

## 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**

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## 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.

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## Arabic summary

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## 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 اليمينيلا 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، وتتوزع جغرافياً على نطاق واسع في العالم، و بخاصةً 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 السمكة وأوراقها بسيطة البديل، النورة تكون مركبة على شكل الخيمة وتحتوي على نورات صغيرة بسيطة كلٍ منها يمثل خيمة صغيرة.

### نبات اليمينيلا أنيسم (اليانسون)

يرجع منشاه إلى آسيا الصغرى واليونان ومصر وينمو بكثرة أيضاً في أسبانيا، الإتحاد السوفيتي، فرنسا وشمال أفريقيا . ومن حيث الوصف النباتي ؛ فهو نبات حولي يصل إرتفاع 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 والعمور 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- مسوعة 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 الليمبنيلا أنيسم (اليانسون) و الفحص المجهري للخلايا المتكونة في الأعضاء النباتية الجديدة من الأنسجة المزروعة.

تم في هذا الجزء زراعة الأنسجة لنباتي أوسيمم بازيليكم و الليمبنيلا 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 البخار 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 امتصاص 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

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"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  
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مقدمة من

ريهام محمد عادل محمد صالح محمد مصطفى

للحصول على درجة

الماجستير في العلوم الصيدلانية

عقاير

موافقون

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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/ المعتز بالله علي النجار

مدرس العقاقير

كلية الصيدلة جامعة دمنهور

د/ هالة حلمي زعطوط

مدرس العقاقير

كلية الصيدلة جامعة الإسكندرية