An -Najah National University

Faculty of Graduate Studies

PHYTOCHEMICAL COMPOSITION AND ANTIDIABETIC, ANTI-OBESITY, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF *SARCOPOTERIUM SPINOSIUM* OIL

By

Eman Mohammad Elsaid

Supervisor

Prof. Raed Alkowni

This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Biotechnology and Biological sciences, Faculty of Graduate Studies, An-Najah National University, Nablus - Palestine.

2025

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This Thesis was Defended Successfully on 07/09/2025 and approved by:

Prof. Raed Alkowni Supervisor	Signature
Dr. Osama Alabdalla External Examiner	Signature
Prof. Nidal Jaradat Internal Examiner	Signature

Dedication

First and foremost, I am filled with gratitude to God for guiding me through this journey, granting me the perseverance to overcome challenges and achieve this work.

Thanks to all who support science and contribute to shaping our paths to knowledge. To the self-made individuals who work hard and believe in their success, to everyone leave a mark in the world.

A heartfelt thank you to my incredibly supportive family: my encouraging father, my kind-hearted mother, and my wonderful sisters and brother, who infuse my life with positivity and love. I am forever grateful for you.

To everyone who has fueled my passion and believed in me, your support is a treasure.

Acknowledgements

I wish to express my gratitude to my supervisor, Prof. Raed Alkowni, who has supported and guided me during my studies. I would like to give special Thanks and gratitude to Prof. Nidal Jaradat from the Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, as well as to Fatima Husain, Linda Esa, and Mohammad Araar from the same department. I appreciate the contributions of the technicians in the Department of Biology at An-Najah National University. Lastly, I sincerely thank my parents, who have been a constant source of support throughout my academic journey.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

PHYTOCHEMICAL COMPOSITION AND ANTIDIABETIC, ANTI-OBESITY, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF *SARCOPOTERIUM SPINOSIUM* OIL

I declare that 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: Emen ElSaid

Signature: 

Date: 7.9.2025

List of Contents

List of Tables

List of Figures

PHYTOCHEMICAL COMPOSITION AND ANTIDIABETIC, ANTI-OBESITY, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF *SARCOPOTERIUM SPINOSIUM* OIL

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Abstract

Background: Obesity, diabetes, oxidative stress, and antimicrobial resistance are concerns negatively impacting individual health and represent an obstacle for the health care system. These problems drive researchers to discover novel solutions to prevent the impact of the issue from spreading worldwide. Medicinal plants are commonly used in traditional medicine to treat and prevent diseases.

This study aims to investigate the antilipase, anti-amylase, antioxidant potential activity and antimicrobial activity against human pathogenic bacteria and phytopathogenic fungi of *Sarcopoterium spinosum* fixed seed oil. Additionally, phytochemical components of *Sarcopoterium spinosum* fixed oil extracted from seeds were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) techniques. Moreover, identified the phytopathogenic fungi by molecular techniques.

Methods: Antioxidant activity was measured by using free radical scavenging activity, with DPPH reagent, and Trolox used as a reference.

Although antilipase for *S. spinosum* oil potential was measured by measuring the amount of hydrolysis of p-nitrophenolate to p-nitrophenol were p nitrophenyl butyrate (PNPB) was utilized, and pancreatic lipase activity was estimated using a spectrophotometer at 405 nm. Orlistat medication was used as a reference.

Where, antiamylase activity of *S. spinosum* fixed oil was evaluated by using DNSA reagents, and acarbose was used as a reference.

Antimicrobial activity was performed against human pathogenic bacteria and phytopathogenic fungi by using disk diffusion methods for antibacterial and microbroth dilution methods assisted with dissecting microscopes for phytopathogenic fungi.

Molecular identification of phytopathogenic fungi was conducted using PCR with ITS primers and sequencing.

Results: *S. spinosum* oil with a composition of nine fatty acids showed significant antioxidant activity with IC_{50} value equal to 10.581 µg/ml, were strong α-Amylase Inhibitory potential with IC_{50} value 222.40 µg/ml, but no activity of pancreatic lipase enzyme.

In addition, *S. spinosum* oil revealed no noticeable activity against bacteria, but activity against important *Paecilomyces niveus, Penicillium expansum. Alternaria alstroemeria Botrytis californica*, and *Fusarium equiseti* phytopathogenic fungi. Additionally, phytopathogenic fungi were identified by molecular methods.

Conclusion: *S. spinosum* fixed seed oil has remarkable antioxidant properties and eco-friendly fungicide function against phytopathogenic fungi. Molecular techniques are effective in determining the precise organisms responsible for plants diseases.

Keywords: *S. spinosum* oil; phytopathogenic fungi; antioxidants; phytochemical composition; antilipase; anti α-amylase.

Chapter One

Introduction

1.1 Overview

Humans throughout ancient history have used the available natural plants along with their products to cover their essential needs, including food, accommodation, clothes, and even natural fragrance. Additionally, humans have used herbs and plants as therapeutic agents, which established the early traditional system for therapeutic potential (1).

In historic times, humans lacked comprehensive knowledge about the underlying causes of illness, which made it troubling to determine the specific plants required to effectively treat diseases. Therefore, the entire process of cure and healing relied on experimental trials and observation. This approach formed the basis of traditional medicinal systems, characterized largely by trial-and-error experimentation (2).

Fossil and archaeological evidence from Mesopotamia, Iraq, dating back approximately 60,000 years, indicates the use of plants such as hollyhock (Alcea rosea L.) for their curative properties and health-promoting effects. Information on the therapeutic effects of plants on human well-being has been passed down through generations (3).

In traditional medicine, Arabs established fundamental principles in pharmaceutical science early on. Notably, the first drug store in the region was founded in Baghdad, Iraq, and its innovative approaches to ingredients, drug extraction, and formulations remain fascinating even today (4).

Jaber Bin Hayan, a well-renowned chemist who lived in Kufa in Iraq, made groundbreaking contributions to the field. Through his pioneering investigations, he discovered various chemical compounds, including alcohol, nitric acid, sulfuric acid, and aqua regia (also known as Royal acid, used to dissolve gold). His work also encompassed the preparation of plant extracts, which were administered in multiple ways: orally, externally, or via inhalation (4).

Al-Razi and Ibn Sina, two well-known physicians who authored a famous medical book: *Al-Qanun fi al-Tibb* (Law of Medicine). They extracted medicinal plants and formulated traditional herbal medicines into powders, teas, and syrups to treat diseases. Their contribution has influenced the development of modern medicine (5).

In Arab culture, including Morocco, Yemen, Egypt, and specifically the Middle Eastern regions, there are more than 2600 documented plant species, with more than 700 recognized for their therapeutic potential or as pesticidal plants. In these area patients start to consult skilled healers with expirties in extraction and preparation medicinal plants for treatments and resolve their symptoms, for example *Alchemilla vulgaris, Anchusa strigosa, Calotropis procera* used for skin diseases, in contrast, *Ammi visnaga, Brassica napus, Glycyrrhiza glabra* for kidney and urinary track proplems, Furthermore, *Ceratonia siliqua, Foeniculum vulgare, Micromeria myrtifolia*, used for stomach and digestive system, additionally, *Anchusa strigosa, Anchusa strigosa, Brassica oleracea*, are used against respiratory and pulmonary illness, even more, *Allium cepa, Arum palaestinum, Brassica oleracea* were used as anticancer potential (6).

According to a report published by the World Health Organization (WHO), 80% of the population in developing countries continues to rely on traditional medicines derived from medicinal plants. There are around 28,187, out of a total of 374,000 known plant species, being used for medicinal purposes by humans. Moreover, the WHO has documented over 20,000 species of medicinal plants, underlining their potential as valuable sources for new drug development (7).

Traditional medicinal plant have been used in developing countries as a natural source and less complicated option tratments which drives the WHO to defined traditional medicinal plants as a natural plants materilas used in absence or minimal industrial drugs production to prevent and treats several diseases in local regoins, later on complementary and alternative medicine in united state (CAM) and traditional Chinese medicine (TCM) is currently preffered options, to covering health systems (8).

Nowadays, around 50% of modern pharmaceutical drugs are derived from medicinal plants.a well-known example are, aspirin derived from *Willow bark*, digoxin formulated

from *Foxglove*, morphine originally from the *Opium poppy*, quinine from *Cinchona skin*, and Pilocarpine from *Maranham Jaborandi* (8).

Phytochemicals are plant-derived components served with supplemented nutrients that are a primary part of the human diet. Although contains biologically active ingredients including polyphenols and anthocyanins, which are associated with of medical health advantages such as reducing the percentage of cardiovascular diseases, protection against cancer, stroke, and type 2 diabetes, and antiobesity potential (9).

Medicinal plants have been known for centuries in multicultural traditional medicine systems as sources of therapeutic medications. They have validation to be essential resources for identifying and developing bioactive compounds that possess a broad spectrum of biochemical effects. Most medicinal plants provide multiple biochemical components that may confer health advantages. Among the bioactive compounds, there are alkaloids, flavonoids, terpenoids, phenolics, and essential oils (10).

The mountainous regions of the Middle East, including those in Palestine, are home to more than 700 plant species identified with their bioactivity, usage, and pesticide potential. This represents a significant portion of the region's original 2,600 (or more) medicinal plant species (4).

In Palestine, particularly in Gaza Strip, several national plants are used to treat different health problems. For example, *Anethum graveolens*, *Amaranthus spinosus* L, *Atriplex semibaccata* R.Br, and *Alhagi graecorum Boiss* are used for urinary tract infection and kidney problems. *Datura innoxia* Mill and *Cyperus esculentus* L are used for treating cancer, while *Hibiscus cannabinus* L has shown a good effect in people with hypertension. *Notobasis syriaca* (L.), *Olea europaea* L, and *Portulaca oleracea* L. have revealed antioxidant activity (11).

In cases of oxidative stress, issues related to obesity, hyperglycemia, and multidrug-resistant microorganisms present significant challenges for the pharmaceutical industry. These challenges have led researchers to seek alternative substances that can combat these issues (12).

Medicinal plants have been used worldwide, especially in developing countries, as an affordable method to prevent and manage diabetes. Several pharmaceutical industries depend on effective compounds derived from natural sources; for example, the widely known antidiabetic drug Metformin is derived from *Galega officinalis*. *Allium sativum* (garlic) and *Momordica charantia* (Bitter Melon) have shown anti-hyperglycemic activity. Moreover, the abundance, low cost, and effectiveness of medicinal plants make them a promising alternative for treating diabetes (13).

Natural antioxidant substances are typically found in edible and natural plants in food and medicinal plants. Antioxidants from plant sources, particularly polyphenols, possess a wide range of bioactivities such as anti-cancer and anti-inflammation. For instance, Pomegranate (*Punica granatum* L.) is notable for its high antioxidant capacity due to its polyphenolic compounds (14).

Essential oil with a combination of esters, ethers, terpenes, saturated and unsaturated hydrocarbons, aldehydes, ketones, alcohol, and oxides phenols, and usually with their pleasant odor and fragrance characteristic, concentrated from flowers, leaves, steam and fruits, gained interest in the 20^{th} century and became more famous in 21^{th}century moreover their health benefits relaed to anti-viral, anti-bacterial, anticancer, anti-inflammatory, immune system enhancing, hormonal balancing, and calming effects are well known documented (15).

The encouraging impact of essential oils due to similarity of the chemical structure of body hormones further penetrates subcutaneous tissues and triggers olfactory receptors signal next to the hypothalamus and stimulates neurotransmitters such as serotonin and endorphins (15).

In December 1945, Alexander Fleming discovered penicillin, the first antibiotic to eliminate bacterial infection. However, he also noted that the use of inappropriate concentrations to kill bacteria could lead to bacterial resistance. Antibiotics misused the primary source to develop antimicrobial resistance (16).

The emergence of new diseases, antibiotic-resistant bacteria, and a growing understanding of the value of herbal medicine, all these factors, support the suggestion about finding alternative therapeutic agents derived from plants. Coumarins, flavonoids,

phenolics, alkaloids, terpenoids, tannins, essential oils and others, all these are bioactive components found in plants (17).

Research challenges occur due to the diverse and highly complex nature of plant extracts. As these extracts contain several bioactive compounds, it is essential to understand the interactions between biologically active compounds. According to multiple studies, a combination of active ingredients in infection treatment minimizes the potential of bacterial resistance occurring, in contrast to treatment based on individual components (18). Certain phytochemically bioactive compounds can directly affect the integrity, permeability, and functionality of the cytoplasmic membrane of microbes. Additionally, protein-protein interaction disruption, biofilm formation inhibition, and induction of cytoplasmic coagulation components are all activities that can affect microbial metabolism. For instance, Osthole compound shows DNA gyrase inhibitor activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and MRSA. While Thymol, which originated from *Thymus vulgaris*, revealed cell membrane disturbance potential against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus flavus,* and *Fusarium oxysporum* (19).

Phyto-pathogenic fungi, such as *Aspergillus, Fusarium*, and *Curvularia*, are responsible for significant economic losses due to the diseases they cause in important agricultural crops like cereals. Chemical fungicides have been used in farming for a long time. This has not only degraded environmental quality but has also contributed to the development of resistance among pathogenic fungi. Given these challenges, it is crucial to seek novel, eco-friendly alternatives for protecting crops (20).

In this study, the essential oil of *Sarcopoterium spinosum* (*S. spinosum*) was selected to assess its antimicrobial activity against various human pathogenic bacteria and phytopathogenic fungi. The objective is to identify the chemical constituents of *S. spinosum* essential oil from Palestinian flora and evaluate its antioxidant properties, as well as its inhibitory effects on porcine pancreatic lipase and α-amylase.

1.2 Literature Review

1.2.1 Fixed and Essential Oils: Composition, Extraction, and Biological Properties

Two types of oils can be produced from plants, fixed and essential oils. The first is triglycerides mainly contain glycerol connected to three fatty acids, while the second type, essential oil, is known as essence, volatile and lipophilic components derived from aromatic plant species. Most common essential oils are colorless or light yellow and liquid at room temperature (21).

Fixed oil, a non-volatile liquid with moderate to high viscosity, is primarily composed of hydrophilic substances rich in fatty acids and ester compounds. Palmitic, stearic, linoleic, and oleic acids are abundant fatty acids found in fixed oils. These oils can be derived from plant sources such as seeds, nuts, leaves, and stems. Moreover, fish oil, meat, and eggs are animal sources of fixed oil (22). Fixed oil is crucial to human well-being. It has various health benefits, including antimicrobial, anticancer, anti-inflammatory, and antioxidant properties. Omega-3 fatty acids, vitamins, and minerals are essential for the human diet and development. Examples of fixed oil rich in fatty acids include maize, safflower, cocoa butter, sunflower, olive oil, and coconut oil (22).

The essential oil concept was first established by Paracelsus German-Swiss scientist. He considered distillation as an effective method to obtain and extract what he referred to as the “plant soul”, considering it the most important and therapeutic chemical component. Steam water distillation, physical or mechanical extraction are all approaches used for essential oil extraction (21).

Different methods for seed oil extraction exist. One example is the Pressurized Liquid Extraction method, also referred to as accelerated solvent extraction. In this method, the biologically active constituents are extracted by both solvent and water at extremely high temperatures and pressure levels. One significant benefit of this procedure is that the extracted oil is rich in polyphenols, terpenoids, and phenolic acids, which contribute to its high antioxidant potential (23).

Cold-pressed extraction employs a mechanical screw press to extract oil under pressure with minimal heat exposure, requiring no additional solvents. This method is highly efficient because 1) it yields a high concentration of phytochemicals, 2) it needs low

temperatures, 3) is an eco-friendly method, 4) it offers highly stable antioxidant activity, and 5) it involves low energy consumption (23). Furthermore, ultrasound-assisted extraction offers advantages in terms of yield percentage and efficiency. This method employs multiple frequences between 20kHz and 40kHz, depending on time duration, physical alteration, and cell wall disruptions (23). Essential oils are formed by secondary metabolites from different plant parts, including seeds, fruits, and stems. The primary purpose is to help the plant to survive. Arab researchers were among the first to utilize hydrodistillation for extracting essential oils (22).

The chemical composition of essential oil can be complex, with 20 to 60 components with different concentrations. However, two or three primary compounds are typically found in abundant percentages, phenylpropanoid derivatives or terpenes, while the others are in low percentages (22).

Consequently, it provides medical and biological effects, including antibacterial and antifungal activity, such as *Citrus sinensis* essential oil shows antifungal activity against *Aspergillus niger*, and causes morphological changes and cytoplasmic alteration. Additionally, permanent damage in membrane structure and fluidity caused by tea tree essential oil and its functional constituents is tested against *Candida albicans.* Furthermore, mitochondria damaged in *Saccharomyces cerevisiae* accelerated after being treated with *Cinnamomum camphora* oil, While oil extracted from *Ocimum americanum* revealed strong anti-biofilm formation capacity in conflict to *Candida albicans* (24).

Furthermore, the high antioxidant properties for essential oils offer the potential for cancer reduction and protection against neurodegenerative diseases (25). Several factors can affect essential oil efficacy. Light exposure can impact their stability and functional properties, while the presence of oxygen can alter their physicochemical characteristics. Additionally, high temperatures can cause severe degradation of essential oil substances, thereby affecting their quality and beneficial activity (26).

1.2.2 *Sarcopoterium spinosum*: Botanical Description and Traditional Uses

Sarcopoterium spinosum (S. spinosum), known as thorny burnet (in Arabic: "natsh" or "bilan"), is a chamaephyte that belongs to the Rosaceae family. Its woody branches feature branched thorns and can reach lengths of 30–40 cm. In the summer, the green

winter leaves at the tips of the branches turn into thorns and are later replaced by smaller leaves(27) *S. spinosum* is a characteristic medicinal plant of the Mediterranean region that is commonly used by Bedouin healers for its antidiabetic properties. This plant holds a significant importance in traditional Arab and Bedouin medicine for alleviating pain, managing diabetes, treating cancer, and addressing digestive problems (28). In Lebanon, people used to consume *S. spinosum* in their diets or as tea because of their therapeutic or preventive diseases benefits in folk medicine (29).

1.2.3 *Sarcopoterium spinosum*: Phytochemistry and Known Bioactivities

S. spinosum possesses a variety of beneficial compounds throughout the plant. Its aerial (above-ground) and root parts are rich in triterpenoids like ursolic acid, tormentic acid, and sitosterol. The leaves, in particular, contain carotenoids and flavonoids. The fruits are packed with polyphenols and flavonoids, which act as strong antioxidants and anti-inflammatory agents (29). Beyond its antioxidant properties, *S. spinosum* has shown promising potential in managing diabetes. The active components in their roots enhance glucose absorption through a distinct mechanism (30). *S. spinosum* antihyperglycemic activity has shown the capability to reduce glucose concentration by activating the insulin signaling pathway in hepatocytes and adipocytes (31). Additional studies have explored the mechanism of *S. spinosum* antidiabetic potential and its capacity to boost insulin sensitivity via the activation of PKB and inhibition of PTEN. This mechanism relies on phosphorylation at Ser473 and Thr308, observed in mice treated with *S. spinosum* extract for six weeks (32).

Prior literature in Greece studied the total phenol content and antioxidant potential of *S. spinosum* methanol extracts from the aerial parts. Data indicated that the IC_{50} was equal to 30 ± 0.6 μg/mL whereas the total phenol content was 364.6 ± 13.5 mg GAE/g. This led to classifying it as a highly antioxidant component rich in phenols(33).

Existing literature also reported antimicrobial activity of *S. spinosum* crude aerial 70% ethanol extracts, demonstrating inhibition effect *against E. coli, S. epidermidi, and C. albicans* with a minimum inhibitory concentration (MIC) value of 2.5 mg/mL, while lower concentrations showed bacteriostatic effects (34).

Helicobacter pylori is one of the most widespread human infections, with various health conditions including gastric cancer, gastric ulcer, and lymphoma. While the treatment of *Helicobacter pylori* infection can be accomplished by using three to four lines of antibiotic along with a suppressing agent, however, the recurrence of infection and bacterial resistance along with financial costs all these reasons increase the need of discovered novel therapeutic agents, recently, *S. spinosum* roots ethanolic extracts shown an intermediate antimicrobial activity against *Helicobacter pylori* (35).

1.2.4 Gas Chromatography–Mass Spectrometry in Plant Oil Analysis

Gas Chromatography–Mass Spectrometry (GC-MS) is a cornerstone analytical technique in plant oil analysis, offering high sensitivity and specificity for the separation and identification of volatile and semi-volatile compounds. GC separates the components of a mixture based on their volatility and interaction with the column's stationary phase, while MS provides structural information by analyzing the mass-to-charge ratio of ionized fragments (36). In the context of plant oils, GC-MS is extensively used to analyze fatty acid methyl esters (FAMEs), essential oils, and minor constituents such as terpenes, sterols, and tocopherols. These compounds are often derivatized—typically via transesterification—to improve volatility and thermal stability, enabling more accurate detection and quantification (36).

Recent innovations, such as comprehensive two-dimensional gas chromatography (GC×GC) coupled with MS, have significantly enhanced the resolution and peak capacity for complex oil matrices. GC×GC-MS enables the separation of hundreds of compounds in a single run, providing deeper insights into the chemical complexity of oils like olive, coconut, and argan (37). GC-MS also plays a critical role in quality control and authentication of plant oils. It helps detect adulteration, assess oxidative stability, and monitor degradation products formed during storage or processing. Additionally, it supports chemotaxonomic studies and geographic origin tracing by identifying characteristic volatile markers (36). The versatility and robustness of GC-MS make it indispensable in both research and industrial settings, ensuring the integrity, safety, and traceability of plant oil products (38).

1.2.5 Challenges and Advances in Plant-Derived Antimicrobials and Antifungals

The growing prevalence of antimicrobial resistance (AMR) has boosted the global search for novel therapeutic agents, with plant-derived compounds emerging as a promising reservoir of bioactive molecules. Medicinal plants, including *Sarcopoterium spinosum*, are rich in secondary metabolites such as flavonoids, tannins, alkaloids, and terpenoids, many of which exhibit potent antimicrobial and antifungal properties (39). Despite their potential, several challenges hinder the development and clinical application of plant-derived antimicrobials:

- **Complexity of Extracts**: Plant extracts often contain hundreds of compounds, making it difficult to isolate and identify the specific bioactive constituents responsible for antimicrobial activity (40).
- **Standardization and Reproducibility**: Variability in plant species, growing conditions, and extraction methods can lead to inconsistent results, complicating standardization and dosage formulation (40)**.**
- **Mechanistic Ambiguity**: The precise mechanisms of action for many plant-derived antimicrobials remain poorly understood, limiting their optimization and integration into modern pharmacotherapy(39).
- **Regulatory and Clinical Validation**: Few plant-based antimicrobials have undergone rigorous clinical trials, and regulatory pathways for their approval remain underdeveloped compared to synthetic drugs (40).
- Recent technological and methodological advances are helping to overcome these challenges.
- **In Silico and High-Throughput Screening**: Computational tools and bioinformatics have accelerated the identification of promising phytochemicals by predicting their binding affinities and biological targets (39).
- **Synergistic Formulations**: Combining plant-derived compounds with conventional antibiotics has shown synergistic effects, enhancing efficacy and reducing resistance development (40).
- **Nanotechnology-Based Delivery**: Encapsulation of plant extracts in nanoparticles improves their stability, bioavailability, and targeted delivery, particularly in antifungal applications (39)**.**

- **Metabolomics and Systems Biology**: These approaches provide a holistic understanding of plant metabolite interactions and their effects on microbial systems, aiding in the rational design of phytotherapeutics (40).

In the case of *Sarcopoterium spinosum*, preliminary studies suggest that its polyphenolic-rich extracts exhibit antimicrobial activity, particularly against Gram-positive bacteria and certain fungal strains. However, further research is needed to isolate active constituents, elucidate mechanisms of action, and evaluate therapeutic efficacy in vivo.

1.3 Problem Statement

1.3.1 Obesity, Diabetes Mellitus, and Oxidative Stress

Obesity is defined as an excess accumulation of fat that might involve health risks and can facilitate chronic disorders such as diabetes and heart disease. Since 1975, global obesity rates have significantly increased, with more than 1.9 billion individuals overweight and 650 million obese by 2016. The WHO predicts a significant increase in obesity, estimating that by 2025, one in five people globally will be obese (41). Many researchers believe that overweight or obesity occurs due to multiple factors. Some are genetic or stem from underlying health conditions, while others are acquired habits. Individual choices, lifestyle, cultural eating habits, and limited physical activity are considered key contributors to obesity (42).

Over the past three decades, weight loss has been recognized as a key strategy and control health side effects associated with abnormal weight gain through reducing food portion consumption, increasing physical activity, and lifestyle changes. However, these interferences considered as ineffective in some extreme weight gain or obese individuals furthermore suffer from regaining weight after five years. The pharmaceutical industry focused on finding novel treatments extracted from herbs against obesity, considered to be effective with minimal side effects (43).

Over fifteen health conditions have been found associated with obesity, including type 2 diabetes, hypertension, polycystic ovarian syndrome, cardiovascular disease, and cartilage health problems. Crucially, thirteen types of cancer are directly linked to being overweight (44). Many studies highlight the relation between obesity and cancer,

suggesting that obesity can cause DNA damage and structural modifications to proteins and carbohydrates because of the formation of reactive oxygen species (ROS). These ROS can bind to and alter nucleic acid structures. An increased risk of breast, ovarian, liver, prostate, renal, thyroid, and colon cancer is highly related to obesity (45). To manage chronic obesity, healthcare professionals typically recommend limiting caloric intake, increasing physical activity, changing lifestyle, and pharmacotherapy intervention (46).

Current pharmacotherapy for obesity centers on three main agents: the lipase inhibitor orlistat, the GLP-1 receptor agonists liraglutide, and semaglutide. Orlistat reduces dietary fat absorption by inhibiting pancreatic lipase, producing modest placebo-subtracted weight losses of about 3–5 % over 1–2 years. However, up to 40 % of patients experience gastrointestinal adverse effects—oily spotting, faecal urgency and flatulence—and long-term use can impair absorption of fat-soluble vitamins A, D, E and K (47).

Liraglutide, a once-daily GLP-1 analogue (3.0 mg), produces mean weight reductions of 5–8% at 56 weeks by enhancing satiety and delaying gastric emptying. However, gastrointestinal side effects have been reported among participants: 44.2% of users report nausea, 24.8% experience vomiting, and 31.5% develop diarrhea. Rare cases of pancreatitis and gallbladder disease have also been reported (48).

Semaglutide, dosed at 2.4 mg once weekly, delivers the most pronounced loss—mean ~15% at 68 weeks—but is similarly marred by nausea (65%) and diarrhoea (48%), plus the need for subcutaneous injections and concerns about thyroid C-cell hyperplasia in animal models. Discontinuation almost invariably triggers rapid weight regain, with two-thirds of lost weight returning within 12 months of therapy (49).

Due to these tolerability, cost, and durability issues, there is an interest in plant-derived compounds—polyphenols, alkaloids, saponins—that inhibit lipase, modulate appetite hormones, or enhance energy expenditure, often with minimal side effects in early studies. Exploring these natural alternatives may yield affordable adjuncts or replacements for current pharmacotherapies (49).

Diabetes mellitus (DM) is a chronic metabolic disorder marked by problems with insulin production, the body's resistance to insulin, or insulin insensitivity, which is evident through sustained hyperglycemia (50). This condition ultimately leads to significant long-term complications such as microvascular and macrovascular issues. The far-reaching consequences of diabetes include a reduced life expectancy, significant morbidity attributed to multiple diabetes-related complications, and a deterioration in overall quality of life (51). According to a report published by the International Diabetes Federation, diabetes was responsible for 4.2 million deaths in 2019. While 463 million people aged 20 to 79 years old have already been diagnosed with diabetes, this number is expected to rise to 700 million by 2045 (52).

There are three major types of diabetes mellitus. Type 1 diabetes is characterized by the breakdown of pancreatic beta-cell islets that produce insulin, resulting in a complete insulin deficiency. It is frequently diagnosed in children, causing significant health conditions in the long term. Type 2 diabetes is a chronic, long-term, controllable disease that affects quality of life through its association with various side effects. Lastly, gestational diabetes mellitus occurs during pregnancy and is associated with a degree of carbohydrate intolerance, an increased risk of type 2 diabetes later in life for the mother, and obesity in their children (53). Despite the advancement in antidiabetic drug formulation, several challenges and disadvantages prevent diabitic patients from achieving optimal effectiveness, including drug toxicity and reduction in effectiveness. For instance, 44% of patients treated with sulfonylureas drugs lose their ability to respond after 6 years of continuous usage. Additionally, glucose-lowering drugs are documented to be unable to manage the hyperlipidemia-associated condition (54).

Oxidative stress is a major concern today because it has the ability to turn healthy cells to malignant ones, through the accumulation of reactive oxygen species (ROS) in the body. Because of its association with serious health problems, oxidative stress has become a crucial focus for medical professionals and scientists alike (55). ROS, in addition to free radicals, are naturally produced during normal cellular activities in physiological conditions including cellular respiration and metabolism (29).

The levels of antioxidant substances and enzymes in the body depend on a careful balance between their rates of production and removal. Once antioxidant defense systems fail to neutralize ROS, these harmful molecules can interact with biological

macromolecules. This can lead to increased lipid peroxidation, trigger DNA damage, and/or alter nucleic acid and protein structures (56)**.**

External factors, including sunlight, UV exposure, and diet, can lead to free radical accumulation. This accumulation contributes to oxidative stress, tissue damage linked to Alzheimer disease, cancer, skin aging, and asthma (57).

1.3.2 Microbial resistance and phytopathogenic fungi

Research studies have believed and estimated that there is approximately 2.2–3.8 million fungal species found on Earth, only 10% nearly have been isolated and identified (58). Phytopathogenic fungi are one of the major infectious organisms in plants, causing significant economic and production losses observed frequently among plant pathogens (53). Fungal infections pose a major threat to agricultural productivity, with 70-80% of productivity losses attributed to microbial diseases. Historically, 8000 fungus species were identified to cause roughly 100,000 different plant diseases around the world. In the last few years, this number has increased to more than 19,000 (60)**.**

Phytopathogenic and symbiotic interactions can be categorized into different classes, firstly, necrotrophic, which kills the plant host and feeds on dead plant tissue (e.g., *Aspergillus flavus, Botrytis cinerea, Alternaria alternata, A. brassicae, A. solani, Claviceps gigantean, Bipolaris sorokiniana, Colletotrichum beeveri, C. gloeosporioides, C. graminicola, C. musae, Sclerotinia sclerotiorum, Zymoseptoria tritici, Stenocarpella maydis*) secondly, biotrophic with their ability to absorbs nutrients from plant host without causing directly harming (e.g., *Melampsora lini, Blumeria graminis, Cladosporium fulvum, Phakopsora pachyrhizi, Puccinia striiformis, Puccinia arachidis, Ustilago maydis, Puccinia graminis, Hemileia vastatrix, Puccinia kuehnii, Sporisorium scitamineum*), thirdly hemibiotrophic were initially starting as a biotroph later on they convert to necrotic manner (e.g., *Fusarium equiseti, F. sacchari F. oxysporum, Ganoderma boninense, Colletotrichum higginsianum, C. trifolii, Phomopsis longicolla, Magnaporthe oryzae*). Moreover, many types of fungi can exist in symbiotic beneficial relations with host plants, for instance *Trichoderma virens, Funneliformis mosseae, Glomus albidum, G. etunicatum, G. mosseae, G. fasciculatum, Glomus albidum* (61).

Pathogenic fungi continuously identify and sense any variations in physical signals, chemical compositions, light intensity, and pH levels in their surroundings. Even more, sensing living cells, plant surface, and underground roots by detecting changes in chemical constituents and hydrophobicity (62).

Plant defence response against fungal invaders can be divided mainly into passive and active mechanisms. Passive consists of rapid response even before direct contact with pathogenic fungi that involve physical barriers, for example, cell walls, as well as chemical defenses, such as pH changes and a shortage in nutrients. Although, active mechanism is activated after fungal infection and recognition, this can be further divided into rapid active mechanism and delayed active mechanism, the rapid responses includes changes in membrane permeability, generation of reactive oxygen species, hydrogen peroxide, hypersensitive cell death a fast, localized kind of programmed cell death. While, delayed rapid mechanism includes wound repair response, pathogen-related proteins expression and salicylic acid biosynthesis (61).

Fungi can overcome plant defences mechanism to establish successful colonization, one of the strategy to evade plant immune system is to avoiding from chitinases production, by masking the chitin in their cell wall or by deacetylating it into chitosan thereby reducing plant immune response. Alternatively, fungi secrete effector proteins that can inhibit plant immune response or change the physical and physiological conditions of host cells, in order to facilitate fungal survival and propagation inside plant host cells (61).

Fusarium, Alternaria, Fusicladium, Neoerysiphe and *Mycosphaerella* are the most common fungal species (63). *Macrophomina tecta* is a fungal plant pathogen soil born pathogen, with a wide spectrum of infections. Firstly, isolated from *Sorghum* plant stem it recognized by specific characteristics like charcoal rot symptoms. More than 400 plant species can be infected by *Macrophomina tecta* which includes crucial crops such as cotton (*Gossypium hirsutum*), peanut (*Arachis hypogaea*), soybean (*Glycine max*), and sunflower (*Helianthus annuus* (64).

One of the most important food spoilages caused by an organism is *Paecilomyces niveus* fungus, with its remarkable heat resistance and mycotoxin production. Notable symptoms are brown colored lesions with concentric rings (65). Ascomycetes, a rice pathogenic *Fusarium fujikuroi* was initially identified in 1890 from infected rice, excessive elongational growth, as well as empty or seed sterility, all caused by its gibberellic acid production capacity. Developed an outstanding dark pigment associated with polyketide synthase (66). Another species of *Fusarum* is *Fusarium equiseti*, soil saprophyte associated with dead plant tissues. and pathogenic to several plants (67). In China, during 2014-2016 a severe wilt of cauliflower (*Brassica oleracea* var. *botrytis* L.) occurred represented by root decay, stem browning, and leaves rotting, all instances for visible indications of infection with *Fusarium equiseti* (68).

Grey mycelia and saprophytic nature are the primary characteristics of the *Botrytis genus*, in addition to the variety of pathogenic fungal species are globally distributed, and it has a narrow plant infection host range but *Botrytis cinerea* determined by a broad host infection spectrum with more than 1400 agriculturally essential plant species. More important, mold gray disease resulting from necrotic activity of fungal and killing plant tissue of the infected plant host (69).

Early stage of infection begins by creating a localized area of decomposed tissue plant after fungal biomass increases and completes the infection stages until it progresses to grey mold disease. Additional specific proteins produced by fungi can promote plant cell death, mimicking regulated plant cell death programmed mechanisms (70).

Fusarium oxysporum, with its huge infection potential across agricultural species, tomato, cereal, maize, banana, and soybeans, can lead to significant economic losses resulting from reduced in production yield from infected crops. Crucially, *Fusarium oxysporum directly* affects human health, 70% of fusariosis cases belong to *Fusarium oxysporum* infection. Three types of fusariosis are found, superficial, locally invasive, and systemic infections, most commonly noticed in people with compromised immune systems people (71).

Penicillium fungi are one of the most common fungal species with a wide range of environmental settings, influencing various disciplines including human health, food spillage, medicine, and agriculture. Moreover, patulin and ochratoxin are well-known mycotoxins produced by *Penicillium* as secondary metabolites causing food decay (58).

Phytopathogenic toxins are damaging non-enzymatic compounds produced by plant pathogens, particularly fungi. For instance, the *Fusarium graminearum* releases cell wall-degrading enzymes such as cellulase, xylanase, and pectinase. These enzymes break down the cell walls of the host plant, helping the pathogen's to penetrate and spread within the tissue(72). Globally, the contamination of food by mycotoxins is considered as a threat to food security and safety. These harmful substances lead to substantial economic losses in trade and agricultural production alike, with severe consequences for developing and less developed countries. It is estimated that mycotoxins can affect between 60% and 80% of crops worldwide, leading to massive economic losses. In addition to spoiling food, mycotoxins can cause serious health problems in humans, including cancer, liver damage, kidney failure, and paralysis (60).

Wilting, blighting, rotting, and the formation of cankers are physiological symptoms of diseases caused by Fusarium in a majority of plants and agricultural crops. The known Panama disease affecting banana plants, also referred to as Fusarium wilt (73).

Bacterial infection can be varied dependent on bacterial types, *Bacillus cereus*, known as Gram Positive bacteria, mesophilic, spore production capability with a broad potential to reproduction in diverse environmental parameters including acidic and alkaline conditions ranging from 5 to 10 pH level, moderate salt tolerance, more over its ability to formation biofilm enhance its capacity to survive in harsh conditions and stick in both biotic and a biotic surrounding by production a complex molecules, proteins, and polysaccharides (74). Food poisoning in particular from dietary products, mainly occurs from contamination with *Bacillus cereus*, diarrhea and other gastrointestinal symptoms, which are health risks associated with *Bacillus cereus,* in severe cases hospitalization is required (74).

Staphylococcus aureus, Gram-Positive bacteria distinguished from other types of bacteria, incorporates multiple virulence factors. Recent research in 2012 documented the rate of infection of *S. aureus* ranging from 20 to 50 cases/100,000 annually, and the mortality percentage among infected patients between 10% - 30% (75).

Immunocompromised, tuberculosis, and chronic illnesses patients, in particular, suffer from a serious risk including death. Also, antibiotic resistance increased the lethality rate notably. The *S. aureus* starts from a symptomatic infection in hospital accommodation or is transferred from other people, even more, zoonotic factors are important in *S. aureus* infection establishment (75).

Several microorganisms showed up as significant public health worries, affecting individual health. *Klebsiella pneumoniae* from *Enterobacteriaceae* contributes to one out of three Gram-Negative bacterial infections, urinary tract infection, septicemia, wound infection, and pneumonia (76). Several virulence factors, such as mucoviscosity related genes and capsular polysaccharides, which provide mucoid encapsulated characteristics, in addition to siderophores facilitating iron absorption from the surrounding environment are essential factor for bacterial survival and propagation in host cell, all these factors lead to accelerated drug bacterial resistance requiring the last resort antibiotics (76).

Theodor Escherich, a German pediatric physician, was the first scientist to describe *Escherichia. coli* as an anaerobic facultative bacterium, Gram-Negative bacteria with a rod shape from *Enterobacteriaceae.* Family, the genus *Escherichia* is named by Honor of Theodor Escherich (77).

Diarrheal disease is considered as a health problem more common in low-income countries and is determined as a mortality risk factor especially in infants and young children. *E. coli* normally colonized in human large intestine and rarely caused serious health conditions, diarrheal diseases, in 2011, Germany suffered from an outbreak of *E. coli* new strain led to more than 900 infected people (70).

One of the virulence pathogenic factors of *E. coli* infection is Shiga toxin produced by *E. coli* this toxin production causes vast symptoms, ranging from mild diarrhea to more dangerous health issues, for instance hemorrhagic colitis and more critical condition called hemolytic uremic syndrome which life threatening syndrome affecting individual (77).

E. coli strains developed methods to overcome antibiotics and acquired their ability to resistant antibiotic, animals and contaminated food are a source of infection. More importantly, hospitals are the major source of bacterial resistance, horizontal gene transfer significantly contributes the bacteria obtain and developing antibiotic resistance potential among strains (78).

Recently, isolates from *E. coli* strains showed resistance to third-generation antibiotic drugs including fluoroquinolones, aminoglycosides, and cephalosporins, while some isolates revealed resistance to all mentioned antibiotics, European Centre for Disease Prevention and Control (ECDC) was reported (78).

Antimicrobial Resistance (AMR) arises when microorganisms such as bacteria, viruses, fungi, and parasites change over time, allowing them to survive and proliferate even when exposed to drugs that used to affect them. Infections caused by AMR lead to serious health complications, prolonged hospital stays, higher healthcare charges, and ultimately, treatment failures (79). The global prevalence of infections caused by antimicrobial-resistant organisms has been escalating at a concerning rate, contributing to over half a million deaths on an annual basis (80).

The inappropriate use or overuse of antibiotics creates abnormal selective pressure in both healthcare settings and the environment, which harms both humans and animals. Resistance develops at the genetic level, either through mutations or the acquisition of new resistance genes via genetic exchange mechanism. It is well-known that antibiotics can trigger resistance mechanisms in pathogens via horizontal gene transfer. Currently, there is a rapid rise in microbial resistance to antibiotics such as third-generation cephalosporins, carbapenems, and polymyxins (81).

Examples of AMR include methicillin-resistant Staphylococcus aureus (MRSA), which is attributed to high mortality rates each year around the world. Furthermore, multi-drug-resistant Gram-negative bacteria have made treating various infections, such as pneumonia and urinary tract infections, much more complicated (79). Over the past few decades, a limited number of new antimicrobials have emerged, including Xerava in 2017 for gram-negative pathogens, Nuzyra in 2019 for community-acquired bacterial pneumonia, and Lefamulin in 2019 for bacterial pneumonia (82).

One strategy from bacteria that enables it to evade antibiotics is altering or destroying the antibiotics by adding a chemical group to the antibiotic, thus blocking the interaction between the antibiotic and the target site in bacteria. Ultimately leading to the loss, antibiotic functional activity. This mechanism presences in both Gram-negative and Gram-positive bacteria. Moreover enzymatic inactivation mechanism in Gram-negative bacteria resistance to aminoglycosides is marked by three distinct types of modifying enzymes, resulting in lowering the affinity of the altered product for RNA, ending up preventing binding to the ribosome and inhibiting protein synthesis (19). Another mechanism used by bacterial resistance strains to reduce antibiotic concentration inside the cell is by efflux pumps, which involve expelling the antibiotic molecule from inside to outside the cell in a non-specific manner, leading to decrease the intracellular concentration of the antibiotic (83).

1.4 Aims and Objectives of the Study

Building upon the recognized health benefits of *Sarcopoterium spinosum* (*S. spinosum*) in traditional herbal medicine, this study seeks to investigate its antimicrobial and antifungal properties. Our primary objectives are to:

- Determine the antimicrobial activity of *S. spinosum* against selected human pathogenic bacteria.
- Determine the antimicrobial activity of *S. spinosum* against selected phytopathogenic fungi.
- Elucidate the phytochemical composition of *S. spinosum* fixed oil using Gas Chromatography-Mass Spectrometry (GC-MS).
- Evaluate its antioxidant capacity for combating oxidative stress.

- Inhibitory effects on porcine pancreatic lipase and its antiobesity potential.
- α-Amylase inhibitory activity, thereby assessing its potential for antidiabetic potential.

Chapter Two

Methodology

2.1 Materials and Methods

2.1.1 Chemical reagents

Tables 1, 2, and 3 lists the chemicals and reagents used for antimicrobial assays and for anti-lipase, antioxidant, and anti-amylase assays, and DNA isolation and molecular detection of phytopathogenic fungi respectively.

Table 1

Chemicals and reagents for antimicrobial methods

Chemicals and Reagents	Suppliers	Suppliers' country
Nutrient agar 28g/L	Himedia Laboratories	Mumbai, India
Mannitol salt agar	Himedia Laboratories	Mumbai, India
MacConkey agar 49.53g/L	Himedia Laboratories	Mumbai, India
Sabouraud's dextrose agar 65g/L	Becton, Dickinson	USA
Mueller-Hinton broth 21g/L	Himedia Laboratories	Mumbai, India
RPMI 1640 medium with L-glutamine without sodium bicarbonate 0.165mol/L (Rose well Park Memorial Institute)	Sigma-Aldrich	USA
Mueller–Hinton (MHA) agar	Himedia Laboratories	Mumbai, India
DMSO 100%	Carlo ERBA	Germany
H2SO4	Merck	Germany
BaCl2	Merck	Germany
Gentamycin	Thermo Fisher	USA

Table 2

Chemicals and reagents for antioxidant, antilipase, and antiamylase activity

Chemicals and Reagents	Suppliers	Suppliers' country
Ethyl alcohol 99.9%	Sun Farm	Nablus, Palestine
Methanol	Backing Self	Israel
Sodium hydroxide 40g/mole	Sun Farm	Nablus, Palestine
DPPH	Sigma-Aldrich USA	Germany
Trolox	Sigma-Aldrich USA	USA
TrisHCl buffer	Sigma-Aldrich USA	USA
PNPB	Sigma-Aldrich USA	USA
Pancreatic lipase	Sigma-Aldrich USA	USA
Acetonitrile	Carlo ERBA	France
Orlistat	Birzeit company	Birzeit, Palestine
DNSA	Thermo Fisher	USA
Na_2HPO4/NaH_2PO_4,	Sigma-Aldrich USA	USA
Sodium Potassium Tartate	MERCK	Germany
NaCl	Sigma-Aldrich USA	USA
Porcine pancreatic-amylase	Sigma-Aldrich USA	USA
Acarbose	Sigma-Aldrich USA	USA
Starch	Sigma-Aldrich USA	USA

Table 3

Chemicals and reagents for fungi DNA isolation and PCR

Chemicals and Reagents	Suppliers	Suppliers' country
Triton-X-100	Sun Farm	Nablus, Palestine
SDS	Backing Self	Israel
EDTA	Sun Farm	Nablus, Palestine
Phenol	Sigma-Aldrich USA	Germany
Chloroform	Sigma-Aldrich USA	USA
Isoamyl alcohol	Sigma-Aldrich USA	USA
Agarose	Thermo Fisher	USA
Boric Acid	Sun Farm	Nablus, Palestine
DNA ladder	Qiagen	Germany
Mix™ Taq PCR Reaction	Thermo Fisher	USA
SYBR Stain	Thermo Fisher	USA

2.1.2 Equipment and Instruments

The Equipment and Instruments used in this study are listed in Table 4

Table 4

Equipment and Instruments

Equipment and Instruments	Supplier
Spectrophotometer	Jenway, U.K
PH meter	Jenway, UK
Sterile syringe filter 0.45µm	Sigma-Aldrich USA
Syringe 5 mL	Sigma-Aldrich USA
Incubator	NÜVE, Turkey
Refrigerator	Ariston, Italy
Multichannel 30–300 µL micropipette	Eppendorf, Germany
Single micropipette 20 -200 µL	Eppendorf, Germany
Tips	Fl medical, Italy
Petri dishes	Fl medical, Italy
96-well plate polystyrene panel	Sigma-Aldrich, USA
Mastercycler Personal	Sigma-Aldrich, USA
Spectrophotometer NanoDrop 1000	Thermo Fisher Scientific, USA

2.2 Plant Collection

The *S. spinosum* seeds were collected and harvested from Jenin region in Palestine. The plant seeds were identified by Pharmacologist Dr. Nidal Jaradat in the Pharmacognosy Laboratory from An-Najah National University with a voucher code Pharm-PCT-2142. A Photograph of *S. spinosum* is shown in Figure 1.

Figure 1

S. spinosum plant and its seeds as pictured from the Palestinian wild

2.3 Oil Extraction

The cold pressing technique was employed to extract oil from the seeds of *S. spinosum* plant for further use. The cold pressing extraction was selected to maintain bioactive compounds like fatty acids , and preserved antioxidant properties despite oil extraction yield (84).

A continuous mechanical screw pressor with low temperature is applied to the dried seeds, this pressure allows the oil to be extract and drained. *S. spinosum* fixed seeds oil extracted from the extraction methods characterized by dark green color.

- **Gas Chromatography–Mass Spectrometry (GC-MS) Evaluation**

Fatty acid methyl esters (FAMEs) of *S. spinosum* fixed oil were prepared following a standard methylation protocol. Initially, 100 mg of oil sample was placed in a 250 mL round-bottom flask along with boiling chips. Subsequently, 10 mL of 0.5 M methanolic sodium hydroxide were added. The mixture was refluxed for 20 minutes. Following this, 10 mL of 13–15% boron trifluoride–methanol complex solution was added, and the reflux continued for an additional 20 minutes. Afterward, 100 mL of HPLC-grade heptane were added, and the mixture was boiled for another 20 minutes. The solution was allowed to cool, and the organic (upper) layer was collected and dried over anhydrous sodium sulfate. A 1 µL aliquot of the resulting sample was injected into the GC-MS system.

GC-MS analysis was performed using a PerkinElmer Clarus 500 gas chromatograph coupled to a PerkinElmer Clarus 560 mass spectrometer. Separation was achieved on an SLB™-5ms fused silica capillary column (30 m × 0.25 mm i.e., film thickness 0.25 µm). The oven temperature was programmed to rise from 100°C at a rate of 3°C/min to a final temperature of 240°C. Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1 mL/min. The injector temperature was set at 290°C, and 1 µL of the methylated oil was injected in split mode (split ratio 1:50). The solvent delay was 0–8 min; mass spectrometer conditions were: source temperature 250°C, scan range m/z 50–500 in EI+ mode, from 8.00 to 65.67 min.

Mass spectra of the components were compared against the National Institute of Standards and Technology (NIST) library and identified by matching with Kovats retention indices from the literature. Kovats retention indices were calculated based on retention times of a C10–C40 hydrocarbon alkane standard mixture.

2.4 DPPH Method

Stock solutions of *S. spinosum* oil and Trolox (used as a positive control) were prepared at a concentration of 0.1 mg/mL in methanol. Working solutions with concentrations of 2, 5, 10, 20, 40, 80, and 100 µg/mL were obtained by serial dilution in methanol.

A freshly prepared 0.002% (w/v) DPPH solution in methanol was used. Equal volumes of DPPH solution, methanol, and the working solutions of *S. spinosum* oil were mixed in a 1:1:1 ratio. A blank solution consisting of DPPH and methanol (1:2) was prepared. All steps were carried out under minimal light exposure to protect DPPH from photodegradation.

The mixtures were incubated in the dark at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using:

$$\%\text{DPHH Inhibition} = \frac{A_B - A_S}{A_B} \times 100 \quad (2.1)$$

Where A_B represents the absorbance of the blank solution, and A_S represents the absorbance of *S. spinosum* oil solutions.

2.5 α-Amylase Inhibitory Test

S. spinosum oil was dissolved in 10% DMSO. A buffer solution (0.02 M Na_2HPO_4/NaH_2PO_4, 0.006 M NaCl, pH 6.9) was prepared fresh. Test solutions with concentrations of 25, 50, 100, 250, 500, and 1000 µg/mL were prepared in the buffer.

To each test sample, 0.2 mL of 2 U/mL porcine pancreatic α-amylase was added, followed by incubation at 30°C for 10 minutes. Then, 0.2 mL of 1% starch solution was added, and the mixture was incubated for 3 minutes. The reaction was stopped by adding 0.2 mL of dinitrosalicylic acid (DNSA) reagent, followed by dilution with 5 mL

of water and boiling at 90°C for 10 minutes. The solutions were cooled to room temperature, and absorbance was measured at 540 nm.

Blank was prepared exactly as the *S. spinosum* oil working solutions, except that the 0.2 ml of *S. spinosum* oil was prepared for the buffer. Antidiabetic drug Acarbose was used as a positive standard reference for the procedure similar as *S. spinosum* oil steps. The following formula is used to calculate the α-Amylase Inhibitory:

$$\%\ \boldsymbol{\alpha} - \text{Amylase Inhibition} = \frac{A_B - A_S}{A_B} \times 100 \qquad (2.2)$$

Where A_B represents the absorbance of the blank solution, and A_S represents the absorbance of *S. spinosum* oil solutions.

2.6 Lipase Inhibitory Assay

The inhibitory activity of *S. spinosum* oil against porcine pancreatic lipase was evaluated by measuring hydrolysis of *p*-nitrophenyl butyrate (PNPB). A stock solution of *S. spinosum* oil (1 mg/mL in 10% DMSO) was prepared and serially diluted to 50, 100, 200, 300, and 400 µg/mL. A PNPB stock solution was prepared by dissolving 20.9 mg in 2 mL of acetonitrile.

Pancreatic lipase (1 mg/mL) was freshly prepared in Tris-HCl buffer (pH 7.4). For each test, 0.2 mL of *S. spinosum* oil solution was mixed with 0.1 mL of enzyme solution and diluted to 1 mL with buffer. The mixture was incubated at 37°C for 15 minutes. Then, 0.1 mL of PNPB solution was added, followed by incubation at 37°C for 30 minutes. Absorbance was measured at 405 nm. Orlistat was used as a positive control. The percentage inhibition was calculated as:

$$\%\ \text{Lipase inhibitory} = \frac{A_B - A_S}{A_B} \times 100 \qquad (2.3)$$

Where A_B represents the absorbance of the blank solution, and A_S represents the absorbance of *S. spinosum* oil solutions.

2.7 Phytopathogenic fungi detection and molecular identification

2.7.1 Fungal Isolation

Plant shows symptoms through stems, leaves, roots, were initially rinsed with tap water, then a small piece approximately 5 mm² was dissected from infected tissue boundaries. These cut tissues were processed with surface sterilization by dipping in 70% ethanol for 30 seconds, then subjected to a 1% sodium hypochlorite solution for 2 minutes. Immediately after, treated pieces were soaked in 99% ethanol for 5 seconds and then into a 3% sodium hypochlorite solution for another 3 minutes without shaking or mixing.

Sterilized tissue segments were aseptically placed on Sabouraud's Dextrose Agar (SDA) media. Supplemented with chloramphenicol in a concentration of 100 mg/L in order to prevent bacterial growth contamination. After that, plates were incubated at 25 ± 2°C in the dark for 5–7 days and their growth rate monitored daily.

To obtain pure culture, fungal colonies were re-cultured into freshly prepared SDA media by using hyphal-tip or single-spore technique. Isolates are individually categorized and retained for further tests.

Initial characterization of fungi isolated based on morphology, coloration, and rate of growth.

Pure colonies were preserved on SDA culture at 4°C for short-term storage and in 15% glycerol solution at –80°C for extended preservation.

2.7.2 DNA-Based Identification of the Phytopathogenic Fungi

2.7.2.1 DNA Extraction Protocols

DNA rapid extraction protocol was used, as mentioned by (85). Starting from 72 h freshly grown isolated culture on SDA media with suitable temperature conditions, around 100-200 mg of mycelium hyphae are harvested with minimal agar media inclusion, and collected into a 1.5- or 2-ml microcentrifugation tubes and, mycelia were completely frozen by incubating the samples in a deep freezer -80°C, for around 1 h or until completely solid, immediately ground the sample using a sterile glass rod until powder.

Breaking buffer solutions comprising 1% SDS, 2% Triton X-100, 100 mM NaCl, 10 mM Tris-HCl (pH 8.0), and 1 mM EDTA, added to each tube after the incubation time was completed, vortexed intensively to ensure homogeneity. Another incubation for 1 hour at 65°C was performed, mix via vortex every 15 min to ensure lysis.

After incubation finished, a phenol: chloroform: isoamyl alcohol mixture (25:24:1) a 500 µL was dispensed to each tube. Two minutes of vortexing were done to form an evenly mixed solution. After that, the samples were centrifuged at 13,000 rpm for 20 minutes, the clear supernatant was collected and transferred to new tubes.

In order to isolate DNA by precipitating, isopropanol was added to the supernatant with an equal volume of supernatant, and the mixture was incubated at -20°C for at least one hour or overnight to improve yield. Then centrifugation at 14,000 rpm for 15 minutes at 4°C. Wash the resulting DNA pellet with 70% cold ethanol and repeat the centrifugation with the same parameters. Discard the supernatant and allow the resulting pellet to air dry under a laminar flow chamber. Lastly, resuspend the DNA in 100 µL of TE buffer or DNase/RNase-free distilled water. DNA concentration was measured by DNA for each sample by utilizing a NanoDrop spectrophotometer, and stored at 20 °C for further use in the PCR test.

2.7.2.2 Polymerase Chain Reaction (PCR) for Fungal Identification

Polymerase Chain Reaction (PCR) was carried out to amplify fungal DNA (86).Using the universal primers ITS1 and ITS4 targeting the ITS (internal transcribed spacer region) fungal region, ITS is a non-coding sequence located between the small-subunit (18S) and large-subunit (28S) rRNA genes, it can be found in chromosomal DNA or as a part of the transcribed region of the polycistronic rRNA precursor.

Primers sequences used as follows:

ITS1: 5’-TCC GTA GGT GAA CCT GCG G-3’.

ITS4: 5’-TCC TCC GCT TAT TGA TAT GC-3’.

PCR performed as following conditions, 25 µL was the final volume of PCR reaction mixture, includes 50–100 ng of genomic DNA (0.5–5 µL), 0.4 µM of each primer (1 µL of a 10 µM stock), 12.5 µL of ReadyMix™ Taq PCR Reaction Mix with $MgCl_2$ (Promega), and nuclease-free distilled water up to the final volume.

PCR amplification step is run in a thermal cycler with a heated lid. The thermal cycling protocol is set up with an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 60 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 60 seconds. A final extension step was completed at 72°C for 10 minutes.

Previously prepared 10X Tris-Borate (TBE) buffer,108g of Tris, and 55 g of Boric acid dissolved in 900 ml of distilled water and stirred until completely dissolved.

Electrophoresis techniques were used to resolve the PCR product according to DNA amplicon size. A 2.0% agarose gel was dissolved in 1X TBE buffer, just before pouring into the gel tray. SYBR green was added to stain DNA amplicons, and run at 100 volts for 40 minutes. DNA bands were visualized under UV light using a gel documentation system.

Biotech Lab Facility Ltd. (Ramallah, Palestine). purified and sequenced the PCR product in forward and reverse directions. To identify fungal species, the resulting sequence analysis by NCBI BLAST tool.

2.8 Antimicrobial Assays

2.8.1 Antibacterial Test

The antibacterial potential of *S. spinosum* oil was evaluated against both Gram- positive and Gram-negative bacteria using strains obtained from the American Type Culture Collection (ATCC), including *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus,* and *Bacillus cereus.*

After filtration with a syringe filter 0.45 µm, antibacterial activity of *S. spinosum* oil against the mentioned bacterial strains was tested using agar disk diffusion method (87),(88).

Bacterial inoculum was prepared by selecting and picking colonies from overnight (16-24 h) growth of bacteria, a sterile loop or cotton swab were used. Moreover, suspend the chosen colony in Mueller–Hinton (MHB) broth media to obtain a visual turbidity equal to 0.5 McFarland standard, roughly equivalent to $1–2 \times 10^8$ CFU/mL

Concisely, culturing each bacterial suspension on a Mueller–Hinton (MHA) agar plate by dipping the sterile cotton swab in the adjusted Mueller–Hinton (MHB) broth media equivalent to 0.5 McFarland, then removing excess fluid, and then evenly and kindly streaking on the agar surface plate. The bacterial suspensions should be used within 15 min of preparation.

After that, filter paper discs about 6 mm in diameter, containing *S. spinosum* oil, are placed on the agar surface, a disc containing sterile distilled water is used as a negative control, and antibiotic gentamycin 10 µg/mL is used as a positive control.

Moreover, the plate was incubated inverted for 16-18 h at 37°C, antibacterial activity was identified with clear inhibition zone around tested discs.

2.8.2 Anti-phytopathogenic Fungi Activity

The influence of *S. spinosum* oil on phytopathogenic fungi was tested against eight significant phytopathogenic fungi, including*: Alternaria alstroemeria, Botrytis californica, Fusarium equiseti, Fusarium foetens, Fusarium fujikuroi, Macrophomina tecta, Paecilomyces niveus, and Penicillium expansum.* They were isolated and identified at An-Najah National University lab.

The minimal inhibitory concentration (MIC) value was determined using microbroth dilution method with a minor modification (89), to evaluate the anti-phytopathogenic fungi activity of *S. spinosum* oil. RPMI 1640 (Roswell Park Memorial Institute) media was used to cultivate the mentioned fungi.

Initially, 100 µg/mL, dissolved in 10% DMSO and RPMI media. Next, in a 96-wells plate polystyrene panel, the prepared *S. spinosum* oil solution was two-fold serially diluted in RPMI media to achieve serial dilutions of 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, etc.

Further, the fungi inocula were prepared comparably to bacteria preparation. Sterile scalpel or needles were used to scratch spores and hyphae from 5-7 days previously cultured fungi on Sabouraud's Dextrose Agar (SDA) media.

Moreover, the spores and hyphae were suspended in RPMI and adjusted to a turbidity equivalent to 0.5 McFarland. However, 0.5 McFarland for fungi is equal to 1×10^6 CFU/ml. This was diluted 1:10 in RPMI media, resulting in 1×10^5 CFU/ml before the inoculum was added to each well containing a certain concentration of *S. spinosum* oil solution, the final inoculum size must 1×10^4 CFU/ml in each well.

Micro well 11 served as a positive control containing RPMI media and tested fungi (*S. spinosum* oil-free), to verify fungi growth. While micro well 12 served as a negative control, the RPMI media was only included to guarantee septic-free conditions. Procedures are required to be performed under sterile conditions.

The panel was covered and kept at room temperature for 7 days. Antifungal activity was assessed by positive growth control, where it should confirm a growth turbidity under a dissecting microscope, in contrast to the negative control with clear media reflecting.

Finally, the MIC determined by visual eye and confirmed with dissecting microscope, were the MIC addressed by the lower concentration of *S. spinosum* oil led to visible inhibition of fungal growth.

Several types of media were prepared for culturing bacteria or phytopathogenic fungi in this study according to manufacturer's instructions. These media are listed as follows:

- **MHB**: 21g media powder in 1000mL distilled water, 300 mL of media was prepared and, then each 5 mL was divided to each test tube and sterilized by autoclave. The media were kept in the refrigerator until work.
- **Mannitol Agar**: For each 1000mL of distilled water, 111g of media powder is needed. 200mL was prepared and placed in an autoclave for sterilization. Once it had cooled down after being poured into petri dishes, gram-positive bacteria were cultured.

- **MacConkey Agar**: To prepare 1000 mL, 51.5 g of media is required. 200mL of media was used. For sterilization, autoclaving is done, then melted agar was poured into petri culture plates, and allowed to cool. This medium was utilized to culture gram-negative bacteria.
- **Nutrient Agar**: This medium was used for either gram-positive or gram-negative bacteria. According to the instructions (28g/L), 5.6 g of agar was dissolved in 00 mL of distilled water was prepared. This was sterilized by using an autoclave and then poured into petri dishes.
- **Sabouraud's Dextrose Agar (SDA)**: 32.5 g dissolved in 500 mL distilled water (65g in 1000 mL), autoclaved, and poured into petri dishes. Moreover, this medium is used to culture fungi.
- **0.5 McFarland solution preparation:** The preparation was initiated by setting up two solutions, the first solution contains 1% of anhydrous barium chloride (BaCl2), and the other contains 1% of sulfuric acid (H2SO4). Then, the two solutions were mixed until the desired turbidity suspension occurred. The resulting $BaSO_4$ solution absorbance measured turbidity is 0.08-0.1 at 600 nm. Finally, the solution was stored at room temperature protected with foil.

Chapter Three

Results

3.1 Phytochemical Profiling

GC-MS technique provides qualitative and quantitative analysis of phytochemical components of *S. spinosum* fixed oil Figure 2. Nine fatty acids, making up the total oil, were identified in Figure 3. Fatty acid composition of *S. spinosum* fixed oil

The abundant fatty acids were cis-11-Eicosenoic acid with 37.48% and Linoleic acid with 23.0%, Elaidic acid and Arachidic acid composed 0.40%, 0.21% respectively. The results are shown in Table 5.

Figure 2

S. spinosum fixed seeds oil

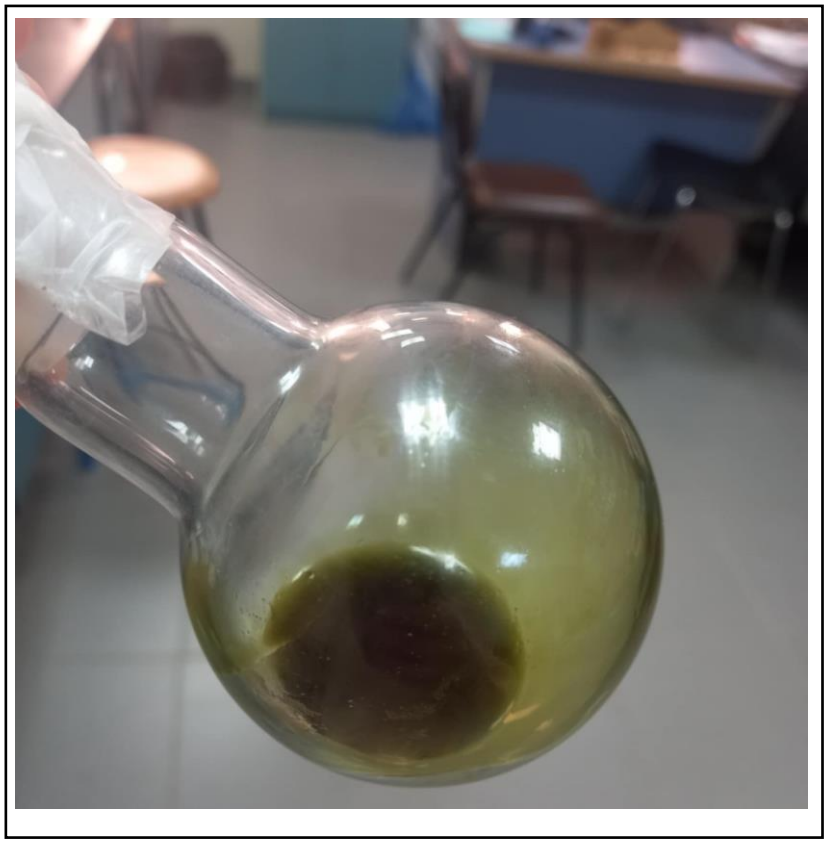


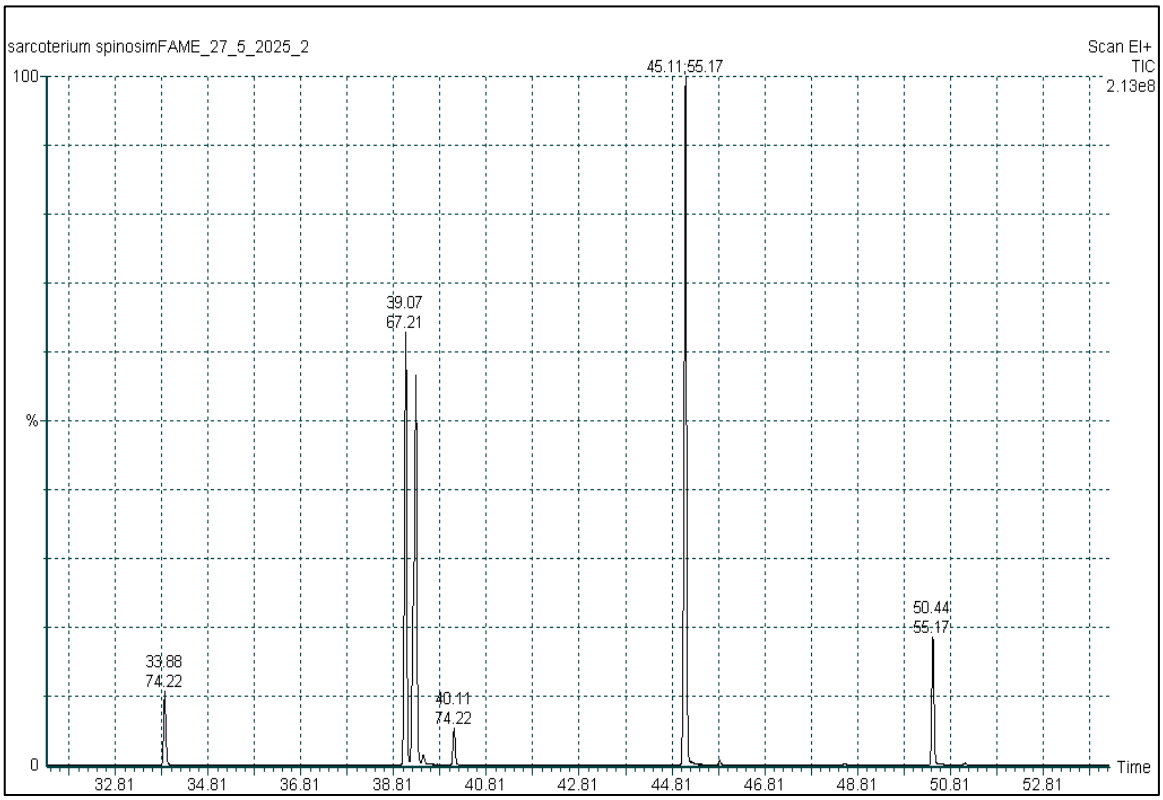
Table 5

Fatty acid composition of S. spinosum fixed oil

Fatty acid Name	Retention Time	Area	% Area	Kovats Index	Retention
Palmitic acid	33.887	1224582	3.61	1927	
Linoleic acid	39.07	7807532	23.05	2093	
Linolenic acid	39.221	4336478	12.8	2098	
Oleic acid	39.307	4759550	14.05	2100	
Elaidic acid	39.45	136121	0.4	2105	
Stearic acid	40.11	616687	1.82	2127	
cis-11-Eicosenoic acid	45.11	12698570	37.48	2294	
Arachidic acid	45.86	71962	0.21	2318	
Erucic acid	50.44	2226161	6.57	2495	
Total		33877643	100		

Figure 3

GC-MS chromatogram of S. spinosum fixed oil

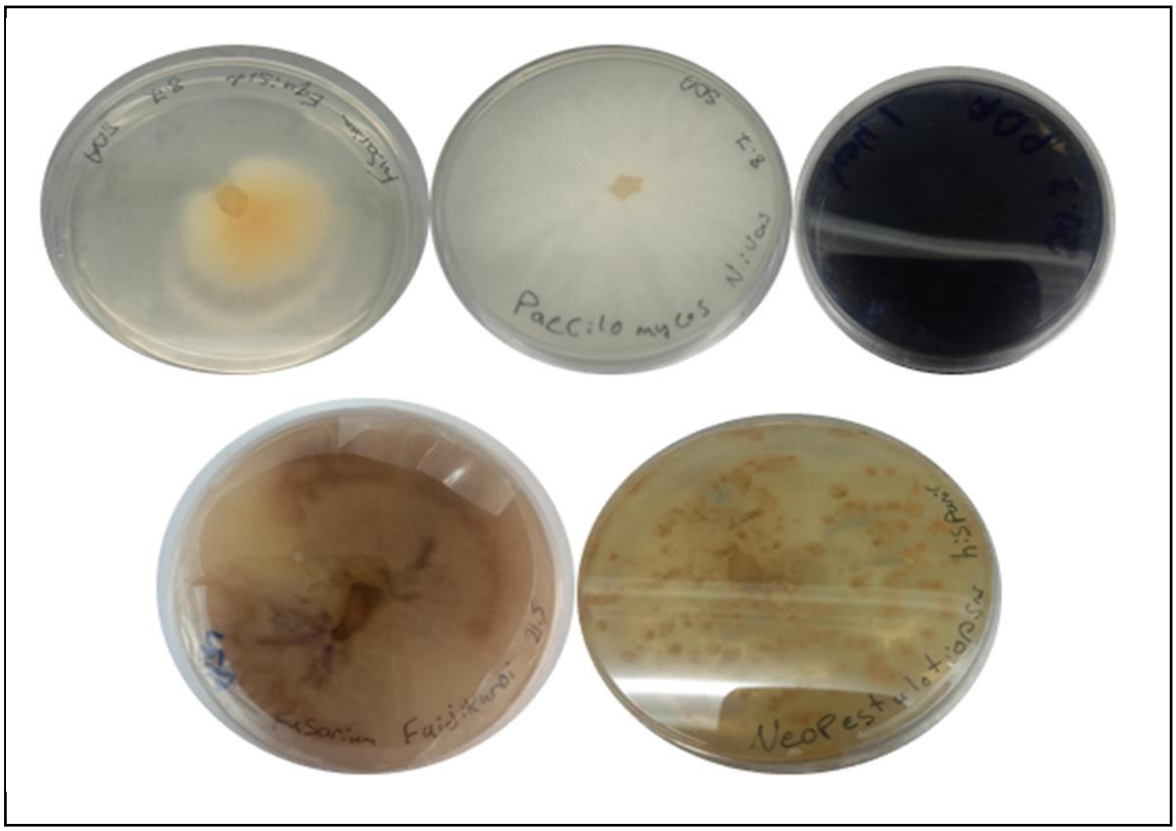


3.2 Morphological Characterization and Molecular Identification of Phytopathogenic Fungi

Selected phytopathogenic fungi were ascertained by morphological and microscopic characterization. For example; *Fusarium equiseti* revealed white with shades of orange-yellow color with fizzy hyphae, while *Fusarium fujikuroi was* recognized with dark purple pigmentation colonies. But *Macrophomina tecta* produced dark black with charcoal colonies, *Neopestalotiopsis hispanica* distinguished by yellow color with stone-like dots with black color, *Paecilomyces niveus.with* White to beige spreading colonies pattern. Figure 4 represents a photograph of isolated fungi.

Figure 4

Morphological characteristics of Fusarium equiseti, Paecilomyces niveus, Macrophomina tecta, Fusarium fujikuroi, and Neopestalotiopsis hispanica respectively From the top left on SDA media



Molecularly, DNA from different fungal isolates was effectively amplified by using ITS1, ITS4 primers targeting the conserved ITS fungi region, a nearly 550 bp amplicon band was visualized in gel electrophoresis, and sequencing in both directions, the consensus sequence was subjected to BLAST analysis against the NCBI GenBank database. The sequence showed the highest similarity to their correspondences.

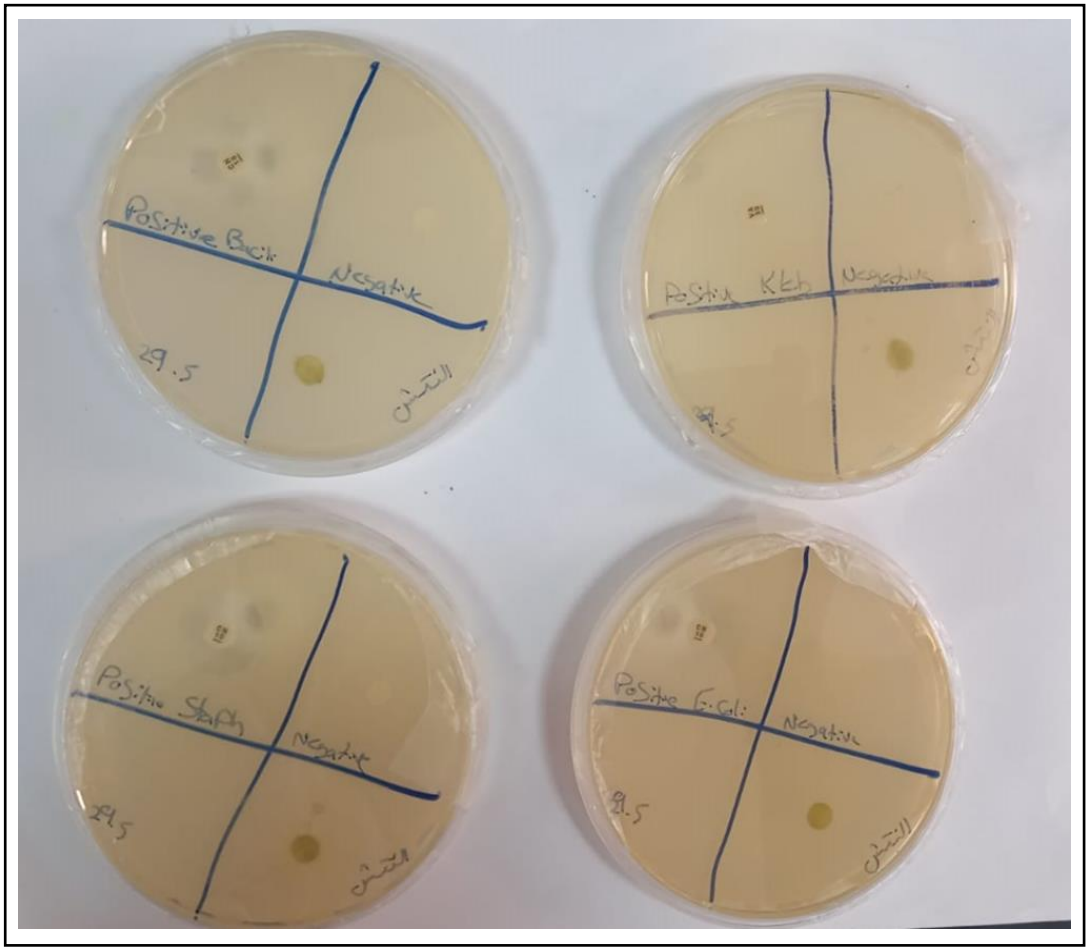
3.3 Antimicrobial Activity

3.3.1 Antibacterial Activity

S. spinosum fixed oil showed no noticeable activity against pathogenic bacteria (Figure 5).

Figure 5

Antibacterial activity of S. spinosum fixed oil disk diffusion methods against pathogenic bacteria included Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, and Bacillus cereus



- **Anti-phytopathogenic fungi**

The MIC values for *S. spinosum* fixed oil against eight types of phytopathogenic fungi were determined as seen in Figure 6 and reported in Table 6. Highest activity was against *Paecilomyces niveus* and *Penicillium expansum* with MIC value of 50µg/mL. Moreover, some phytopathogenic fungi such as *Alternaria alstroemeria* in addition to *Botrytis californica* reported limited susceptibility towards *S. spinosum* fixed oil, while *Fusarium foetens, Fusarium fujikuroi* and *Macrophomina tecta* showed no noticeable response.

Table 6

MIC values for S. spinosum fixed oil activity against phytopathogenic fungi

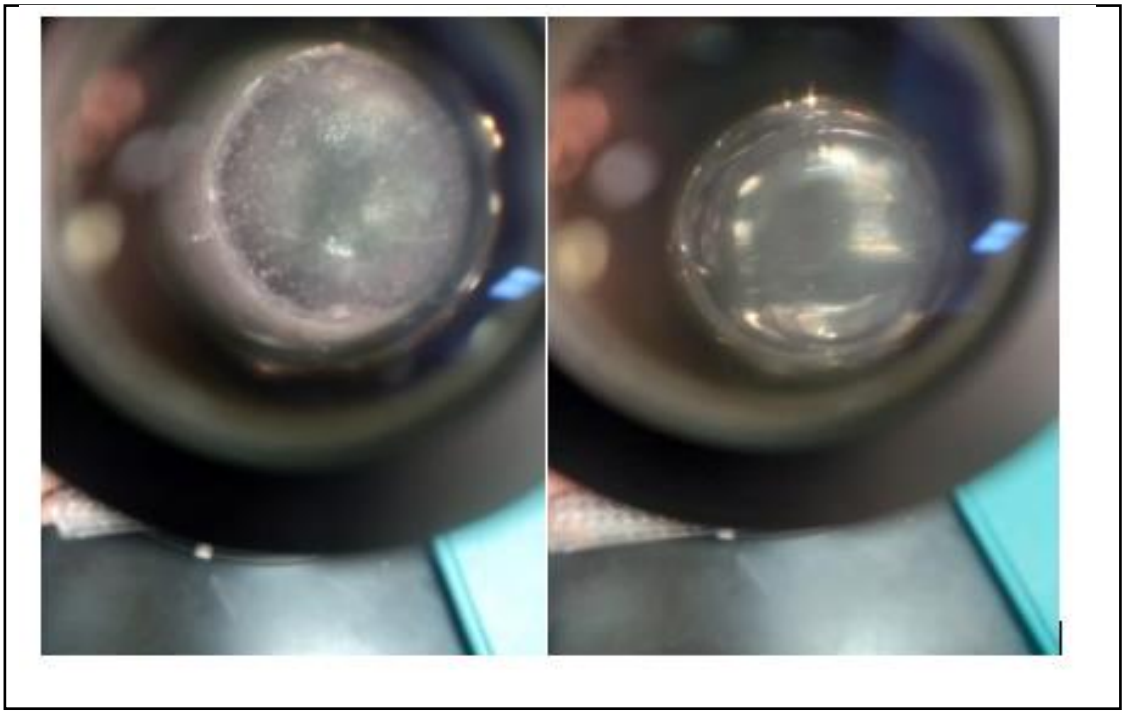
phytopathogenic fungi Name	MIC value (µg/mL)
Paecilomyces niveus	50
Penicillium expansum.	50
Alternaria alstroemeria	100
Botrytis californica	100
Fusarium equiseti	100
Fusarium foetens	Not Detected
Fusarium fujikuroi	Not Detected
Macrophomina tecta	Not Detected

Figure 6

MIC results of S. spinosum against phytopathogenic fungi

Figure 7

Positive fungi growth control on the left and negative growth control on the right under dissecting microscope

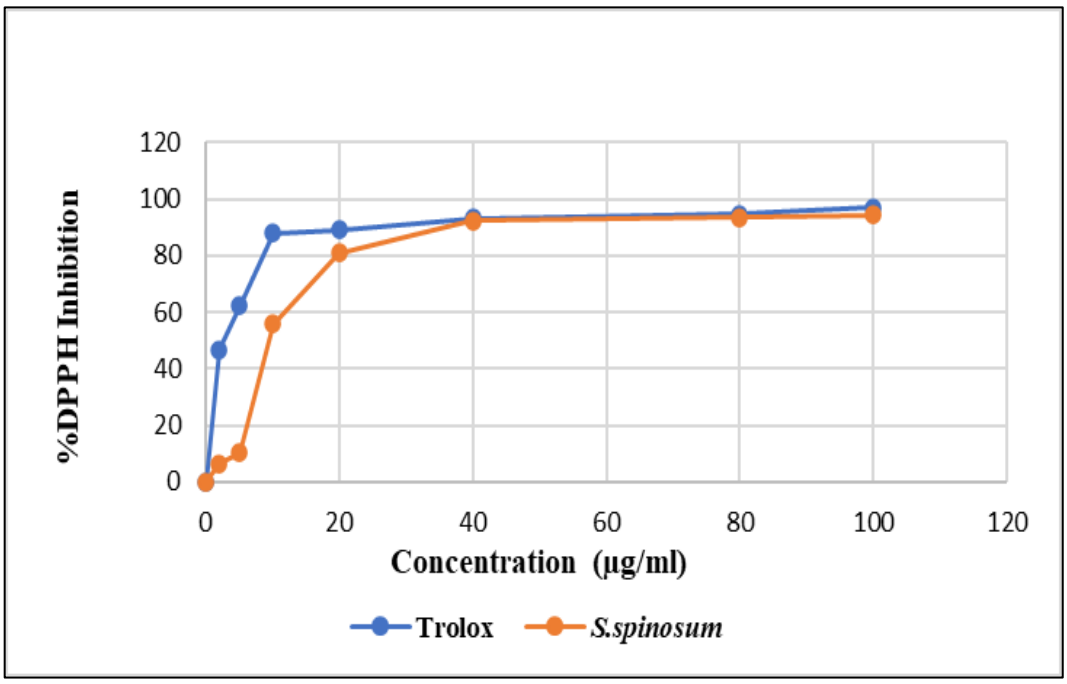


3.4 Antioxidant Activity

Free radical scavenging activity of *S. spinosum* seeds fixed oil was determined by %DPPH inhibition methods in Figure 8. Trolox was used as a standard reference for DPPH free radical activity. *S. spinosum* showed potent antioxidant potential with IC50 value equal to 10.581 μg/ml, compared to an IC50 value of 3.855 μg/ml Trolox.

Figure 8

Antioxidant activity for S. spinosum fixed oil

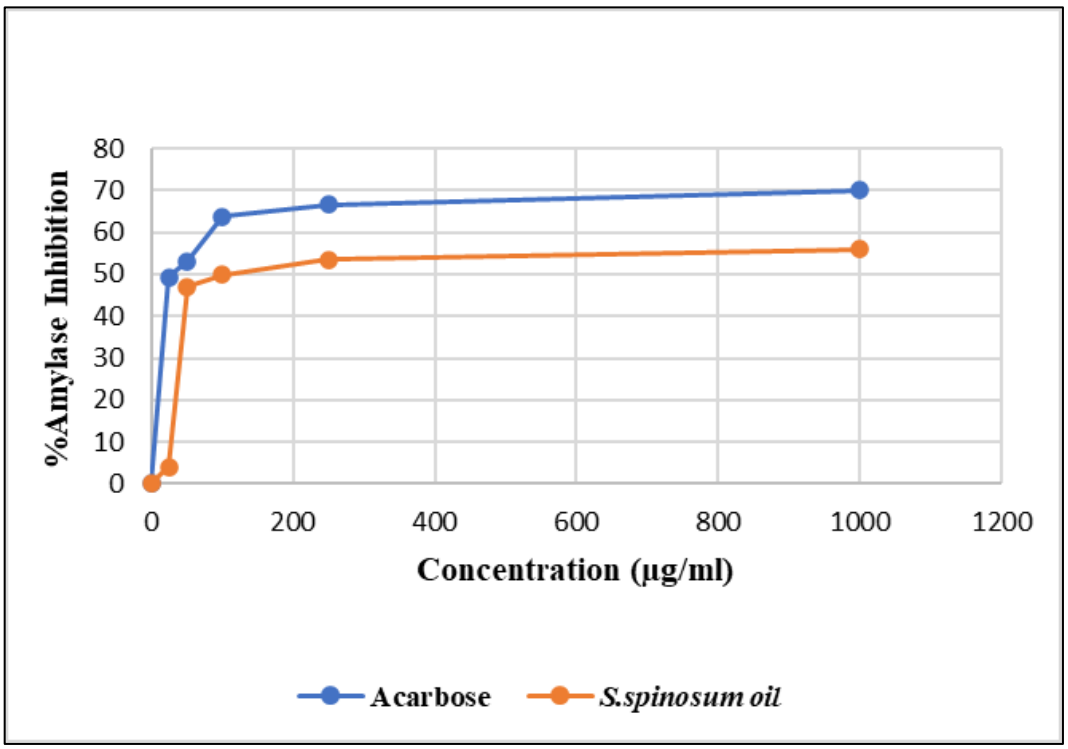


3.5 α-Amylase Inhibitory Activity

α-Amylase Inhibitory activity of fixed oil extracted from *S. spinosum* seeds was evaluated by porcine pancreatic α-amylase testing method in Figure 9. Acarbose was used as a control standard for α-Amylase Inhibitory activity. *S. spinosum* showed moderate to strong potential α-Amylase Inhibitory with IC50 value 222.40 µg/ml, against Acarbose with a 47.72µg/ml IC50 value.

Figure 9

α-Amylase inhibitory activity profile by acarbose and S. spinosum oil

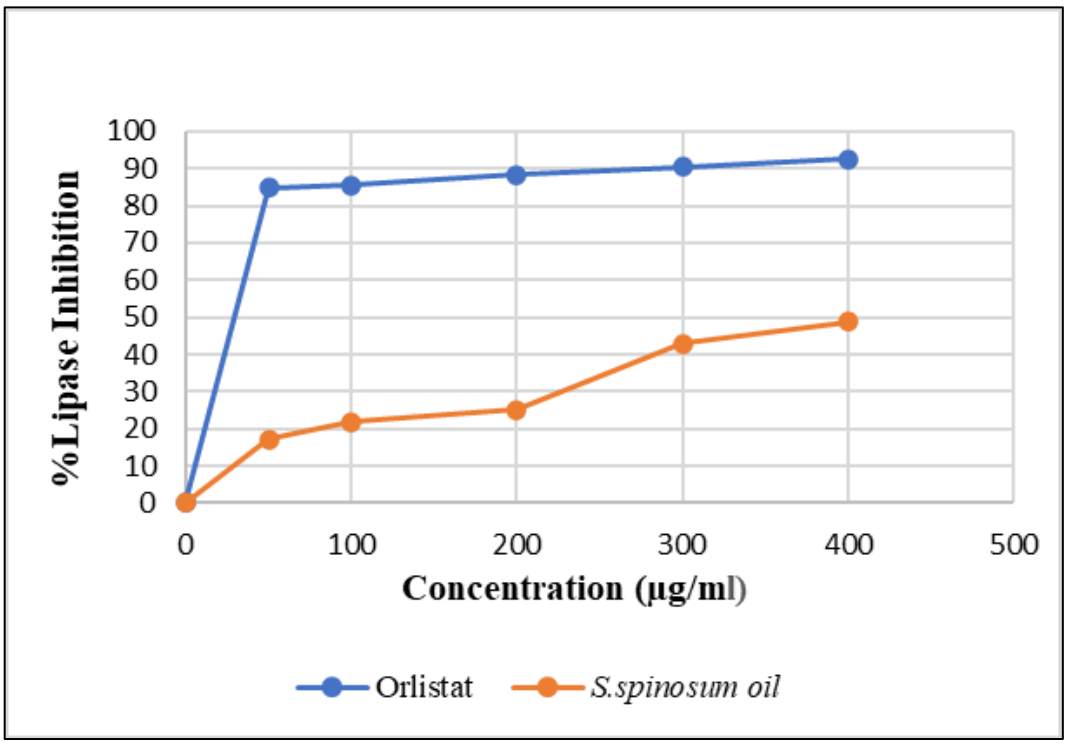


3.6 Lipase inhibition potential

The porcine pancreatic lipase inhibitory activity of *S. spinosum* oil was measured and shown in Figure 10. The findings provided that *S. spinosum* oil has no notable lipase inhibition activity against porcine pancreatic lipase enzyme $IC_{50} = 2022.1$ µg/ml in contrast with the orlistat standard $IC_{50} = 15.62$ µg/ml.

Figure 10

Porcine pancreatic lipase inhibitory activity of the standard and S. spinosum oil



Chapter Four

Discussion and Conclusion

4.1 Discussion

GS-MS technique is used to determine the phytochemical composition of the tested sample, based on comparing the mass spectra and retention time of analyzed phytochemicals with a reference library (90)**.** A study from Syria identified the phytochemical composition of *S. spinosum* roots essential oil. Fifteen phytochemicals were recognized, 99% of the oil was oxygenated sesquiterpenes, with 66.65 % elemol as the major component and the other represents eudesmol with 33.26 % (91). Another research focused on essential oil from *S. spinosum* roots, leaves and stems growing in the Turkish region, shows that the main composition were aldehydes with 51.6 %,42.5 % and 39.6 % respectively, more compounds were found in smaller proportion, such as Oxygenated sesquiterpenes and aromatic substances (92). However, no prior data available identified the phytochemical component of *S. spinosum* seeds fixed oil, this work presents for the first time the phytochemical composition of *S. spinosum* seeds fixed oil.

A composition of nine fatty acids were formed in the total *S. spinosum* seeds fixed oil. The major fatty acids were cis-11-Eicosenoic acid (37.48%), Linoleic acid (23.0%), Oleic acid (14.05%), Linolenic acid (12.80%), and Erucic acid (6.57%). While an insignificant portion of fatty acids was Palmitic acid (3.61%), Stearic acid (1.82%), Elaidic acid (0.40%), and Arachidic acid (0.21%). The provided data shows strong antidiabetic activity, which agrees with previous studies. For instance, the activity of alpha-glucosidase activity was reduced after being treated with the methanol and acetone extracts of *S. spinosum* (93).

Moreover, a study of *S. spinosum* root extract conducted by pancreatic β-cells demonstrated that the *S. spinosum* root extract can mimic the insulin effect by lowering glucose uptake and elevating insulin secretion (27)**.** In addition**,** *S. spinosum* extract documented that an enhancement in insulin sensitivity and insulin signaling pathway by phosphorylation of PKB pathway in high-fat diet-induced KK-Ay mice model (94). Erucic acid is a well-known antidiabetic effect working by enhancing insulin resistance by working on downregulation of peroxisome proliferator-activated receptor (PPAR),

which is recognized by its roles in insulin resistance and plays a major role in adipocyte development (95). Linoleic acid, unsaturated fatty acids contain antidiabetic activity, a recent study showed that increased linoleic acid consumption in the diet can decrease the risk association with diabetes mellitus type 2.

This relation can be explained due to unsaturated fatty acids can enhance cell membrane fluidity and increase insulin receptor binding, which promotes insulin sensitivity (96)**.** Free radical scavenging activity is a method used to determine antioxidant potential activity to certain substances. DPPH reagent is distinguished by its purple color, free radical stability molecule with maximal absorption at 512nm. When it reacts with hydrogen or electron doner substances purple color diminish, this resulting from by converting DPPH to 2,2-diphenyl-1-picryl hydrazine and lowering the absorbance reading. A Previous study on *S. spinosum* leaves extracts from Rammalah region, resulting in 70% ethanol leaves plant extract, revealed the strongest antioxidant activity arising from the ability to solubilize phenolic and antioxidant components, in contrast to water leaves extract, which shows weak antioxidant potential (97).

Pronounced antioxidant activity with IC50= 10.581µg/ml of *S. spinosum* seeds fixed oil obtained in this study aligns with literature data. Increased oil concentration is directly correlated with DPPH inhibition. Elevated levels of linoleic acid and linolenic acid in *S. spinosum* seeds fixed oil can explain the reliable antioxidant potential. Unsaturated linoleic acid and linoleic acid are well known and documented for their antioxidant activity (98).

Studies reported and confirmed results of the new isolated phytopathogenic plant, data from (99) mentioned the morphological characteristics of *Fusarium equiseta,* causing chilli wilt in Kashmir. While Malaysian Pineapple (*Ananas comosus*) suffers from fusariosis symptoms and which caused reduce in pineapple yield, after isolation and molecular identification the main cause was *Fusarium. Fujikuroi* (100). (101), and (102) also confirmed our finding with *Neopestalotiopsis hispanica* and *Paecilomyces niveus*, respectively.

Few studies mention antimicrobial potential of *S. spinosum*, a study from turkey done on *S. spinosum*, leaves, steams and roots methanol and hexane extracts reported and indicate that hexane leaves extract had antimicrobial activity regarding *Staphylococcus*

aureus and *Bacillus cereus*. While hexane stems showed activity against *S. aureus* only. Although, Methicillin Resistant *S. aureus* (MRSA), *S. aureus* and *Micrococcus luteus* were inhibited by the methanol extracts from stems and leaves. Continue from this study, Essential oil extracted from *S. spinosum* mentioned activity in opposite to *Candida krusei*, *candida. Parapsilosis*, and MRSA (92).

Our results demonstrated no significant antimicrobial efficacy reported of *S. spinosum* fixed seeds oil toward either Gram-positive or Gram-negative *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* no interesting inhibition activity observed by disk diffusion methods.

S. spinosum fixed seeds potential activity toward phytopathogenic fungi has been demonstrated for the first time, *Paecilomyces niveus* and *Penicillium expansum* with *an* MIC value 50 µg/mL. *Paecilomyces niveus* is a wound infection phytopathogenic fungi, and it inhabits soil and food products, its the ability to infect and spread within fruits (103). Postharvest disease of apples is widely associated with *Paecilomyces niveus*, causing crop damage and juice contamination, peaches, pears, citrus, and cherries are also affected by *Paecilomyces niveus* (104). Infected fruits show epidermal discoloration with white or brown while brown lesions with necrotic tissues are noticed in infected fruits (104). Although patulin, mycotoxin produced by *Paecilomyces niveus* is a potent mycotoxin that affects individual health and induces pathological problems involving, immunological responses, the digestive system, and neurological integrity (105). The main concern of the food industry and post harvested market is the contamination with *Paecilomyces niveus,* which leads to food spoilage due to the ability to produce heat-resistant ascospores to survive in high temperatures up to 85°C.

One of the main agricultural and economic global concerns is blue mold disease caused by *Penicillium expansum* infections, initially established by bruises or puncture in stem, the infected plants, particularly fruits revealed brown discoloration, blue- green hypha in fruits surface and soft tissue texture and finally decay, this costs millions of dollars as an economic loss (106). *Alternaria alstroemeria* in addition to *Botrytis californica* reported limited susceptibility against *S. spinosum* fixed oil, *Alternaria alstroemeria* with their wide range of growth environments, including soil, atmosphere, and living plants, is responsible of leaves dark spots leading to leaves defoliation (107). Grey mold disease is caused by *Botrytis californica*, a necrotrophic fungal pathogen responsible for

shoot blights and grey mold of diverse plant species, usually the infection starts from stem and spreads, dark coloration and grey mycelia appeared in the infected area in compared to the uninfected part, a fungal nest is formed by connected hypha allow to spreads the infection to the adjacent plant (108).

The term botryose" or grape cluster-like", referring to the structure of the conidiophores, has traditionally represented the key to morphological characteristics within the genus, the documented data revealed that the economic losses from *Botrytis* plant diseases cost from 10 to 100 billion USD yearly worldwide (109).

While *Fusarium foetens*, *Fusarium fujikuroi* and *Macrophomina tecta* showed no noticeable response stearic acid interferes with fungal mycelium of *Botrytis cinerea*, contributing vital inhibition in growth and notable changes in morphology and prancing pattern, which affect nutrient intake and oxygen (110)**.**

Chemical conventional fungicides are associated with their toxicity, and non-targeting binding sites contribute to their effect on non-specific organisms. For instance, the carboxylic acids functional group in chemical fungicides inhibit DNA topoisomerase II enzyme, which is an essential enzyme for cellular division and fungal replication, but this enzyme is present in prokaryotes which are considered as a threat to other beneficial microorganisms found in environments (111).

While streptomycin fungicides function by inhibiting protein synthesis the mechanism of action involves amino acid synthesis interfering with, streptomycin also has been reported of its function to prevent amino acid synthesis in Gram-negative bacteria *E. coli* (111).

More important, direct or indirect changes in microbiota impacts plant-associated microbial community structure and influence plant metabolic processes (111).

This agrees with GC-MS analysis of *S. spinosum* fixed seeds, which contain stearic acid. *S. spinosum* fixed oil from the seeds potential activity toward phytopathogenic fungi has been demonstrated for the first time, *Paecilomyces niveus* and *Penicillium expansum* L have been more sensitive to *S. spinosum* fixed seeds, while *Alternaria alstroemeria* , in addition to *Botrytis californica* reported less effectiveness regarding *S.*

spinosum fixed oil, while *Fusarium foetens, Fusarium fujikuroi,* and *Macrophomina tecta* showed no observable response.

S. spinosum fixed seeds show activity against porcine pancreatic lipase enzyme. Phytochemical composition differs in *S. spinosum* plants during four seasons: winter, summer, autumn, and spring. Also, the soil content and the interested plant parts play an important role in phytochemical content (112)**.** Environmental factors, including climate change, environmental concern, Soil nutrients, and water limitation, are essential environmental aspects of the concentration of secondary metabolites in plants (113)**.**

4.2 Conclusion and Recommendation

Data from this conducted study shows that *S. spinosum* fixed oil extracted from the plant seeds, with its olive -green color, slightly odder, and rich in fatty acids consists of nine fatty acids composing a total oil remarkable antioxidant activity compared to Trolox standard reference, strong inhibition activity against α-amylase enzyme. In contrast, no porcine pancreatic lipase activity was detected, although intermediate antimicrobial potential was reported. More importantly, the ability to isolate and identify by using molecular techniques in Palestine and determine the accurate phytopathogenic fungi was conducted.

To conclude, *S. spinosum* seeds fixed oil has an intense antioxidant potential and antidiabetic efficacy, but modest efficacy against antiobesity and antimicrobial activity. Our results highlighted the health promotion of *S. spinosum* extracted from plant seeds and its ability to be used in the pharmaceutical industry. More investigation should be conducted, focused on specific bioactive components, potential therapeutic doses, toxicity, and side effects. In like manner.

Conventional chemical agents used to control plant pathogens should be replaced with Plant derived products and their oils with their promising biological activity against several plant pathogens, the chemical hazardous toxins and their ability to contaminates soil, water and even health individual along with nonspecific targeting activity of synthetic fungicides highlights the importance to discovered plant derived components, more effective, specific targeting, less hazardous, and more environmentally friendly.

Additional research aimed at phytopathogenic fungi should be studied, due to its importance in the agricultural sectors, which reflects food security and human health.

Additionally, the identification and discovery of new biologically based agents for plant disease management is highly recommended to provide highly effective and eco-friendly replacements to the conventional synthetic fungicides.

List of Abbreviations

Abbreviation	Meaning
AMR	Antimicrobial Resistance
ATCC	American Type Culture Collection
CFU	Colony forming unit
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNSA	Dinitrosalicylic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FAMEs	Fatty acid methyl esters
GC-MS	Gas Chromatography-Mass Spectrometry
GLP	Glucaagon-like peptide receptor
HPLC	High-Performance Liquid Chromatography
IC50	Half-maximal inhibitory concentration
MHA	Mueller–Hinton agar
MHB	Mueller–Hinton broth
MIC	Minimal inhibitory concentration
MRSA	Methicillin-resistant Staphylococcus aureus
PCR	Polymerase Chain Reaction
PKB	Protein Kinase B
PNPB	p-Nitrophenyl butyrate
PPAR	Peroxisome proliferator-activated receptor
PTEN	Phosphatase and tensin homolog
ROS	Reactive oxygen species
RPMI 1640	Roswell Park Memorial Institute
SDA	Sabouraud's Dextrose Agar
TBE	Tris-Borate buffer
Tris-HCl	Tromethamine –HCl
WHO	World Health Organization

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جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

التركيب الكيميائي النباتي والانشطة المضادة لمرض السكر والسمنة ومضادات الاكسدة والمضادات الميكروبية لزيت نبات ساركوبوتريوم سبينيوسيوم العطري

إعداد

ايمان محمد السعيد

إشراف

أ. د. رائد الكوني

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في التقنيات الحيوية والعلوم الحياتية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2025

التركيب الكيميائي النباتي والانشطة المضادة لمرض السكر والسمنة ومضادات الاكسدة والمضادات الميكروبية لزيت نبات ساركوبوتريوم سبينيوسيوم العطري

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إشراف

أ. د. رائد الكوني

الملخص

السمنة، مرض السكري، الاجهاد التأكسدي والكائنات الدقيقة المقاومة للادوية المتعددة هي تحديات تؤثر على حياة الافراد و النظام الصحي. مما دفع العلماء الى ايجاد حلول جديدة من اجل منع انتشار هذه المشكلات وتعد النباتات الطبية عرفت تقليديا بمعالجتها الامراض ومنع حدوثها.

الهدف من هذه الدراسة هو دراسة نشاط المضاد لإنزيم lipase البنكرياسي، والمضاد لإنزيم amylase البنكرياسي، والنشاط المضاد للأكسدة، والفعالية البيولوجية المضادة للبكتيريا الممرِضة للإنسان والفطريات المسببة لأمراض النبات لزيت بذور نبات *Sarcopoterium spinosum*.تحليل المكونات الكيميائية النباتية المكونة للزيت *S. spinosum* بواسطة تقنيات الكروماتوغرافيا الغازية المتحدة مع مطياف الكتلة كما تم تعريف الفطريات الممرضة للنبات باستخدام تقنيات بيولوجية جزيئية.

الطريقة: تم تحليل النشاط المضاد للأكسدة بواسطة اختبار الجذور الحرة الممتصة بواسطة مادة DPPH، وتم استخدام Trolox كمرجع.

أما بالنسبة للنشاط المضاد لإنزيم Lipase فقد تم قياس مقدار تحلل p-nitrophenolate بواسطة nitrophenyl butyrate (PNPB)، حيث تم حساب نشاط إنزيم lipase البنكرياسي باستخدام جهاز قياس الطيف عند طول موجي 405 نانوميتر. وتم اسنخدام دواء Orlistat كمرجع.

كما تم حساب نشاط زيت *S .spinosum* المضاد لإنزيم Amylase من خلال DNSA وتم استخدام Acarbose كمرجع.

تم دراسة الفعالية البيولوجية المضاد ضد البكتيريا الممرضة للإنسان والفطريات الممرضة للنبات باستخدام طريقة Disk diffusion للبكتيريا، Microdilution method بمساعدة المجهر التشريحي للفطريات الممرضة للنبات.

تم تحديد الفطريات الممرضة للنبات باستخدام تقنية تفاعل البوليميراز المتسلسل باستخدام بادئات ITS .

النتائج: أظهر زيت *S. spinosum*، والذي يتكون من تسعة أحماض دهنية، نشاطًا قويًا كمضاد للأكسدة بقيمة IC50 = 10.581 ميكروغرام/مل، كما أظهر قدرة ملحوظة كمثبط لإنزيم α-Amylase بقيمة IC50 = 222.40 ميكروغرام/مل، ولكنه لم يظهر أي نشاط تجاه إنزيم Lipase البنكرياسي.

بالإضافة إلى ذلك، لم يُظهر زيت *S. spinosum* نشاطًا ملحوظًا ضد البكتيريا، لكنه أظهر فعالية ضد بعض الفطريات النباتية المهمة مثل *Paecilomyces niveus*، *Penicillium expansum*، *Alternaria alstroemeria*، *Botrytis californica*، و*Fusarium equiseti*. كما تم تحديد الفطريات الممرضة للنبات بدقة باستخدام الطرق الجزيئية.

الاستنتاج: يتمتع الزيت المستخلص من بذور *Sarcopoterium spinosum* بخصائص مضاد للأكسدة قوية، ويُعد مبيدًا فطريًا صديقًا للبيئة ضد الفطريات الممرضة للنبات. كما أن التقنيات الجزيئية فعّالة في تحديد الكائنات الدقيقة المسؤولة عن أمراض النباتات بدقة.

الكلمات المفتاحية: زيت *S. spinosum*؛ الفطريات الممرضة للنبات؛ مضادات الأكسدة؛ التركيب الكيميائي النباتي؛ مضاد لإنزيم Lipase ؛ مضاد لإنزيم α-amylase.