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Research article

Assessment of antioxidant, antimicrobial, and anticancer activities of *Sisymbrium officinale* plant extract

Mahmoud Khalid [a], Mousa Amayreh [b], Saadi Sanduka [a], Zaidoun Salah [a], Fuad Al-Rimawi [c], Ghassab M. Al-Mazaideh [d], Abdulkareem A. Alanezi [e], Fadel Wedian [f,*], Fawaz Alasmari [g], Mohammed Helmy Faris Shalayel [h]

[a] *Al Quds-Bard College, Al-Quds University, Abu Dies, Jerusalem, Palestine*
[b] *Department of Chemistry, Palestine Technical University-Kadoorie, Tulkarm, Palestine*
[c] *Department of Chemistry, Faculty of Science and Technology, Al-Quds University, Jerusalem, Palestine*
[d] *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hafr Al-Batin, Hafr Al-Batin, Saudi Arabia*
[e] *Department of Pharmaceutics, College of Pharmacy, University of Hafr Al Batin, Hafr Al Batin, Saudi Arabia*
[f] *Department of Chemistry, Faculty of Science, Yarmouk University, P.O. Box 560, Irbid, 22163, Jordan*
[g] *Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*
[h] *Professor of Biochemistry and Consultant Dermatologist, University of Hafr Al-Batin, Saudi Arabia*

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ABSTRACT

The most efficient and safe source of medications is the natural and traditional medications which are produced from plants and herbs. In this study, *Sisymbrium officinale* (*S. officinale*) was tested to explore its total phenolic and flavonoids contents. Antioxidant, antimicrobial, and anticancer activities were assessed as well. *S. officinale* was bought from a local Palestinian market, air-dried, and extracted with 99% ethanol with the aid of ultrasonication. The extract was tested on three types of bacteria using well diffusion method. The anti-microbial testing included three different types of bacteria, two gram-positive bacteria, *Streptococcus* and *Staphylococcus* and *E. coli* as a gram-negative bacterium. Antioxidant activity of the plant extract was conducted using DPPH method, while total phenolic and flavonoids contents were performed using a well-known assay chemical method. Anticancer activity of the extract was conducted against two cancer cell lines (breast (MCF7) and colon (HCT116) cancer cell lines). Results showed that the extract is rich polyphenolic and flavonoids and has strong antioxidant activity reflected by inhibition of free radicals (DPPH) (193.7 ± 3.4). The plant extract showed also strong antimicrobial activity against both *E. coli* and *Streptococcus* bacteria with of inhibition of 10 and 14 mm respectively. The extract of this plant also showed anticancer activity (about 6%) against MCF7 (breast cancer cell line).

1. Introduction

Traditional medicine is known as the knowledge, skills, and practices based on previously done theories and experiments on maintaining health and finding treatments for physical and mental illness through the use of herbs [1]. The Medicinal plant is the use of plants in herbalism and for medicinal purposes. Plants have been a source for medical use for so many years. Herbal medicine varies from using traditional to popular medicines that were extracted from herbs and plants. However, herbal medicine is evaluated according to its safety, efficiency, and efficacy. Therefore, every extracted herb must be investigated and studied professionally before putting it to public use.

Chemical drugs have so many toxic side effects, the side effects of the chemotherapeutic drugs in the long-term cause serious health complications, also many types of bacteria start developing resistance against synthetic antibiotics drugs, new treatments should be developed and investigated instead, the most efficient and safe source of medications is the natural and traditional medications which are produced from plants and herbs. A medicinal plant is any plant that contains one or more substances in its organs that might be used for therapeutic purposes, or investigated in the synthesis of useful drugs [2]. Plant extracts can be an alternative to manufactured drugs and act as an antimicrobial agent such as; *Artemisia annua* which has antimalarial activity [3]. Medicinal plants can play vital roles in the prevention of diseases. Several studies were

* Corresponding author.
E-mail address: alwedian@yu.edu.jo (F. Wedian).

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done on different plants and the results were promising. *Sisymbrium officinale* (commonly known as hedge mustard) is a medical and herbal plant that was used since the Greeks' age in medicine (Figure 1). It can also be used in cuisines such as salads and mustard sauces. However, *S. officinale* was not only used for culinary preparations. It was mainly used as an herb in traditional medicine in southwest Asia, Arabia, northwestern India, and northeast Africa. It helped in treating respiratory tract diseases, such as pharyngitis, laryngitis, cough, aphonia, common cold, sore throat, and other asthma [4].

S. officinale is an annual or biennial herbaceous plant from the family Brassica Mediterranean area [5]. It commonly grows on disturbed sites such as roadsides, wastelands, disturbed habitats, and field margins [6]. The wild plants were always important in the traditional medicine history in the Mediterranean region. Some researchers tested the anti-inflammatory antioxidant and antimicrobial activities of *S. officinale* [7]. However, despite its traditional use, this plant was not investigated deeply.

S. officinale has a single stem erect branched at right angles, and opaque green or purplish [4]. This stem showed antimicrobial, muscle relaxant, antimutagenic, and antioxidant activities in some researches [8]. *S. officinale* starts to emerge from late autumn until early spring [9]. It has been used for many centuries but only has been studied and explored scientifically in the last decades. It possesses interesting therapeutic properties, especially for throat diseases and that is why it is commonly called “the herb of singers".

Humans are always exposed to many radiations and causing oxidative damage to DNA, protein, and lipids and thus causing chromosomal mutations. For example oxidative stress activities may lead to other diseases such as cancer, diabetes, and even neurological and cardiovascular diseases. *S. officinale* was used in many experiments that proved its antioxidant activity. *S. officinale* can block biochemical reactions which potentially leads to genetic material damage. These antioxidative activities can support the consumption of the plant by people who work in highly polluted environments or even smokers [10].

The increased use of antimicrobial agents in clinical practices leads to bacterial resistance against antibiotic agents, where this mechanism is developed by bacteria to promote such resistance to survive [11]. However, a high MIC above the susceptibility threshold to an antibiotic will be reported as resistance. Bacteria also may develop resistance through gaining the resistance genes from other bacteria or in developing a mutation that results in the reduction of antibiotic component [9].

Cancer is widely cured by targeted therapy such as radiation and chemotherapy. However, the side effects of chemotherapy in the long-term cause serious concerns [12]. It may affect the patient by causing nausea and vomiting through the shot-run, and ulceration, anorexia, malabsorption, anemia, and sepsis in the long run [13]. On the other hand, the overuse of antibiotics has been a serious issue the past decade causing microbial, and even cancerous diseases to be resistant to drugs [14]. Therefore, innovative and alternative therapeutic strategies must be found through investigating the use of herbs to reduce the use of chemotherapy and antibiotics.

Despite the significance of *Sisymbrium officinale* being a natural herbal plant and its use to cure many respiratory tract diseases, its antioxidant and biological activities are not thoroughly investigated. The objectives of this work are therefore to investigate antioxidant, antimicrobial and anticancer activities of *S. officinale* as well as its total phenolic and flavonoids contents.

2. Materials and methods

2.1. Plant material

The plant *S. officinale* was bought from the local market in Jerusalem Palestine in January 2021. The plant was air-dried (Figure 1) in a shade room for 16 days and crushed to obtain a fine powder. The dried plant material was stored in a plastic bag in the refrigerator, until extraction.

2.2. Extraction

About 3 g of dried *S. officinale* leaves were soaked in 50 ml of ethanol (99%) and placed in the ultrasonicater for 90 min. The mixture was then filtered by passing through Whatman filter paper. After that, the ethanol solvent was evaporated using rotary evaporator at reduced pressure. The obtained viscous crude extract was collected and transferred into small bottles and stored in the fridge to be used for analysis. For analysis, the extract was dissolved in a minimum amount of ethanol to get a concentration of 1.0 mg per ml.

2.3. Determination of total phenolic content

A volume of 1.8 ml of Folin-Ciocalteu (5ml of Folin-Ciocalteu reagent were diluted with 50 ml distilled water) was added to 50µl of sample extract. Then 1.2 ml of sodium carbonate (7.5%) was added after 5 min. The mixture was left for 120 min and absorbance was measured at 760 nm. Calibration curve using gallic acid was used for total phenolic content determination, and the results of total phenolic content in the extracts were expressed as mg gallic acid per gram of crude extract.

2.4. Determination of total flavonoid content

To 100 µl of crude extract, 4 ml of distilled water, 0.3 ml of 10% $AlCl_3$, and 0.3 ml of 5%$NaNO_2$ were added. After 6 min, 2 ml of 1 N NaOH, and 2.5 ml of distilled water were added to the mixture. The absorbance was then measured at 510 nm. Calibration curve using Catechin was used for total flavonoids content determination, and the results of total flavonoids content in the extracts were expressed as mg catechin per gram of crude extract.

2.5. Antioxidant activity (free radical scavenging activity using DPPH)

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) assay is focused on the calculation of antioxidants' scavenging capacity against the stable radical DPPH. 4 ml aliquot of 0.0634 mM of DPPH solution in methanol was mixed with aliquots of extract (50 µL) at several concentrations (0.1–2 mg/ml), then incubated at 25 °C for 30 min in the dark area. A positive standard using vitamin C was prepared by the same procedure. The absorbance of the different samples and standards was

Figure 1. *Sisymbrium officinale*, Hedge Mustard leaves (fresh) and dried plant.

measured at 515 nm. The scavenging ability of DPPH radicals was assessed according to scavenging activity (%) = Abs (Control) – Abs (Sample)/Abs (Control) × 100, where Abs control is the absorbance of the control (it is containing all reagents except the plant extract), and Abs (sample) is the absorbance of the tested plant extract. The extract concentration that gives 50% inhibition (IC_{50}) is calculated based on the plot of inhibition (%) against extract concentration and compared with the IC_{50} of vitamin C value as a positive control.

2.6. HPLC analysis

The HPLC analysis of the extracts was conducted on the ODS column of Waters (XBridge, 4.6 ID x 150 mm, 5 μm). The mobile phase is a mixture of water (0.5 percent) acetic acid (solvent A) and acetonitrile (solvent B) (80:20, v/v). 100% (solvent A) decreased to 70% (solvent A) in 40 min, then to 40% (solvent A) in 20 min and eventually to 10% (solvent A) in 2 min and would remain there for 6 min and then return to initial conditions in 2 min. The HPLC system shall be balanced with the initial acidic mobile water phase (solvent A) for 7 min before the next sample is injected. With a 0.45 μm PTFE filter, all samples were filtered. The range of PDA wavelengths is 210–500 nm. Flow rate is 1 ml/min. The volume of injection is 20 μL and the temperature of the column is 25 °C.

2.7. Antimicrobial activity using well diffusion method

The well diffusion method was used to test the antimicrobial activity of the *S. officinale* extracts against three different types of bacteria, two gram-positive bacterium (*Streptococcus,* and *Staphylococcus aureus)* and 1-g negative bacteria (*E. coli)*. Müller-Hinton agar (Oxoid, UK) was prepared and poured into different sterilized petri dishes, the bacterial dilutions were prepared using the 0.5 McFarland turbidity standard and streaked separately on different agar plates. DMSO was used as negative control against the bacterial cell lines. Wells were made in the agar plates using aseptic techniques in order to add the *S. officinale* plant extract. The petri dishes were then incubated at 37 °C for 24 h, before measuring the zone of inhibition.

2.8. Anti-cancer activity

Anti-cancer activity was performed using two types of cancer cell lines, the breast (MCF7) and colon (HCT116) cancer cell lines. The cancer cells were cultured in RPMI media and incubated for 24 h before the treatment with the *S. officinale* plant extract. 100μl of the extracts were diluted with DMSO and then added separately at four different concentrations (50 μg/ml, 100 μg/ml, 200 μg/ml, and 400 μg/ml), in addition to a negative control of DMSO. After the treatment, the cells were incubated for 24, and 48 h before assessing the effect of the different concentrations of the plant extract on the different cancer cell lines.

2.9. Statistical analysis

All data analysis were performed using the Microsoft excel 2013. Results were reported as mean of three samples ±standard deviation (SD).

3. Results and discussion

3.1. Total phenolic contents (TPC)

Total phenolic content of the *S. officinale* extract was determined using Folin method using calibration curve of different concentrations of gallic acid and the results are expressed as mg gallic acid per gram of crude extract (Table 1). TPC of *S. officinale* plant extracts when extracted with ethanol was found to be 101.3 ± 2.4 mg/g. These results show that the plant ethanolic extract is rich in polyphenolic compounds.

3.2. Total flavonoid content (TFC)

Total flavonoids in the extracts were determined using aluminum chloride method using calibration curve of different concentrations of catechin and the results are expressed as mg catechin per gram of crude extract. TFC for the plant material, when extracted with ethanol extract, was found to be 29.3 ± 1.2 mg catechin/g, which shows that the plant is rich in flavonoids (Table 1).

3.3. Antioxidant activity

An antioxidant is a term that is used for a chemical that prevents oxygen consumption. However, antioxidants act as a radical scavenger, electron donor, hydrogen donor, peroxide decomposer, enzyme inhibitor, synergist, and metal chelating agent [15]. Free radicals and oxidants play a dual role since they can be harmful and helpful to the body. They could be produced either in situ from normal cell metabolism or ex-situ from pollution, smoking, radiation, and medications. When an overload of free radicals cannot be destroyed it would be oxidative stress. Oxidative stress may cause many illnesses such as autoimmune disorders, cancer, aging, cardiovascular and neurodegenerative diseases. However, the body counteracts oxidative stress through several mechanisms which are either naturally produced in situ or ex situ gained from food or supplements [15].

Antioxidant Activity accounts for the availability of antioxidants like phenolic compounds and flavonoids as efficient oxygen radical scavengers. Phenolic antioxidant activity is primarily due to their redox properties which make them serve as reduction agents, donors of hydrogen and singlet oxygen quenchers. Results showed that the plant extract has strong antioxidant activity reflected by DPPH test (193.7 ± 3.4 μmol Vitamin C/g) which also implies that this extract is rich with antioxidants reflected by inhibition of the free radical DPPH. Results were expressed as μmol vitamin C per gram extract. Different concentrations of Vitamin C were prepared for the calibration curve.

The % inhibition of DPPH was also calculated. Results showed that the DPPH radical scavenging activity increased in a concentration-dependent manner at 3–30 μg/mL with 8–74% inhibition of DPPH (Figure 2). 20 μg/ml *S. officinale* extract was associated with 50% inhibition of the DPPH radical, whereas the ascorbic acid (as a reference) was associated with a 50% inhibition of the DPPH radical, at 6.2 μg/ml concentration.

3.4. HPLC-PDA profiles of the extracts

Figure 3 shows the chromatogram of the crude extract of *S. officinale* extract at 340 nm. This wavelength was selected because the major peaks showed maximum absorption at this wavelength. The eluted compounds were detected in the range of 3-5- and 10-25-minutes indicating compounds with different polarities.

3.5. Antimicrobial activity

In order to test the antimicrobial activity of *S. officinale* extract, a well diffusion method was used. Results showed strong antibacterial activity of *S. officinale* extracts against *E. coli* and *Streptococcus* with 10 mm, and 14 mm zone of inhibition, respectively (Figure 4). On the other hand, no activity was observed for *S. officinale* plant extract against *Staphylococcus* bacteria.

Table 1. Total phenolic and flavonoids contents, and antioxidant activity of ethanolic extracts of *S. officinale*.

Concentration of extract	Total phenolic content (mg gallic acid/g)	Total flavonoids content (mg ruting/g)	Antioxidant activity (μmol Vitamin C\g)
0.2 mg/ml	101.3 ± 2.4	29.3 ± 1.2	193.7 ± 3.4

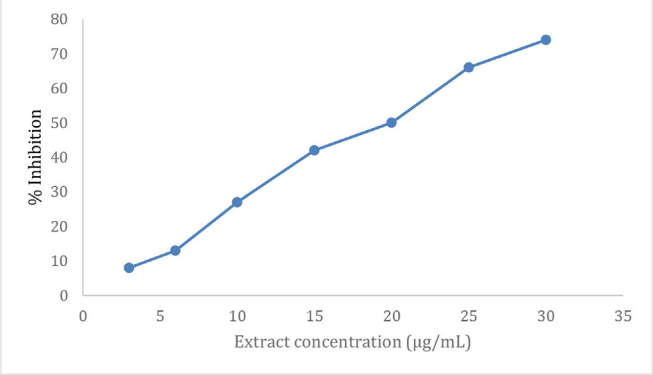

Figure 2. Inhibition (%) of DPPH against extract concentration of *S. officinale.*

3.6. Anticancer activity

Anticancer activity was noticed at 100µL of the plant extract on the MCF7 (breast cancer cells) with 6% cancer cell death (Figure 5). However, no results were shown on the 50µL, 200 µL, or 400 µL concentrations. No anticancer results were shown on the HCT116 (colon cancer cells) at all concentrations studied of the plant extract. Negative control using DMSO was used for the two cancer cell lines investigated in this study, where results show no anticancer activity against these two cancer cell lines.

4. Discussion

The tests of total phenolic content, flavonoids, and antioxidant activity of ethanolic extracts of dried *S. officinale* prove that there are medicinally active constituents in the studied ethanolic extract of *S. officinale*. It has been observed that the plant has a good antioxidant activity, and the extract is rich in flavonoids and phenolic compounds. Additionally, the results of the HPLC were shown in the chromatogram of the *S. officinale* plant extracts at 340 nm, which revealed mixed polarities of compounds in the plant's extract.

The antioxidant properties of the plant *S. officinale* extracts might be connected to the chemical composition which includes bioactive phytochemicals, high levels of phenolic acids, and flavonoids. *S. officinale* could be considered as a good source of antioxidants that will help in the prevention of many diseases that are caused by oxidative stress e.g.

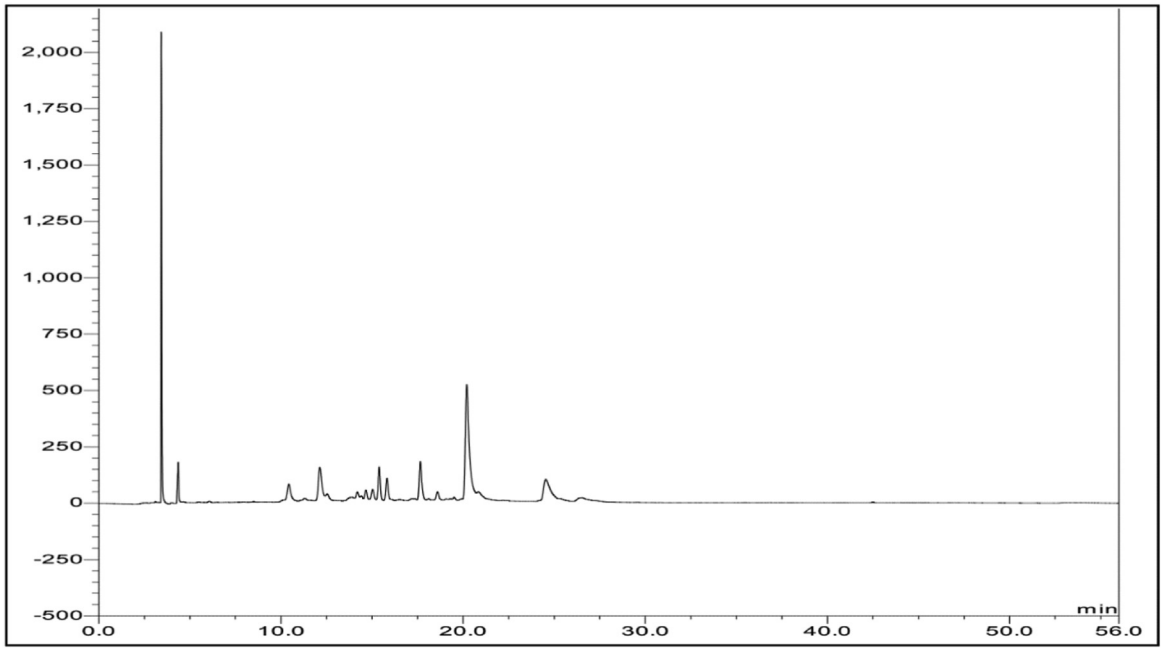

Figure 3. HPLC-PDA chromatogram of crude extract of *S. officinale* at 340 nm.

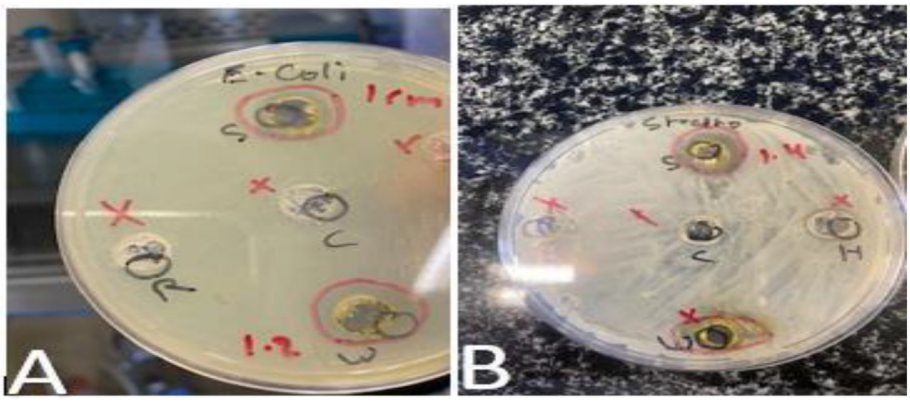

Figure 4. Antimicrobial activity of *S. officinale* (A): the plate of *E. coli* bacteria (S) shows (10 mm) zone of inhibition. (B): the plate of *Streptococcus* bacteria (S) shows (14 mm) zones of inhibition.

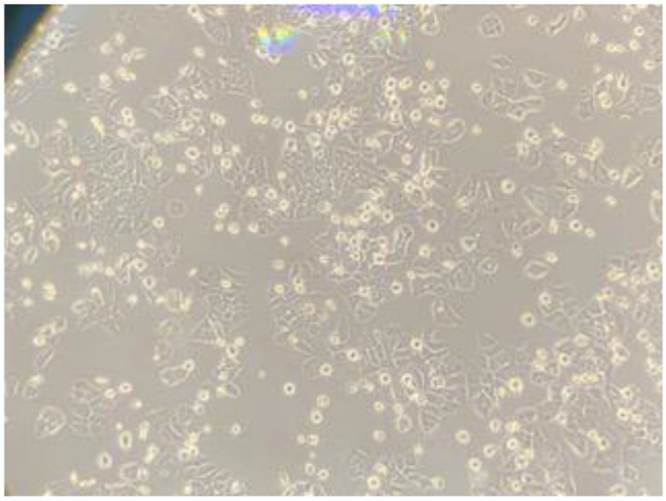

Figure 5. Anticancer activity of *S. officinale* on MCF7 (breast cancer cells) showing 6% of breast cancer cell death.

diabetes mellitus, carcinomas, neurodegenerative disorders and cardiovascular diseases.

Recently, many studies were done about phenolic and flavonoid-rich natural diets with antioxidants activities. Natural phenolic and flavonoid compounds hold an aromatic ring bearing with at least one hydroxyl group for this reason it is considered as a plant secondary metabolite [16]. The phenolic compounds are considered a good electron donor because their hydroxyl group can contribute to antioxidant actions directly. According to previous investigations phenolic compounds prevent oxidative disease burden, exhibit free radical inhibition, and peroxide decomposition [16]. Recent studies have shown that the consumption of leafy plant vegetables that contain phenolic and flavonoid compounds with potent antioxidant activity is associated with lowering the incidence of cancer, cardiovascular diseases, diabetes, and neurodegenerative diseases [17].

Antimicrobial activity has shown strong activity against *E. coli,* and *Streptococcus bacteria,* but no activity against *Staphylococcus* bacteria. This result shows the selectivity of this extract against 1-g positive bacteria (*Streptococcus bacteria*) and gram-negative bacteria (*E. coli*). Previous studies showed antimicrobial activity for both gram-positive and gram-negative bacteria and especially for *E. coli* bacteria [18]. The well diffusion method was done in this experiment using different concentrations of the *S. officinale* extract. More investigations should be done for using the *S. officinale* plant as an antimicrobial agent. *S. officinale* plant extracts have been also used for microbial infections and as a source of antioxidants. Since pathogenic microorganisms have been building resistance to many antibiotics and antimicrobial medications, new drug discovery is needed. Ethyl acetate extracts of *S. officinale* had also a good antimicrobial activity [18, 19].

The anticancer activity was shown for the breast cancer cells on a concentration of 100μL of the plant extraction. Previous studies revealed the anticancer activity of the *S. officinale* plant extract [20]. The *S. officinale* plant extract is promising for the anticancer activities since it showed a 6% of cell death when it was incubated for 24 hrs, after the addition of 100μL of the plant extract.

5. Conclusion

Traditional medicine is widely used to cure diverse diseases, and now researchers are exploiting it in new research and medical drug synthesis. *S. officinale* was used in traditional medicine for wounds and some diseases for a long time. However, serious issues have been evolving lately such as bacteria gaining resistance against antibiotics. Therefore, scientists were encouraged to look for new medicine. Our research about the *S. officinale* plant through our experiments and data analysis suggests that the leaf part of the plant can be used as an antioxidant, anti-bacterial, and anticancer agent. The completion of this study provided further information about the antioxidant, antimicrobial, and anticancer activity of *S. officinale* in particular. We recommend further investigations on the leaves of *S. officinale* which has shown a potential anticancer activity by causing apoptosis to stop cell-proliferation. Also, antioxidant activity was observed highly in this research. The *S. officinale* has shown also an antimicrobial activity against the *E. coli* and the *Streptococcus* bacteria.

Declarations

Author contribution statement

Mahmoud Khalid; Mousa Amayreh; Saadi Sanduka: Performed the experiments; Wrote the paper.

Zaidoun Salah: Analyzed and interpreted the data; Wrote the paper.

Fuad Al-Rimawi; Abdulkareem A. Alanezi: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ghassab M. Al-Mazaideh; Fadel Wedian: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mohammed Helmy Faris Shalayel; Fawaz Alasmari: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

The authors are unable or have chosen not to specify which data has been used.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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