**An-Najah National University**

**Faculty of Graduate Studies**

# Characterization of Bioactive Phytochemical Ingredients from Sarcopoterium Spinosum Plant as an Approach to Develop Natural-Based Drug Leads

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# Dedication

**To my parents for helping, taking care and praying for me.**

**To my husband's family which is my second family to help, care and pray for me.**

**To my husband Ameer Masri for his support, love, and encouragement.**

**To all who prayed for me.**

**To all whom I loved and knew.**

# Acknowledgments

**Praise to almighty Allah who guided my steps in all my work till the very end. I would like to express my gratitude.**

**Thanks for my supervisors, Prof. Mohammed Al-Nuri and Dr. Ibrahim Abu-Reidah, who always supported me with their knowledge and experiences. I would also like to thank Mr. Nafith Dweikat, for facilitating my work in the labs and helping me during research.**

**Specially thanks for my parents.**

**Thanks, are also due to my friends, particularly Ms. Anwar Afeef, who always has been my intimate friend. Finally, thanks to chemistry department at my University.**

## الإقرار

انا الموقعة ادناه، مقدم الرسالة التي تحمل العنوان:

**Characterization of Bioactive Phytochemical Ingredients from Sarcopoterium spinosum Plant as an Approach to Develop Natural-Based Drug Leads**

**توصيف المواد الكيميائية النباتية النشطة بيولوجياً من نبات البلان**

**كنهج لتطوير أدوية من أصل طبيعي (Sarcopoterium spinosum) الشوكي**

اقر بأن ما اشتملت عليه الرسالة انما هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة اليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل اي درجة او بحث علمي او بحثي لدى اي مؤسسة تعليمية او بحثية أخرى.

## Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work and has not been submitted elsewhere for any other degree or qualification.

**Student's Name:** **اسم الطالب:**

**Signature:** **التوقيع:**

**Date:** **التاريخ:**

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## List of Abbreviations

| Symbol | Abbreviation |
|---|---|
| **R.T** | retention time (Min) |
| **R.I** | retention index |
| **M/F** | molecular formula |
| **M.W** | molecular weight (g/mol), |
| **Ref.** | reference |

**Characterization of Bioactive Phytochemical Ingredients from Sarcopoterium Sspinosum Plant as an Approach to Develop Natural-Based Drug Leads**

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# Abstract

Three parts (seed, leave, root) from Sarcopoterium spinosum plant used in traditional medicine in Palatine were tested for their biological activity against four types of bacteria and one type of fungi {S. sonnie (ATCC 25931), S. aureus (ATCC 25923), Escherichia coli (E. coli), MARSA, and Candida albicans (C. albicans)(ATCC 90028) }respectively . They were collected from Tubas region. The family name of plant is Sarcopoterium spinosum (L) and is known as **natish** in Arabic. The ethanolic, hexane and distilled water extracts of those parts of the plants were tested for their antioxidant activity through DPPH assay the D.W from leave has got the highest percentage inhibition (89%).

Some constituents were detected from ethanolic and hexane plants extracts using GC-MS/MS spectrophotometer and separated by flash chromatography.

Also, some elements were detected from distilled water plant extracts using ICPMS analysis, these elements are Fe, Zn, and Sr with deferent percentage per extract part of plant.

# Chapter One

# Introduction

Since thousands of years, plants were used in traditional medicine in treating diseases **[1]**. Plant analysis has shown that there are 500,000 plant species in our world. Many species of plant have been found to contain biologically active ingredients. Which are used in treatment of many diseases **[2]**. Nowadays, a large number of different medicinal plants are available in markets and being sold as remedies in Folk medicine **[3]**.

Many plant products are being used as a cure for various types of disease without any side effects and with high biological activity. Such reasons attempted scientists to focus on the medicinal plants subject. Large numbers of papers have been published dealing with the biological activity of medicinal plant **[3, 4 and 6]**. Recently, many of methods for separation, identification, characterisation and determination of chemical content have been established. Furthermore, biological activity tests were made on plant extract and chemical constituent **[5]**.

## 1.2 Main chemical constituents in medicinal plant

According to chemical analysis, the plant extracts have shown to contain large number of chemical constituents found in the plant. Such constituents are having different functional groups **[7]**. Flavonoids and phenolic compounds and their derivatives are found in most of plants. They are considered very important chemical groups for both human and plants **[8]**.

They have many functions in plants. Flavonoids and phenolic acids also have antioxidative and anticarcinogenic effects **[9]**. Phenolic compounds present in plants as hydroxylated derivatives of benzoic and cinnamic acids **[10]**. They are important in the defense mechanisms of plants under different environmental stress conditions such as wounding, infection, and excessive light or UV irradiation **[11].**

The main two groups of phenolic compounds are listed below:

**1. Hydroxybenzoic acids:** They are derived from benzoic acid directly. Four acids are commonly: syringic acid, m-hydroxy benzoic salicylic acid, vanillic acid and protocatechuic acid as shown below figure (1.1) **[12]**.

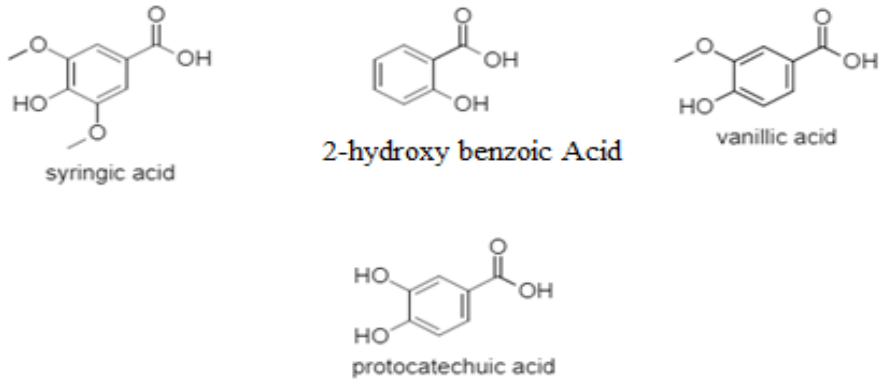


**Figure 1.1:** Common Hydroxybenzoic acids

**2. Hydroxycinnamic acids:** The four common are distributed widely in plants are p-coumaric acid, sinapinic acids, ferulic acid and caffeic acid shown in figure (1.2)**[ 13].**

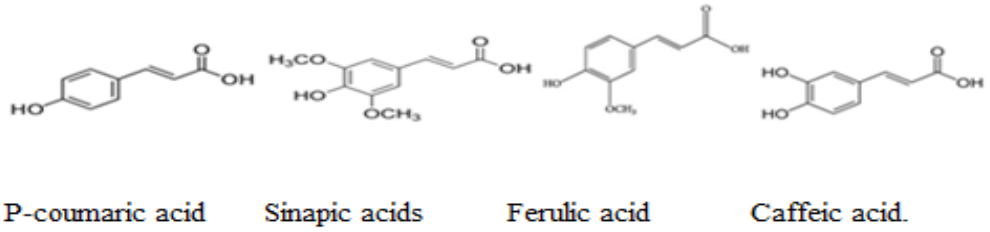


**Figure 1.2:** Structures of Hydroxycinnamic acids

## 1.3 Methods of isolation and quantitative determination

### 1.3.1 Extraction

Extraction is the next step in study of medicinal plants after collection of the plant. Several methods of extraction plant contents. There are many factors effects on extract like, pH of the medium of extract, stability of the effective constituent, and biological activity of the constituent of the plants and type of solvent either organic or aqueous.

Popular methods of extraction **[14]**:

A. Decoction: It is traditional extraction methods, in which the extract is prepared by placing the part of plant in cold water, then boiling for several minutes, after that the extract is filtered or decanted.

B. Soaking

The part of plant is soaked in organic or aqueous solvent for three days, and then the extract decanted and dried, and takes it for analysis.

### 1.3.2 Chromatographic types and techniques

A chromatographic technique was used to separate coloured pigments, and also; used to separate mixtures which have a high number of organic compounds. Chromatography is used to avoid any change of the structure of the contents in plant, so it is considered as physical method for separation**[15].**

Several types of chromatographic techniques were being used in our research for separation as thin layer chromatography (TLC), column chromatography (CC), HPLC, and GC **[16, 17, 18, 19, 20, 21, 22 and23].**

## 1.4 Importance of medicinal plants

In general, plants and medicinal herbs are used not only in the treatment of mild diseases, such as colds, flu, headache and other ailments, but also in the long-term treatment of chronic and incurable diseases, (diabetes, hypertension or cancer) **[24].** Most of plants contain large and different ingredients which have anticancer, antibacterial, and antifungal activities. Others have antioxidant properties **[25, 26]**.

## 1.5 Medicinal plants in Palestine

In Palestine, the screens of plants for active constituents begun in late of last century **[27].** Sarcopoterium spinosum plant was used in this study **[28]**.

Sarcopoterium spinosum is a genus of flowering plants in the rose family. Its length between 30 to 40 cm, and its presence in the Mediterranean region.

The active material is called Sarcopoterium Spinosum **[29, 30]**. Sarcopoterium spinosum is a medicinal plant that has antioxidant activity, and it is abundant in Palestine mountains.S. Spinosum contains natural phenolic compounds in leaves and fruits which are known as anti-oxidant, which have valuable importance in pharmaceutical, food, and cosmetics industries **[31, 32].**

Sarcopoterium spinosum has been used as a traditional medicine for treatment of diabetes by Bedouins. Its roots have been used in the treatment of diabetes, toothaches, gastrointestinal problems and infections **[33]**.

Since ancient times the use of many medicinal plants has been used as a substitute for industrial medicines **[34]**.

### 1.6 Biological Activities

Biological activity is related to constituents that make change in process of biological behavior of the tissue **[35].**

#### 1.6.1 Anti-microbial (antibacterial)

The microscope used to see microbes which are tiny microorganisms. These are existing in water, soil, air and plants which are spread and replicate rapidly. These of microbial organisms like viruses, fungi and bacteria **[36]**. Medicinal plants are rich with antimicrobial agents. These plants in many countries are being used as remedies for curing many ailments medicinally **[37, 38].** Large number of parts of medicinal plants are extracted and used in

drugs synthesis. These parts include flowers, stems, roots, fruits and leaves **[39].** Antimicrobial drugs make inhibition for the microbe without any side effects **[40]**.

### 1.6.2 Antifungal activities

Medicine of antifungal makes selectivity to reduce fungal pathogens. Unlike diseases of bacteria, diseases of fungi are more difficult to treat. Often oral and topical treatments are long term and may be partially successful in controlling the fungus. Fungal infections are the most spread on skin of all mycoses **[41, 42].**

### 1.6.3 Anti-oxidants

Anti-oxidant "free radical scavengers" are substances that may delay or sometimes prevent types of cell damage by blocking the activity and reacting with free radicals and preventing them from causing the damage of scavengers so as to prevent or delay different diseased states. These free radicals are considered as highly reactive species which have an odd number of electrons, which gives them high potentials to cause damages to cells called cellular pathologies. Some of these damages may lead to cancer. In the biological system, oxygen gives rise to a large number of free radicals and other reactive species collectively known as "reactive oxygen species" (ROS). "Reactive nitrogen species"(RNS) are another groups of reactive species that plays a dual role as both deleterious and beneficial species **[43, 44 and 45].** Two types of free radicals exist. One of them synthesized

naturally in the body. The second type is introduced through external sources to our bodies. Sources of radicals are exposure to the sun, and tobacco smoke. These sources make endogenous antioxidants, which neutralize free radical **[46]**. However, the body needs external sources of antioxidants sources like fruits and vegetables. The high potential of free radicals gives them high reactivity which harms the cell. They are created when a molecule or an atom either loses or gains an electron **[47]**.

## 1.7 Aims of study

The main objectives of this study are the followings:

1- Identification and characterization of bioactive phytochemical compound from Sarcopoterium spinosum plant.

2- Evaluate the biological activity (antifungal and antibacterial activities) and anti-oxidant for Sarcopoterium spinosum plant extract of different part of it in Palestine.

# Chapter Two

# Experimental Part

All chemicals were purchased from Sigma-Aldrich Chemical Company and used without further purifications. The antibacterial activity of the extracts was determined against the following microorganisms; Escherichia coli, Shigella, MRSA, S. aureus. On the other hand, the antifungal activity test (Yeast) was done against Candida.

## 2.1 Collection of plants

The leaves, fresh fruits, and roots of the plants were obtained during April 2019 from Tubas.

The leaves, fresh fruits, and root were placed at room temperature in a shaded area away from direct sunlight, well ventilated until is completely dry.

## 2.2 Extraction

The dry leaves, roots, and fruits were grinded partially and soaked in hexane for 5 days at room temperature.

Then, we got the hexane extract by suction filtration. The solvent was removed by evaporation (rotary evaporator) to collect the hexane extract (crude product) at 35$^{o}$C, and then the chosen part of the Sarcopoterium spinosum plant was soaked in ethanol for 5 days then finally the Sarcopoterium spinosum plant was soaked in water for 5 days. And solvents were removed by suction filtration and water was removed by freeze drying for 3 days.

After the extracts were dried, each extract of the plant (leaves, fresh fruits, roots) was chemically and biologically tested.

**GC-MS/MS Analysis**

**2.3 Sample preparation00 for GC-ANALYSIS**

2 mg of Organic Sarcopoterium spinosum plant extracts (ethanol and hexane extracts) were dissolved in 2ml of acetonitrile.

**2.4 GC-MS/MS techniques**

Gas chromatography-mass spectrometry (GC-MS) is an important technique for qualitative and quantitative analysis for plant extract. It is fast and sensitive, provides a high peak capacity and allows determination of thermally stable and volatile compounds **[48]**.

It is used for the separation of a mixture that contains a large number of organic compounds. Chromatography is a physical method that helps to avoid any reaction that may change the structure of the original compounds in the plant **[49]**.

**2.5 GC-MS/MS Apparatus and Conditions**

Instrument conditions: Fat (Total, Saturated, and Monounsaturated) in Food AOAC996.06.

The Clarus 500GC MS used in the analysis employed a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30 m × 0.25 mm ID × 0.25µm df) and the components were separated using Helium as carrier gas

at a constant flow of 1.1ml/min. The µL sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 62.5 minute GC extraction process, the oven was maintained at a temperature of 50$^{o}$C with 5 minutes holding. The injector temperature was set at 250$^{o}$C (mass analyzer).

The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200$^{o}$C; Source temperature: 200$^{0}$C). Mass spectra were taken at 4 min; a scan interval of 0.2 s and fragments from 50 to 500 Da.

**Figure 2.1:** GC-MS apparatus used for analysis

## 2.6 Results and Discussion for GC-ANALYSIS

In GC-MS analysis carried out, the compounds were identified from the flow rate of hexane extract of Sarcopoterium spinosum plant as shown in **figure 2.2, 2.3** respectively in page (11and12).

There are similar compounds in the hexane seed extract but with a different percentage. The presence of these compounds in the seed is caused this part of Sarcopoterium spinosum plant is highly effective when the biological activities and antioxidants have been examined.

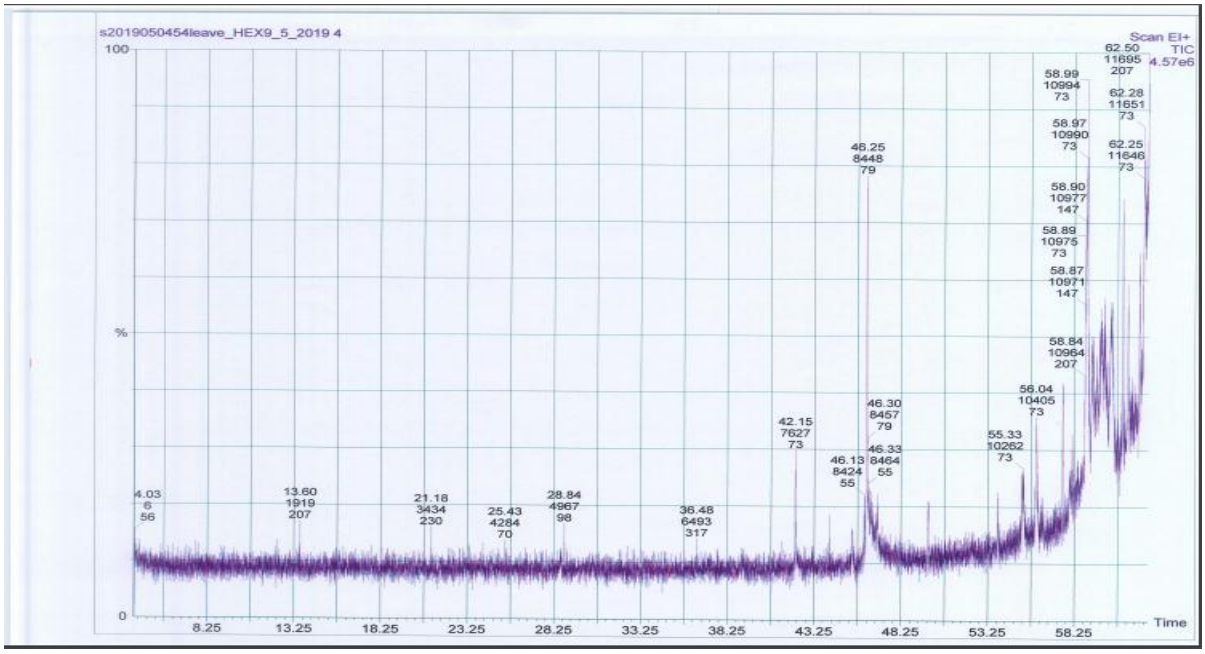


**Fig.2.2:** GC-MS Chromatogram of the leaf hexane extract of Sarcopoterium spinosum plant.

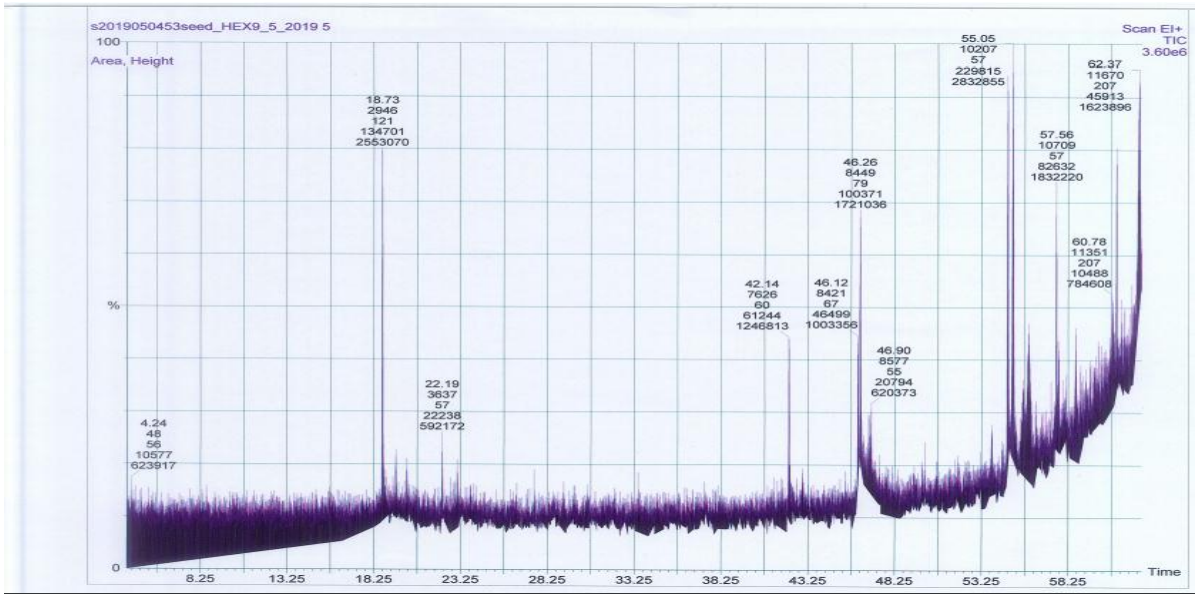


**Fig.2.3:** GC-MS Chromatogram of the seed hexane extract of Sarcopoterium spinosum plant.

**Table 2.1: The compound isolated from GC-MS/MS analysis of leaf and Seed hexane Sarcopoterium spinosum extract respectively.**

| Extract Type | R.T | R.I | Compound Name | High | Area | %High | Area% | M/F | M.W (g/mol) | Biological Study |
|---|---|---|---|---|---|---|---|---|---|---|
| Leaf Extract | 42.15 | 517 | Pentadecanoic acid, 15-bromo- | 53350 | 1041254 | 22.63 | 24.85 | $C_{15}H_{29}BrO_2$ | 321.23 | Anti-oxidant [50] |
| | 46.25 | 82.3 | Methyl 11, 14, 17-eicosatrienoate | 182440 | 3149272 | 77.36 | 75.15 | $C_{21}H_{36}O_2$ | 320.5 | Antimicrobial, antioxidant, antidiabetic [51] |
| | 18.73 | 798 | 1–Formyl-2,2,6-trimethyl-3-cis-(3methylbut-2- | 134701 | 2553070 | 15.81 | 19.97 | $C_{15}H_{24}O$ | 220.33 | Anti-oxidant [52] |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | enyl)-5-cyclohexene | | | | | | | |
| Seed Hexane | 46.13 | 695 | (E)-9,11 – dodecadien-1-ol | 46499 | 1003356 | 5.56 | 7.85 | $C_{12}H_{22}O$ | 182.30 | Antimicrobial activity [53] |
| | 46.26 | 759 | Methyl 8,11,14-heptadecatrienoate | 100371 | 1721036 | 11.78 | 13.46 | $C_{18}H_{30}O_2$ | 278.44 | Antimicrobial activity [54] |
| | 54.76 | 860 | Hentriacontane (isomer) | 178437 | 2677000 | 20.94 | 20.94 | $C_{31}H_{64}$ | 436.85 | anti-inflammatory [55] |
| | 55.05 | 790 | Hentriacontane (isomer) | 229815 | 2832855 | 26.97 | 22.70 | $C_{31}H_{64}$ | 436.85 | anti-inflammatory [55] |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 57.56 | 603 | Sulfurous acid, Octadecyl 2-propyl ester | 82632 | 1832220 | 9.70 | 14.33 | $C_{21}H_{44}$ | 376.64 | antibacterial activity [56] |
| Seed | 57.56 | 600 | Sulfurous acid, 2-propyl tetradecyl ester | 82632 | 1832220 | 9.70 | 14.33 | $C_{17}H_{36}O_{35}$ | 320.53 | antibacterial activity [56] |
| Hexane | 61.04 | 821 | Hentriacontane | 79677 | 1670855 | 9.35 | 1.31 | $C_{31}H_{64}$ | 436.85 | anti-inflammatory [55] |
| Seed Hexane | | | | | | | | | | |

**R.T: retention time (Min), R.I: retention index, M/F: molecular formula, M.W: molecular weight (g/mol), Ref: reference.**

In the GC-MS chromatogram of leaf hexane extract, two compounds were identified, but in seed hexane, eight compounds were identified. As shown in **Table 2.1**.

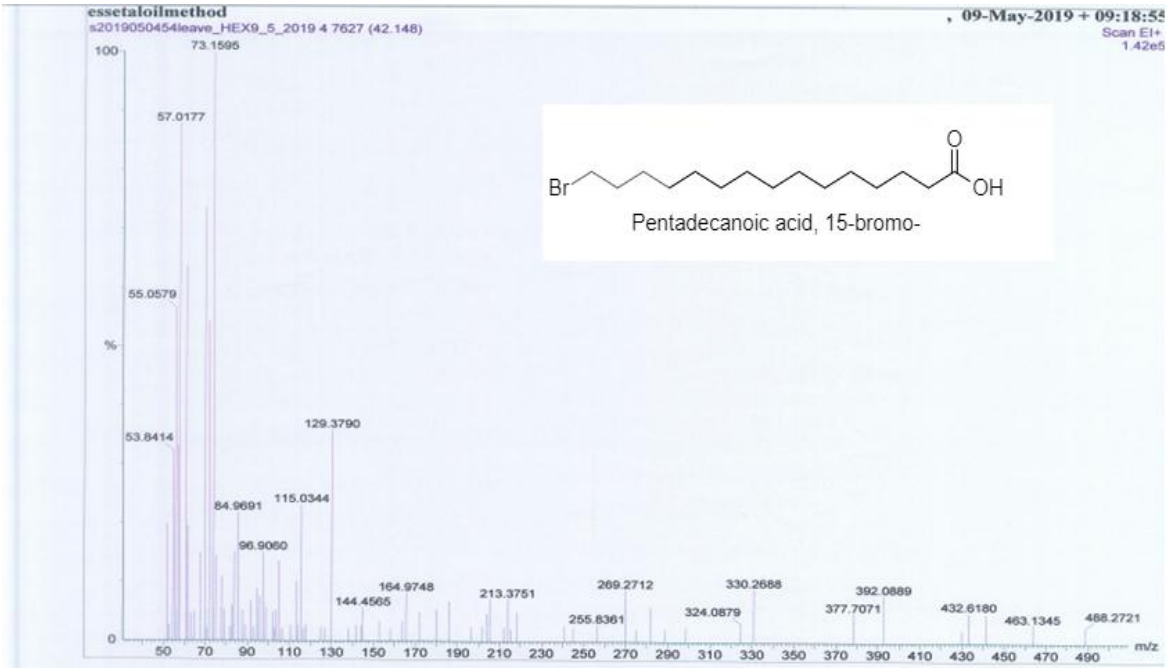


**Fig.2.4:** GC-MS Chromatogram of the Pentadecanoic acid, 15-bromo- compound from leaf hexane extract of Sarcopoterium spinosum plant at 42.15 minute.

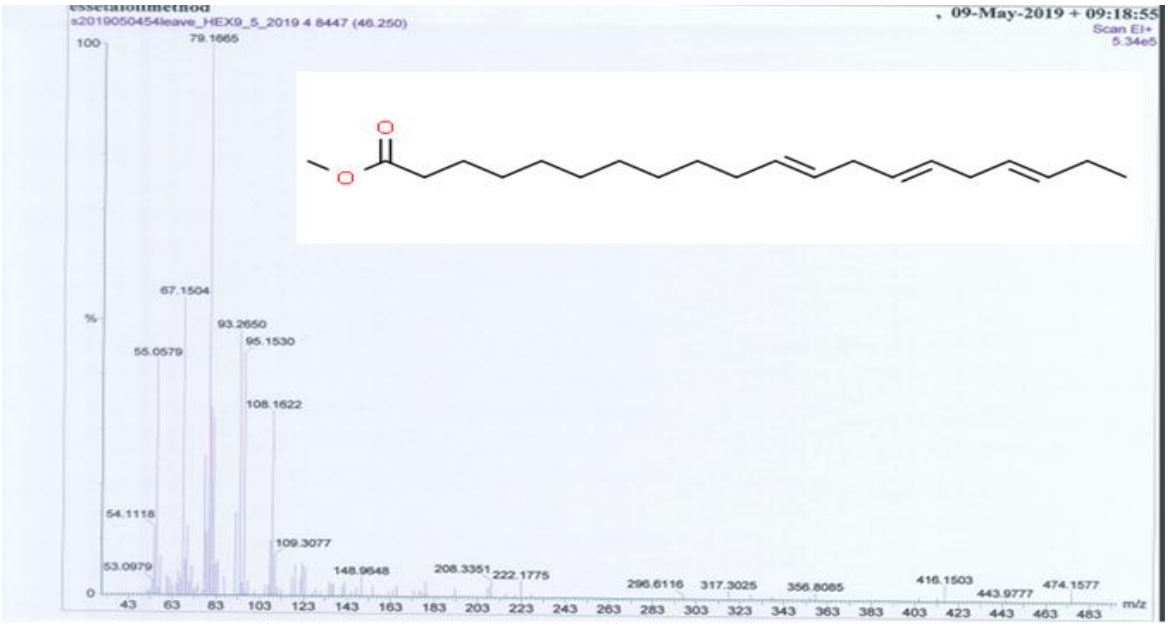


**Fig.2.5:** GC-MS Chromatogram of the Methyl 11, 14, 17-eicosatrienoate compound from leaf hexane extract of Sarcopoterium spinosum plant at 46.25 minute.

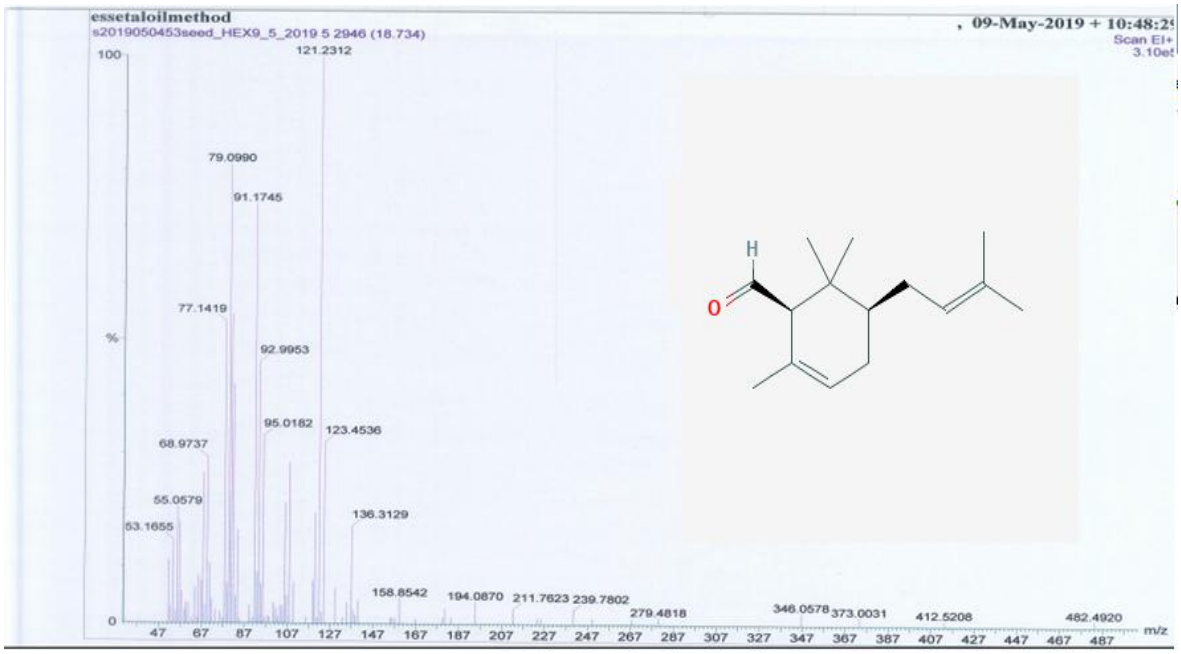


**Fig.2.6:** GC-MS Chromatogram of the 1 –Formyl-2, 2, 6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclohexene compound from seed hexane extract of Sarcopoterium spinosum plant at 18.73 minute.

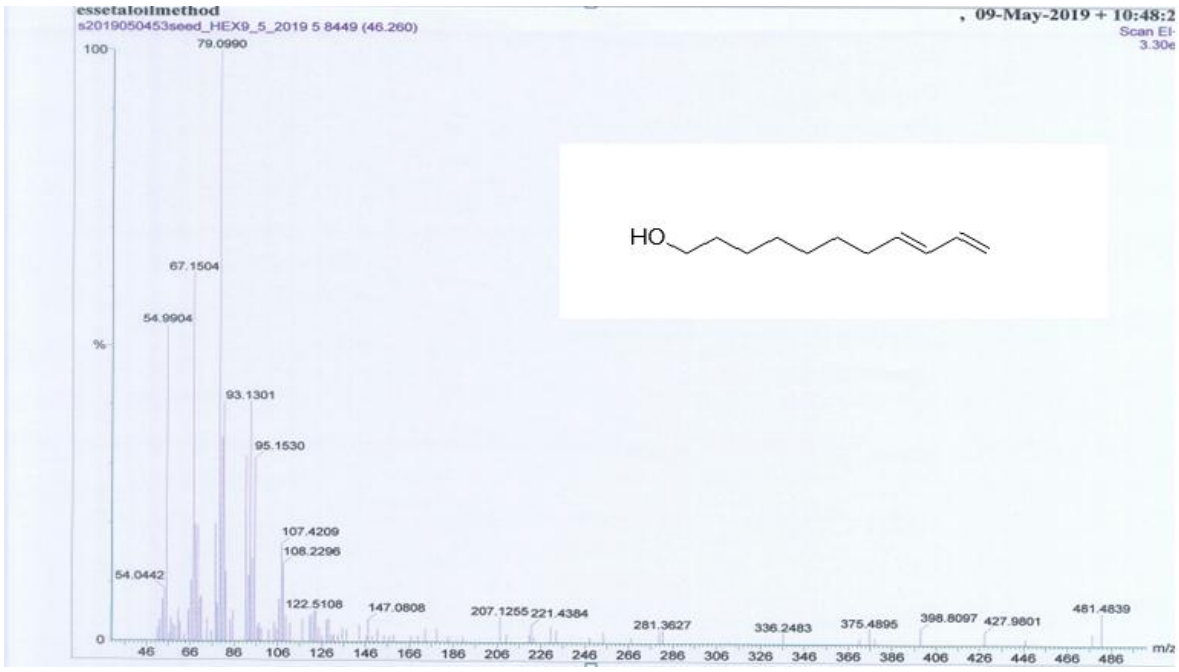


**Fig.2.7:** GC-MS Chromatogram of the (E)-9, 11 –dodecadien-1-ol compound from seed hexane extract of Sarcopoterium spinosum plant at 46.13 minute.

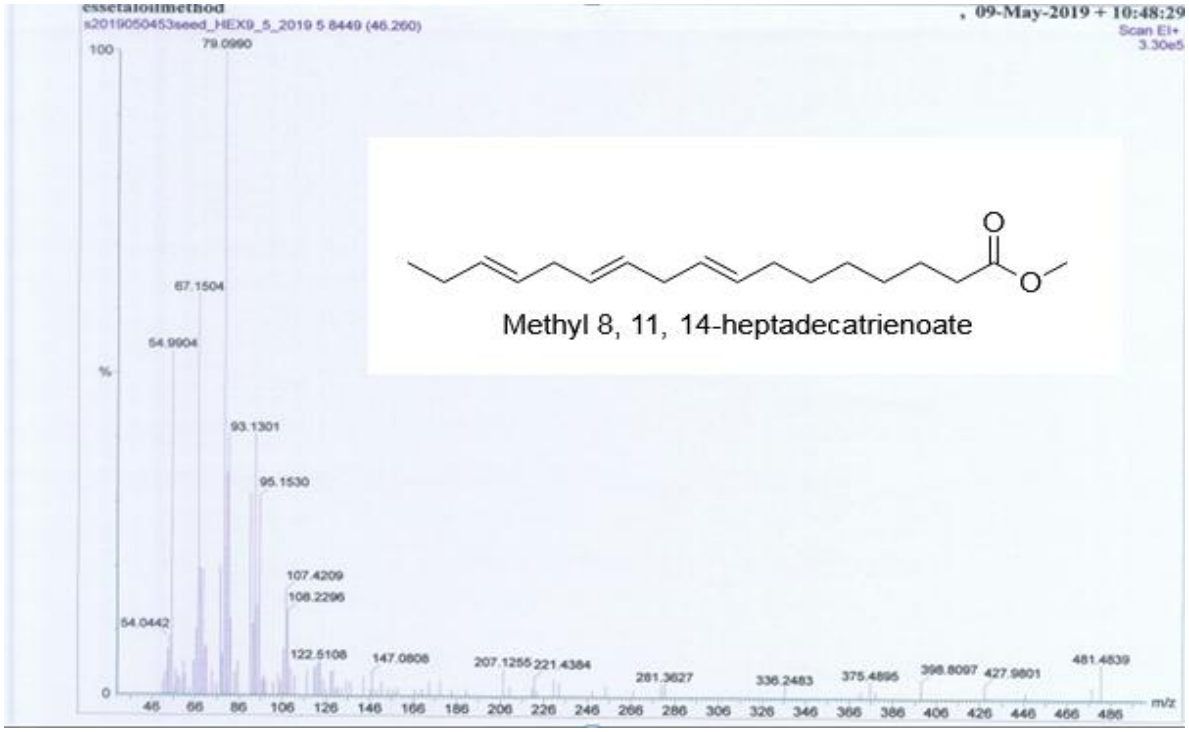


**Fig.2.8:** GC-MS Chromatogram of the Methyl 8, 11, 14-heptadecatrienoate compound from seed hexane extract of Sarcopoterium spinosum plant at 46.26 minute.

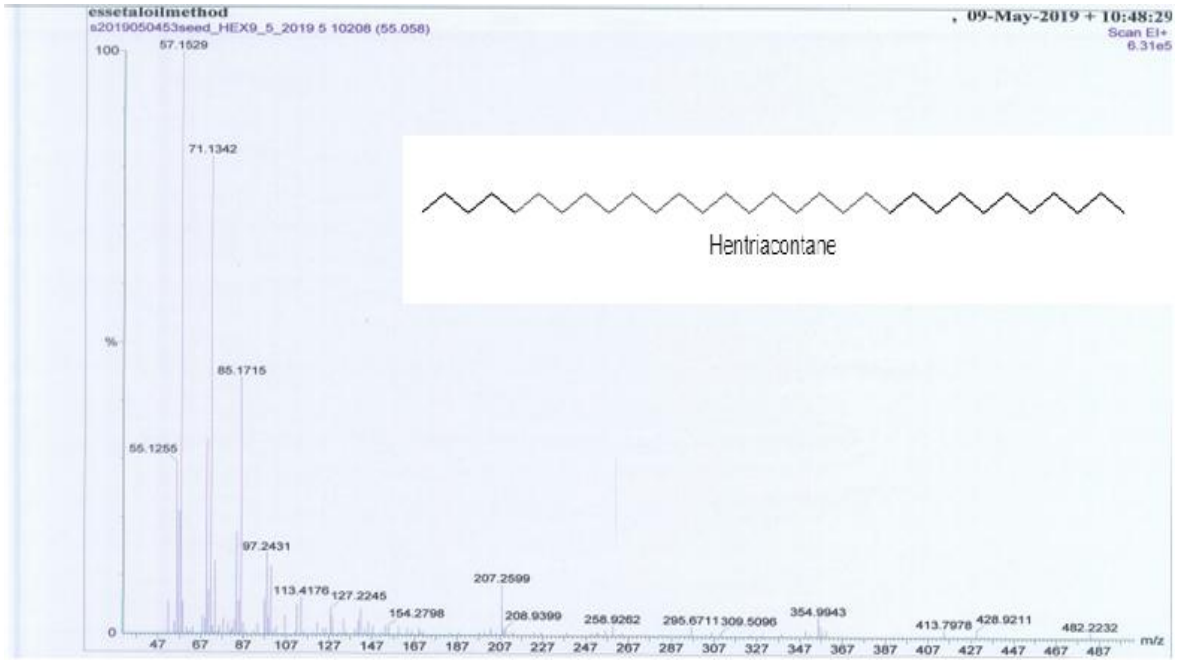


**Fig.2.9:** GC-MS Chromatogram of the Hentriacontane compound from seed hexane extract of Sarcopoterium spinosum plant at 61.04, 54.67 and 55.05 minute.

The compound Hentriacontane is found three times at different retention times in seed hexane. This compound has anti-inflammatory effect **[56].**

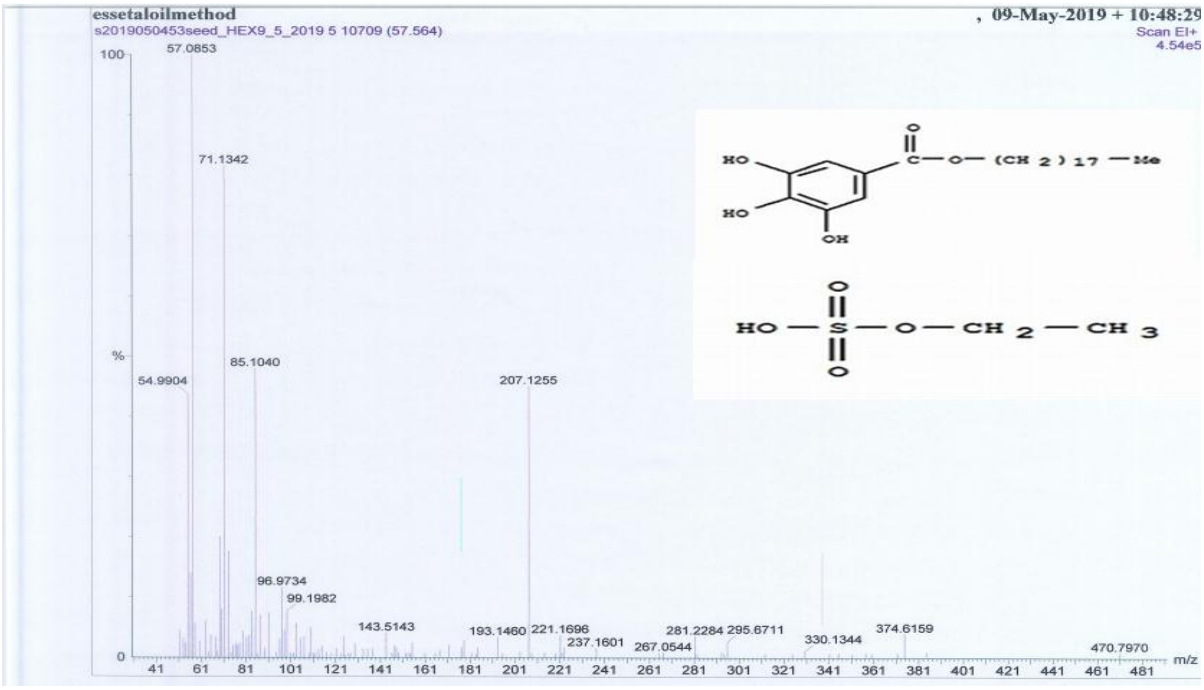


**Fig.2.10** GC-MS Chromatogram of the Sulfurous acid, Octadecyl 2-propyl ester and Sulfurous acid, 2-propyl tetradecyl ester compound from seed hexane extract of Sarcopoterium spinosum plant at 57.56

## ICPMS Analysis

## 2.7 Sample preparation for ICPMS-Analysis

50mg of aqueous extract plant (Distilled water) were dissolved in 50 ml deionized distilled water.

## 2.8 ICPMS Techniques

Metals analysis by ICP-MS is used to measure the presence and quantity of minute.

various elements present in a sample. Samples are decomposed to neutral elements in high-temperature argon plasma and analyzed based on their mass to charge ratios using a mass spectrometer system.

Most elements can be analyzed at the parts-per-million (ppm) to parts per-trillion (PPT) concentration levels. ICP-MS is also capable of monitoring isotopic specification for the ions of choice.

## 2.9 Results and Discussion for - ICPMS ANALYSIS

As shown in **Table 2.1** some elements had large concentration in leave D.W extract Sarcopoteriumspinosum like Fe, Al, Ba-1, and Sr (324, 50.1, 31.2, and 29.6ppb) respectively.

But in roots D.W extract the metals Fe, Mn, Al, and Zn (255.7, 55.1, 37.9and 31.6 ppb) respectively. As shown in **Table 2.2**.

But in fruit D.W extract the metals Fe, Zn and Mn (164.7, 66.9 and 31.3 ppb) respectively. As shown in **Table 2.3**.

**Table 2.2: The concentration results of leave D.W extract from the ICPMS analysis.**

| Extract type | Metal | Conc. (ppb) | Dilution factor |
|---|---|---|---|
| Leave D.W | Ag | 0.244 | 244 |
| | Al | 50.156 | 50156 |
| | Ba | 31.291 | 31291 |
| | Sr | 29.667 | 29667 |
| | Bi | 0.022 | 22 |
| | Cd | 0.257 | 257 |
| | Cr | 8.513 | 8513 |
| | Co | 0.566 | 566 |
| | Cs | 0.015 | 15 |
| | Cu | 8.439 | 8439 |
| | Fe | 324.672 | 324672 |
| | Ga | 0.259 | 259 |
| | V | 0.259 | 259 |
| | Li | 0.403 | 403 |
| | Mn | 40.032 | 40032 |
| | Mo | 1.095 | 1095 |
| | Ni | 7.037 | 7037 |
| | Pb | 1.449 | 1449 |
| | Rb | 9.145 | 9145 |

**D.W:** distilled water, Conc.: Concentration (ppb).

In leave D.W extract (Fe, Al, and Mn) is the highest concentration.

**Table 2.3: The concentration results of Root D.W extract from the ICPMS analysis.**

| Extract type | Metal | Conc. (ppb) | Dilution factor |
|---|---|---|---|
| Root D.W | Ag | 0.084 | 84 |
| | Al | 37.979 | 37979 |
| | Ba | 22.326 | 22326 |
| | Cd | 0.050 | 50 |
| | Cr | 7.767 | 7767 |
| | Co | 1.645 | 1645 |
| | Cu | 13.379 | 13379 |
| | Fe | 255.702 | 255702 |
| | Ga | 0.20 | 200 |
| | Li | 0.299 | 299 |
| | Mn | 55.128 | 55128 |
| | Mo | 1.420 | 1420 |
| | Ni | 6.985 | 6985 |
| | Pb | 0.902 | 902 |
| | Rb | 8.444 | 8444 |
| | Sr | 29.450 | 29450 |
| | V | 0.478 | 478 |
| | Zn | 31.606 | 31606 |

***D.W: distilled water, Conc. : Concentration (ppb).**

In root D.W extract (Fe and Mn) is the highest concentration.

**Table 2.4: The concentration results of Fruits D.W extract from the ICPMS analysis.**

| Extract type | Metal | Conc. (ppb) | Dilution factor |
|---|---|---|---|
| Fruit D.W | Ag | 0.248 | 248 |
| | Al | 24.612 | 24612 |
| | Ba | 11.461 | 11461 |
| | Cd | 0.063 | 63 |
| | Cr | 6.715 | 6715 |
| | Co | 0.326 | 326 |
| | Cu | 9.237 | 9237 |
| | Fe | 164.724 | 164724 |
| | Ga | 0.117 | 117 |
| | Li | 0.176 | 176 |
| | Mn | 31.379 | 31379 |
| | Mo | 0.884 | 884 |
| | Ni | 4.437 | 4437 |
| | Pb | 0.976 | 976 |
| | Rb | 12.179 | 12179 |
| | Sr | 0.045 | 45 |
| | V | 0.045 | 45 |
| | Zn | 66.979 | 66979 |

***D.W: distilled water, Conc. : Concentration (ppb).**

In fruit D.W extract (Fe, Al, and Mn) are the highest concentration.

# Chapter Three

# Biological activities

## 3.1 Antimicrobial activity tests

### 3.1.1 Preparation of samples for testing

Organic plant extracts (ethanol and hexane extracts) were dissolved in Sterile 10 %( DMSO) Dimethyl sulfoxide to make the concentration of 100 mg/ml. While aqueous extracts were dissolved in sterile (D.W) distilled water to make the concentration 100mg/ml. These dissolved extracts were stored at 4°C for other assays.

### 3.1.2 Media and Solutions

#### 3.1.2.1. Nutrient Broth

Nutrient broth (ACUMEDIA) was prepared according to instructions labeled on the bottle. In a 0.5 L bottle, 250 ml deionized water was mixed and then boiled with 2 g of nutrient broth medium to dissolve. The broth was distributed to tubes to have 5-10 ml on each and plugged with cotton. These tubes were autoclaved for 15 min at 121°C, cooled and refrigerated.

#### 3.1.2.2. Mueller-Hinton Broth

Mueller-Hinton broth (HI Media Laboratories) was prepared according to instructions labeled on the bottle. In a 0.5 L bottle, 250 ml deionized water was mixed and then boiled with 5.25 g of Mueller-Hinton broth medium to dissolve. The broth was distributed to tubes to have 5-10 ml in each then

plugged with cotton. These tubes were autoclaved for 15 min at 121°C, then allowed to cool and kept in refrigerator at 4-6°C.

### 3.1.2.3. Meullar Hinton Agar (MHA)

Meullar Hinton agar (BD) was prepared according to instructions that labeled on the bottle. In a bottle (1 L), 0.5 L of the deionized water was mixed with medium (19 g), stirred and heated until the agar dissolved. The solution boiled for 1 minute, then autoclaved at for 15 minutes 121°C. Then it was allowed to cool to about 55°C, and then the media was poured into Petri dishes which sterile to have (25-30) ml in each, the plates were left overnight at the room temperature. After 24 hours the Petri dishes were turned upside down and kept in refrigerator at 4-6°C.

### 3.1.2.4 Sabouraud Dextrose Agar

Sabouraud dextrose agar (Hi Media Laboratories) was prepared according to instructions that labeled on the bottle. In a bottle (0.5 L), deionized water (0.25 L) was mixed with Sabouraud dextrose agar (16.25 g), then stirred and heated to dissolve the medium. Then the solution boiled for 1 minute, and then put into autoclaved for 15 minutes at 121°C. After that it was cooled to about 55°C, and then the medium was poured to sterile Petri dishes to have (25-30) ml in each, then the plates were left at room temperature overnight **[57]**. Next day morning the Petri dishes were turned upside down and kept in refrigerator at 4-6°C.

### 3.1.2.5. Normal Saline (0.9% NaCl)

Normal saline solution (0.9% NaCl, MWt 58.44) was prepared in the bottle (0.5 L); deionized water (250 ml) was mixed with NaCl (2.25 g) to dissolve. The saline solution was distributed into tubes have to 5-10 ml in each and then plugged with cotton. The tubes were autoclaved for 15 min at 121°C, then allowed to cool and kept in refrigerator at 4-6°C.

### 3.1.2.6. Preparation of McFarland turbidity standard No. 0.5

McFarland 0.5 turbidity standard was prepared by mixing 50 µl of a 1.175% (w/v) barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$) solution with 9.95 ml of 1% (v/v) sulfuric acid. The tube had the 0.5 McFarland standards was sealed in parafilm to stop evaporation and then stored at room temperature in the dark. The 0.5 McFarland standards were vigorously mixed on a vortex mixer before use. As with the barium sulfate standards, a 0.5 McFarland standard is comparable to a bacterial suspension of 1-5 x $10^8$ colony-forming units (CFU)/ml or 0.5 McFarland standard is comparable to Candida albicans suspension of 1-5 x $10^6$ yeast cells/mL **[58]**.

Three to four colonies from each bacteria were transferred into tubes had 5.0-10 mL of sterile normal saline; the turbidity of the bacterial suspensions was adjusted to have similar turbidity of 0.5 McFarland standards with bacterial suspension of about 1.5 x$10^8$ cfu/ml. Few colonies from C. albicans sub-cultured on Sabouraud Dextrose Agar were transferred into tube had 5.0-10

mL of sterile normal saline. The turbidity of the yeast C. albicans suspension of about 1-5 x $10^6$ yeast cells/ml**[58].**

### 3.1.3. Test Microorganisms

Microorganisms used in this study represent pathogenic species commonly associated with infections. The microorganisms were stored in the Microbiology research laboratory at An-Najah National University, Nablus-Palestine. These microorganisms consisted of 2 Gram-positive strains Staphylococcus aureus (S. aureus) (ATCC 25923), clinical isolate of methicillin resistant staphylococcus aureus (MRSA), two Gram-negative strains, Shigella sonnie (S. sonnie)(ATCC 25931) and multidrug clinical Escherichia coli (E. coli)isolate and one yeast strain Candida albicans (C. albicans) (ATCC 90028). All the bacterial strains were sub cultured on Mueller-Hinton agar while C. albicans was sub-cultured on Sabouraud Dextrose Agar **[59].**

### 3.1.4. Determination of Minimum Inhibitory concentration (MIC)

#### 3.1.4.1. Determination of Minimum Inhibitory concentration (MIC) against bacteria

Minimum Inhibitory concentration (MIC) of aqueous and organic plant extracts (ethanol and hexane) was calculated by the broth micro dilution method in sterile 96- wells microtiter plates according to standard method described previously by the Clinical and Laboratory Standards Institute [60].The organic and aqueous plant extracts were dissolved in 10% DMSO

which sterile and distilled water that sterile, respectively, to make a final concentration 100mg/ml. Both extracts water and organic 10% DMSO (negative control) were two fold-serially diluted in nutrient broth in the wells of the plates in a final volume of 100µL. After that, a bacterial inoculum size of $10^5$ CFU/ml was added to each well. Other negative control wells containing either 100µL nutrient broth only, or organic plant extracts (or aqueous plant extracts) and nutrient broth without bacteria was included in these experiments. Each plant extract was run in duplicate. The microtiter plates were covered and incubated for 24 h at 37°C. The MIC was considered as the lowest concentration of the plant extract which inhibited the bacterial growth.

### 3.1.4.2. Determination of Minimum Inhibitory concentration (MIC) against yeast

Minimum Inhibitory concentration (MIC) of organic plant extracts (ethanol and hexane extracts) and aqueous of plant extracts was determined by the method called broth micro dilution method in sterile 96- wells microtiter plates according to standard method described previously .The organic plant extracts and aqueous plant extracts were dissolved in sterile 10% DMSO and sterile distilled water, respectively, to a final concentration 100mg/ml. Both extracts organic and water and DMSO10% (negative control) were two fold-serially diluted in Mueller- Hinton broth in the wells of the plates to the final volume of 100µL. Then, a C. albicans inoculum size of $0.5 \times 10^5$ to $2.5 \times 10^5$ CFU/ml was added to well. Other negative control wells containing either 100µL Mueller-Hinton broth only, or aqueous plant extracts (or organic) and

Mueller-Hinton broth without bacteria was included in these experiments. Each plant extract was run in twice. The microtiter plates were covered and incubated for 48 h at 37°C. The MIC was considered as the lowest concentration of the plant extract which inhibited the yeast growth.

## 3.2 Results and Discussion of biological activities

The antibacterial activities of the extracts obtained from the Sarcopoterium spinosum Plant extracts under study by the broth microdilution method against different pathogens are shown in **Table 3.1** and **Figures 3.1 , 3.2, and 3.3**. Micro dilution method against different pathogens is shown in **Table 3.1 and Figures 3.1, 3.2, and 3.3.**

**Table 3.1: Minimum Inhibitory concentration values (µg/ml) for different Sarcopoterium spinosum Plant extracts types against different pathogens.**

| Microorganism | Type of extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leaf extract | | | Root extract | | | Seed extract | | |
| | W | E | H | W8 | E | H | W | E | H |
| | MIC (µg/ml) | | | | | | | | |
| E. coli | 3.125 | 100 | 100 | 3.125 | 100 | 100 | 3.125 | 25 | 50 |
| S. sonnie (ATCC 25931) | 3.125 | 6.250 | 100 | 3.125 | 50 | 100 | 3.125 | 12.5 | 100 |
| MRSA | 3.125 | 3.125 | 100 | 3.125 | 3.125 | 50 | 3.125 | 3.125 | 50 |
| S. aureus (ATCC 25923) | 1.563 | 0.391 | 100 | 1.563 | 3.125 | 100 | 1.562 | 12.5 | 50 |
| C. albicans (ATCC 90028) | 3.125 | 100 | 100 | 3.125 | 25 | 100 | 3.125 | 12.5 | 100 |

*** W: aqueous extract, E: ethanol extract, H: hexane extract.**

Results of the current study showed that ethanolic leaf extract had the highest antimicrobial activity against S. aureus (ATCC 25923) and MARSA, while the D.W leaf extract had the highest antimicrobial activity against S. aureus.

Minimum Inhibitory concentration of different leaf extract types against different pathogens are presented in **Figure 3.1**.

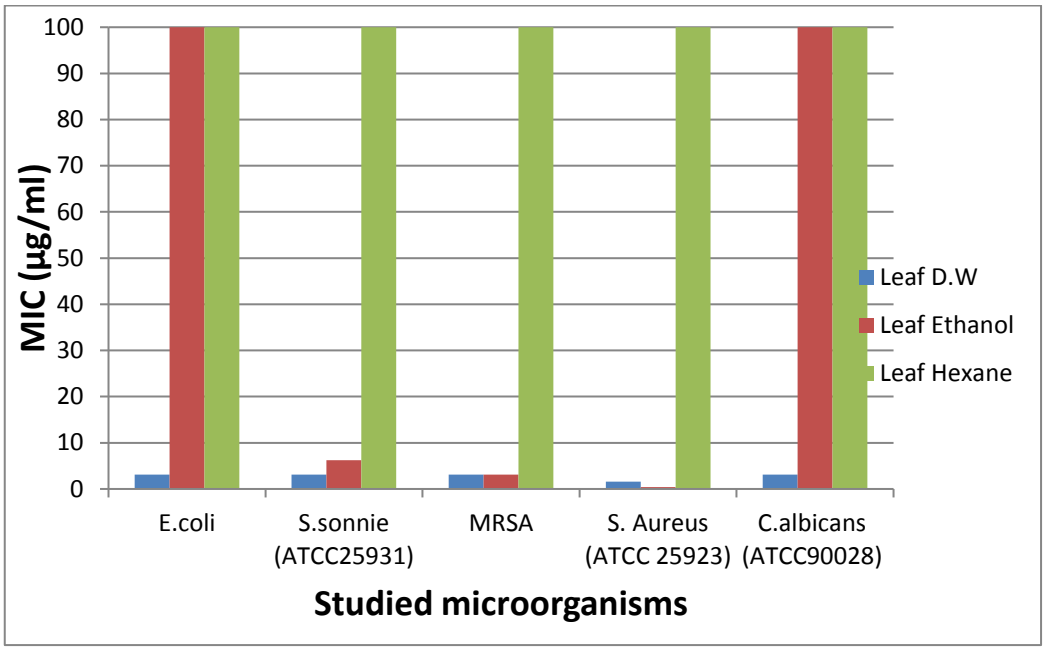


**Figure 3.1:** Minimum Inhibitory concentration (µg/ml) of different leaf extract types against different pathogens.

Results of this study showed that aqueous root extract had the highest antimicrobial activity against S. aureus (ATCC 25923), and then against MARSA, S. sonnie (ATCC 25931) and E.coli, while ethanolic extract had the highest antimicrobial activity against S. aureus (ATCC 25923) and MARSA. Aqueous root extract had the highest activity against C. albicans (ATCC 90028) compared with hexane and ethanolic root extracts.

Minimum Inhibitory concentration of different root extract types against different pathogens are presented in **Figure 3.2**.

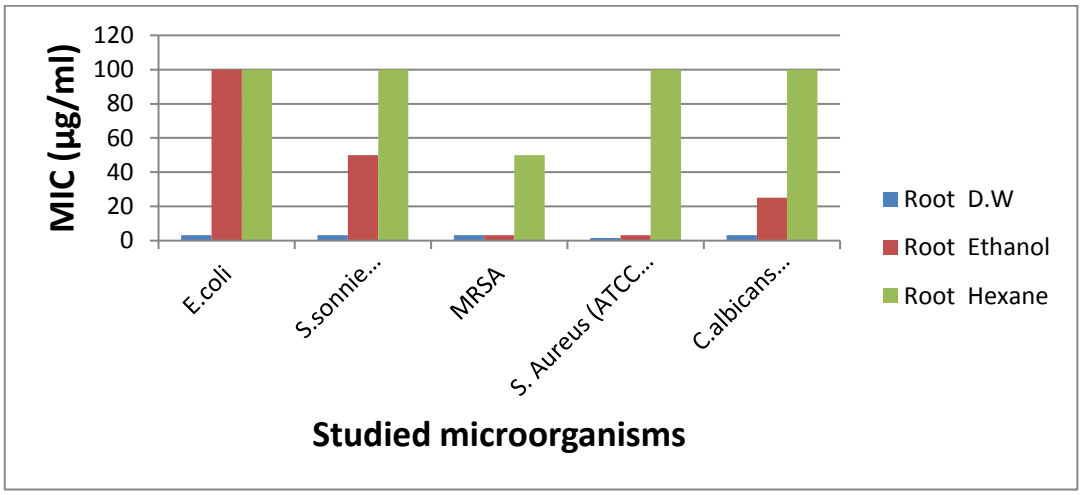


**Figure 3.2:** Minimum Inhibitory concentration (µg/ml) of different root extract types against different pathogens.

Results of the current study showed that aqueous seed extract had the highest antimicrobial activity against S. aureus (ATCC 25923)and the highest activity against C. albicans (ATCC 90028) . While ethanolic extract had the highest antimicrobial activity against MARSA. Hexane extract had the highest antimicrobial activity against E.coli.

Minimum Inhibitory concentration of different seed extract types against different pathogens are presented in **Figure 3.3**.

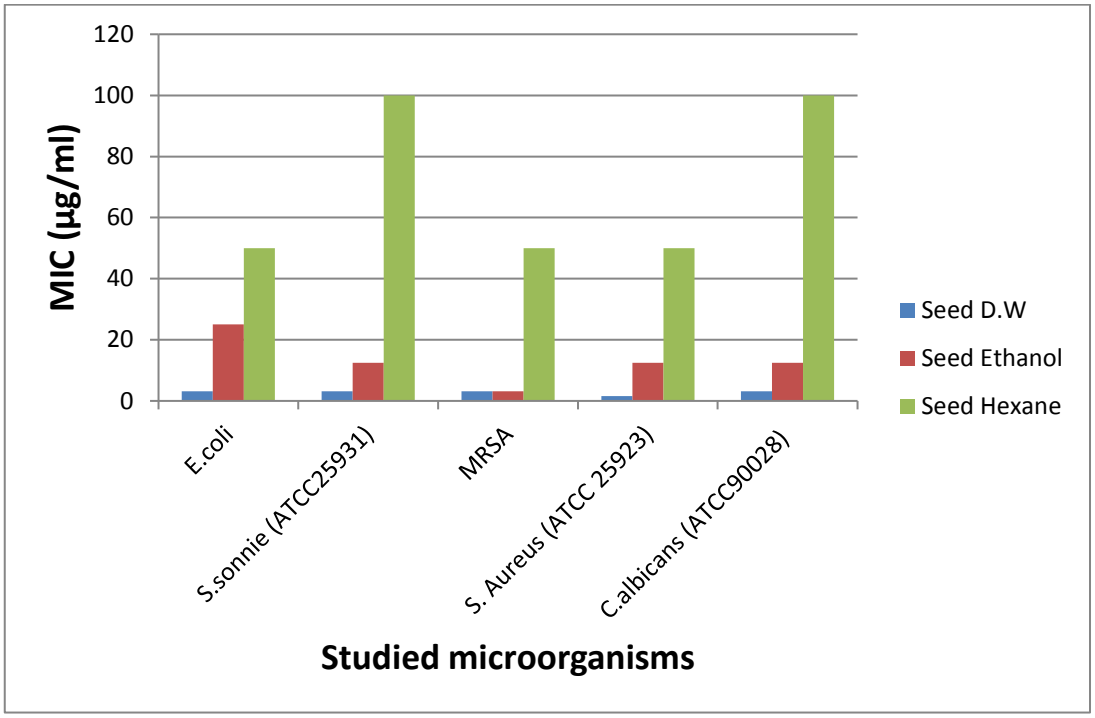


**Figure 3.3:** Minimum Inhibitory concentration (µg/ml) of different seed extract types against different pathogens.

# Chapter Four

# Anti-oxidant activity (free radical scavenging activity)

## 4.1. General procedure of anti-oxidant test for Sarcopoterium-spinosum plant extract.

The electron or hydrogen atom donation abilities of the compounds were calculated from the disappearance of the violet-colored in methanolic solution (1, 1- Diphenyl -2-picryl-hydrazyl) of DPPH. This spectrophotometric assay uses the radical DPPH that stable as a reagent.

The solution of a concentration of 1mg/ml (stock solution) in methanol was prepared for the extract of the plant. Stock solutions was used to make working solutions with the concentrations (2, 5, 10, 30, 50, 80, 100 μg/ml) by make serial dilution with methanol.

DPPH solution was prepared freshly with a concentration of 0.002% w/v. After that, it was mixed with methanol with each of the working concentration in ratio of 1:1:1.

The spectrophotometer was zeroed by a blank solution as methanol. The first solution was DPPH with methanol. The solutions were still at room temperature and dark cabinet for 30 minutes. Then, the optical densities were calculated by spectrophotometer at 517nm wavelength.

The percent Inhibition of free radical I (%) by DPPH was finding as below:

$$(\mathrm{I}\%) = \frac{\text{A blank} - \text{A sample}}{\text{A blank}} * 100\% \quad \text{Equation 1}$$

**Equation4. 1** ： I (%): The percent Inhibition of free radical by DPPH

A blank：The absorbance of the control reaction (containing all reagents except the test compound),A sample: the absorbance of the test compound.

Extract concentrations providing 50% inhibition ($IC_{50}$) were measured by plotting of inhibition (%) against concentration. Tests were made in triplicates.

**4.2. Results and discussion**.

Values of %Inhibition and IC50 for the Sarcopoteriumspinosum plant extract shown in **Table 4.1**. In D.W and ethanolic extract, as concentration increase % Inhibition will increase. But in hexane extract no change in % Inhibition.

As shown in **Figures 4.1, 4.2 and 4.3**.

**Table 4.1: %Inhibition and IC50 for D.W, Ethanolic and Hexane extracts.**

| Conc. | %Inhibition for Different extracts | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | D.W from leaf | D.W from root | D.W from seed | Hexane from leaf | Hexane from root | Hexane from seed | Ethanol from leaf | Ethanol from root | Ethanol from seed |
| **1** | 9.4 | 40.5 | 40.6 | 47.4 | 38.6 | 38.6 | 37.7 | 5.5 | 5.5 |
| **2** | 10.5 | 42.6 | 42.6 | 46.5 | 34.8 | 35.6 | 44.2 | 5.8 | 14.7 |
| **5** | 15.1 | 50.6 | 44.4 | 45.9 | 35.6 | 34.8 | 63.1 | 6.3 | 7.7 |
| **7** | 30.4 | 53.2 | 51.4 | 41 | 36 | 36 | 77 | 7.2 | 7.4 |
| **10** | 48.8 | 59.1 | 52.6 | 40.8 | 35.7 | 35.8 | 86.5 | 7.2 | 15.5 |
| **20** | 77.7 | 68.5 | 43.7 | 39.6 | 36 | 36.2 | 89.7 | 6.7 | 22.1 |
| **30** | 92.1 | 65.7 | 64.7 | 40.7 | 36.6 | 38 | 92 | 26.4 | 29.5 |
| **50** | 95.2 | 72 | 67.2 | 44.6 | 39 | 39.4 | 92 | 47.9 | 42.6 |
| **80** | 93.1 | 78.1 | 72 | 44.2 | 39.5 | 40.3 | 92.7 | 65.4 | 69.4 |
| **100** | 91.6 | 81.8 | 79.4 | 42.6 | 42.7 | 43.6 | 92.7 | 74.5 | 74.5 |
| **IC50** | 10.678 | 28.258 | 397.551 | 5.312 | 158.58 | 148.043 | 5.155 | 44.741 | 87.628 |

*** D.W: aquesous extract.**

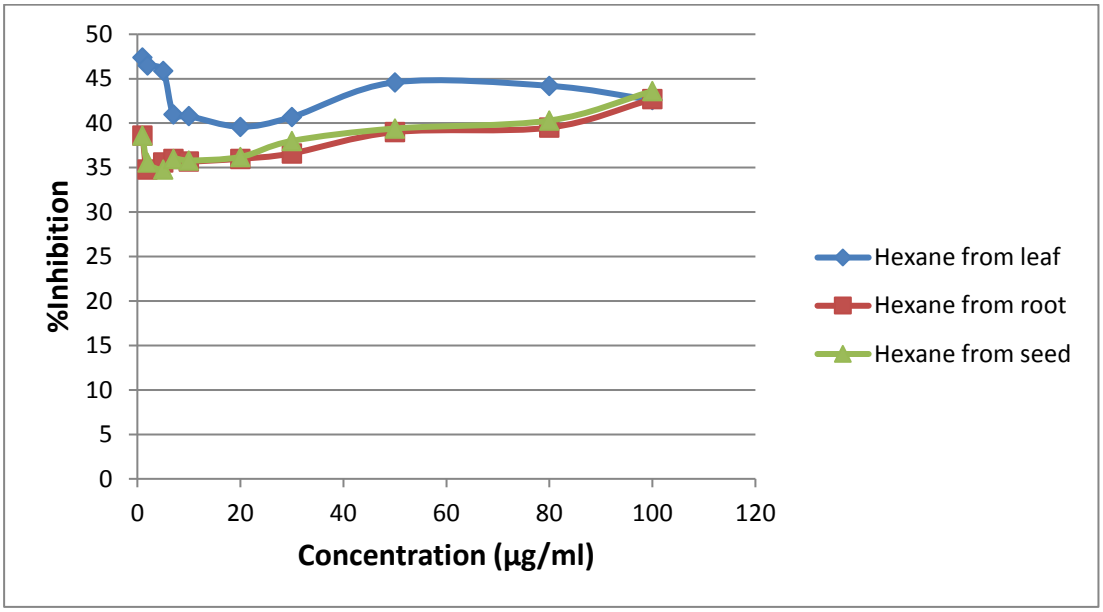


**Figure 4.1:** Anti-oxidant % Inhibition concentration (µg/ml) of different Hexane extract types.

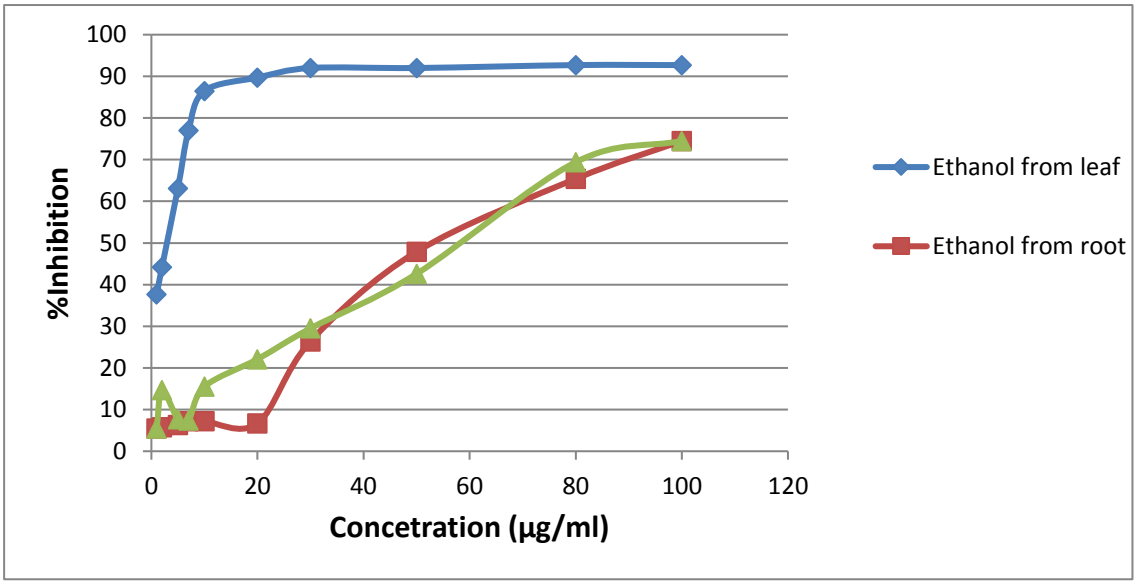


**Figure 4.2:** Anti-oxidant % Inhibition concentration (µg/ml) of different Ethanol extract types.

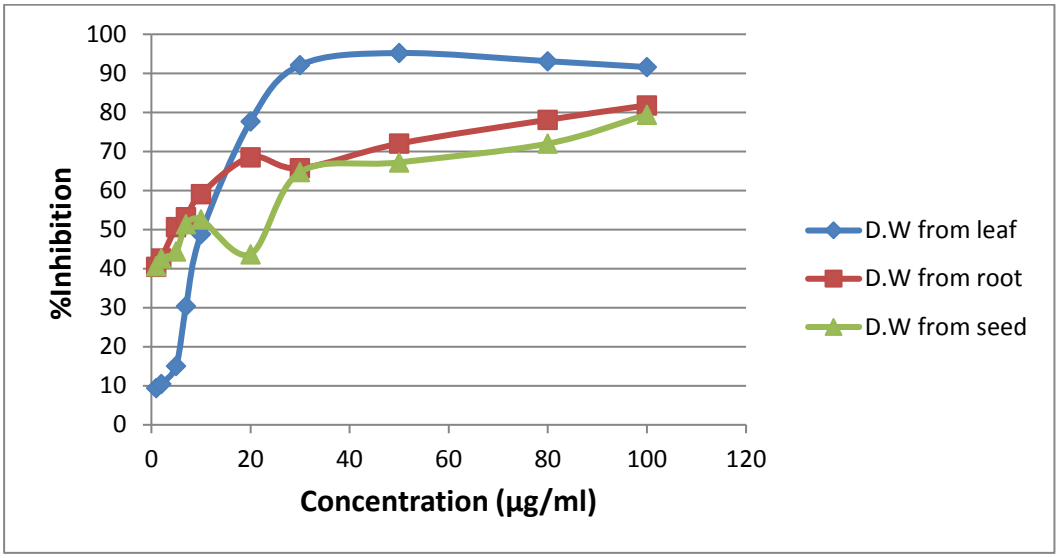


**Figure 4.3:** Anti-oxidant % Inhibition concentration (µg/ml) of different D.W extract types.

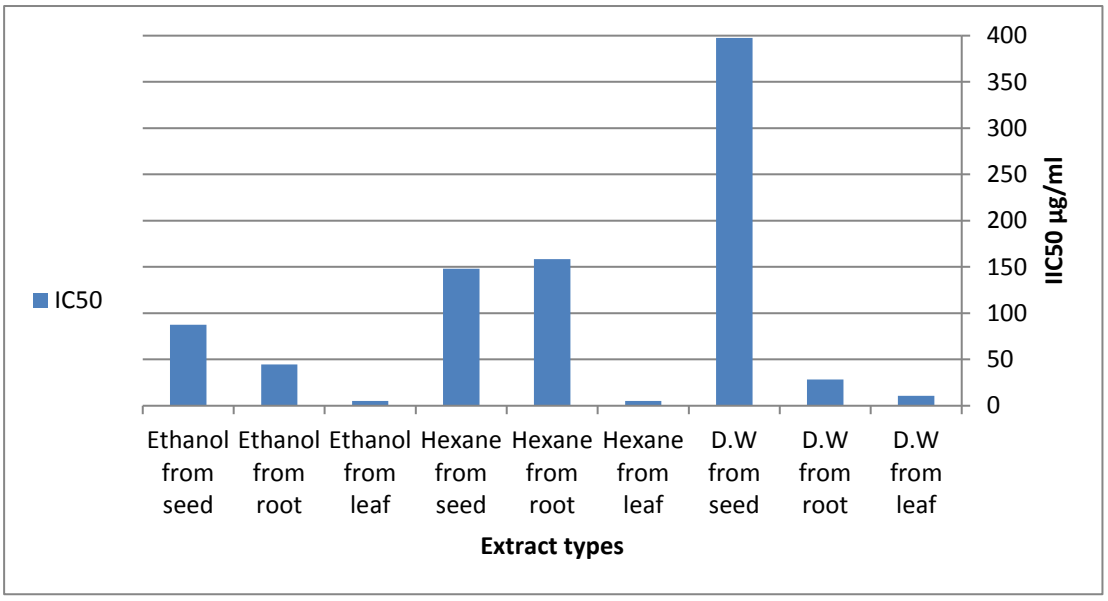


**Figure4.4:** Free radical scavenging capacities IC50 of Sarcopoterium spinosum plant extract.

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**جامعة النجاح الوطنية**

**كليــة الدراسات العليــا**

# توصيف المواد الكيميائية النباتية النشطة بيولوجياً من نبات البلان الشوكي (Sarcopoterium spinosum) كنهج لتطوير أدوية من أصل طبيعي"

**إعداد الطالبة**

**سوار موفق حسين صوافطه**

**إشراف**

**أ.د محمد النوري**

**د. أبراهيم ابو ريدة**

**قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس- فلسطين.**

**2019**

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# الملخص

في هذا البحث قمنا بعمل اكثر من مستخلص لجزء الاوراق والبذور والجذور كل على حدى لنبته البلان الشوكي المتعارف عليها باسم النتش مستخلص بالماء ومستخلص بالايثانول وبالهكسان لأنها تستخدم في الطب الشعبي في فلسطين لعلاج امراض مختلفة واجراء بعض الفحوصات على مستخلصاتها الثلاث على امكانيتها بوقف او منع عمليه التأكسد باستخدام DPPH، وايضا اثر هذه المستخلصات على انواع مختلفة من البكتيريا والفطريات التي تصيب الانسان منها E. coli, S. sonnie (ATCC 25931), MRSA, S. aureus(ATCC25923) and C. albicans (ATCC90028).

وايضا تم تحليل المستخلصات ومعرفه بعض مركباتها باستخدام GC-MS ودراسة كل تأثير لكل ماده ، وايضا تم دراسة المعادن ونسبه وجودها في المستخلصات باستخدام ICP-MS ..