Phytochemical and Biological Studies on Certain Traditional Apiaceous Plants Having Potential Biological Activity

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بسم الله الرحمن الرحيم

"وَهُوَ الَّذِي أَنزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُّخْرِجُ مِنْهُ حَبًّا مُّتَرَاكِبًا وَمِنَ النَّخْلِ مِن طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِّنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ ۗ انظُرُوا إِلَىٰ ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ ۚ إِنَّ فِي ذَٰلِكُمْ لَآيَاتٍ لِّقَوْمٍ يُؤْمِنُونَ "

(الأنعام: 53)

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Noha Hassan Khalil, 2015

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATCC	American Type Culture Collection
ANOVA	One-way Analysis of Variance
CC	Column Chromatography
CCl_4	Carbon tetrachloride
CFU	Colony Forming Unit
CPP	Conditioned Place Preference
cm	centimeter
^{13}C-NMR	Carbon-13- Nuclear Magnetic Resonance
CNS	Central Nervous System
CVS	Cardiovascular System
conc.	concentrated
2-DR	2-deoxyribose enzyme
d	doublet
dd	Double doublet
dq	Double quartet
D. carota	*Daucus carota*
DMEM	Dulbecco's Modified Eagle Medium
DMSO-d_6	Deuterated Dimethylsulfoxide
2D-NMR	Two-dimensional Nuclear Magnetic Resonance
DPPH•	2,2-diphenyl-1-picrylhydrazyl radical
EDTA	Ethylenediaminetetraacetic Acid
EI-MS	Electron ionization-Mass spectrometry
FBS	Fetal Bovine Serum
Fig.	Figure
5-FU	5-Flouro-Uracil
FRAP	Ferric reducing ability of plasma
GPX	Glutathione peroxidase
GST	Glutathione-S-transferase
g	gram
GC-MS	Gas chromatography-Mass spectrometry
GC-FID	Gas chromatography-Fluid ionization detector
Hep-G2	Hepatocellular carcinoma cell line
HL-60	Acute myeloid leukemia cell line
^{1}H-^{1}H COSY	Homonuclear Correlated Spectroscopy
^{1}H-NMR	Proton Nuclear Magnetic Resonance
hrs	hours
IC_{50}	The half maximal inhibitory concentration
IR	Infrared
J value	Coupling consatnt

Kg	Kilogram
KI	Kovat's index
l	Litre
5-LO	5-Lipoxygenase enzyme
L929 cells	Murine aneuploid fibrosarcoma cell lines
m	meter
MES	Maximal Electroshock Seizures
MCF-7	Breast cancer cell lines
m/z	Mass to charge ratio
mg/kg	Milligram/kilogram
mg/ml	Milligram/millilitre
MIA-$PaCa_2$	Pancreatic cancer cell lines
MIC	Minimum Inhibitory Concentration
min.	minutes
ml	millilitre
mm	millimeter
mM	millimole
MTT	3-(4,5-dimethyl-thiazoyl)-2,5-diphenyl-tetrazolium bromide
Neuro-2a	Neuroblastoma cell lines
Nm	nanometer
PBS	Phosphate buffered saline
ppm	Part per million
PTZ	Phenylene tetrazole
PTLC	Preparative Thin Layer Chromatography
R_f	Retardation factor
ROS	Reactive Oxygen Species
s	Singlet
SAR	Structure Activity Relationship
SGPT	Serum Glutamic Pyruvic Transaminase
SGOT	Serum Glutamic Oxaloacetic Transaminase
SOD	Superoxide Dismutase
S.D.	Standard Deviation
STZ	Streptozotocin
TBARS	Thiobarbituric Acid Reactive Substances
TLC	Thin Layer Chromatography
µg	Microgram
µl	Microlitre
µg/ml	Microgram/millilitre
UV	Ultraviolet
v/v	Volume per volume
v/w	Volume per weight
δ	Chemical shift
λ	Wave length

Introduction

Historically, natural products have been used since ancient times and in folklore for the treatment of many diseases and illnesses. Classical natural product chemistry methodologies enabled a vast array of bioactive secondary metabolites from terrestrial and marine sources to be discovered. Many of these products have gone on to become current drug candidates. Natural products (secondary metabolites) have been the most successful source of potential drug leads **(Haefner, 2003, Butler, 2004, Mishra and Tiwari, 2011, Rey-Ladino *et al.*, 2011)**. Their products continue to provide unique structural diversity in comparison to standard combinatorial chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds.

Since less than 10% of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery with the challenge being how to access this chemical diversity of secondary metabolites **(Kingston and Newman, 2005)**. The use of natural products as medicines has been described throughout history in the form of traditional medicines, remedies, potions and oils with many of these bioactive natural products still unidentified. The dominant source of knowledge of natural product uses from medicinal plants is a result of man experimenting by trial and error for hundreds of centuries through palatability trials or untimely deaths, searching for available foods for the treatment of diseases **(Hicks, 1966, Kinghorn *et al.*, 2011)**.

Natural antimicrobial agents derived from sources such as plant oils have been recognized and used for centuries in food preservation. Aromatic plants and spices have been used in folk medicine as antibacterial and antifungal agents since ancient times. Essential oils and spices were used by the early Egyptians and have been used for centuries in Asia; China and India **(Cowan, 1999, Grayer and Kokubun, 2001)**. The antimicrobial and antifungal properties have been attributed to plants' secondary metabolites with complex molecular structures. These metabolites include alkaloids, flavonoids, tannins, coumarins, glycosides, terpenes and phenolic compounds **(Belletti *et al.*, 2007)**. Essential oils from aromatic plants are known to possess antibacterial and antifungal activity **(Burt, 2004)**. They constitute, in this way, complementary or alternative therapeutic options that are increasing in popularity, yet still have scant scientific credibility.

Many hundreds of plants contain well known anti-inflammatory agents. They can be used as the sole therapy in autoimmune disease or as complementary corticosteroid-sparing therapies allowing patients to take smaller doses or shorter courses of corticosteroids **(Spelman *et al.,* 2006)**. Many plant-based foods have anti-inflammatory effects, whether we know it or not, which is one reason why a diet rich in fruits and vegetables is generally beneficial for health.

The *Apiaceae* Family also known under the name of *Umbelliferae* family, due to the monopodial inflorescences in the shape of an umbel, consists of a large number of representatives (more than 300 genera and 3700 species), of which the majority are aromatic plants **(Downie *et al.,* 2000)**.

The *Apiaceae* family provides a large number of plants that can be used for both medicinal and alimentary purpose and are also called nutraceutical plants **(Parry *et al.,* 2005)**. Nutraceutical is commonly defined by the dietary supplement industry as any non-toxic food component that has scientifically proven health benefits, including disease treatment and prevention. Based on many studies, it seems that several species in this family are good sources for phytocompounds with antimicrobial and anti-inflammatory properties. Many members of this family have culinary uses and are very common in Egypt. Egyptian medicine was widely respected throughout the ancient Mediterranean world. The earliest written records of its practices are to be found in the Ebers Papyrus, dating from the sixteenth century BC. This is historically of value since in itself, it represents a compilation of earlier works that contain a large number (877) of prescriptions and recipes. Many of the plants currently used by herbalists are mentioned, including coriander, cumin, fennel, carrot, anise and celery. Coriander was one of the herbs offered to the temple by the king, and seeds were found in the tomb of Tutankhamun and in other ancient burial sites.

In our work, we selected the most common Apiaceous members used traditionally in Egypt. The work was on the essential oil fraction since this family is popular for its richness in the essential oil content; that is mentioned traditionally to have several medicinal values, and the hexane fraction as it contains the essential oil as well as the fixed oil fractions.

Aim of Work:

1. Collection and identification of selected Apiaceous fruits cultivated in Egypt [*Pimpinella anisum* L. (anise), *Carum carvi* L. (caraway), *Apium graveolens* L. (celery), *Coriandrum sativum* L. (coriander), *Cuminum cyminum* L. (cumin), *Anethum graveolens* L. (dill), *Foeniculum vulgare* L. (fennel), *Petroselinum crispum* L. (parsley), *Daucus carota* L. var. *sativus* (yellow carrot) and *Daucus carota* L. var *boissieri* (red carrot)].

2. Bio-guided Fractionation of the hexane extract of red carrot fruits
   2.1. Preliminary cytotoxic investigation of the hexane extract on MCF-7 cell lines using MTT assay.
   2.2.Investigation of the hexane extract to separate its pure components.
   2.3.Identification of the isolates using different spectroscopic methods of analysis (IR, EI-MS, ^{1}H-NMR, ^{1}H-^{1}H NMR and ^{13}C NMR).
   2.4.Cytotoxic studies on the isolated compounds and the essential oil of red carrot fruits.

3. Antimicrobial screening of the essential oils hydro-distilled from ten selected Apiaceous fruits.

4. Comparing the biological activity (antioxidant and anti-inflammatory) of the essential oils of the fruits of Yellow Carrot (*Daucus carota* L. var. *sativus*) and Red Carrot (*Daucus carota* L. var *boissieri*).

5. Chemical analysis of the most active essential oils using GC-MS and GC-FID.

Literature Review

This chapter provides an overview for the selected Apiaceous species, including their chemical constituents, mainly in their essential oils as well as the literature collected on their biological activity.

1. Anise

Name: Anise

Synonymous: *Pimpinella anisum L.*

(0.15x) (1x)

Figure 1: *Pimpinella anisum* herb and fruits (wikipedia.com)

Folk medicinal uses:

Pimpinella anisum L. (also known as anise), is one of the oldest medicinal plants. It is an annual grassy herb with 30–50 cm high, white flowers, and small green to yellow fruits, which grow in the Eastern Mediterranean Region, West Asia, the Middle East, Mexico, Egypt, and Spain **(Shojaii and Abdollahi Fard, 2012)**. Anise is primarily grown for its fruits that are harvested in August and September. Anise fruits contain 1.5–6% essential oil and used as flavoring, digestive, carminative, and relief of gastrointestinal spasms. Consumption of anise in lactating women increases milk production and also reliefs their infants from GI problems **(Penagos Tabares *et al.*, 2014)**. In the food industry, anise is used as a flavoring for fish products, ice cream, sweets, and gums **(Kosalec *et al.*, 2005)**.

Chemical review:

Essential Oil

Anise fruit contains 1.5- 6 % of essential oil consisting primarily of *trans*-anethole. Different studies dealing with the analysis of anise essential oilare summairzed in Figure (2) **(Sabrina *et al.*, 2012, Gradinaru *et al.*, 2014)**.

Other Constituents:

Anise also contains as much as 8–11 % of lipids rich in fatty acids, such as palmitic and oleic acids. It contains approximately 4 % carbohydrates, and 18 % protein **(Rodrigues *et al.*, 2003)**. In a study by Fujimatu *et al*., four aromatic glucosides, an alkyl glucoside, and a glucide were isolated as new compounds from the polar portion of methanolic extract of anise fruits **(Fujimatu *et al.*, 2003)**. It is also rich in flavonoids **(Kunzemann and Herrmann, 1977, Waumans *et al.*, 2003, Kosalec *et al.*, 2005, Denev *et al.*, 2011, Abdallah *et al.*, 2013)**.

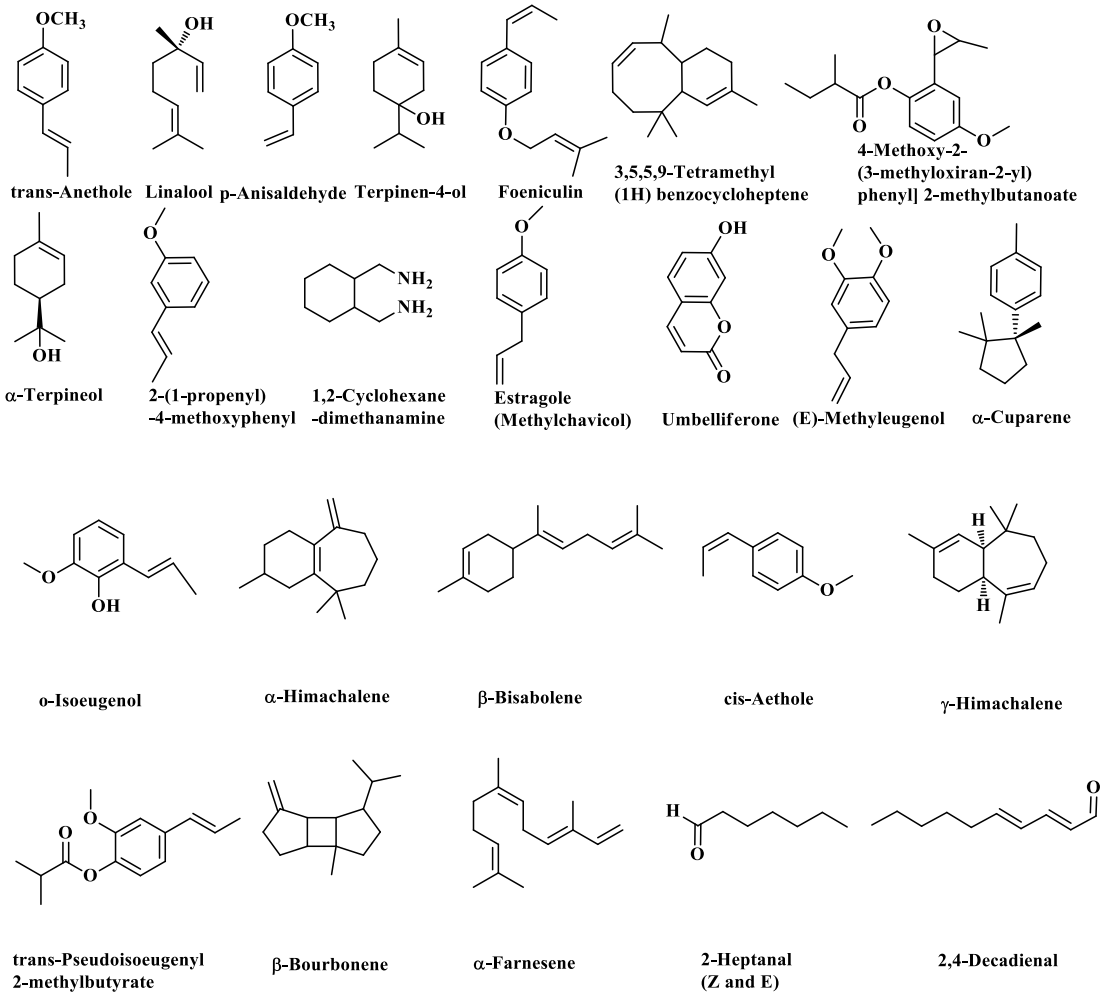

Figure 2: Essential Oil Components of *Pimpinella anisum*

Biological Review:

1. Antibacterial and Antifungal Effects

Aqueous and methanolic extracts of anise fruits exhibited fair antibacterial activity (Gradinaru *et a*l., 2014). Ethanolic extracts showed significant inhibitory activity against 10 Gram +ve bacterial species as well as *Candida albicans* (Glucin *et al*.,