Deanship of graduate studies

Al-Quds University

Antibacterial and Anticancer activity of *Cupressus sempervirens* L. fruit extraction

Hiba Metib Hussain Salah

M.Sc. Thesis

Jerusalem - Palestine

1444 / 2022

Antibacterial and Anticancer activity of *Cupressus sempervirens* L. fruit extraction

Prepared by:

Hiba Metib Hussain Salah

B.Sc.: Food Technology. Al -Quds University. Palestine.

Supervisor: Dr. Mohannad Qurie

Co-Supervisor: Dr. Mahmod Alkhatib

A thesis Submitted in Partial fulfillment of requirement for the degree of the Master of Applied and Industrial Technology, Department of Chemistry & Chemical Technology Faculty of Science and Technology, Al-Quds University.

1444 / 2022

Al- Quds University

Deanship of Graduate Studies

Applied and Industrial Technology Program

Faculty of Science and Technology

Thesis Approval

Antibacterial and Anticancer activity of *Cupressus sempervirens* L. fruit extraction

Prepared by: Hiba Metib Hussain Salah

Registration No: 21420286

Supervisor: Dr. Mohannad Qurie

Co-Supervisor: Dr. Mahmod Alkhatib

Master thesis submitted and accepted, Date: 3/9/2022

The name and signatures of the examining committee are as follows:

1.Head of committee: Dr. Mohannad Qurie Signature .....

2.Co-Supervisor: Dr. Mahmod Alkhatib Signature .....

3.Internal Examiner: Dr. Fuad Rimawi Signature .....

4.External Examiner: Dr. Murad Abu Hasan Signature.....

1444 / 2022

Dedication

Thank and blessing for Allah

To my country, my capital, and my beautiful university

To the soul of my dear father…

To my mother

My mother, all the love, thanks and gratitude to you for your support and for being by my side

To my brothers

My brothers Hussain and Ahed, without you, I would not have achieved this dream. Thank you for your support and encouragement for me to reach this stage.

To my friends

To all my friends, thank you very much for your support and encouragement.

Declaration

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledges, and that this thesis (or any part of the same) has not been submitted for the higher degree to any other university or institute.

Signed: 

Hiba Metib Hussein Salah

Date: 3/9/2022

Acknowledgments

After all these long years, I finally achieved this dream.

At the outset, I thank and extend gratitude to God Almighty, who stood with me and guided me to this stage of my life, and to all the people who supported me and stood by my side from the beginning of the work on this research until its end.

I would like to thank my supervisors, Dr. Mohannad Qurie and Co-supervisors Dr. Mahmod Alkhatib, for standing by me and providing all possible facilities for the completion of this research.

Also, a special thanks to Dr. Sawsan Salameh who spared no effort to help me, and Dr. Zaidoun Salah, who provided all possibilities to complete this research.

And a special thanks to Dr. Mohammed Abu-Alhaj in particular, for his continuous support and encouragement.

I would like to thank the internal examiner, Dr. Fuad Rimawi, and the external examiner, Dr. Murad Abu Hasan, for their honorable role.

I also extend my thanks to everyone who had a role in getting me here, and a special thanks to all the laboratory technicians in the Faculties of Science and Pharmacy, especially Mr. Sameh Nusseibeh, Mr. Ibrahim Ayyad and Mrs. Samya Al-Khatib.

I extend all my gratitude and appreciation to everyone, who supported me and stood by me during this stage of my life.

Hiba Salah

Abstract

C. sempervirens L is one of the medicinal plants widely found in Palestine. It is used to treat many health problems. The leaves and fruits are used to obtain *C. sempervirens* L. extract in addition to *C. sempervirens* L. essential oil. In this research, *C. sempervirens* L. fruits were collected from Ramallah in Palestine in January 2021, it was dried and blended, and then *C. sempervirens* L. extracts were prepared with ethanol and water using several methods, namely the Sonicator, Soxhlet and boiling.

The Ethanolic *C. sempervirens* L. extract was analyzed using GC/MS, and two main compounds were identified: δ-3-carene and α-pinene.

One of the objectives of this research is to investigate the antibacterial activity of *C. sempervirens* L. fruit extracts against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*E.coli*) and anaerobic bacteria (*Porphyromonas gingivalis*), using the disc diffusion method and the well diffusion method for *Porphyromonas gingivalis*. Ethanolic extract had an effect on *Staphylococcus aureus* and *Porphyromonas gingivalis* at various concentrations. where the diameter of the inhibition zone for *Staphylococcus aureus* at the highest concentration was (15 mm), and the diameter of the inhibition zone for *Porphyromonas gingivalis* was (22 mm). Water extract had no effect on bacteria except *Staphylococcus aureus* at the highest concentration, where the inhibition zone was (14 mm). For *E. coli* bacteria, neither extract had any effect.

Also, the effect of *C. sempervirens* L. fruit extracts was revealed as anticancer properties against HT29 colon cancer cells and Mcf7 breast cancer cells. The effect of the Ethanolic extract on the percentage of live cells of the two types of cancer cells, as the percentage of live colon cancer cells HT29 at the highest concentration of the extract was (25%), and the percentage of live breast cancer cells Mcf7 at the highest concentration of the extract was (19%). As for *C. sempervirens* L. Water extract, it had no significant effect on the two types of cancer cells, as the percentage of live colon cancer cells HT29 at the highest concentration was (94%), and the percentage of live breast cancer cells Mcf7 at the highest concentration was (96%).

Table of the Content

- Declaration..... I
- Acknowledgments..... II
- Abstract..... III
- Table of the Content..... IV
- List of Table..... VII
- List of figure..... VIII
- List of abbreviations, Symbols and terminology..... X

Chapter one: Introduction..... 1

1.1 Synthetic medicine 2

1.2 Medicinal plants 3

1.3 Natural Compounds (Phytochemicals)..... 4

1.3.1 Terpenoids 4

1.3.2 Phenolic Compounds 6

1.3.2.1 phenolic acids 6

1.3.2.2 Flavonoids..... 7

1.3.2.3 Tannins..... 7

1.3.3 Alkaloids..... 8

1.4 Palestinian medicinal plant 8

1.5 Overview of the study plant *Cupressus sempervirens* L. (Cypress) 9

1.6 Research Questions..... 11

1.7 Research Aim and objective 11

Chapter two: literature review 12

2.1 Anti-bacterial activity..... 13

2.2 Anti-cancer activity. 14

2.3 GC/MS Analyses of *C. sempervirens* L. 15

Chapter Three: Experimental Work..... 17

3.1 Chemicals, Instrumentations and Plant materials..... 18

3.1.1 Chemicals and reagents..... 18

3.1.2 Instrumentation 18

3.1.3 Plant materials. 18

3.2 Methodology..... 18

3.2.1 Preparation of plant samples..... 18

3.2.2 Crude extraction..... 19

3.2.2.1 Extraction by sonication. ……. 19
3.2.2.2 Extraction by Soxhlet…… 19
3.2.2.3 Extraction by Boiling…… 20
3.2.3 Analysis of crude ethanolic extracts using GC/MS…….. 20
3.2.4 Antibacterial activity…….. 20
3.2.4.1 Bacterial Strain……. 20
3.2.4.2 Preparation of bacterial medium…….. 21
3.2.4.3 Culture of bacteria…… 21
3.2.4.4 Antimicrobial assay…… 21
3.2.4.5 Determination of Inhibition zone IZ …… 22
3.2.5 Anti cancer activity…….. 22
3.2.5.1 Cell Culture …….. 22
3.2.5.2 Cell Count Colon……... 22
3.2.5.3 Cell Count Breast……..……... 23

Chapter four: Result and Discussion……. 24
4.1 Plant extraction …… 25
4.2 Characterizing of crude ethanolic extract ……... 25
4.2.1 GC/MS analysis …….. 25
4.3 Antibacterial activity……...……..... 28
4.3.1 Effect of DMSO and Ethanolic *C. sempervirens* L. extract on different bacteria strains …….. 28
4.3.1.1 Effect of DMSO on bacteria growth……. 29
4.3.1.2 Effect of Ethanolic *C. sempervirens* L. extract on *Staphylococcus aureus* as gram positive bacteria……. 29
4.3.1.3 Effect of Ethanolic *C. sempervirens* L. extract on E. coli as gram-negative bacteria……..... 30
4.3.1.4 Effect of Ethanolic *C. sempervirens* L. extract on *Porphyromonas gingivalis*.…… 31
4.3.2 Effect of Water *C. sempervirens* L. extract on different bacteria strains…….. 33
4.3.2.1 Effect of water *C. sempervirens* L. extract on *Staphylococcus aureus* as gram positive bacteria……. 33
4.3.2.2 Effect of water *C. sempervirens* L. extract on *E. coli* as gram-negative bacteria……... 34
4.3.2.3 Effect of water *C. sempervirens* L. extract on *Porphyromonas gingivalis* ……. 35
4.4 Anticacer activity ……37
4.4.1 Effect of Ethanolic *C. sempervirens* L. extract on different cancer cells..... 37

4.4.1.1 Breast cancer cell lines (Mcf7 cells)……..... 37
4.4.1.2 Colon cancer cell lines (HT29 cells)…….. 40
4.4.2 Effect of Water *C. sempervirens* L. extract on different cancer cells…….. 42
4.4.2.1 Breast cancer cell lines (Mcf7 cells)…….. 42
4.4.2.2 Colon cancer cell lines (HT29 cells)…….. 45

Chapter five: Conclusions and Future work……... 50
Conclusion……... 50
Future work……. 51
References…… 52
الملخص..... 60

List of Table

Table 4.1 Antimicrobial activity of ethanolic extract of *C. sempervirens* L…… 32

Table 4.2 Antimicrobial activity of water extract of *C. sempervirens* L……..... 36

Table 4.3 Total count cells, count live cells, and percentage of live cells at various extract concentrations…….. 48

List of Figures

Figur1.1 Chemical Structures of various terpenes, depending on the carbon number, terpenes are classified as A. Mono-terpenes, B. Sesqui- terpenes and C. Di-terpenes…… 5

Figur1.2 Basic Chemical Structures of phenolic acids, flavonoids, condensed and hydrolyzable tannins. A. Hydroxybenzoic acid, B. Hydroxycinnamic acids, C. Flavonoids, D. Condensed tannins and D. Hydrolyzable tannins……... 7

Figure 1.3 Image of Cupressus sempervirens L. tree and fruits…….. 10

Figure 3.1 Fresh *C. sempervirens* L. fruit before & after grinding……... 19

Figure 3.2 Anaerobic bacteria jar……..... 21

Figure 4.1 The Total ion chromatogram of ethanolic C. sempervirens L. extract…….. 26

Figure 4.2 The chromatogram of the ethanolic C. sempervirens L. extract…… .. 27

Figure 4.3 Chemical structure of α-pinene δ-3-carene……. 27

Figure 4.4 Effect of DMSO and ethanolic C. sempervirens L. extract on Staph. aureus growth by disc diffusion methods. At different concentration (12.5, 25, 50, 75, 100 mg/ml).. 30

Figure 4.5 Effect of DMSO and ethanolic C. sempervirens L. extract on E. coli growth by disc diffusion methods. At different concentration (12.5, 25, 50, 75, 100 mg/ml……. 31

Figure 4.6 Effect of DMSO and ethanolic C. sempervirens extract on P. gingivalis bacteria, a. disc diffusion method, b. well diffusion method. At different (12.5, 25, 50, 75, 100 mg/ml)…… 32

Figure 4.7 Antimicrobial activity of Ethanolic extract on different types of bacteria (E.coli, Staph. aureus, P. gingivalis) and effect of DMSO……. 33

Figure 4.8 Effect of Water C. sempervirens extract on Staph. aureus by disc diffusion methods. At different concentration (12.5, 25, 50, 75, 100 mg/ml)……. 34

Figure 4.9 Effect of water C. sempervirens extract on E.coli by disc diffusion methods. At different concentration (12.5, 25, 50, 75, 100 mg/ml)…….. 35

Figure 4.10 Effect of water C. sempervirens L. extract on P. gingivalis bacteria, a. disc diffusion methods, b. agar well diffusion methods. At different concentration (12.5, 25, 50, 75, 100 mg/ml)……. 35

Figure 4.11 Antimicrobial activity of water extract on different types of bacteria (E.coli, Staph. aureus, P. gingivalis)……. 36

Figure 4.12 Mcf7 cell live% with Ethanolic C. sempervirens L. extract. At different concentration (5, 15, 25, 50 mg/ml), DMSO and control sample…… 38

Figure 4.13 Chart to investigated IC50 value for Mcf7 cell live% with Ethanolic C. sempervirens L. extract. At different concentration (5, 15, 25, 50 mg/ml)…… 39

Figure4.14 Efficiency of the Ethanolic C. sempervirens L. extract for Mcf7 cancer cells at different concentrations (A-D), (E) Control, (F) DMSO, observed after 72 hours…… 39

Figure 4.15 HT29 cell live% with Ethanolic C. sempervirens L. extract. At different Concentration (5, 15, 25, 50 mg/ml), DMSO and control sample…….. 41
Figure 4.16 Chart to investigated IC50 value for HT29 cell live% with Ethanolic C. sempervirens L. extract. At different concentration (5, 15, 25, 50 mg/ml)……..... 41
Figure 4.17 Efficiency of the Ethanolic C. sempervirens L. extract for HT29 cancer cells at different concentrations(A-D), (E) Control, (F) DMSO, observed after 72 hours..... 42
Figure 4.18 Mcf7 cell live% with Water C. sempervirens L. extract. At different concentration (50, 100 ,250, 500 mg/ml) …….. 43
Figure 4.19 Chart to investigated IC50 value for (Mcf7) cell live% with water C. sempervirens L. extract. At different concentration (50, 100 ,250, 500 mg/ml)…..……... 44
Figure 4.20 Efficiency of the water C. sempervirens L. extract for Mcf7 cancer cells at different concentrations (A-D), (E) Control, observed after 72hour…….. 44
Figure 4.21 HT29 cell live% with Water C. sempervirens extract. At different concentration (50, 100 ,250, 500 mg/ml)…….. 46
Figure 4.22 Chart to investigated IC50 value for (Mcf7) cell live% with water C. sempervirens L. extract. At different concentration (50, 100 ,250, 500 mg/ml…… 46
Figure 4.23 Efficiency of the water C. sempervirens L. extract for HT29 cancer cells at different concentrations (A-D), (E) Control, observed after 72 hours…….…….. 47

List of abbreviations, Symbols and terminology

Abbreviation	**Description**
C.	Concentration
C. sempervirens L.	*Cupressus sempervirens L.*
D.W	Distilled water
DMSO	Dimethyl sulfoxide
E.coli	*Escherichia coli*
GC/MS	Gas chromatography-mass spectrometry
HT29	Colon cancer cell
IZ	Inhibition zone
Mcf7	Breast cancer cell
Ml	Microliter
min.	Minute
Mg	Milligram
N/D	Not Detected
P.gingivalis	*Porphyromonas gingivalis*
Staph. aureus	*Staphylococcus aureus*

Chapter one:

Introduction

Plants are the fundamental components of medicine; many important medicines are still in use and are derived from medicinal plants. The pursuit of new medicines has led ethnobotany and ethnopharmacology down a new path as a valuable source of information, leading to a variety of sources and classes of compounds. (Aslam.S.M. and Ahmad.S.M. 2016) Synthetic organic and medicinal chemistry, like organic chemistry a century ago, is reorienting the field of medicine development. Researchers' most focused and difficult task is synthesizing new drugs, designing, developing, and applying them. (Perez-Nueno, V. 2016)

1.1 Synthetic medicine

Synthetic drugs are medicines that are not found in nature and are manufactured in laboratories using various methods. Non-toxicity, specificity, potency, stability, and potency are the ultimate criterion for any drug (natural or synthetic). (Tanner, J. 2006). Basic research into the medicinal and chemical nature of the diseased state is required for drug discovery. This necessitates the involvement of organic and medicinal chemists, as well as academic researchers. Synthesis of newly developed organic molecules with more appealing therapeutic effects, as well as their conversion to derivative products with efficient medicinal actions, as well as the synthesize the novel molecule or drug by combining different heterocyclic rings, and the synthetic pathway followed must be economical, safer, and less time consuming in order to create combinational molecule and also improved formation against deadly diseases such as malaria and tuberculosis. (H.M. Patel et al 2014; B. Gervais, 2016).

With the passage of time and advances in the field of medicine, synthetic medicines began to gradually replace natural medicines, despite the fact that the former have some side effects. Many synthetic medicines benefit humanity and are used safely, but many have been linked to serious side effects. (W.P. Sherman and J. Billing 1999).

Many examples of side effects caused by synthetic drugs have been reported in the literature, for example; paracetamol is a well-known antipyretic drug, but it can also cause liver poisoning as a major side effect (Tanne.J 2006). Most commonly used medications, such as aspirin, ibuprofen, clopidogrel, diclofenac, warfarin, naproxen, and enoxaparin, are widely available and cause mild to severe symptoms (ranging from back pain to headaches) (excessive bleeding, hemorrhage, and difficulty

breathing, among others). (W.P. Sherman and J. Billing 1999) Naproxen also has gastrointestinal side effects. (E.M. Hay et al. 1999)

1.2 Medicinal plants

Over 35,000 plant species are used medicinally (Lewington.A. 1993). In addition to basic health care Approximately 80% of the world's population relies on traditional medicines, which almost always include the use of plant extracts (Sandhya et al. 2006). Following isolation and identification, different combinations of these plants' active components can be created, and their synergistic effects must be studied further (Singh.R. 2015). The effectiveness of traditional medicines is now assumed due to their better compatibility with the human body, greater cultural acceptability around the world, and fewer side effects. (Verma, S., and Singh, S.P. 2008) However, just because a product is "natural" does not always mean it is safe. Even though there is limited evidence to suggest that disadvantages associated with natural compound consumption are less likely to occur than those associated with conventional drugs, they do occur, though they are usually mild and affect a small number of people. Recent evidence suggests that some herbs previously thought to be safe have been linked to health risks. (George, P. 2011)

Several plants have long been known to have anti-infective properties, which have recently been discovered to act as antimicrobials against human pathogens due to the availability of secondary metabolites. Phytochemicals have received a lot of attention in the last decade for their antibacterial activity, particularly against multidrug-resistant Gram-negative and Gram-positive bacteria (Borges.A et al 2015). Over the last decade, there has been a significant revival of interest in the use of herbal medicines in both developed and developing countries. Almost half of today's medicines are entirely plant-based. (Haq.I. 2004) Nature has created an amazing variety of secondary metabolites over time. According to empirical observations, natural product extracts were the first and, for a long time, the only medicines available to mankind. Despite the fact that crude extracts remain the primary source of healthcare for the vast majority of the world's population, active pharmaceutical ingredients have largely replaced them in the Western world. (Ganesan.A2008) Because of the unrivaled availability of chemical constituents, natural products

derived from medicinal plants, whether pure or standardized extracts, provide limitless opportunities for new drugs.

Since ancient times, natural products have been used all over the world to treat and cure chronic diseases such as cancer, diabetes, asthma, anti-inflammatory, analgesic, and as substitutes for hormone replacement therapy. (Rasul, M.G. 2018) Furthermore, medicinal plants are frequently used as raw materials for extracting active components used in the synthesis of various drugs. Plant-derived ingredients can be found in laxatives, blood thinners, antibiotics, and anti-malaria medications. (Hassan, B. 2012)

1.3 Natural Compounds (Phytochemicals)

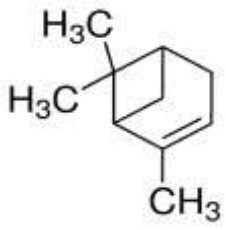
Plants' medicinal properties are usually the result of different combinations of these natural compounds known as phytochemicals. Primary and secondary phytochemicals are the two types of phytochemicals. Chlorophyll, proteins, and sugars are examples of primary compounds, while terpenoids, alkaloids, flavonoids, and phenolic acids are examples of secondary compounds. (Saboon et al. 2019) Phytochemicals are used in routine healthcare systems all over the world. Terpenoids, alkaloids, phenolics, and tannins, for example, have the potential to prevent diseases while also acting as anti-microbial, anti-inflammatory, anti-oxidant, anti-cancer, detoxifying agent, immunity potentiating agent, and neuropharmacological agents. Each class of these functional agents is composed of a distinct blend of chemicals with varying potencies. Each class of these functional agents is made up of a unique combination of chemicals with varying potencies. (Koche.D et al 2016) The following are some examples of phytochemicals found in plants:

1.3.1 Terpenoids

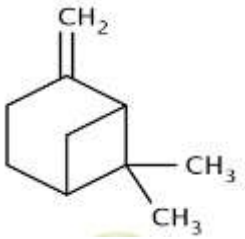
Terpenoids are a large class of antimicrobial compounds that plants produce. Terpenoids are made up of five-carbon isoprene units, and the majority of them have multi-cyclic structures with various functional groups and basic carbon skeletons. Mono-terpenes are terpenes made up of two isoprene units that are found in essential oils extracted from various plants. These compounds have been shown to have antimicrobial activity. These compounds' antimicrobial activity has been studied over the last two decades (Barbieri.R et al 2017). Terpenes are the most common organic

compound class produced by plants. Historically, terpene-containing plant oil has been used to treat a wide range of diseases, despite the fact that the precise functions or mechanisms of action of the individual bioactive compounds were unknown. (Cho.K.S, et al 2017), the most abundant class of naturally occurring organic compounds, with over 40,000 structures reported so far. (Gershenzon 2007, Chappell 2002) Terpenes are classified as mono-, sesqui-, or di-terpenes based on the number of isoprene units (C10, C15, and C20, respectively). (Kirby, J. 2009) The chemical structures of some of these compounds are depicted in Figure 1.1.

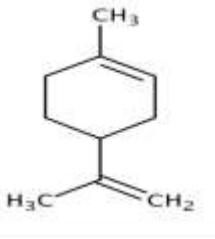
Mono- terpenes



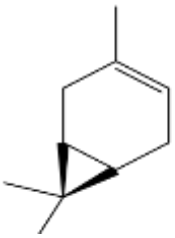
α—Pinene



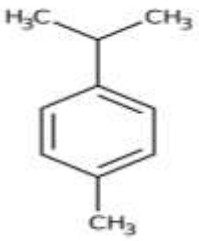
β-Pinene



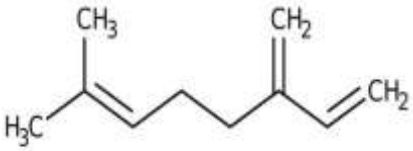
limonene



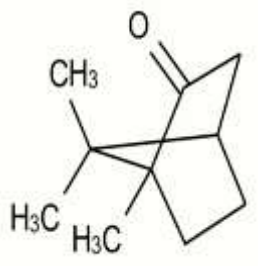
δ-3-carene



p-cymene

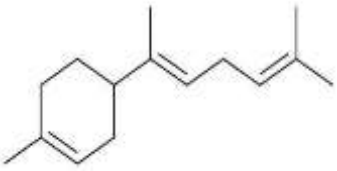


Myrcene

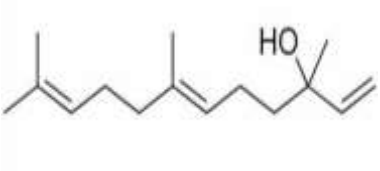


Camphor

A) Sesqui- terpenes

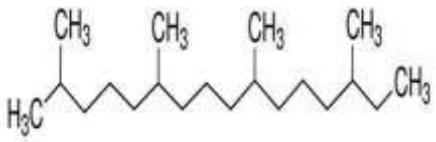


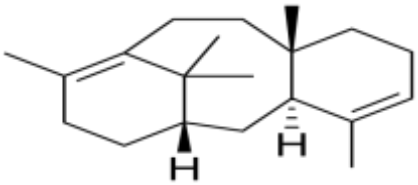
Bisabolene



nerolidol

B) Di-terpenes





Phytane Taxadiene

Figure 1.1 Chemical Structures of various terpenes, depending on the carbon number, terpenes are classified as A. Mono-terpenes, B. Sesqui- terpenes and C. Di-terpenes.

1.3.2 Phenolic Compounds

The most abundant phytochemicals are phenolic compounds, which are found throughout the plant kingdom. (Walton.N.J. et al., 2003) Phenolics are chemical compounds that have hydroxyl groups (-OH) that are directly bonded to aromatic hydrocarbon groups. The most basic type of natural compound in this category is phenol (C6H5OH). They are important in defense because they are secondary metabolites. Phenolics have a variety of health-promoting properties, and their antioxidant properties are critical in determining their role as anti-free radical-mediated disease processes. Flavonoids and phenolicacids are the most important phenolic groups. 2016 (Koche.D et al)

1.3.2.1 phenolic acids

Phenolic acids are aromatic secondary plant metabolites found all over the plant kingdom. The potential role of phenolic acids in preventing oxidative damage diseases such as coronary heart disease, cancer, and stroke through fruit and vegetable consumption has sparked renewed interest in them. (R.J. Robbins 2003) Many agricultural, biological, chemical, and medical studies have been conducted on phenolic acid compounds and functions. These compounds are part of a large family that includes the widely used hydroxybenzoic and hydroxycinnamic acids. Hydroxycinnamic acid-containing compounds are frequently synthesized as simple esters with glucose or hydroxy carboxylic acids. (Mandal.S.M 2010)

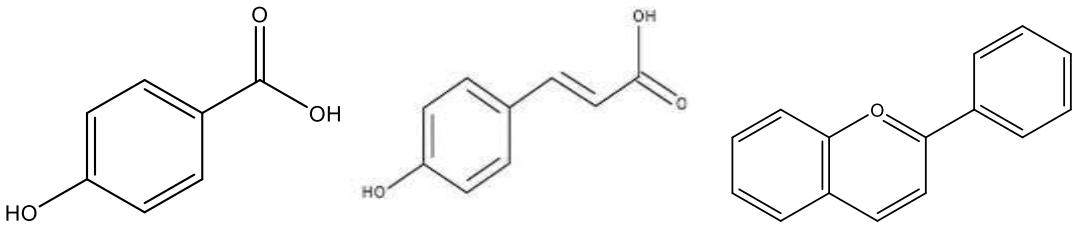
1.3.2.2 Flavonoids

Flavonoids are the most abundant type of polyphenol in plants, accounting for at least 2000 different compounds. (Shahidi.F and Tang Ho.C, 2005) Flavonoids An aromatic ring with one or more hydroxyl groups is the basic structural feature of phenolic compounds. (Chirinos.R et al 2009) Flavonoids are a naturally occurring class of substances with varying phenolic structures that can be found in fruits, vegetables, stems, bark, roots, grains, flowers, and tea. Flavonoids are now recognized as an important component in a wide range of nutraceutical, pharmaceutical, medicinal, and cosmetic applications. (Panche.A.N et al 2016)

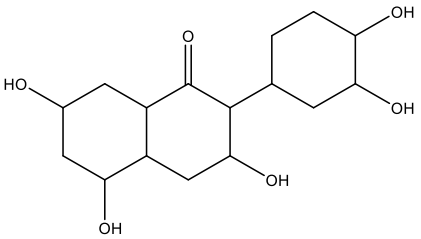
1.3.2.3 Tannins

Tannins are a heterogeneous group of polyphenolic compounds with high molecular weight that can form reversible and irreversible complexes with proteins (primarily), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids, and minerals (Schofield .P et al 2001). Tannins are classified into four major groups based on their structural characteristics: gallotannins, ellagitannins, complex tannins, and condensed tannins. Tannins are found in fruits like grapes, persimmon, blueberry, tea, chocolate, legume trees like Acacia spp. and Sesbania spp., and grasses like sorghum and corn. (Koche.D et al 2016)

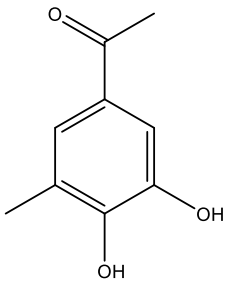
Figure 1.2 shows basic structures of phenolic acids, flavonoids, condensed and hydrolyzable tannins.



A) Hydroxybenzoic acid B) Hydroxycinnamic acids C) Flavonoids



D) Condensed tannins



E) Hydrolyzable tannins

Figure 1.2 Basic Chemical Structures of phenolic acids, flavonoids, condensed and hydrolyzable tannins. A. Hydroxybenzoic acid, B. Hydroxycinnamic acids, C. Flavonoids, D. Condensed tannins and D. Hydrolyzable tannins

1.3.3 Alkaloids:

Alkaloids are a diverse group of natural compounds produced by secondary metabolism. They are usually kept separate from plants. Alkaloids are important chemical components that can be used to create new medications. Several alkaloids isolated from natural herbs have been shown in vitro and in vivo to have anti-proliferation and anti-metastasis effects on various cancer types. Camptothecin and vinblastine are two anticancer drugs that have already been developed. (Lu.J et al. 2012) Alkaloids have a wide range of pharmacological properties, including antihypertensive and antimalarial effects. These are just a few examples of how economically significant this group of bioactive constituents is. Caffeine and nicotine are stimulants, while morphine is an analgesic and quinine is an anti-malarial medication (Wink.M, Schmeller.T 1998). Pyrrolidine alkaloids, Pyridine-piperidine alkaloids, and Isoquinoline alkaloids are the various classes of alkaloids based on the heterocyclic ring system they contain. (Koche.D et al., 2016)

1.4 Palestinian medicinal plant

Throughout history, various natural materials have been used as remedies for various diseases. Synthetic drugs based on modern chemistry and biotechnology have largely replaced natural products in recent decades (Eisenberg et al.1998). In Palestine, numerous medicinal plants have been described for the treatment of various diseases. Herbal medicine is regarded as an important part of Palestinian culture, and it plays an

important and necessary role in current public healthcare. Over 2600 plant species grow on Palestine's hills and mountains, with over 700 of them used as medicinal herbs or botanical pesticides (Silva and Abraham 1981).

Many medicinal plants used in folk medicine in Palestine for the treatment of various diseases such as cancer, injuries, and chronic diseases have been documented through ethnobotanical field surveys conducted in the area. Plants and their parts have medicinal value and can be used to treat, prevent, or relieve a variety of human diseases. Plants contain a variety of phytochemicals, which can help to reduce the occurrence of many diseases by supporting various organ functions in the human body (Jamous.R.M et al 2015).

They are also thought to have minor environmental effects and have the potential to be used as biological control agents. Some medicinal herbs, on the other hand, have not been widely used for a variety of reasons and are sometimes referred to as "forgotten plants." Given the growing demand for natural ingredients that can be used as food additives, functional food components, plant disease prevention, and nutraceuticals, it is reasonable to reconsider the "forgotten plants" by evaluating their applicability and benefits using modern scientific analysis methods (Jouda.M.M et al 2016).

1.5 Overview of the study plant

Cupressus sempervirens L. (Cypress)

Cupressus sempervirens L., also known as "Cypress," is a medium-sized evergreen tree native to the eastern Mediterranean region that grows up to 35 m tall (Alkurdi.MIS and Supuka.J. 2015). *C. sempervirens* L. is found all over the world, from the Mediterranean region to subtropical Asia to North America (Rawat et al 2010). Male and female strobili (cones) bear separately at the ends of short branches of *C. sempervirens* L. C. sempervirens L. varieties horizontal's, with spreading branches and a broad conical crown, is the most common in natural areas. C. *sempervirens* L. variety pyramidalis, the most common ornamental, is distinguished by erect branches parallel to the trunk, which give the tree its distinctive columnar shape. (Giovanelli and De Carlo 2007).

The dried leaves are used to treat stomach pain and diabetes, and the dried fruits are used to treat throat infections and dental infections, as well as a contraceptive (Mascolo et al 1987). The essential oil of *C. sempervirens* L. is used internally to treat whooping cough, blood spitting, spasmodic coughs, colds, flu, and sore throats. It is also used as a skin lotion (Koriem.K 2009). Phyto-preparations derived from the core and young branches of *C. sempervirens* L. have antiseptic, aromatherapeutic, astringent, balsamic, and anti-inflammatory properties.

C. sempervirens L. has also been reported to have antispasmodic, astringent, antiseptic, deodorant, and diuretic properties, as well as to improve bladder tone and act as a co-adjuvant in the treatment of urinary incontinence and enuresis. *C. sempervirens* L. has also been used as an antiseptic (Rawat et al 2010, Selim.S et al 2014).

Figure 1.3 Image of *Cupressus sempervirens* L. tree and fruits.

1.6 Research Questions

- Does *C. sempervirens* L. fruit extract have antibacterial properties against *Staphylococcus aureus, E.coli* and *Porphyromonas gingivalis* bacteria?
- Do the *C. sempervirens* L. fruits extract have anti-cancer properties against Breast and Colon cancer cells?

1.7 Research Aim and objective

Aim:

Investigate the biological activity of *C. sempervirens* L. fruit extract.

Objective

- To extract the active substance from *C. sempervirens* L. fruit using ethanol and water.
- To characterization the constituents of the *C. sempervirens* L. extract using GC/MS.
- To test the antibacterial activity of *C. sempervirens* L. extract against *Staphylococcus aureus, E.coli* and *Porphyromonas gingivalis*.
- To test the anticancer activity of *C. sempervirens* L. extract against Breast and Colon cancer cells.

Chapter Two
Literature review

There are several scientific papers that discuss the anti-bacterial and anti-cancer activity of *C. sempervirens* L. oil and extract, in addition to articles that examined the constituents of C. sempervirens L. extract.

2.1 Anti-bacterial activity:

One of the most serious global public health challenges of the twenty-first century is antimicrobial resistance. Antimicrobial resistance develops naturally when bacteria are exposed to antibiotics. Antibiotics selectively kill or inhibit susceptible bacteria, whereas bacteria that are naturally resistant to antibiotics or have acquired antibiotic-resistant traits have a better chance of survival and multiplication (Prestinaci.F et al 2015). Plants are a rich source of natural products that have been used for centuries to treat a variety of diseases. The idea behind plant-derived medicines is that they contain natural compounds that can improve health and alleviate illness. As a result, a return to natural compounds is an absolute necessity in our time.(Masoud.E.A and Gouda.H.A 2012)

As a result, we presented some research on the effect of *C. sempervirens* L. as a natural antibacterial. Here some of articles that investigated the antibacterial activity of *C. sempervirens* L.

Examples of articles that investigated the antibacterial activity of *C. sempervirens* L. (Selim.S et al 2014) The purpose of this study was to determine the antimicrobial activity of *C. sempervirens* L. essential oil and methanol extract. The antimicrobial test results revealed that, with the exception of yeast species, the methanol extract of *C. sempervirens* L. strongly inhibited the growth of the test bacteria studied, while the essential oil had moderate antibacterial activity.

Furthermore, (Qaralleh.H et al 2021) used disc diffusion methods to assess the antibacterial efficacy of methanol extract and essential oil against bacteria. A methanol extract of *C. sempervirens* L. inhibited *Bacillus subtilis, Micrococcus luteus, Staphylococcus epidermidis,* and *Staphylococcus aureus* at 2000 μg/disc, with inhibition zones ranging from 12 to 15mm.

In addition, (Zhang.J et al 2012) used the agar well diffusion method to test methanolic, ethanolic, and ethyl acetate extracts of *C. sempervirens* L. leaf for antibacterial activity against six bacteria (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,*

Escherichia coli, Klebsiella pneumoniae, and *Salmonella typhimurium*). Methanolic extracts had the highest antimicrobial activity among the plant extracts, followed by ethyl acetate and ethanol extracts. The methanolic extract was most effective against *K. pneumonia, B. subtilis,* and *S. aureus*. The ethanolic extract was more effective against *P. aeruginosa. C. sempervirens* L. ethyl acetate extract had higher inhibitory activity against *S. typhimurium* and *E. coli.*

Another study discovered antimicrobial activity against ten food-spoilage yeasts in *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Halomonas elongate, Salmonella typhimurium, Enterococcus hirae, Aspergillus niger, Candida albicans,* and *Trichoderma reesei*. According to the findings, the essential oil of *C. sempervirens* L. could be used as a readily available source of antimicrobial agent (Boukhris.M et al 2012).

C. sempervirens L. essential oil was also evaluated for antimicrobial activity against five bacteria (3 Gram-positive, 2 Gram-negative) and three fungi (Mazari.K et al 2010). Antibacterial and antifungal activity was found to be moderate in the oils.

2.2 Anti-cancer activity:

Cancer is the world's second cause of death, properly accounted for over 6 million deaths every year (Loizzo et al 2008).The increased occurrence of cancer suggests that new powerful and effective medications derived from plants are urgently needed (Singh et al 2015). Therefore, we went to study the effect of *C. sempervirens* L. as an anticancer, there are some previous studies that were conducted on the effect of *C. sempervirens* L. as an anti-cancer, and they will be presented here.

The methanol extract of *C. sempervirens* L. leaf reduced the viability of several cell lines tested, including human hepatocellular carcinoma cell (HEPG2), lung carcinoma cell (A549), human Caucasian breast adenocarcinoma (MCF7), and especially human colon cancer cell (HCT116). (Abd Alhady.M et al 2020)

Furthermore, (Fayed.S 2015) the essential oil extracted from *C. sempervirens* L. leaves was tested in vitro and in vivo for antioxidant and anticancer activity. In vitro anticancer activity was assessed in two human promyelocytic leukemia cell lines (HL-60 and NB4),

as well as an experimental animal cancer cell line (EACC). With an LC50 of 333.79 g mL-1, *C. sempervirens* L. essential oil had the highest cytotoxic activity against NB4 cell lines, followed by HL-60 and EACC cell lines (LC50s of 365.41 and 372.43 g mL-1, respectively). In an in vivo anticancer study, the essential oil outperformed the initiation and post-initiation treatments on tumor (EACC) transplanted female mice, increasing lifespan (percent), decreasing total EACC number, and increasing dead cells.

The sulphorhodamine B assay was used to assess the antiproliferative activity of essential oils from *C. sempervirens* ssp. pyramidalis on amelanotic melanoma C32 cells and renal cell adenocarcinoma cells. *C. sempervirens* ssp. pyramidalis leaf oil had the highest cytotoxic activity against C32, with an IC50 value of 104.90 micro g /ml (Verma.V et al 2014).

2.3 GC/MS Analyses of *C. sempervirens* L.:

The chemical composition of a hydro-distilled essential oil of *C. sempervirens* L. was investigated using a GC and GC/MS system (Selim.S et al 2014). -pinene (48.6%), -3-carene (22.1%), limonene (4.6%), and -terpinolene (4.5%) were identified as constituents, accounting for 98.1 percent of the oil.

(Chanegriha.N et al 1993) also determined the chemical composition of *C. sempervirens* L. essential oil from Algeria using GC, GC/MS, and GC/FTIR. 70 components were detected or tentatively identified. The main compounds discovered were a-pinene (47.00-52.76%), δ-3-carene (19.35-21.13%), α-terpinyl acetate (4.10-6.47%), cedrol (2.03-3.92%), myrcene (3.11- 3.48%), and limonene (2.28-3.31%).

Alternatively, (Boukhris.M et al 2012) investigated the chemical composition of essential oil from *C. sempervirens* L. in Tunisia. Essential oils were examined using gas chromatography-mass spectrometry (GC-MS), and 24 compounds were identified.

Furthermore, (Ismail.A et al 2013) used (GC) and (GC/MS) to determine the chemical composition of essential oils extracted from Tunisian *C. sempervirens* L. leaves, branches, and female cones, were 52 compounds identified, and there were qualitative and quantitative differences between the oils. All oils contained a high concentration of mono-terpene hydrocarbons, with the major constituents being α-pinene (27.5 to 35.8

%), cedrol (7.7 to 19.3 %), δ-3-carene (5.8 to 13.2 %), and germacrene D. (3.9 to 12.1%).

GC-MS was also used to determine the chemical composition of essential oil isolated from the leaves of *C. sempervirens* L. via steam distillation (Mazari.K et al 2010). The oil contained 75.7 percent mono-terpene hydrocarbons, with -pinene (60.5%) being the most abundant. The second most important constituent in *C. sempervirens* L. oil, cedrol (8.3%), was discovered.

Furthermore, (Leandri.C et al 2003) investigated Cypress essential oils extracted from the leaves of seven different types of Cypress trees found throughout France. Essential oils were analyzed using GC/MS. The main constituents of *C. atlantica Gaussen* essential oil were -pinene and -3 carene, while -pinene and sabinene were also present. found in the oil of *C. chengiana Hu, and C. funebris Endl, C. cashmeriana Royle* and α-pinene and cadinol for *C. macnabiana. C. guadalupes Hickel, A. Murr, S. Wats,* limonene, and cadinol for *C. duclouxiana* α-pinene, terpinen-4-ol, and a sesquiterpene are all present.

Chapter Three

Experimental Work

3.1 Chemicals, Instrumentations and Plant materials

3.1.1 Chemicals and Reagents

Ethanol 99%, Methanol, 99.9%, Ethyl acetate, Dimethyl sulfoxide (DMSO) 99%, were purchased from Sigma-Aldrich. Ultrapure water was generated from an ultrapure water system. Anaerobic bacteria (*Porphyromonas gingivalis* serial number 20097410), and aerobic bacteria (*Escherichia coli* (gram-negative bacteria) and *(Staphylococcus aureus* (gram-positive bacteria)), were obtained from the laboratories of Al-Quds University. And Muellar Hinton agar for aerobic bacteria purchased from Himedia, Brucella agar for anaerobic bacteria purchased from OXOID, normal saline, horse serum. Colon cancer cell lines (HT29), Breast cancer cell lines (Mcf7), DMEM medium, RPMI 1640 medium from GibcoThermofisher.

3.1.2 Instrumentation

The analytical GC/MS Perkin Elmer Clarus 500 gas chromatograph, Rotary evaporator from IKA WEREK RV06-ML, Ultrasonic cleaner (Sonicator) from mrc, UV light, Autoclave, Centrifuge, Soxhlet, Hot plate, laboratory water bath, Incubator, Incubator 5% CO2, Refrigerator, Anaerobic jar, Automated Cell Counter (BIO RAD), Fluorescent microscope, Blender, Micro pipette, Micro filter 0.45 μm, Test tubes, Petri dish, Sterile Discs 6 mm Filter Paper, Buchner.

3.1.3 Plant materials

Cupressus sempervirens L. fruit samples were obtained and collected from Ramallah city, Palestine, in January 2021.

3.2 Methodology

3.2.1 Preparation of plant materials

The fruits of *C. sempervirens* L. samples were collected and washing with tap water to remove leaves, dust or any other impurities, it was dried in the oven at 30°C, then grinding with blender to extract with variation solvents in last stage.

Figure 3.1 Fresh *C. sempervirens* L. fruit before & after grinding.

3.2.2 Crude extraction

Three different methods were used for after preparing a sample of *C. sempervirens* L. fruits powder extract. The crude extract, obtained using sonication, Soxhlet, and boiling. The sonication method was chosen because it produced the highest extract percentage compare to other methods. The percentage of ethanolic extract of *C. sempervirens* L. sample was 7.4%. The ethanol used in the extraction was completely evaporated, in order to be sure that the results that would appear due to the effect of the extract, not the effect of ethanol. DMSO was used as a solvent for the ethanolic extract to make different concentrations.

3.2.2.1 Extraction by sonication

20 gm of the dried powdered plant material was sonicated with 100 ml of different solvents (D.W, 99.9% Ethanol), extracted for 180 min. at 37 °C, and filtered. Then the crude extracts was concentrated using a rotary evaporator under reduced pressure and finally was stored in Refrigerator at 4C° until analysis.

3.2.2.2 Extraction by Soxhlet

An ethanolic extract was prepared using a Soxhlet apparatus. 15 gm of dry *C. sempervirens* L. was extracted in 99 % ethanol for 180 min. using the Soxhlet apparatus method. *C. sempervirens* L. crude filtered, then concentrated using a rotary evaporator under reduced pressure . and the final extracts was stored at 4°C.

3.2.2.3 Extraction by Boiling

20 gm of dried *C. sempervirens* L. sample with 100 ml D.W was boiling it for an hour, at 100 °C, on hot plate.

3.2.3 Analysis of crude ethanolic extracts using GC/MS analysis

The chemical composition was determined using GC-MS. (Makari.K et al., 2010) The ethanolic extract was gas chromatography-mass spectrometry (GC/ MS) analyzed using a Perkin Elmer Clarus 500 gas chromatograph. Rtx-5 capillary columns (60 m 0.32 mm, 0.25 m film thickness) were used. At a flow rate of 1.0mL/min, the carrier gas was helium (He). The GC was linked to a mass detector (Perkin Elmer Clarus500) in EI+ mode. Using an ionizing voltage of 70 eV, the mass spectra were recorded over 40-500 amu, revealing the total ion current (TIC) chromatograms. The injector, transfer line, and ion source temperatures were kept at 210°C, 210°C, and 200°C, respectively. (Lohania.H et al 2015)

3.2.4 Antibacterial activity

The antibacterial testing was carried using the agar disc diffusion method (Qaralleh.H, Khleifat.K et al 2021). DMSO was used to make negative control, Ethanolic *C. sempervirens.*L extract was diluted with DMSO. Antibacterial activity was measured by measuring the diameter of the inhibition zone around the disc against the tested bacteria.

3.2.4.1 Bacterial Strain

Aerobic gram-negative and gram-positive bacteria (*E.coli* and *Staphylococcus aureus*), as well as anaerobic *Porphyromonas gingivilis* bacteria, were used to determine the antibacterial activity of *C. sempervirens* L. extracts. The *P.gingivilis* bacteria was grown in anaerobic conditions and placed in an anaerobic jar, Figure 3.2 shows image of the anaerobic jar.

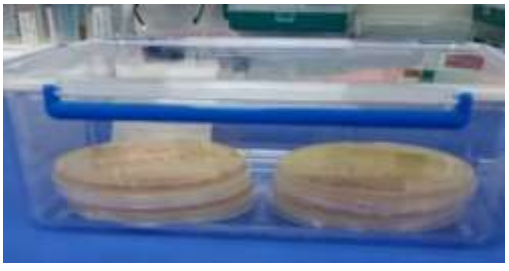


Figure 3.2 Anaerobic bacteria jar.

3.2.4.2 Preparation of bacterial medium

Bacteria growth requires specific environmental conditions, as well as specific types of nutrients that are available in the media designated for bacteria, and the appropriate temperatures that bacteria need for growth and reproduction. Therefore, Mueller Hinton agar was used for aerobic bacteria and Brucella agar for anaerobic bacteria in Petri dishes. The two types of media were prepared in accordance with the instructions on the bottle, where the media was prepared and then placed in the autoclave for 15 minutes at 121 °C. The media was then poured into Petri dishes after it had been cooled to about 50 °C. Brucella requires a type of supplement, which is Horse serum. The dishes were inverted after the media had solidified to avoid the accumulation of steam on them, then refrigerated until use.

3.2.4.3 Culture of bacteria

The bacteria test was prepared by activating the different types of bacteria, which were cultured on nutrient media (Mueller Hinton and Brucella agar), and they were left for 24 hours in the incubator at 37 °C. Then a part of the bacteria was taken and diluted in normal saline to obtain (0.5) McFarland, and then the different types of bacteria were distributed on the surface of the agar by using cotton swab.

3.2.4.4 Antimicrobial assay

The antibacterial activity of both aerobic bacteria was studied, In addition to anaerobic bacteria (*P.gingivalis*), the test was carried out using disc diffusion methods. Sterile discs with a diameter of 6 mm were used. Furthermore, to deliver the extracts to anaerobic bacteria, also well diffusion method was used, holes were made in the media and the extracts was poured directly into the holes. Ethanolic *C. sempervirens* L. extract has been diluted with DMSO to make different concentration (12.5, 25, 50, 75, 100 mg/ml), 50 µl of these concentrations were added to the discs, and 50 µl DMSO was added to ensure its effectiveness as an antibacterial. In addition to well diffusion method, poured directly on the holes on the surface of the media, and placing *C. sempervirens* L. extracts directly in them. Then the bacteria were placed in the incubator for 24 hours at 37 °C. For anaerobic bacteria, they were placed in a jar designated for anaerobic bacteria and then placed in the incubator.

3.2.4.5 Determination of Inhibition zone IZ

The zone of inhibition IZ is a circular area surrounding the antibiotic spot where bacteria colonies do not grow. (Bhargav.H.S et al 2016) The disc diffusion method was used to determinate the zone of inhibition growth of bacteria by *C. sempervirens* L. extracts. In addition to the well diffusion method, for *P.gingivalis* bacteria. 50 µl of *C. sempervirens* L. extract were added to sterilized filter paper discs with a diameter of 6 mm. DMSO was added as a negative control. Then it was incubated for 24 hours at 37 °C. Then the diameter of the Inhibition Zone was measured in mm. This test was repeated three times.

3.2.5 Anti cancer activity

The purpose of this experiment is to determine the efficiency of *C. sempervirens* L. extracts on cancer cells. Two types of cancer cell lines were used in this study: Colon cancer cell lines (HT29) and Breast cancer cell lines (Mcf7). HT29 Colon cancer cells were grown in DMEM, while Mcf7 Breast cancer cells were grown in RPMI medium, both of which were supplemented with 10% FBS (Gibco), 1% glutamine, and penicillin/streptomycin. Cancer cells were cultured in dishes before being placed in a 5% CO2 incubator, different concentrations of water and ethanol *C. sempervirens* L. extracts were used to determine the lowest concentration that may affect cancer cells. After 48 hours, a fluorescent microscope was used, to observe the effect of the extracts on the cells, in addition to taking pictures of the cells. The number of live and dead cells, as well as the percentage of each, were determined using a cell counting device.

3.2.5.1 Cell Culture

Colon cancer cell lines (HT29) and Breast cancer cell lines (Mcf7) were grown in RPMI media (GibcoThermofisher), supplemented with 10% FBS (GibcoThermofisher), 1% glutamine, and 1% penicillin/streptomycin (Biological Industries). All Cells were incubated in humidity chamber on 37°C with 5% CO2.

3.2.5.2 Cell Count Colon

To determine the effect of *C. sempervirens* L. extracts on cell growth and cell death, 0.5 million of the different cells were cultured in 60 mm plates in triplicates. One day afterwards, cells were treated with different concentrations of *C. sempervirens* L. extracts, water extract concentrations were (50,100,250,500 mg/ml) and ethanolic

extract concentrations were (5,15,25,50 mg/ml) . At 72 h post treatment, both adherent and floating cells were collected and counted using a trypan blue exclusion assay to detect the percentage of dead cells.

3.2.5.3 Cell Count Breast

3x10^4 cells were seeded in 6 well plate in triplicates and cells were counted. Cells were first trpsynized and collected into 15 ml conical tubes and centrifuged at 1600 RPM for 10 min. Then the supernatant was removed and cells were resuspended in 1 mL media. Next, 10 µl of the homogenous supernatant was counted using counting chamber slides.

Chapter Four

Result and discussion

The *C. sempervirens* L. fruits were collected, dried at room temperature, milled, extracted with water and ethanol then filtrated. Samples of crude extracts were analyzed using GC/MS, crude extract was investigated as antibacterial activity included aerobic gram-negative bacteria (*Escherichia coli* (*E. coli*)) and gram-positive bacteria (*Staphylococcus aureus* (*Staph. aureus*)), as well as anaerobic gram-negative bacteria (*Porphyromonas gingivalis* (P. *gingivalis*)), in addition to anticancer activity included (Colon and Breast cancer cells).

4.1 Plant extraction

The crude extracts of *C. sempervirens* L. plants were prepared through maceration of fruit powder in solvents, ethanol and water. Several extraction methods were used, including a Sonicator and Soxhlet for ethanol extraction, as well as a Sonicator and Boiling for water extraction. The sonicator extraction method was adopted because it gave the best amount of extract. After filtration, the crude ethanolic *C. sempervirens* L. extract was concentrated using a rotary evaporator under reduced pressure. The final extracts were kept at 4°C in the refrigerator (Selim.S et al 2014), until GC/MS analysis in addition to anti-bacterial and anti-cancer tests.

4.2 Characterizing of crude ethanolic extract

4.2.1 GC/MS analysis

Figure 4.1 shows Total ion chromatogram of ethanolic extract *C. sempervirens* L. It shows separation of different compounds in the range of 4 to 61 minutes using the chromatographic conditions and MS described in materials and methods section. The major peaks are at 11.56, 16.71, 24.28, 26.12, 34.31, 52.34, 58.19, and 60.7 minutes. The compounds detected were identified according to NIST library.

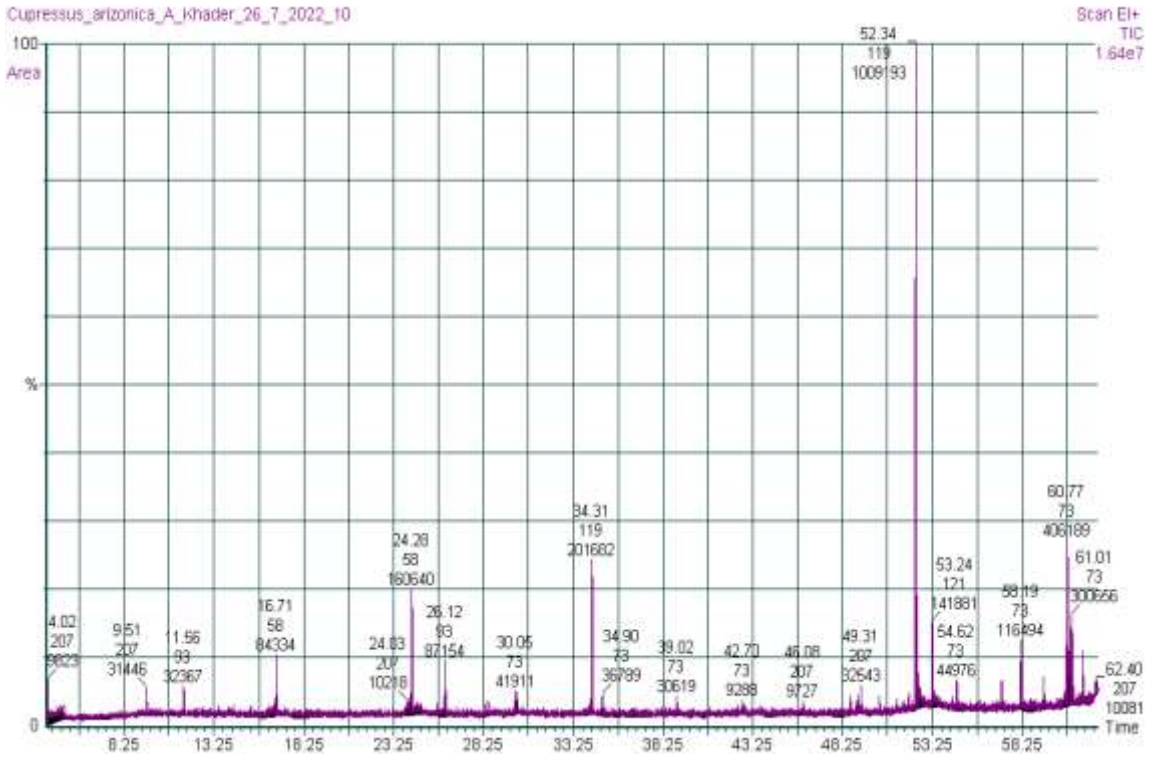


Figure 4.1 the Total ion chromatogram of ethanolic *C. sempervirens* L. extract.

To identify the main compounds in ethanolic *C. sempervirens* extract, GC/MS instrument was used, we were able to identify at least two main compounds in this extract. The results of *C. sempervirens* L. had several peaks in its profile. The findings confirmed the difference in chemical composition. A number of compounds have been identify depending on the molar mass that appeared for the main compounds. Figure 4.2 shows the chromatogram of the *C. sempervirens* L. crude ethanol extract.

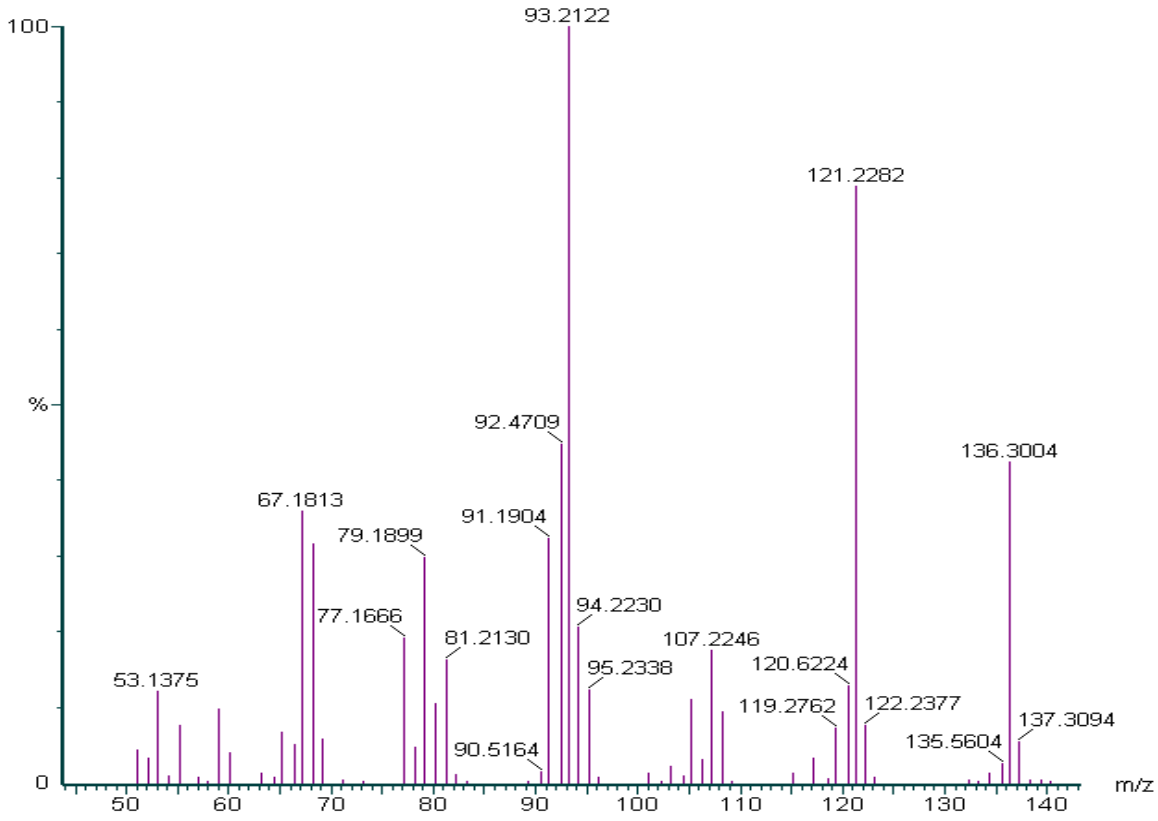


Figure 4.2 The mass spectrum of the ethanolic *C. sempervirens* L. extract.

The main constituents of our essential oil has the upper mass spectrum. This mass spectrum fits well with both α-pinene and δ-3-carene, which are the main constituents of Algerian cypress essential oil (Chanegriha et al. 1993). Both have the formula $C_{10}H_{16}$, and molar mass 136 g/mole. The base peak of both is at 93 m/z. Figure 4.3 show the chemical structure to these main constituents.

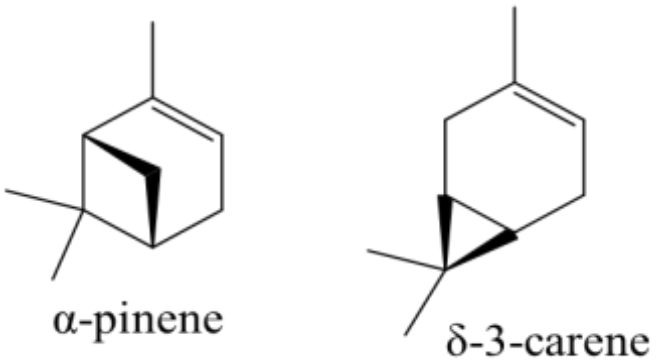


Figure 4.3 Chemical structure of α-pinene δ-3-carene.

Our result is consistent with many previous studies, where the GC/MS was used in the analysis of *C. sempervirens* L. oil. to determine the chemical composition of *C. sempervirens* L. essential oils from leaves, branches, and cones. The major constituents of all oils were α-pinene, δ-3-carene, germacrene D. and α-cedrol. (Ismail.A, Hamrouni.L et al 2013).

Also, the chemical composition of a hydro-distilled essential oil of *C. sempervirens* L. was investigated using GC/MS system. α-pinene, δ-3-carene, limonene, and α-terpinolene were identified as constituents, accounting for 98.1 percent of the oil. (Selim.S et al 2014)

4.3 Antibacterial activity

The disc diffusion test results revealed that extracts of both ethanol and water have different effects on the inhibition of bacterial growth. *C. sempervirens* L. essential oil was also tested for antimicrobial activity against five bacteria, three Gram-positive (*Staphylococcus aureus, Enterococcus* feacalis and *Bacillus cereus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*), antibacterial activity was found to be moderate in the oils. (Mazari.K, Bendimerad.N et al 2010). *Bacillus subtilis, Micrococcus luteus, Staphylococcus epidermidis*, and *Staphylococcus aureus* were inhibited by a methanol extract of *C. sempervirens* L. at 2000 µg/disc, with inhibition zones ranging from 12 to 15mm. (Qaralleh.H, Khleifat.K et al 2021)

While in this study, we have noticed the effect of *C. sempervirens* L. extracts differs according to different factors, first the bacteria types gram-positive or gram-negative *(Staphylococcus aureus, E.coli* and *P.gingivalis* bacteria) and secondly the extraction solvent if it ethanol or water extract. This difference will be clarified in the following.

4.3.1 Effect of DMSO and Ethanolic *C. sempervirens* L. extracton different bacteria strains:

The effect of DMSO and the activity of the Ethanolic *C. sempervirens* L. crude extracts obtained by sonication against gram-positive bacteria (*Staphylococcus aureus)* and gram-negative bacteria (E.coli) and anaerobic gram-negative bacteria (*Porphyromonas gingivalis*) was shown in following sections.

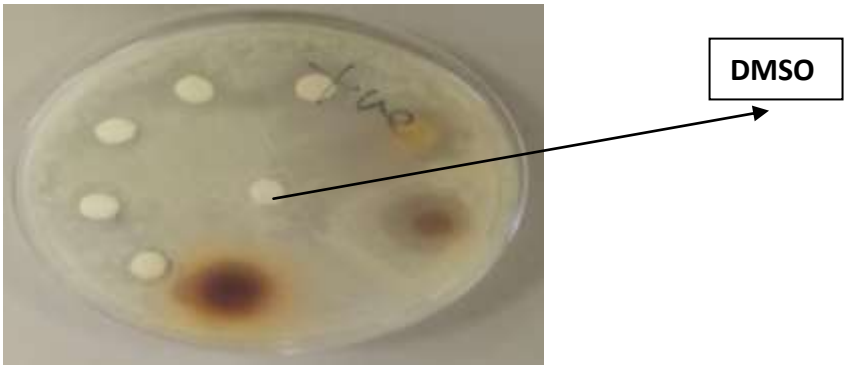
4.3.1.1Effect of DMSO on bacteria growth

The DMSO solvent was used for dilution of ethanolic *C. sempervirens* L. extract, to prepare different concentrations in the anti-bacterial experiment. Dimethylsulfoxide (DMSO) is one of the most commonly used solvents.(Carlos de Brito.R, Silva.N.G et al 2017) DMSO was also applied directly to various bacterial strains to test its effect on bacterial growth. The number of related studies on the antimicrobial activity of essential oils and their constituents is increasing. However, the hydrophobic nature of the essential oils has complicated the experiments, necessitating the use of organic solvents in the tests to avoid such complications.

Figures (4.4, 4.5. and 4.6) summarizes the effect of DMSO on bacterial growth. The results indicated that there had no DMSO effect on bacterial growth, this results was consistent with previous studies that have found that at lower concentrations of DMSO apparently there no affect on the bacterial growth significantly. (Wadhwani.T et al 2009)

4.3.1.2 Effect of Ethanolic *C. sempervirens* L. extract on *Staphylococcus aureus* as gram-positive bacteria:

Figures 4.4 shows disc diffusion assay of ethanolic *C. sempervirens* L. extract on *Staph. aureus* growth. The results indicated that there are a clear effect of *C. sempervirens* L. extract on the growth of *Staph. aureus* at several concentrations (12.5, 25, 50, 75, 100 mg/ml). This result is consistent with the result of previous studies (Chaudhary.H.J et al 2012) that examined the effect of ethanolic *C. sempervirens* L. extract on growth of *Staph. aureus*. Moreover, The result consistent with result obtained by (Selim.S et al 2014) where the effect of *C. sempervirens* L. extract with methanol in same gram-positive bacteria (*Staph. aureus*). This affectivity result may indicate the possibility that the active substances present in the methanol extract are similar to the ethanolic extract. The effect of variation concentration in inhibition zone show that the diameter of the inhibition zone increased as the concentration of the extract increased, were the concentration (12.5, 25, 50, 75, 100 mg/ml) and the inhibition zone (0, 10, 11, 12, 15 mm) respectively, the results enhancement there are no DMSO effect in bacterial growth.



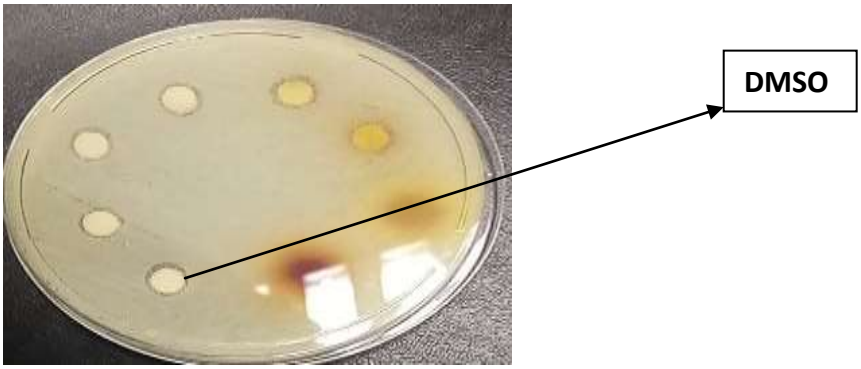
Figures 4.4 Effect of DMSO and ethanolic *C. sempervirens* L. extract on *Staph. aureus* growth by disc diffusion methods. At different concentration (12.5, 25, 50, 75, 100 mg/ml).

4.3.1.3 Effect of Ethanolic *C. sempervirens* L. extract on E. coli as gram-negative bacteria:

The activity of the ethanolic *C. sempervirens* L. crude extracts against gram-negative bacteria was shown in figure 4.5. It shows disc diffusion assay of ethanolic *C. sempervirens* L. extract on *E. coli* growth as gram-negative bacteria using variation concentration. DMSO was used as dilution solvent.

Ethanolic *C. sempervirens* L. extract had no inhibition zone was recorded on (*E. coli*) and in control DMSO. The experiment was repeated more than once to confirm the validity of the result, but no effect was shown. Our results consistent with (Chaudhary.H.J et al 2012) study that show, there was a small effect of the ethanolic extract on *E.coli* bacteria.

This result indicates that the active substances from ethanolic *C. sempervirens* L. extract ineffective in inhibiting bacterial growth, which could be attributed to the composition of Gram-negative bacteria and their ability to resist the active substances present in the extract. Gram-negative bacteria have a variety of mechanisms that prevent the action of several antimicrobials being used clinical medicine. (Oliveira.J , Reygaert.W.C 2022) The composition of the cell wall of gram-negative bacteria made of three layer that effect as an effective barrier that control the passage of large molecules into the cell such as antibiotic. (Lakna, 2017)



Figures 4.5 Effect of DMSO and ethanolic *C. sempervirens L.* extract on *E. coli* growth by disc diffusion methods. At different concentration (12.5, 25, 50, 75, 100 mg/ml).

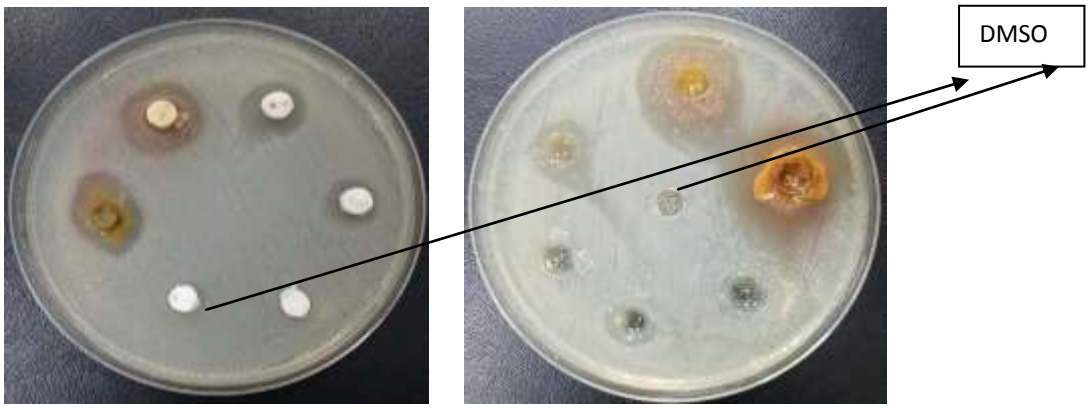
4.3.1.4 Effect of Ethanolic *C. sempervirens* L. extract on *Porphyromonas gingivalis*:

Figure 4.6 and figure 4.7 shows the effect of DMSO and ethanolic *C. sempervirens* L. extract on P. *gingivalis* bacteria by disc diffusion methods and well diffusion methods using different concentrations (12.5, 25, 50, 75, 100 mg/ml). Table 1 shows the effect of ethanolic *C. sempervirens* L. extract by the diameter of the inhibition zone in different bacteria strains *(E.coli, Staph. aureus and P. gingivalis*)

The experiment was carried out on *P. gingivalis* bacteria, which is an anaerobic and Gram-negative bacteria, and it indicated as one of the bacteria that causes periodontal infections. The results revealed the ethanolic *C. sempervirens* L. extract had a clear effect in several concentrations, The diameter of the inhibition zone increased as the concentration of the extract increased, were concentrations (12.5, 25, 50, 75, 100 mg/ml) and the inhibition zone (0, 0.9, 14, 18, 22 mm) respectively for disc diffusion methods, and (0, 11, 13, 21, 23 mm) respectively for well diffusion methods.

Also in this experiment DMSO had no effect on bacterial growth. This is Indicating the ability of Ethanolic *C. sempervirens* L. extract to act as an antibacterial for certain types of bacteria, and the mechanism of action varies depending on the type of bacteria. The highest inhibition zones among ethanolic extracts were recorded in 100 mg/ml for both bacteria types (*Staph. aureus* and *P. gingivalis*), and the inhibition zones were (15) and (22, 23) mm respectively. While no any inhibition zone was revealed in E.coli and DMSO whereas the lowest inhibition zone was recorded in low

concentration for both bacteria types using disc diffusion methods with 10 and 0.9 mm respectively. Our results consistent with (Selim.S et al 2014) study. that reported the dried *C. sempervirens* L. fruit is used to treat inflammation and toothache.



a) b)

Figure 4.6 Effect of DMSO and ethanolic *C. sempervirens* extract on *P. gingivalis* bacteria, a. disc diffusion method, b. well diffusion method. At different (12.5, 25, 50, 75, 100 mg/ml).

Table 4.1 shows the effect of ethanolic *C. sempervirens* L. extract by the diameter of the inhibition zone.

Table 4.1 Antimicrobial activity of ethanolic extract of *C. sempervirens* L.

Zone of inhibition in mm				
concentration mg/ml	**E.coli**	**Staphylococcus aureus**	**Porphyromonas gingivalis**	
	Disc diffusion methods			Well diffusion method
100	ND	15	22	23
75	ND	12	18	21
50	ND	11	14	13
25	ND	10	0.9	11
12.5	ND	ND	ND	ND
DMSO	ND	ND	ND	ND

*ND: Not detected.

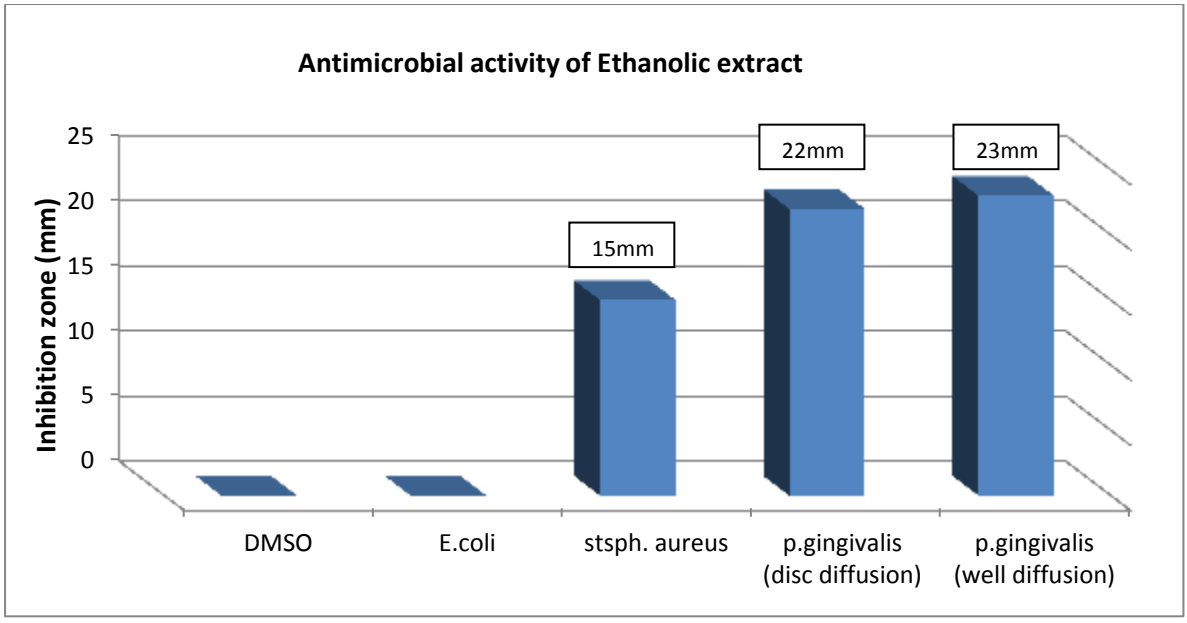


Figure 4.7 Antimicrobial activity of Ethanolic extract on different types of bacteria (*E.coli, Staph. aureus, P. gingivalis*) and effect of DMSO.

4.3.2 Effect of Water *C. sempervirens* L. extract on different bacteria strains

The activity of the crude water extract of *C. sempervirens* L. obtained by sonication against gram-positive bacteria (*Staphylococcus aureus)*, gram-negative bacteria (E.coli) and anaerobic gram-negative bacteria (*Porphyromonas gingivalis*) was shown in following sections.

4.3.2.1 Effect of water *C. sempervirens* L. extract on *Staphylococcus aureus* as gram-positive bacteria:

Figure 4.8 show the effect of water *C. sempervirens* extract on *Staph. aureus* as gram-positive bacteria by disc diffusion methods using different concentration. The result clear effect on *Staph. aureus* bacteria at the highest concentration only, the diameter of the inhibition zone was 15 mm. This indicates that the concentration of the active substances present in the water *C. sempervirens* L. extract affects the efficiency of the extract in acting as an antibacterial. The antibacterial effect increased as the concentration of the extract increased. (Haghgoo.R et al 2017) And this is consistent with what was mentioned at (Al-Snafi.A.E 2016), where water extract of *C. sempervirens* L. was tested for antibacterial activity against *Staph. aureus*, this extract had antibacterial activity.

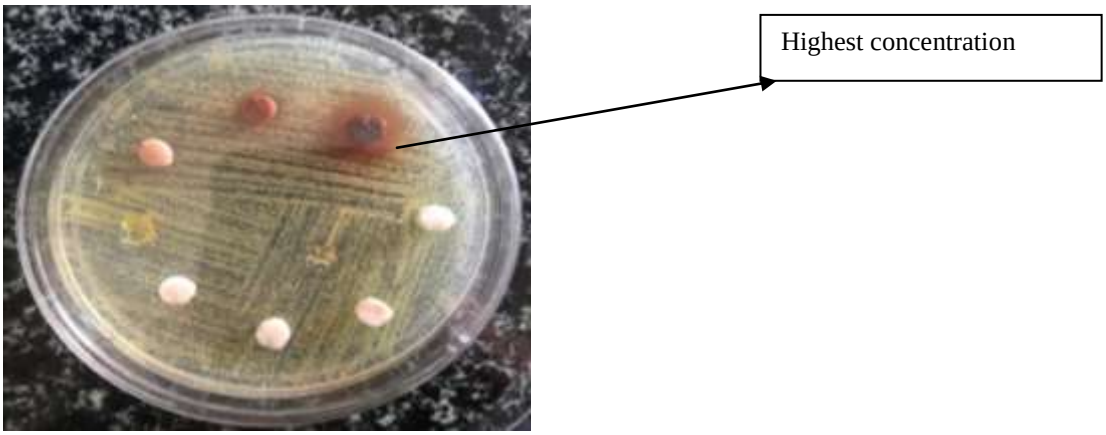


Figure 4.8 Effect of Water *C. sempervirens* extract on *Staph. aureus* by disc diffusion methods. At different concentration (12.5, 25, 50, 75, 100 mg/ml).

4.3.2.2 Effect of water *C. sempervirens* L. extract on *E. coli* as gram-negative bacteria:

Figures 4.9 shows disc diffusion assay of water *C. sempervirens* L. extract on *E. coli* growth. Water *C. sempervirens*.L extract had no inhibition zone was recorded on Gram-negative bacteria (*E. coli*). This experiment was repeated more than once to confirm the validity of the result, but no effect was shown at any concentration.

This indicates that the different types of bacteria play a role in the result, as we observed in gram-positive bacteria the effect of the highest concentration of the extract with water, while in gram-negative bacteria we did not observed any effect. This is also due to the high resistance of these bacteria to the active substances present in the extract. This is consistent with what was mentioned at (Al-Snafi.A.E 2016) where water extract of *C. sempervirens* L. was tested for antibacterial activity against *E.coli* . This extract had low antibacterial activity against *E.coli*. this is because Gram-negative bacteria have a variety of mechanisms that prevent the action of several antimicrobials being used clinical medicine. (Oliveira.J , Reygaert.W.C 2022)

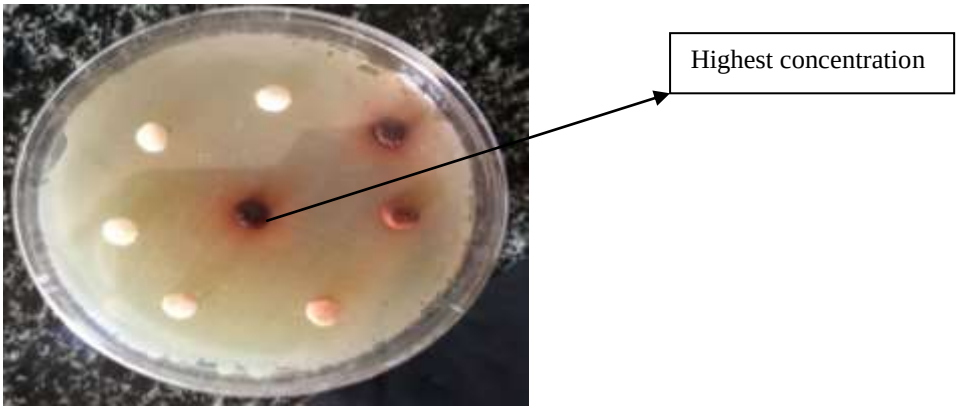


Figure 4.9 Effect of water *C. sempervirens* extract on *E.coli* by disc diffusion methods. At different concentration (12.5, 25, 50, 75, 100 mg/ml).

4.3.2.3 Effect of water *C. sempervirens* L. extract on *Porphyromonas gingivalis*:

Figures 4.10 shows disc diffusion and well diffusion methods assay of water *C. sempervirens* extract on *P. gingivalis* bacteria growth using variation concentration. water *C. sempervirens* L. extract had no inhibition zone was recorded on *P.gingivalis* bacteria, and no effect was shown at any concentration. This also indicates that the different types of substances present in the extract with different solvents play a role in the result, as we noticed that the ethanolic *C. sempervirens* L. extract has the ability to act as an antibacterial against *P.gingivalis* bacteria whereas the water extract, it had no or limited effect.

a) **b)**

Figure 4.10 Effect of water *C. sempervirens* L. extract on *P. gingivalis* bacteria, a. disc diffusion methods, b. agar well diffusion methods. At different concentration (12.5, 25, 50, 75, 100 mg/ml).

Table 4.2 shows the effect of water *C. sempervirens* L. extract by the diameter of the inhibition zone.

Table 4.2 Antimicrobial activity of water extract of *C. sempervirens* L.

Zone of inhibition in mm				
concentration mg/ml	**E.coli**	**Staphylococcus aureus**	**Porphyromonas gingivalis**	
	Disc diffusion methods			Well diffusion method
100	ND	14	ND	ND
75	ND	ND	ND	ND
50	ND	ND	ND	ND
25	ND	ND	ND	ND
12.5	ND	ND	ND	ND

*ND: Not detected.

Figure 4.11 show the antimicrobial activity of water extract on different types of bacteria. *E.coli, Staph. aureus, P. gingivalis*.

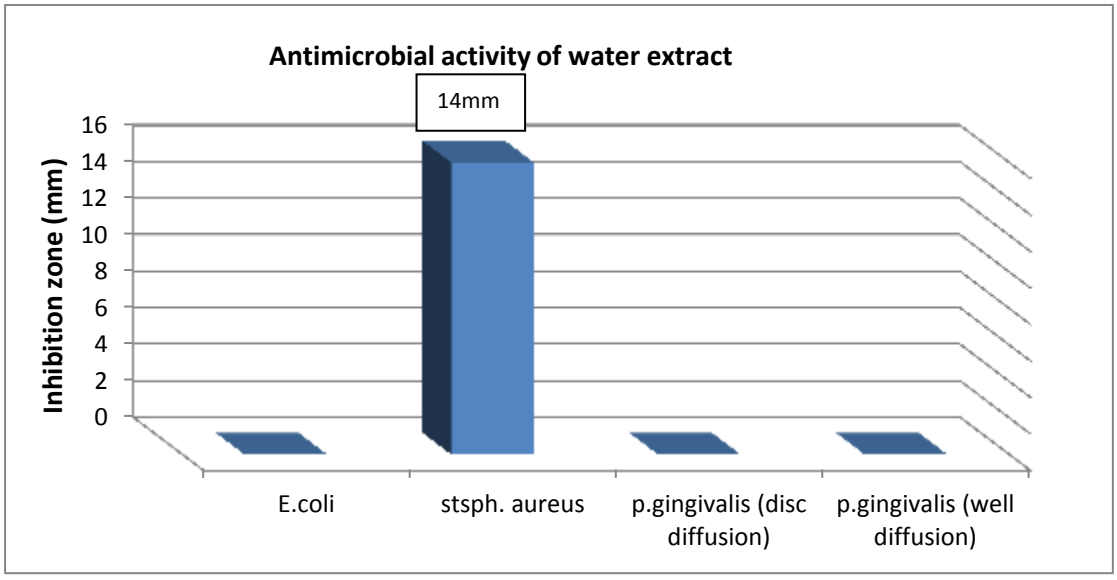


Figure 4.11 Antimicrobial activity of water extract on different types of bacteria (*E.coli, Staph. aureus, P. gingivalis*).

The result that appeared in the GC/MS analysis identified two main compounds α-pinene and δ-3-carene. Some studies showed the ability of α-pinene compound to act as an antibacterial, and it was effective against *Staph. aureus*. (Pichette.A et al 2006)

α-pinene has antibacterial and antibiotic modulating properties, making it useful in the development of new antibacterial drugs.(Freitas.P.R. et al 2020)

The steadily increasing resistance of microorganisms to conventional antibiotics, as well as the lack of new chemical entities, are major public health concerns. As a result, α-pinene could be used as a natural antibacterial alternative.(Allenspach.M, Steuer.C 2021), in addition the other substances that identified in *C. sempervirens* L. extracts by GC/MS are usually associated with antibacterial properties such as 3-carene, cedrol and limonene. Therefore, it gives *C. sempervirens* L. extract efficiency in acting as an anti-bacterial. (Gupta.A, Jeyakumar.E et al 2021, Boukhris.M, Regane.G et al 2012).

4.4 Anticacer activity

The activity of for *C. sempervirens* L. extracts with water and ethanol on Colon and Breast cancer cells at different concentrations was investigated. The results of both extraction and application in colon and breast cancer cell was described in the following. ,Based on the results of previous studies, such as (Fayed.S 2015) and (Abd Alhady.M et al 2020), it was expected that *C. sempervirens* L. extract would have an anti-cancer effect.

Inhibitory concentrations (IC50s) used early in the discovery process to evaluate the suitability and the performance of drugs.(Sebaugh.J.L 2010) Each of the following figures (4.13, 4.16, 4.19, 4.22) will show the IC50 value for the effect of *C. sempervirens* L extracts on the cancer cells that were used in the anticancer test.

4.4.1 Effect of Ethanolic *C. sempervirens* L. extract on different cancer cells

The ethanolic *C. sempervirens* L. crude extract was diluted for different concentration (5, 15, 25, 50 mg/ml) using DMSO for the anti-cancer experiments, DMSO was injected directly on cancer cells, to test its effect on the cells . The results was shown in figures (4.12-4.19) and table 4.3.

4.4.1.1 Breast cancer cell lines (Mcf7 cells)

The effect of different Ethanolic *C. sempervirens* L. extract concentrations (50, 25, 15, 5 mg/ml) on the percentage number breast cancer Mcf7 was investigated using Automated Cell Counter (BIO RAD). Figure 4.12 shows the effect of each concentration on the percentage of living cells. And figure 4.13 shows the images of

cancer cells after 72 hrs treatment cells, were observed under inverted fluorescent microscope (Olympus CKX 41) and imaged. The results showed that ethanolic *C. sempervirens* L. extract had good effect on the Mcf7 cancer cells . It observed that an increasing in the concentration of ethanloic extract, the number of living breast cancer cells Mcf7 was decreased. as it is reduced the number of living cells using 50mg/ml as highest concentration to less than 25% whereas the lowest concentration 5mg/ml was reduced to 72%. This result is consistent with (Abd Alhady.M et al 2020) study that shows the effect of methanol *C. sempervirens* L. extract on human Caucasian breast adenocarcinoma (MCF7) reduced the viability of cell line.

The images show the difference between the effects of each concentration, in addition to the effect of DMSO, compared to the control sample. Where the difference in the shape and number of cancer cells appears in the different concentration images compared to the control sample.

The result shows DMSO had no effect on Mcf7 cells cancer. Also, a sample of cells without any additives was used to be taken as a control sample to confirm and to compares' the result of experiment. The percentage of live cells in the control sample for Mcf7 cancer cells was 96%, while the percentage of live cells for the following concentrations, respectively, 5, 15, 25, 50 mg/ml, was 96%, 72%, 30%, 35%, 19%. For the sample in which DMSO was used, there was no effect, as the percentage of live cells was 96% .

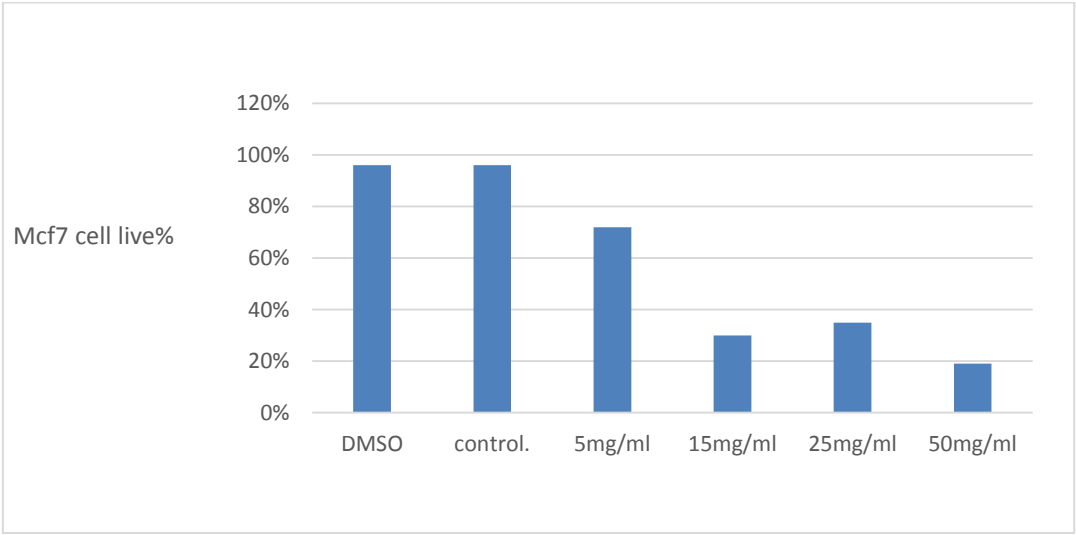


Figure 4.12 Mcf7 cell live% with Ethanolic C. sempervirens L. extract. At different concentration (5, 15, 25, 50 mg/ml), DMSO and control sample.

IC50 value for breast cancer (Mcf7) was investigated using IC50 Calculator. Its result, based on the following law, is equal to:

$$Y = \text{Min} + \frac{\text{Max} - \text{Min}}{1 + \left(\frac{X}{IC50}\right)^{\text{Hill coefficient}}} \qquad Y = 22.6904 + \frac{96.2026 - 22.6904}{1 + \left(\frac{X}{6.821}\right)^{2.1715}}$$

$IC_{50} = 6.821$ mg/ml

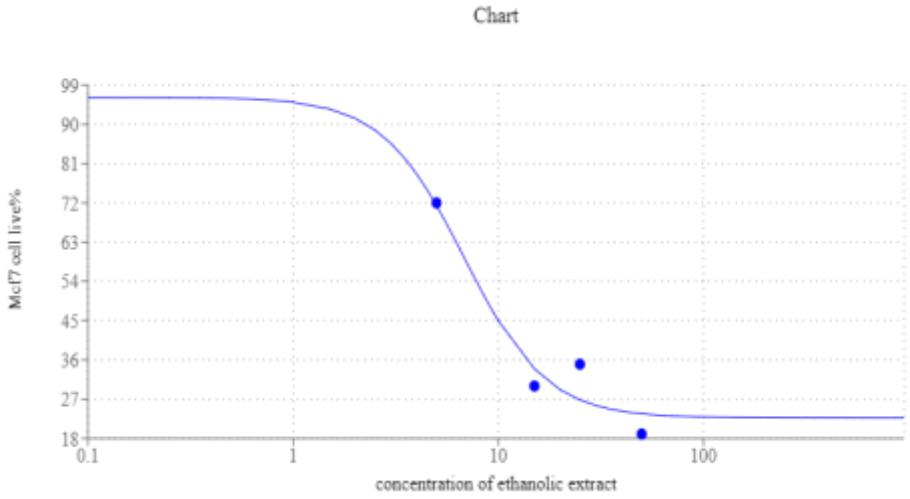
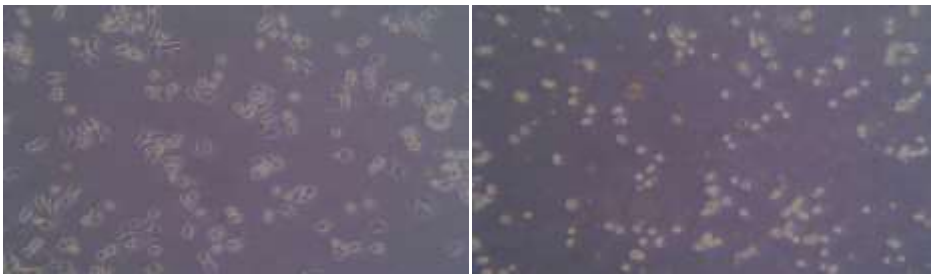
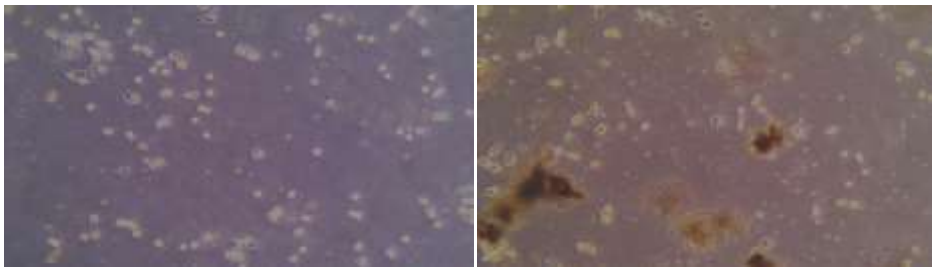


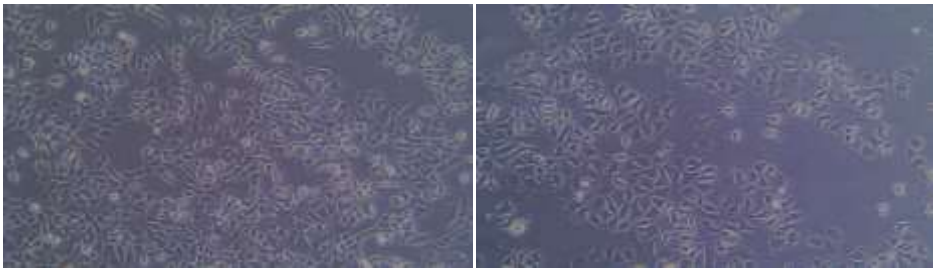
Figure 4.13 Chart to investigated IC50 value for Mcf7 cell live% with Ethanolic C. sempervirens L. extract. At different concentration (5, 15, 25, 50 mg/ml)



A- 5 mg/ml B- 15 mg/ml



C – 25 mg/ml D – 50 mg/ml



E – control F - DMSO

Figure 4.14 Efficiency of the Ethanolic C. sempervirens L. extract for Mcf7 cancer cells at different concentrations (A-D), (E) Control, (F) DMSO, observed after 72 hours.

4.4.1.2 Colon cancer cell lines (HT29 cells):

Figure 4.14 summarizes the effect of variation concentration of Ethanolic C. sempervirens extract on the percentage of living colon cancer cells. Figure 4.15 shows the images of HT29 cancer cells after 72 hrs treatment cells, were observed under inverted fluorescent microscope (Olympus CKX 41) and imaged. The effect of different Ethanolic *C. sempervirens* L. extract concentrations (50, 25, 15, 5 mg/ml) on the percentage number colon cancer cells HT29 was investigated using Automated Cell Counter (BIO RAD).

The results showed that ethanolic C. sempervirens L. extract had an effect on HT29 cancer cells, the number of living colon cancer cells was reduced as the concentration of the extract increases. The 50 mg/ml concentration of Ethanolic C. sempervirens extract shows only 25% of living cells decreases with 75% educed from total living cells.

The images shows the difference between the effect of each concentration on the shape of cancer cells, in addition to the effect of DMSO, compared to the control sample. Where the difference in the appearance of cancer cells appears in the different concentration images compared to the control sample. DMSO used as control experiment, to investigated the effect of DMSO on cancer cells. Also, a sample of cells without any additives was used as a control sample to confirm and to compares with variation concentration and DMSO experiments.

The percentage of HT29 living cells in the control sample was 89%, while the percentage of living cells for the variation concentrations, 5, 15, 25, 50 mg/ml, were 64%, 52%, 44%, 25%, respectively. Also, the result of DMSO experiment observed no effect on HT29 cancer cells showing 96% percentage of living cells.

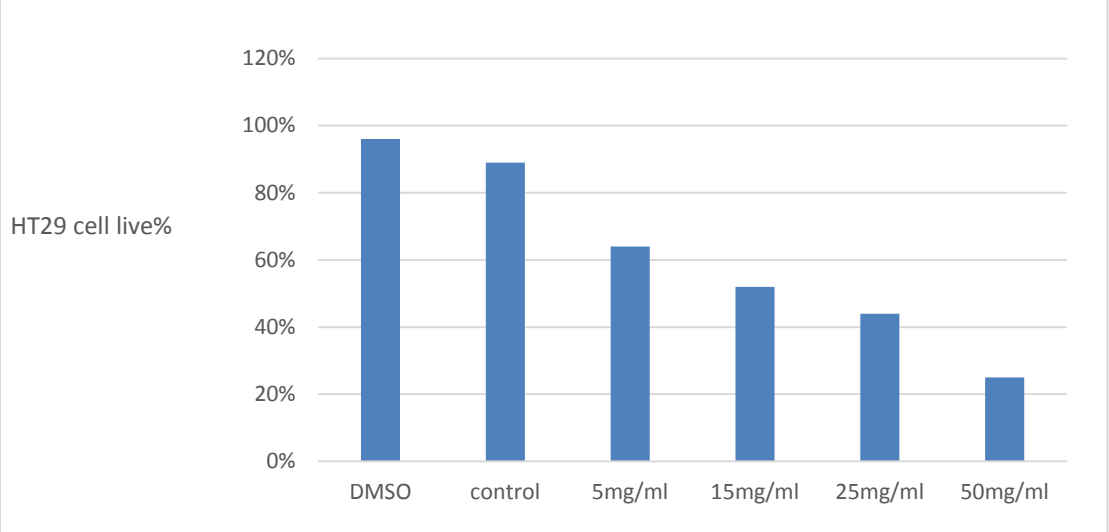


Figure 4.15 HT29 cell live% with Ethanolic *C. sempervirens* L. extract**.** At different Concentration (5, 15, 25, 50 mg/ml), DMSO and control sample.

IC50 value for colon cancer (HT29) was investigated using IC50 Calculator. Its result, based on the following law, is equal to:

$$Y = -122.9105 + \frac{88.5809 + 122.9105}{1 + \left(\frac{X}{253.4234}\right)^{0.5423}}$$

$IC_{50} =$ 253.4234 mg/ml

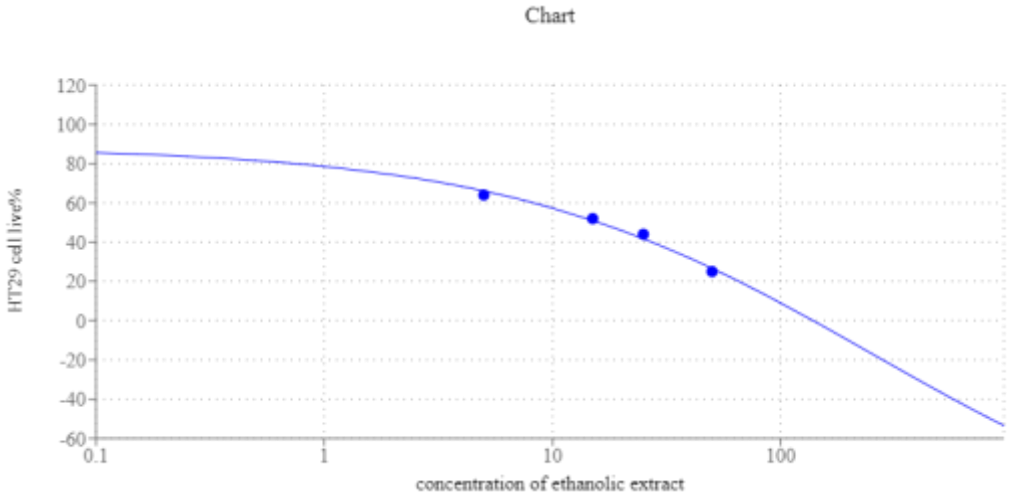
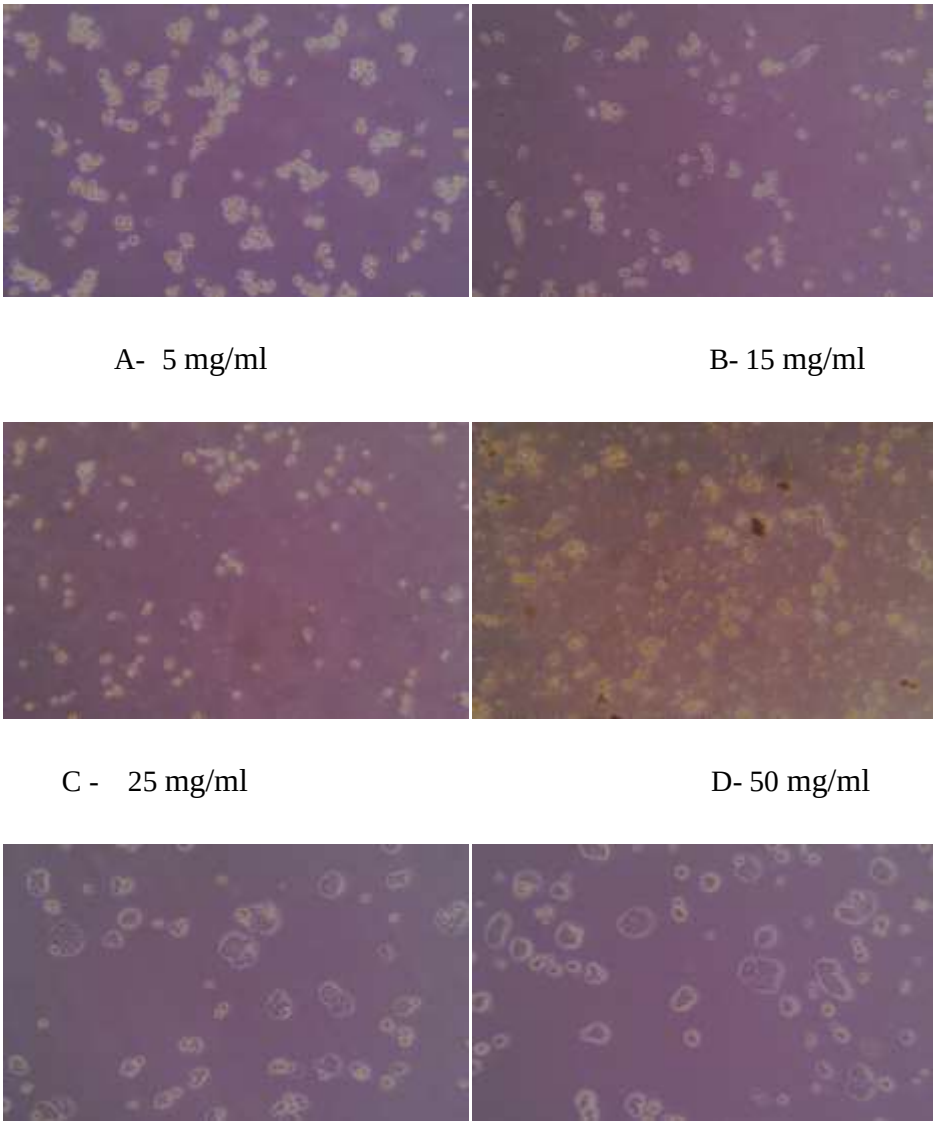


Figure 4.16 Chart to investigated IC50 value for HT29 cell live% with Ethanolic C. sempervirens L. extract. At different concentration (5, 15, 25, 50 mg/ml)



A- 5 mg/ml B- 15 mg/ml

C - 25 mg/ml D- 50 mg/ml

E- control F- DMSO

Figure 4.17 Efficiency of the Ethanolic C. sempervirens L. extract for HT29 cancer cells at different concentrations(A-D), (E) Control, (F) DMSO, observed after 72 hours.

4.4.2 Effect of Water *C. sempervirens* L. extract on different cancer cells:

4.4.2.1 Breast cancer cell lines (Mcf7 cells):

Figure 4.16 shows the effect variation water C. sempervirens L. extract on the percentage of living breast cancer cells Mcf7. Different concentrations of the water C. sempervirens L. extract as following 50, 100 ,250, 500 mg/ml were used. The effect of extract on the percentage number breast cancer cells Mcf7 was investigated using Automated Cell Counter (BIO RAD).

The results showed that water *C. sempervirens* L. extract had no clear effect on Mcf7 cancer cells, no effect on the number of living cells, when the concentration of the extract increases. The results obtained shows that the percentage of live cancer cells Mcf7, in the control sample was 96%, and the percentage of live cells of the following concentrations, respectively, was 50, 100 ,250, 500 mg/ml are 93%, 97%, 95%, 96%. Figure 4.17 shows the images of Mcf7 cancer cells after 72 hrs treatment cells were observed under inverted fluorescent microscope (Olympus CKX 41) and imaged, these images show the difference between the shape of cells at each concentration compared control sample.

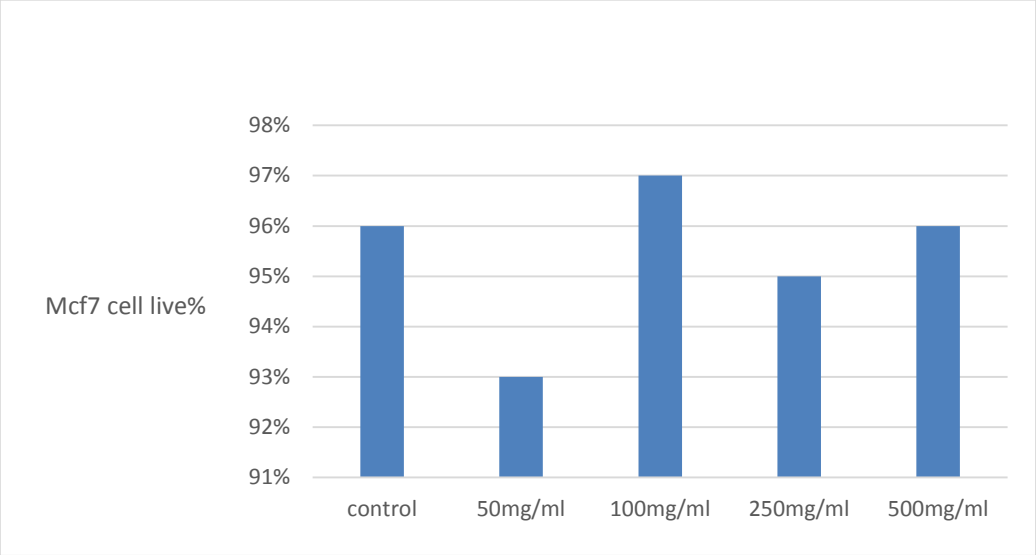


Figure 4.18 Mcf7 cell live% with Water *C. sempervirens* L. extract. At different concentration (50, 100 ,250, 500 mg/ml)

IC50 value for breast cancer (Mcf7) was investigated using IC50 Calculator. Its result, based on the following law, is equal to:

$$Y = -38.2641 + \frac{96.001 + 38.2641}{1 + \left(\frac{X}{33.9097}\right)^{-9.7309}}$$

IC_{50} =33.9097 mg/ml

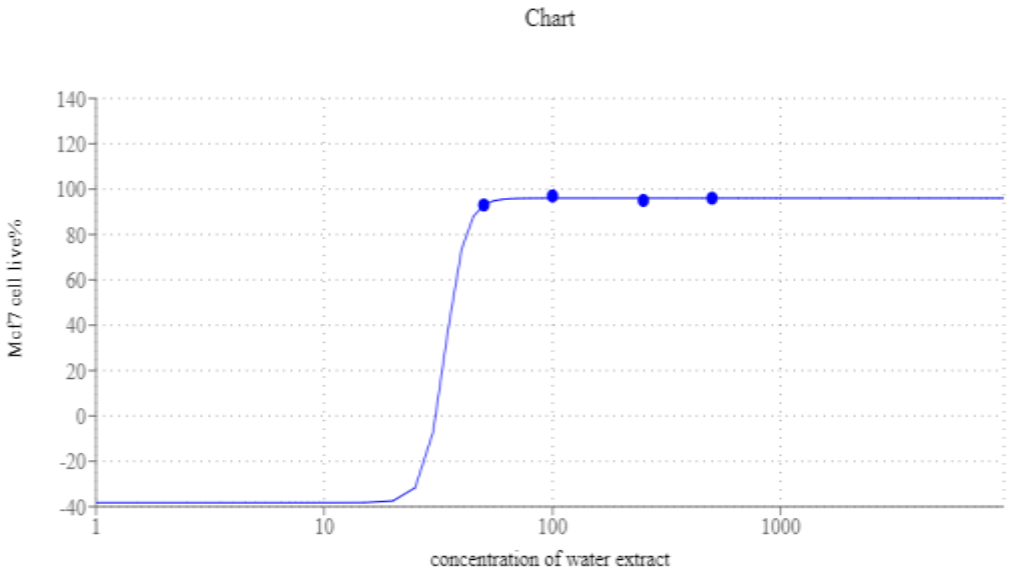
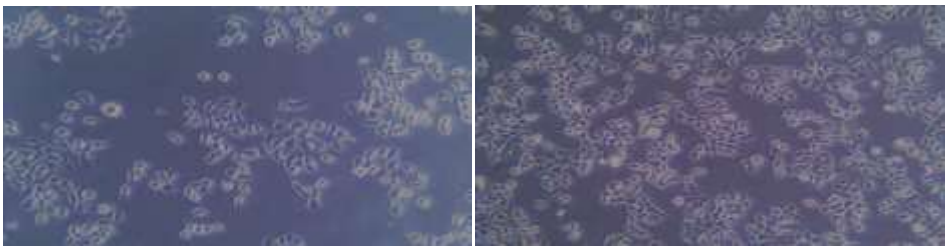


Figure 4.19 Chart to investigated IC50 value for (Mcf7) cell live% with water C. sempervirens L. extract. At different concentration (50, 100 ,250, 500 mg/ml)



A - 50 mg/ml

B – 100 mg/ml

C – 250 mg/ml

D – 500 mg/ml

E -Control

Figure 4.20 Efficiency of the water *C. sempervirens* L. extract for Mcf7 cancer cells at different concentrations (A-D), (E) Control, observed after 72hour.

4.4.2.2 Colon cancer cell lines (HT29 cells):

Figure 4.18 summarizes the effect of variation concentration water *C. sempervirens* L. extract vs. the percentage of HT29 living cells. The effect of extract on the percentage number of Colon cancer cells HT29 was investigated using Automated Cell Counter (BIO RAD). Figure 4.19 shows the images of HT29 cells, after 72 hrs treatment cells were observed under inverted fluorescent microscope (Olympus CKX 41). Different concentrations of the water *C. sempervirens* L. extract as following 50, 100 ,250, 500 mg/ml were injected against HT29 cells. The results showed that water *C. sempervirens* L. extract had no clear effect on HT29 cancer cells, no effect on the number of living cells. There are no relation between increasing the concentration against the living cells. On the other hand the results observed for that the percentage of live cancer cells HT29, in the control sample was 96%, and the percentage of live cells of the following concentrations, respectively, was 50, 100 ,250, 500 mg /ml are 99%, 92%, 75%, 94% showing no effect of water *C. sempervirens* L. extract against HT29 cells. The images show the effect of each concentration compared control, there is no difference in the shape of the cancer cells compared with the control sample, this indicates that the extract has no effect on cancer cells.

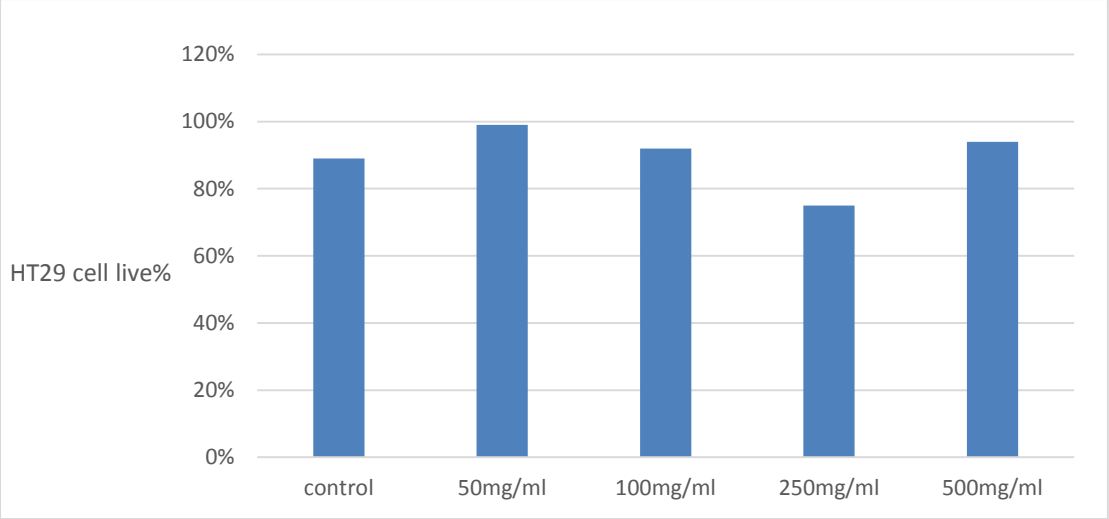


Figure 4.21 HT29 cell live% with Water *C. sempervirens* extract. At different concentration (50, 100 ,250, 500 mg/ml).

IC50 value for colon cancer (HT29) was investigated using IC50 Calculator. Its result, based on the following law, is equal to:

$$Y = 84.5002 + \frac{99.007 - 84.5002}{1 + \left(\frac{X}{100.6178}\right)^{10.9682}}$$

$IC_{50=}$ 100.6178 mg/ml

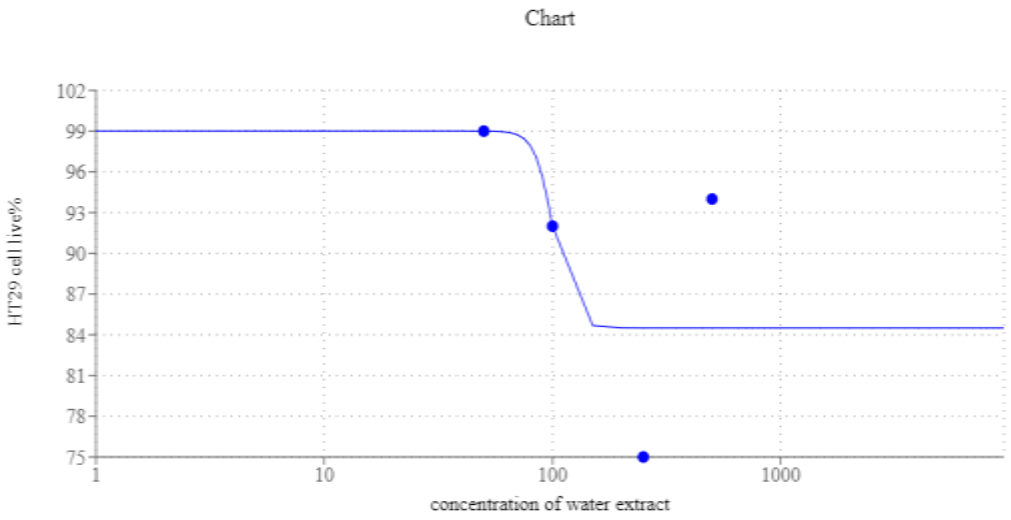
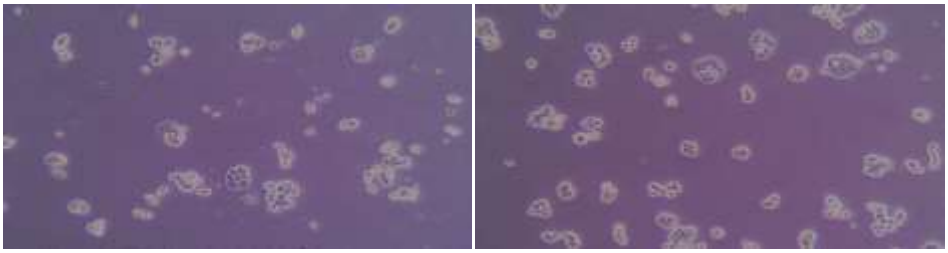
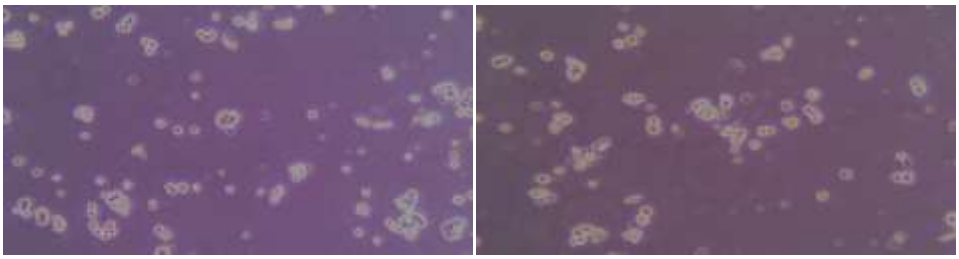


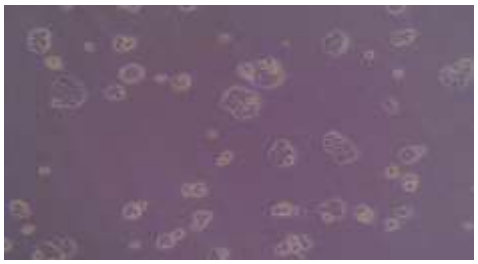
Figure 4.22 Chart to investigated IC50 value for (Mcf7) cell live% with water C. sempervirens L. extract. At different concentration (50, 100 ,250, 500 mg/ml)



A- 50 mg/ml B – 100 mg/ml



C – 250 mg/ml D – 500 mg/ml



E – Control

Figure 4.23 Efficiency of the water *C. sempervirens* L. extract for HT29 cancer cells at different concentrations (A-D), (E) Control, observed after 72 hours.

The result that appeared in the GC/MS analysis identified two main compounds, α-pinene and δ-3-carene. Some studies showed the α-pinene has cancer prevention properties. (Zhao.Y et al 2017) found that α-pinene significantly inhibited prostate cancer cell in a Mouse. This confirms the result obtained in the anti-cancer experiment.

The variation of compounds in different *C. sempervirens* L. extraction causing different effect of both water and ethanol extract against colon and breast cancer cells. The Ethanol extract of have different active compounds as α-pinene and δ-3-carene compare to water extract, this compound shows higher biological and cancer activity.

The results that appeared in the anticancer test for the ethanolic extract are consistent with the results that appeared for cypress oil on other types of cancer cells, as appeared with (Fayed.S 2015), where C. sempervirens L. essential oil had the highest cytotoxic activity against two human promyelocytic leukemia cell lines. The research found that methanol C. sempervirens L. leaf extract reduced the viability of several cell lines, human Caucasian breast adenocarcinoma (MCF7), and particularly human colon cancer cell (HCT116). (Abd Alhady.M et al 2020), Based on the results of previous studies, it was expected that C. sempervirens L .extract would lead to the death of cancer cells and have an anticancer effect.

Table 4.3 Total count cells, count live cells, and percentage of live cells at various extract concentrations.

Cells type	**Extraction solvent**	**Concentration**	**Total count cells**	**Live cells count**	**Live cells %**
HT29	Water	500ul	8.64*10^4	8.1*10^4	94%
HT29	Water	250ul	3.5*10^5	2.65*10^5	75%
HT29	Water	100ul	4.48*10^5	4.11*10^5	92%
HT29	Water	50ul	6.00*10^5	5.94*10^5	99%
HT29	Ethanol	5ul	4.11*10^5	2.65*10^5	64%
HT29	Ethanol	15ul	7.56*10^5	3.94*10^5	52%
HT29	Ethanol	25ul	6.81*10^5	3.03*10^5	44%
HT29	Ethanol	50ul	1.59*10^6	4.00*10^5	25%
HT29	Control	-	4.59*10^5	4.11*10^5	89%
HT29	DMSO	50ul	4.38*10^5	4.21*10^5	96%
Mcf7	Ethanol	50ul	1.66*10^6	3.08*10^5	19%
Mcf7	Ethanol	25ul	1.66*10^6	5.78*10^5	35%
Mcf7	Ethanol	15ul	1.31*10^6	3.89*10^5	30%
Mcf7	Ethanol	5ul	2.86*10^5	2.05*10^5	72%
Mcf7	Control	-	7.89*10^5	7.56*10^5	96%
Mcf7	DMSO	50ul	4.38*10^5	4.21*10^5	96%
Mcf7	Water	500ul	5.51*10^5	5.29*10^5	96%
Mcf7	Water	250ul	5.94*10^5	5.67*10^5	95%
Mcf7	Water	100ul	4.92*10^5	4.75*10^5	97%
Mcf7	Water	50ul	5.08*10^5	4.70*10^5	93%

Chapter Five

Conclusions and Future Work

Conclusions

The effect of *C. sempervirens* L. fruit extract in Palestine was analysis by using GC/MS was used to determine the main components of the ethanolic extract. *C. sempervirens* L. was studied as an antibacterial and an anticancer.

By using GC/MS, two main compounds in the ethanolic extract were identified, namely α-pinene δ-3-carene.

The result of antibacterial experiment it was found that;

Ethanolic *C. sempervirens* L. extract had antibacterial activity as it had a strong effect on gram-positive bacteria (*Staphylococcus aureus*).

Ethanolic *C. sempervirens* L. extract had antibacterial activity as it had a strong effect on anerobic gram-negative bacteria (*P.gingivalis*).

Ethanolic *C. sempervirens* L. it had no effect on aerobic gram-negative bacteria (*E.coli*).

Water *C. sempervirens* L. extract had no effect on gram-negative bacteria except for gram-positive bacteria (*Staphylococcus aureus*) it had effect at highest concentration.

Ethanolic *C. sempervirens* L. fruit was found to be the strongest anticancer activity against Colon cancer cell lines (HT29) and Breast cancer cell lines (Mcf7). As for water C. sempervirens L. extract, it had no effect on cancer cells.

These results indicate that the most effective substances dissolve in organic solvents (ethanol). While these substances are insoluble in inorganic solvents (water).

Future Work

This work was mainly focused on the antibacterial and anticancer activity of *C. sempervirens* L. extracts, studying the antibacterial activity of a type of bacteria related to periodontal infections. so we suggest focusing on this aspect and conducting more experiments to separated the main constituent a-pinene, δ-3-carene, and apply the constituent on the varieties bacteria and cancer cells. And application the *C. sempervirens* L. extracts in different bacteria and cancer cells.

References

Ref.

- Abd Alhady.M, Abo El-Fadl.R, Hegazi.G and Desoukey.S (2020).(In vitro production of some secondary metabolites from Cupressus sempervirens) J. Adv. Biomed. & Pharm. Sci. Vol.(3), P. 127-134.
- Alkurdi.MIS and Supuka.J. (2015).(Assessment of Cupressus sempervirens L. hardiness through carbohydrates and pigments content in the leaves). Acta Scientiarum Polonorum Formatio Circumiectus. Vol.14 (2), 3-16.
- Al-Snafi.A.E (2016).(Medical importance of Cupressus sempervirens- A review) IOSR Journal Of Pharmacy. Vol.(6), P. 66-76.
- Aslam.S.M, Ahmad.S.M. (2016).(Worldwide Importance of Medicinal Plants: Current and Historical Perspectives). Recent Advances in Biology and Medicine, Vol.(2), P.88-93,89.
- Barbieri.R, Coppo.E, Marchese.A, Daglia.M, Sanchez.E.S, Nabavi.S.F and Nabavi.S.M. (2017).(Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity). Microbiological Research Vol. (196), P. 44-68.
- Bhargav.H.S, Shastri.S, Purushothama.P.S, Darshan.K.M, Nayak.M (2016).(Measurement of the Zone of Inhibition of an Antibiotic) IEEE. 978-1-4673-8286-1/16.
- Boukhris.M, Regance.G, Sayadi.S and Bouaziz.M. (2012).(Chemical Composition and Biological Potential of Essential Oil from Tunisian Cupressus sempervirens L.). Journal of Arid Land Studies Vol. 22(1), P.329 - 332.
- Bravo.L (1998).(Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance). Nutr. Rev., Vol.(56), P. 317-33.
- Chanegriha.N, Badiouamer.A and Meklati.B.Y (1993).(Chemical Composition of Algerian Cypress Essential Oil) J. Essent. Oil Res., Vol. (5), P 671- 674.
- Chappell.J (2002).(The genetics and molecular genetics of terpene and sterol origami) Current Opinion in Plant Biology.Vol. (5), P.151-157.
- Chaudhary.H.J, Shahid.W , Bano.A , Ullah.F , Munis.F , Fahad.S and Ahmad.I (2012).(In vitro analysis of Cupressus sempervirens L. plant extracts antibaterial activity) J. Medicinal Plants Research Vol. (6), P. 273-276.

- Chirinos.R, Pallardel.I.B, Huamán.A, Arbizu.C, Pedreschi.R and Campos.D (2009).(HPLC-DAD characterisation of phenolic compounds from Andean oca (Oxalis tuberosa Mol.) tubers and their contribution to the antioxidant capacity) Food Chemistry Vol.(113), P. 1243-1251.
- Cho.K.S, Lim.Y, Lee.K, Lee.J, Lee.J.H and Lee.I.S (2017).(Terpenes from Forests and Human Health) Toxicol. Res. Vol. (33), P. 97-106.
- Duraipandiyan.V, Ayyanar.M and Ignacimuthu.S. (2006).(Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India). BMC Complement Altern Med. Vol. (6), P. 35.
- Eisenberg.DM, Davis.RB, Ettner.SL, Appel.S, Van Rompay.M and Kessler.RC. (1998).(Trends in alternative medicine use in the United States 1990 – 1997 results of a follow-up national survey). Journal of American Medical association 280, P. 1569-1575.
- Fayed.S. (2015).(Chemical Composition, Antioxidant, Anticancer Properties and Toxicity Evaluation of Leaf Essential Oil of Cupressus sempervirens). Notulae Botanicae Horti Agrobotanici Cluj-Napoca.Vol. 43(2), P. 320-326.
- Freitas.P.R, De Araújoa.A.C.J, Barbosa.C.R.S, Muniz.D.F, Silva.A.C.A, Rocha.J.E et al.(2020).(GC-MS-FID and potentiation of the antibiotic activity of the essential oil of Baccharis reticulata (ruiz & pav.) pers. and α-pinene) Industrial Crops & Products Vol. (145), P. 112-106.
- Ganesan.A (2008).(The impact of natural products upon modern drug discovery) Current Opinion in Chemical Biology, Vol. (12), P. 306–317.
- George.P. (2011).(Concerns regarding the safety and toxicity of medicinal plants - An overview) Journal of Applied Pharmaceutical Science Vol. (1), P. 40-44.
- Gershenzon.J and Dudareva.N (2007).(The function of terpene natural products in the natural world) Nat. Chem. Bio. Vol.(3), P. 408-414.
- Gervais.B (2016).(An overview of molecular hybrids in drug discovery) Expert Opin Drug Discov. Vol. (11), P. 281-305.
- Giovanelli.A, and .De Carlo.A. (2007).(Micropropagation of Mediterranean cypress(Cupressus Sempervirens L.)). Protocols for Micropropagation of Woody Trees and Fruits ,P. 93–105.

- Haghgoo.R, Mehran.M, Afshari.E, Zadeh.H.F, Ahmadvand.M (2017).(Antibacterial Effects of Different Concentrations of Althaea officinalis Root Extract versus 0.2% Chlorhexidine and Penicillin on Streptococcus mutans and Lactobacillus (In vitro)) J. Int. Soc. Prev. Community Dent. Vol.(7), P. 180-185.
- Haq.I. (2004).(Safety of medicinal plants). Pakistan J. Med. Res. Vol.(43) No.4.
- Hassan.B (2012).(Medicinal Plants (Importance and Uses)) Pharmaceut Anal Acta. Vol.(3), Issue 10.
- Hay.E.M, Paterson.S.M, Lewis.M, Hosie.G and Croft.P (1999).(Pragmatic randomised controlled trial of local corticosteroid injection and naproxen for treatment of lateral epicondylitis of elbow in primary care) BMJ.Clinical Research Vol. (319), P. 964-968.
- Ismail.A, Hamrouni.L, Hanana.M and Gargouri.S. (2013).(Chemical composition, bio-herbicidal and antifungal activities of essential oils isolated from Tunisian common cypress (Cupressus sempervirens L.). Journal of medicinal plant research Vol.(7), P. 1070-1080.
- Jamous.R.M, Abu Zaitoun.S.Y, Husein.A.I , Qasem.I.P.Y and Shtayeh.M.S.A (2015).(Screening for Biological Activities of Medicinal Plants Used in Traditional Arabic Palestinian Herbal Medicine). European Journal of Medicinal Plants Vol. 9(1): 1-13, P. 1-13.
- Jouda.M.M, Elbashiti.T, Masad.A and Albayoumi.M (2016).(The Antibacterial effect of some Medicinal Plant extracts and their synergistic effect with Antibiotics). World Journal of Pharmacy and Pharmaceutical Science. Vol. (5), Issue 2. P. 23-33.
- Kirby.J, Keasling.J.K (2009).(Biosynthesis of Plant Isoprenoids: Perspectives for Microbial Engineering) Annu. Rev. of Plant Bio. Vol.60(1), P. 335-55.
- Koche.D, Shirsa.R and Kawale.M (2016).(An overerview of major classes of phytochemicals: their types and role in disease prevevtion) Hislopia J. Vol. 9 (1/2), ISSN:0976-2124.
- Kondratyuk.T.P and Pezzuto.J.M (2004).(Natural Product Polyphenols of Relevance to Human Health) Pharmaceutical Biology. Vol. (42), P. 46-63.

- Koriem.K. (2009).(Lead toxicity and the protective role of Cupressus sempervirens seeds growing in Egypt). National research center. Vol.(37), P.231-242
- Leandri.C and Fernadez.X Cuvelier.L and Loiseau.A. (2003).(Chemical Composition of Cypress Essential Oils: Volatile Constituents of Leaf Oils from Seven Cultivated Cupressus Species). Journal of Essential Oil Research Vol. 15(4), P. 242-247.
- Lewington.A. (1993).(A review of the importation of medicinal plants and plant extracts into Europe). Cambridge, UK, Traffic International. P. 36-37.
- Lohania.H, Bhandaria.U , Gwaria.G , Haidera.S.Z and Chauhan.N.K (2015).(Constituents of essential oils of Cupressus arizonica Greene from Uttarakhand Himalaya (India)) J.of Essential Oil Research. Vol. (27).5, P. 459-463.
- Loizzo.MR, Tundis.R, Menichini.F, Saab.AM and Statti.GA (2008).(Antiproliferative effects of essential oils and their major constituents in human renal adenocarcinoma and amelanotic melanoma cells). Cell Prolif. Vol.41(6), P. 1002-1012.
- Lu.J, Bao.J.B, Chen.X.P, Huang.M and Wang.Y.T (2012).(Alkaloids Isolated from Natural Herbs as the Anticancer Agents) Vol.(2012), 12 pages.
- Mandal.S.M, Chakraborty.D, Dey.S (2010).(Phenolic acids act as signaling molecules in plant-microbe symbioses) Plant Signal Behav . Vol.5(4), P. 359-68.
- Mascolo.N, Autore.G, Capasso.F, Menghini.A,Fasulo.MP. (1987).(Biological screening of Italian medicinal plants for anti-inflammatory activity). Phytother Res 1987,Vol. 1(1), P. 28-31.
- Masoud.E.A and Gouda.H.A (2012).(Effect of some natural plant extracts against gram-negative bacteria in Njran Area, Saudi Arabia)Egypt. Acad. J. Biolog. Sci., Vol.4(1), P. 85-92.
- Mazari.K, Bendimerad.N, Bekhechi.C and Fernande.X. (2010).(Chemical composition and antimicrobial activity of essential oils isolated from Algerian Juniperus phoenicea L. and Cupressus sempervirens L). J.Med.Plants Res. Vol.4(10), P. 959-964.

- Oliveira.J, Reygaert.WC. (2022).(Gram-negative Bacteria) StatPearls [Internet]. Treasure Island (FL) PMID: 30855801.
- Panawala.L (2017).(Difference Between Gram-positive and Gram-negative Bacteria) pediaa.com.P.1-13.
- Panche.A.N, Diwan.A.D and Chandra.S.R (2016).(Flavonoids: An overview) J. of Nutritional Science, Vol. (5), P.1-15.
- Patel.H.M, Noolvi.M.N, Sharma.P, Jaiswal.V, Bansal.S, Lohan.S, Kumar.S.S, Abbot.V, Dhiman.S and Bhardwaj.V (2014).(Quantitative structure–activity relationship (QSAR) studies as strategic approach in drug discovery) Med. Che. Res. Vol. (23), P.4991-5007.
- Perez-Nueno.V (2016).(Towards the integration of Quantitative and Pharmacology into Drug Discovery: a systems level understanding of therapeutic and toxic effects of drugs) Current Pharmaceutical Design, Vol. (23) 22, P.6881-6884.
- Pichette.A, Larouche.P.L, Lebrun.M, Legault.J (2006).(Composition and antibacterial activity of Abies balsamea essential oil) Phytotherapy Research Vol. (20), P.371-373.
- Prestinaci.F, Pezzotti.P and Pantosti.A (2015).(Antimicrobial resistance: a global multifaceted phenomenon) Pathog. Glob Health.Vol. 109(7), P.309-18.
- Qaralleh.H, Khleifat.K, Alhroob.A and Alsaudi.A. (2021).(Chemical Composition, Antioxidant and Inhibitory Effect of Cupressus sempervirens Essential Oils and Methanolic Extract on Beta-lactamase producing Isolates). Research J. Pharm. and Tech.Vol. 14(9), P.4673-4679.
- Rasul.M.G (2018).(Extraction, Isolation and Characterization of Natural Products from Medicinal Plants) IJBSAC. Vol.(2), P.2394-367.
- Rawat.P, Khan.M.F, Kumar.M, Tamarkar.A.K, Srivastava.A.K, Arya.K.R, Maurya.R. (2010).(Constituents from fruits of Cupressus sempervirens.). Fitoterapia. Vol. (81), P.162-166.
- Robbins.R.J (2003).(Phenolic Acids in Foods: An Overview of Analytical Methodology) J. Agric. Food Chem. Vpl. (51), P. 2866-2887.
- Saboon, Arshad.S, Amjad.M.S and Akhtar.M.S (2019).(Natural Compounds Extracted from Medicinal Plants and Their Applications) Natural Bio-active Compounds. P.193-207.

- Sandhya.B, Thomas.S, Isabel.W and Shenbagarathai.R (2006).(Ethnomedicinal plants used by the Valaiyan community of Piranmalai Hills (Reserved Forest), Tamil Nadu, India. A pilot study). Afr. J. Traditional Complementary Altern, Med. Vol. 3(1), P.101-114.
- Schofield .P, Mbugua.D and Pell.A.N (2001).(Analysis of condensed tannins: A review) Animal Feed Science and Technology Vol. 91(1), P.21-40.
- Sebaugh.J.L (2010).(Guidelines for accurate EC50/IC50 estimation) Pharmaceutical Statist. 10.1002. pst.426
- Selim.S, Adam.M, Hassan.S and Albalawi.A. (2014).(Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean cypress (Cupressus sempervirens L.)). BMC Complementary and Alternative Medicine Vol. 2(14), P.179.
- Shahidi.F and Tang Ho.C (2005).(Phenolics in Food and Natural Health Products: An Overview) In Phenolic Compounds in Foods and Natural Health Products. ACS Symposium Series. Vol.(909), P.1-8.
- Sherman.W.P, Billing.J (1999).(Darwinian Gastronomy: Why We Use Spices: Spices taste good because they are good for us). Bio. Science, Vol. 49(6), P.453-463.
- Silva.F and Abraham.A. (1981).(The potentiality of the Israeli flora for medicinal purposes). Fitoterapia.Vol. (52), P.195-200.
- Singh.R (2015).(Traditionally used Medicinal Plants as Alternative Source for Future Anticancer Drugs).The International Journal Of Science & Technoledge. Vol. 3(9), P.111.
- Soto-Vaca.A, Gutierrez.A, Losso.J.N, Xu.Z, and Finley.J.W (2012).(Evolution of Phenolic Compounds from Color and Flavor Problems to Health Benefits) J. Agric. Food Chem. Vol. (60), P.6658-6677.
- Srivastava.K.C, Bordia.A, Verma.S.K (1995).(Curcumin, a major component of food spice turmeric (Curcuma longa) inhibits aggregation and alters eicosanoid metabolism in human blood platelets) Prostaglandins Leukot Essent Fatty Acids .Vol. 52(4), P.223-7.
- Tanne.J (2006).(Paracetamol causes most liver failure in UK and US) BMJ Vol.332(7542), P.628.

- Verma.S and SinghS.P. (2008).(Current and future status of herbal medicine). Veterinary World Vol. 2(2), P.347.
- Verma.V, Sharma.V, Singh.V, Kumar.R, Khan.M.F, Singh.A.K, Sharma.R, Arya.K.R, Maikhuri.J.P, Dalela.D, Maurya.R and Gupta.G (2014).(Labda-8 (17),12,14-trien-19-oic acid contained in fruits of Cupressus sempervirens suppresses benign prostatic hyperplasia in rat and in vitro human models through inhibition of androgen and STAT-3 signaling).Phytother Res. Vol. 28(8), P.1196-1203.
- Wadhwani.T, Desai.K, Patel.D, Lawani.D, Bahaley.P and Joshi.P (2009).(Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials) The Inter. J. Micro.. Vol. 7, N.1.
- Walton.N.J, Mayer.M.J and Narbad.A (2003).(Molecules of Interest: Vanillin) ChemInform Vol. 34(38).
- Wink.M and Schmeller.T (1998).(Modes of Action of Allelochemical Alkaloids: Interaction with Neuroreceptors, DNA, and Other Molecular Targets) J. Chemical Ecology Vol. 24(11), P.1881-1937.
- Zhang.J, Rahman.A, Jain.S and Jacob.M (2012).(Antimicrobial and antiparasitic abietane diterpenoids from Cupressus sempervirens). Research and Reports in Medicinal Chemistry. Vol. 2(2), P.1-6.
- Zhao.Y, Chen.R, Wang.Y, Yang.Y (2017).(α-Pinene Inhibits Human Prostate Cancer Growth in a Mouse Xenograft Model) Chemotherapy. Vol. 63(1), P.1-7.

النشاط المضاد للبكتيريا وللسرطان لمستخلصات ثمار السرو

اعداد: هبة متعب حسين الصلاح

اشراف: د. مهند قريع و د. محمود الخطيب

الملخص:

يعتبر السرو *C. sempervirens* L من النباتات الطبية الموجودة بشكل واسع في فلسطين، وهو يستخدم لعلاج العديد من المشاكل الصحية, حيث يتم استخدام الاوراق والثمار للحصول علي مستخلص السرو بالإضافة الي الحصول علي زيت السرو وهو احد الزيوت الأساسية. في هذا البحث تم جمع ثمار السرو من مدينة رام الله في فلسطين في شهر كانون الثاني 2021, وقد تم تجفيفها وطحنها, من ثم تم تحضير مستخلصات السرو بالايثانول والماء باستخدام عدة طرق وهي ال Sonicator و Soxhlet والغلي. تم تحليل مستخلص ثمار السرو الايثانولي باستخدام ال GC/MS وقد تم تحديد مركبين رئيسيين وهما δ-3-carene و α-pinene.

في هذا البحث تم الكشف عن فعالية مستخلصات ثمار السرو للعمل كمضادات بكتيرية ضد بكتيريا E.coli، *Staphylococcus aureus* والبكتيريا اللاهوائية سالبة غرام *Porphyromonas gingivalis*، باستخدام طريقة القرص وطريقة الحفر للبكتيريا *Porphyromonas gingivalis* وقد تم العثور علي تأثير المستخلص الايثانولي على البكتيريا موجبة غرام *Staphylococcus aureus* وعلى البكتيريا *Porphyromonas gingivalis* عند عدة تراكيز, حيث كان قطر منطقة التثبيط للبكتيريا *Staphylococcus aureus* عند أعلى تركيز (15 ملم)، وكان قطر منطقة التثبيط للبكتيريا *Porphyromonas gingivalis* (22 ملم) اما مستخلص السرو بالماء فلم نلاحظ له تأثير سوى على البكتيريا *Staphylococcus aureus* عند اعلى تركيز حيث كان قطر منطقة التثبيط (14 ملم)، أما بالنسبة للبكتيريا E .coli فلم يكن هناك أي تأثير لكلا المستخلصين.

وأيضا تم الكشف عن تأثير مستخلصات ثمار السرو كمضادات للسرطان ضد خلايا سرطان القولون HT29 وخلايا سرطان الثدي Mcf7. وقد لاحظنا تأثير المستخلص الايثانولي على نسبة الخلايا الحية لنوعي الخلايا السرطانية حيث كانت نسبة خلايا سرطان القولون HT29 الحية عند أعلى تركيز للمستخلص (% 25) وكانت نسبة خلايا سرطان الثدي Mcf7 الحية عند أعلى تركيز للمستخلص (% 19)، أما مستخلص السرو بالماء لم يكن له تأثير كبير على نوعي الخلايا السرطانية حيث كانت نسبة خلايا سرطان القولون HT29 الحية عند أعلى تركيز (% 94) وكانت نسبة خلايا سرطان الثدي Mcf7 الحية عند اعلى تركيز (% 96). توصي نتائج البحث بأهمية استخدام مستخلص السرو كبديل طبيعي للمواد الكيميائية لامتلاكه نشاط مضاد للبكتيريا ونشاط مضاد للسرطان.