidemiology of hepatocellular carcinoma in USA*. *Hepatol Res*, 2007. **37**(suppl 2): S88-S94.
172. Nadia, M., G.I., Eman, A. , *Cancer Pathology Registry: 2003-2004, and Time Trend Analysis*. National Cancer Institute, Cairo University, 2007.
173. Ezzat, S., et al., *Association of pesticides, HCV, HBV, and hepatocellular carcinoma in Egypt*. *Int. J. Hyg. Environ Health*, 2005. **208**(5): p.329-339.
174. Cao, H., Phan, H., and Yang, L., *Improved Chemotherapy for Hepatocellular Carcinoma*. *Anti cancer Research*, 2012. **32** (4):p. 1379-1386.
175. American Cancer Society. *Cancer Facts & Figures 2014 Atlanta*. Ga:American Cancer Society, 2014.
<http://www.cancer.org/acs/groups/cid/documents/webcontent/003096-pdf.pdf>.
176. DM., P., *Global cancer statistics in the year 2000*. *Lancet Oncol*, 2001. **2**(9): p.533-543.

177. Ferrari, P., et al., *Lifetime and baseline alcohol intake and risk of colon and rectal cancers in the European prospective investigation into cancer and nutrition (EPIC)*. Int. J Cancer, 2007. **121**:p. 2065-2072.
178. Boyle, P., and Levin, B., *World Cancer Report 2008*. Lyon, France: World Health Organization. International Agency for Research on Cancer, 2008.
179. Mandel, J.S., Bond, J.H., and Church, T.R., *Reducing mortality from colorectal cancer by screening for fecal occult blood*. N Engl J Med, 1993. **328**: p. 1365-1371.
180. Lambert, R., Sauvaget, C., and Sankaranarayanan, R., *Mass screening for colorectal cancer is not justified in most developing countries*. Int J Cancer, 2009. **125**: p. 253-256.
181. N., M., *Cancer pathology registry 1985-1989*. National Cancer Institute: Cairo, Al Asdekaa press, 1991.
182. Soliman, A.S., Bandy, M.L., Levin, B., Hamza, M.R., Ismail, K., Ismail, S., Hammam, m., and ELHattab, O.H., *Coloectal cancer in Egyptian patients under 40 years of age*. Int. J Cancer, 1997. **71**(1): p. 26-30.
183. Gill, S., Thomas, R. R., and Goldberg, R. M., *Review article: colorectal cancer chemotherapy*. Aliment Pharmacol Ther, 2003. **18**(7) p. 683-692.
184. Pandey, Govind, *An overview on certain anticancer natural products*. Journal of Pharmacy Research, 2009. **2**(12): p.1799-1803.
185. *Mohamed Abdulla and Per Gruber, Role of diet modification in cancer prevention*. BioFactors, 2000. **12**(1-4): p. 45-51.
186. Mukherjee, A.K., Basu, S., Sarkar, N., and Ghosh, A.C., *Advances in cancer therapy with plant based natural products*. Current Medicinal Chemistry, 2001. **8**(12): p. 1467-1486.
187. Zheng, S.H., Yang, H., Zhang, S., Wang, X., Yu, L., Lu, J., and Li, J., *Initial study on naturally occurring products from traditional Chinese herbs and vegetables for chemoprevention*. Journal of Cellular Biochemistry, 1997. **67** (27): p. 106-112.
188. [Kathirvel, P.](#), and [Ravi, S.](#), *Chemical composition of the essential oil from basil (*Ocimum basilicum* Linn.) and its in vitro cytotoxicity against HeLa and HEP-2 human cancer cell lines and NIH 3T3 mouse embryonic fibroblasts*. Natural Product Research: Formerly Natural Product Letters, 2012. **26**(12): p. 1112-1118.
189. Borenfreund, E., and Puerner, J., *A simple quantitative procedure using monolayer cultures for cytotoxicity assay*. J. Tissue Culture Methods, 1984. **9** (1): p. 7-9.
190. Fotakis, G. and Timbrell, J., A., Toxicol. Lett., 2006. **160**(2): p. 171-177.
191. Boyum, A., *Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1g*. Scand. J. Clin. Lab. Invest. Suppl. 1968, **97**: p.77-89.
192. GraphPad InStat Version 3.0, GraphPad Software, San Diego, California, USA.
www.graphpad.com.
193. Mie-Yin Change and Yi-ling Shen, *Linalool exhibit cytotoxic effects by activating antitumor immunity*. Molecules, 2014. **19**: p. 6694-67706.

194. Choo, E.J., Rhee, Y.H., and Jeonge, S.J., *Anethole exerts antimetastatic activity via inhibition of matrix metalloproteinase 2/9 and AKT/Mitogen-Activated Kinase/Nuclear Factor Kappa B signaling pathways*. *Biol. Pharm. Bull*, 2011. **34**(1): p. 41-46.
195. Devasagayam, T.P.A., Tilak, J.C., Boloor, K.K, Sane, S., Ghaskadbi, S., and Lele, R.D., *Free radicals and antioxidants in human health current status and future prospects*. Japi, 2004. **52**: p. 794-804.
196. Sies, H., *Biochemistry of Oxidative Stress*. *Angew Chem Internat Ed Eng*, 1986. **25**: p.1058-1071.
197. Tilak, J.C., Devasagayam, T.P.A., and Lele, R.D., *Antioxidant activities from Indian medicinal plants: A review - Current status and future prospects*. *International Conference on Natural Antioxidants and Free Radicals in Human Health and Radiation Biology (NFHR)*, 2001. P. 5.
198. Krishnaswami, K., *Indian functional food: role in prevention of cancer*. *Nutr Rev*, 1996. **54**: p. 127-131.
199. Hussain, A., Anwar, F., Sherazi, S.T., and Przybylski, R., *Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations*. *Food Chemistry*, 2008. **108**(3): p. 986-995.
200. Politeo, O., Jukic, M., and Milos, M., *Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil*. *Food Chemistry*, 2007. **101**(1): p. 379-385.
201. Bozin, B., Mimica-Dukic, N., *Characterization of the Volatile Composition of Essential Oils of Some Lamiaceae Spices and the Antimicrobial and Antioxidant Activities of the Entire Oils*. *J. Agric. Food Chem.*, 2006. **54**(5): p. 1822-1828.
202. Nahar. L., Rusell, W., Middleton, M., Shoeb, M., and Sarker, S., *Antioxidant phenylacetic acid derivatives from the seeds of *Ilex aquifolium**. *Acta Pharm*, 2005. **55**(2): p.187-193.
203. Miguel, M.G., *Antioxidant activity of medicinal and aromatic plants. A review*. *Flavour Fragr. J.*, 2010. **25**: p. 291-312.
204. Smail, A., Badia L., and Maria G., *Antioxidant and anticholinesterase activities of some commercial essential oils and their major compounds*, *Molecules*, 2011. **16**: p. 7672-7690.
205. Iris, H., Dorman, H.J., and Hiltunen, R., *Antioxidant activities of extracts from culinary herbs and spices*. *Food Chemistry*, 2006. **97**: p. 122-129.
206. Ahrens, J., *Scientific Proceedings OM Laboratories*. Geneva, 1984: p. 29-39.
207. Klausen, B., and Evans, R., *Oral Microbial. Immunol*, 1991. **6**: p.193-201.
208. Maestrony, C., and Losa, G., *Scientific Proceedings OM Laboratories*. Geneva, 1984: p.56-65.
209. Marinova, S., L., and Tchorbadijska, *Int. J. Immunopharmac.*, 2000. **22**: p.843-854.
210. Petrunov, B., Nenkov, P., and Shekerdjiisky, R., *The role of immunostimulants in immunotherapy and immunoprophylaxis*. *Biotechnol.*, 2007: p. 454-462.
211. Ciului, I., Grigorescu E., Stanescu U., *Plante medicinale, fitochimie și fitoterapie*, vol I – II, Ed. Medicală, București, 1993.
212. Grigorescu, E., MLazăr, I., Stănescu, U., and Ciulei, I., *Index fitoterapeutic*, Ed. Cantes, and Iași, 2001.
213. Stanescu, U., et al., *Bazele farmaceutice, farmacologice și clinice ale fitoterapiei*, vol II, Ed. UMF “Gr.T.Popa” Iași, 2002. p. 105-145.

214. Hashemi, S.R., et al., *The effect of selected sterilization methods on antibacterial activity of aqueous extract of herbal plants*. J. Boil. Sci., 2008. **8**: p.1072-1076.
215. Shan, B.E., Yoshida, Y., Sugiura, T., Yamashita, U., *Stimulating activity of Chinese medicinal herbs on human lymphocytes in vitro*. Int. J. Immunopharmacol., 1999. **21**: p. 149-159.
216. Cao, L.Z., and Lin, Z.B., *Regulatory effect of Ganoderma lucidum polysaccharides on cytotoxic T-lymphocytes induced by dendritic cells in vitro*. Acta Pharmacol. Sin., 2003. **24**: p. 312-326.
217. Lin, Z.B., and Zhang, H.N., *Anti-tumor and immunoregulatory activities of Ganoderma lucidum and its possible mechanisms*. Acta Pharmacol. Sin., 2004. **25**: p. 1387-1395.
218. Tan, B.K.H., and Vanitha, J., *Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: A review*. Curr. Med. Chem., 2004. **11**: p. 1423-1430
219. Li, X.Y., *Immuno-modulating components from Chinese medicines*. Pharm. Biol., 2000. **38**: p. 33-40.
220. Oda, K., et al., *Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants*. Biol. Chem., 2000. **381**: p. 67-74.
221. Jie, Y.H., Cammisuli, S., and Baggiolini, M., *Immunomodulatory effects of Panax ginseng ca MEYER in the mouse*. Agents Actions, 1984. **15**: p. 386-391.
222. Kensil, C.R., *Saponins as vaccine adjuvants*. Crit. Rev. The. Drug. Carrier. Syst., 1996. **13**: p. 1-55.
223. Maharaj, I., Froh, K.J., and Campbell, J.B., *Immune responses of mice to inactivated rabies vaccine administered orally: Potentiation by Quillaja saponin*. Can. J. Microbiol., 1986. **32**: p.414-420.
224. Lee, J.H., Choi, Y., Kang, h., and Choi, B., *An aqueous extract of Platycodi radix inhibits LPS-induced NF-kappa B nuclear translocation in human cultured airway epithelial cells* Int. J. Mol. Med., 2004. **13**: p. 843-847.
225. Recchia, J., Lurantos, MHA., Amsden, JA., and Storey, J., *A semisynthetic Quillaja saponin as a drug delivery agent for aminoglycoside antibiotics*. Pharm. Res., 1995. **12**: p. 1917-1923.
226. Dashputre, L., Naikwade, S., *Preliminary Immunomodulatory Activity of Aqueous and Ethanolic Leaves Extracts of Ocimum basilicum Linn in Mice*. International Journal of PharmTech Research, 2010. **2** (2): p.1342-1349.
227. Walford, R., Gallagher, R. and Sjaarda j., *Serologic typing of human lymphocytes with immune serum obtained after homografting*. Science, 1964.**144**: p. 868-870.
228. Repetto, G., Peso, A. and Zurita, J., *Neutral red uptake assay for the estimation of cell viability/cytotoxicity*. Nature Protocols, 2008. **3**: p. 1125-1131.

Arabic summary

الملخص العربي

تتضمن هذه الدراسة زراعة الأنسجة لإثنين من النباتات الطبية المنتجة للزيوت الطيارة وهما نبات أوسيمم بازيليكم (الريحان الحلو) وينتمي إلى العائلة الشفوية، و نبات الليمبنيلا أنيسم (اليانسون) وينتمي إلى العائلة الخيمية.

العائلة الشفوية

تشتهر هذه العائلة بالنباتات ذوات الزيوت الطيارة، و تحتوي هذه العائلة على مائة و ثمانين جنساً و ثلاثة آلاف وخمسمائة نوعاً، وتوزع جغرافياً على نطاق واسع في العالم، و خاصةً في منطقة حوض البحر الأبيض المتوسط، وتتميز هذه العائلة بالأعشاب البرية والشجيرات الصغيرة.

نبات أوسيمم بازيليكم (الريحان الحلو)

جنس الأوسيمم يتكون من حوالي خمسون إلى مائة و خمسون نوعاً من الأعشاب والشجيرات التي توجد في المناطق الإستوائية في آسيا، أفريقيا، ووسط وجنوب أمريكا. ومن حيث الوصف النباتي ؛ فهو نبات حولي يصل ارتفاع ساقه من ثلاثين إلى ستين سنتيمترات، الأوراق تكون متعكسة الترتيب على الساق على شكل بيضاوي وأحياناً تكون مسننة، الزهور تكون مخنثة صغيرة الحجم بيضاء وأحياناً مزكرشة بالأرجواني ولها تويج مسنن الأوراق وإثنين من البتلات على شكل الشفاه والثمار تكون شبه كروية صغيرة.

العائلة الخيمية

وهي عائلة واسعة الإنتشار تتكون من أربعمائة جنساً و ثلاثة آلاف و خمسمائة نوعاً، ويكثر إنتشارها في المناطق ذات الطقس المعتدل. و تتسم هذه العائلة بالأعشاب ذات الساق السمكية وأوراقها بسيطة البديل، النورة تكون مركبة على شكل الخيمة وتحتوي على نورات صغيرة بسيطة كلٍ منها يمثل خيمة صغيرة.

نبات الليمبنيلا أنيسم (اليانسون)

يرجع منشاه إلى آسيا الصغرى واليونان ومصر وبنمو بكثرة أيضاً في أسبانيا، الإتحاد السوفيتي، فرنسا وشمال أفريقيا . ومن حيث الوصف النباتي ؛ فهو نبات حولي يصل ارتفاع ساقه إلى خمسين سنتيمترات، الأوراق بيضاوية الشكل تكون متعكسة الترتيب على الجزء العلوي من الساق و متبادلة في الجزء السفلي. اما النورة فتكون مركبة على شكل الخيمة لها ساق طويلة تحتوي على عدد يصل إلى ثمانية حتى أربعة عشر فرعاً حاملةً نورات صغيرة بسيطة كلٍ منها يمثل خيمة صغيرة. الزهور صغيرة بيضاء اللون وثمارها صغيرة بنية اللون.

وتمثل نظم زراعة الأنسجة النباتية مصدراً متجدداً للمركبات الطبية، والنكهات والطور والملونات، والتي لا يمكن أن تنتجها الخلايا الميكروبية أو التركيب الكيميائي. ولذلك فإن زراعة الأنسجة لإثنين من النباتات الطبية الهامة ودراسة بعض الأنشطة البيولوجية للزيوت الطيارة المتكونة في الأنسجة الجديدة هونصب أعيننا و قمة أهدافنا البحثية.

من المعلومات البحثية السابقة الرسالة- بين الأيدي- مسوغة للنقاط التالية:

- الظروف المناسبة التي تسمح لخلايا النبات المتكونة لإنتاج الزيوت الطيارة ذات الفائدة الطبية.
- متابعة إنتاج هذه الزيوت الطيارة وفقاً للزمن البحثي.

- دراسة تكون اعضاء نباتية جديدة من الأنسجة المزروعة.
- تحديد مكونات الزيوت الطيارة الناتجة من الأنسجة المزروعة ونسبتها المئوية .
- إجراء مسح حيوي لهذه الزيوت الطيارة، عن طريق اختبار التأثيرات المثبطة للخلايا و المنشطة للمناعة ومضادات الأكسدة.

تتكون هذه الرسالة من ثلاثة أجزاء:

الجزء الأول: زراعة الأنسجة لنباتي أوسيمم بازيليكم (الريحان الحلو) و اليبمبنيلا أنيسم (اليانسون) و الفحص المجهري للخلايا المتكونة في الأعضاء النباتية الجديدة من الأنسجة المزروعة.

تم في هذا الجزء زراعة الأنسجة لنباتي أوسيمم بازيليكم و اليبمبنيلا أنيسم في وسط غذائي يحتوي على هرمونات نباتية مختلفة، ونتج عن ذلك أنسجة نباتية جديدة والتي نتج منها أعضاء جديدة وحدث أيضاً تميز خلوي بدرجة عالية نتج عنه نسيج الخشب المميز، وكل ذلك تم فحصه مجهرياً بدقة.

الجزء الثاني: عزل الزيوت الطيارة المتكونة من الأنسجة المزروعة للنباتين والتعرف علي مكوناتها عن طريق المسح الطيفي للمواد الطيارة.

تم عزل هذه الزيوت الطيارة باستخدام طريقة التقطير بالبخار ومتابعة إنتاج هذه الزيوت الطيارة من معلق الخلايا للنباتين وفقاً للزمن البحثي، وتم التعرف على مكوناتها ومعرفة نسب تواجدتها باستخدام المسح الطيفي للمواد الطيارة.

الجزء الثالث: دراسة بعض التأثيرات الحيوية للزيوت الطيارة المتكونة، ويتكون هذا الجزء من ثلاثة فصول وهم كالاتي:

الفصل الأول: التأثير المثبط للخلايا

تمت دراسة التأثير المثبط للخلايا للزيوت الطيارة المتكونة على كل من خلايا الدم الخارجية وحيدة النواة (الطبيعية) خارج جسم الكائن الحي، ونوعين من ال خلايا السرطانية(سرطان الكبد وسرطان القولون)، وأظهرت النتائج أن الزيوت الطيارة المختبرة لها تأثير آمن على خلايا الدم أحادية النواة ولها تأثير مثبط لخلايا السرطان السابق ذكرها.

الفصل الثاني: التأثير المضاد للأكسدة

أظهرت النتائج أن الزيوت الطيارة المختبرة لها تأثير مضاد للأكسدة عن طريق تقليل إمتصاص الضوء لمركب 1,1- ثنائي الفينيل-2- بيكريل هيدرازيل المحتوي على إلكترون حر مما يدل على اختزاله و تقليل قدرته على الأكسدة .

الفصل الثالث: التأثير المنشط للمناعة

أظهرت النتائج أنه فقط الزيت الطيار المستخلص من الأنسجة المزروعة لنبات الأوسيمم بازيليكم له تأثير منشط للمناعة يعتد به .

المخلص العربي

"بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ"

دراسة زراعة الأنسجة النباتية لبعض النباتات
التي تنتمي للعائلة الخيمية والعائلة الشفوية.

رسالة مقدمة إلى

كلية الصيدلة- جامعة الإسكندرية

كجزء من متطلبات الحصول على

درجة الماجستير في العلوم الصيدلانية

(عقاير)

مقدمة من

ريهام محمد عادل محمد صالح محمد مصطفى

بكالوريوس في العلوم الصيدلانية

(جامعة الإسكندرية 2009)

ج.م.ع

2015م

لجنة الإشراف

أ.د/فتحي قنديل الفقي

أستاذ العقاقير

كلية الصيدلة - جامعة الإسكندرية

د/ حاتم محمد مكي

مدرس العقاقير

كلية الصيدلة - جامعة الإسكندرية

د/ المعتز بالله علي النجار

مدرس العقاقير

كلية الصيدلة - جامعة دمنهور

د/ هالة حلمي زعطوط

مدرس العقاقير

كلية الصيدلة - جامعة الإسكندرية

دراسة زراعة الأنسجة النباتية لبعض النباتات
التي تنتمي للعائلة الخيمية والعائلة الشفوية.
مقدمة من

ريهام محمد عادل محمد صالح محمد مصطفى

للحصول على درجة

الماجستير في العلوم الصيدلانية

عقاير

موافقون

لجنة المناقشة والحكم على الرسالة

الأستاذ الدكتور/ فتحي قنديل الفقي

أستاذ بقسم العقاقير

كلية الصيدلة

جامعة الإسكندرية

الأستاذ الدكتور/ نبيلة محمد غازي

أستاذ بقسم العقاقير

كلية الصيدلة

جامعة الإسكندرية

الدكتور/ هاني نشأت بركة

أستاذ مساعد بقسم العقاقير

كلية الصيدلة

جامعة المنصورة

الدكتور/ حاتم محمد مكي

مدرس بقسم العقاقير

كلية الصيدلة

جامعة الإسكندرية

التاريخ / /

لجنة الإشراف

أ.د/فتحي قنديل الفقي

أستاذ العقاقير

كلية الصيدلة جامعة الإسكندرية

د/ حاتم محمد مكي

مدرس العقاقير

كلية الصيدلة جامعة الإسكندرية

د/ المعتر بالله علي النجار

مدرس العقاقير

كلية الصيدلة جامعة دمنهور

د/ هالة حلمي زعطوط

مدرس العقاقير

كلية الصيدلة جامعة الإسكندرية