

RESEARCH

Open Access



# Phytochemical analysis, antioxidant capacities, and in vitro biological activities of the extract of seed coat as by-products of pea

Hanaa S. S. Gazwi<sup>1\*</sup>, Maha O. A. Omar<sup>2</sup> and Magda E. Mahmoud<sup>1</sup>

## Abstract

Converting seed coat peas (hulls) (SCP) into beneficial products provides a solution for waste treatment. This study aimed to investigate the phytoconstituents and biological activities of SCP extract. Phytochemical screening, total bioactive compounds, and GC–MS analysis were evaluated. Then, the antioxidant, antibacterial, anticancer, and antiviral activities of SCP extract were determined. The results demonstrate that SCP extract has bioactive compounds such as carbohydrates ( $29.53 \pm 4.23$  mg/mL), protein ( $0.24 \pm 0.02$  mg/mL), phenolics ( $27.04 \pm 0.94$  mg GAE/g extract), and flavonoids ( $17.19 \pm 0.48$  mg QE/g extract). The existence of more than 16 substances was determined using GC–MS analysis. The extract showed potential antioxidant activities, with the maximum activity seen for extract ( $IC_{50} \mu\text{g/mL}$ ) =  $79.16 \pm 1.77$  for DPPH,  $67.40 \pm 5.20$  for ORAC, and  $61.22 \pm 4.81$  for ABTS assays. The SCP extract showed potent antimicrobial activity against four gram-positive bacteria (*Bacillus cereus*, *Streptomyces sp.*, *Staphylococcus aureus*, and *Salmonella sp.*) and two gram-negative bacteria (*Escherichia coli* and *Pseudomonas sp.*). SCP extract exhibited potential anticancer activity against lymphoma U937 and leukemic cells (THP1). The extract exhibited potential antiviral activity, with a selectivity index (SI) equal to 11.30 and 18.40 against herpes simplex-II (HSV-2) and adenovirus (Ad7), respectively. The results demonstrate more accurate information about peas by-products' chemical and antioxidant activities in various applications. The chemical components of peas by-products were found to have an in vitro antioxidant, antibacterial, and antiviral activity against leukemia and lymphoma.

**Keywords** Seed coat pea, Antioxidant, Antimicrobial, Antiviral, Leukemia, Lymphoma

## Introduction

The widespread use of antibiotic-resistant bacteria is a major public health concern [1]; this is mostly the consequence of deliberate efforts to genetically modify germs to be resistant to a certain medicine. Meanwhile, studies have shown that viruses are among earth's most common

forms of life [2, 3]. Millions of individuals are sick with viruses, and many do not get treatments or vaccinations, particularly for retrovirus infections [4]. Infections with the hepatitis C virus (HCV), adenovirus type 7 (ADV7), or herpes simplex virus type 1 (HSV1) are universally devastating illnesses that may cause a variety of liver complications, as well as mortality and genital herpes [5]. Constant effort is put towards discovering a more effective material derived from nature that may mitigate this threat to public health. The potential of several plants as antibacterial and antiviral agents has been studied [6].

One study found that lymphoma accounts for 3% of all cancers globally [7]. Since lymphoma is linked to immune system damage, some hereditary illnesses,

\*Correspondence:

Hanaa S. S. Gazwi  
hanaa.saleh@mu.edu.eg

<sup>1</sup> Department of Agricultural Chemistry, Faculty of Agriculture, Minia University, El-Minya, Egypt

<sup>2</sup> Department of Microbiology, Faculty of Agriculture, Minia University, El-Minya, Egypt



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

immunosuppressive medication regimens, and specific viruses are recognized as risk factors [7]. Myeloid blood cells proliferate too quickly and unisexually, giving rise to the hematological malignancy known as acute myeloid leukemia (AML) [8]. About 351,000 new instances of leukemia are diagnosed annually across the globe, accounting for 2.8% of all cancers and 3.4% of all cancer deaths [9]. Clinical therapies such as bone marrow transplant, radiation, and chemotherapy are used to treat leukemia patients; however, most treatments are not yet suitable due to the significant side effects that are still encountered. Because of their ability to prevent tumor development at several stages, phytochemicals are gaining significance as a source [10].

The pea (*Pisum sativum* L.) is a nutritious leguminous crop that is extensively farmed [11]. Pea has a good balance of macronutrients, like dietary fibers, carbohydrates, high-quality proteins, minerals, and vitamins, and is good for health [12]. Peas also have a lot of antioxidants, which mostly come in the form of phenols, carotenoids, and tocopherols [12]. Legume seed coats are natural antioxidants with anti-inflammatory, heart-protective, anticancer, and other beneficial properties that work with protein-rich seeds to improve health [13]. Pea seed coats have a lot of bioactive phenolic compounds. Moreover, it has a lot of flavonoid compounds, which are becoming known for their ability to fight free radicals and act as antioxidants [14].

On the other hand, the waste products of the peas industry processing must be considered to avoid pollution. As a result, it is vital to examine their chemical composition and biological activities to maintain appropriate usage of these wastes in various applications [15]. Therefore, this study is the first to validate the phytochemical analysis, antioxidant capacities, and in vitro biological activities of the extract of SCP.

## Methodology

### SCP prepared extract

SCP was acquired from the vegetable processing sector in El-Minya, Egypt, and identified by professor Raga A. Taha, Horticulture Department, Faculty of Agriculture, Minia University. These samples were dried, blended, homogenized by grinding to a fine powder, then passed through a 1 mm sieve. SCP powder was extracted by water (1:10 w/v) after three hours of stirring at room temperature (25 °C). Whatman No. 1 filter paper was used for filtration. Then, the filtrate was filtered again to maximize the extract. The solution was filtered and dried in an oven at a temperature of 45–50 °C. The extract was kept at 4 °C for future use. The collection of SCP complies with relevant institutional, national, and international guidelines.

### Phytochemical screening

The major phytochemical compounds of the extract were tested qualitatively, according to Jamil et al. [16]. Alkaloids, flavonoids, glycosides, tannins, saponins, terpenes, and phenolics were among the significant studied components.

### Bioactive compounds

#### Total soluble carbohydrate content

Total soluble carbohydrate analysis was performed using the method of Gerhardt et al. [17]. The total soluble carbohydrates of the samples are presented as  $\mu\text{g}$  glucose/mg sample.

#### Total protein content

10 mg of sample was dissolved in 10 mL phosphate buffer saline pH 7.4. The sample was sonicated for 30 min. The sample was then filtrated using a 0.45  $\mu\text{m}$  syringe filter. According to the manufacturer's instructions, the BCA protein assay kit (Novagen) was used to test the sample.

#### Total phenolic content (TPC) and total flavonoid content (TFC)

The TPC of the extract was defined spectrophotometrically according to the Folin-Ciocalteu colorimetric technique reported by Haq et al. [18]. The sample results are presented as  $\mu\text{g}$  Gallic acid equivalent/mg extract. The TFC of the extract was measured by the aluminum chloride colorimetric technique [19]. The sample results are presented as  $\mu\text{g}$  quercetin equivalent/mg extract.

### Gas chromatography–mass spectrometry (GC–MS) analysis

The GC–MS analysis of the SCP extract was performed according to a previously described method [20]. GC–MS was conducted at the National Center Dokki's mass spectrometry laboratory in Giza, Egypt, using a Thermo Scientific Trace GC Ultra ISQ single Quadrupole MS, TG-5MS fused silica and capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$  film thickness). The column oven was set to 50 °C, then 200 °C at 7 °C/min, held for 2 min, then 290 °C, increased at 15 °C/min, and held for 2 min. After a 4-min solvent delay, an AS3000 autosampler and GC in the split mode were employed to inject 1  $\mu\text{L}$  of the diluted sample automatically. An electron ionization device with an ionization energy of 70 eV was used. Helium gas was used as the carrier gas and flowed at a constant rate of 1 mL/min. The injector and the MS transfer line were adjusted to 270 °C and 250 °C,

respectively. The percent relative peak area was employed to test the possibility of quantifying the components.

### Evaluation of the in vitro biological activities

#### *In vitro antioxidant activity*

Three complimentary in vitro assays were applied to determine the antioxidant activity of the SCP extract: DPPH-free radical scavenging, the oxygen radical absorbance capacity (ORAC), and ABTS+ radical cation scavenging. Lu et al. [21] technique was utilized to measure DPPH, and the Hao et al. [22] method was used to assess the ability to neutralize the radical ABTS+. The ORAC test was conducted using the technique of Liang et al. [23].

#### *Antibacterial activity*

Antibacterial activity of SCP extract against four gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Streptomyces* sp., and *Salmonella* sp.) and two gram-negative bacteria (*Escherichia coli* and *Pseudomonas* sp.) was evaluated using an optimized method by Manoj et al. [24]. Three concentrations of SCP extract were prepared (40, 60, and 80%). A single colony of each bacterium was transferred into a 5 mL nutrient broth medium. The bacterial inoculums were adjusted to  $10^6$  CFU (Colony Forming Units)/mL. One milliliter of each bacterial inoculum was poured with 10 mL of nutrient agar medium [25] into a 10 cm Petri plate. Five-millimeter diameter sterile filter paper discs were saturated with SCP extract concentrations. Sterilized water was used for control discs. Four paper discs were placed into each Petri dish, one for control and three representing the three concentrations. The plates were incubated at 37 °C for 24 h. Following incubation, the diameter of each growth inhibition zone was measured in mm. Three replicates were used for each treatment, and the experiment was repeated twice.

#### *Antiviral activity*

Nawah-Scientific, Egypt, provided the Herpes simplex virus type II and Vero cells. Vero cells were cultured in DMEM media containing 10% fetal bovine serum and 0.1% antibiotic/antimycotic solution. The trypsin–EDTA antibiotic, antimycotic solution, DMEM medium, and fetal bovine serum were provided by Gibco BRL (Grand Island, NY, USA). The crystal violet method evaluated the antiviral activity and cytotoxicity assays using the recently reported cytopathic (CPE) inhibition effect [26]. In brief, Vero cells were seeded at a density of  $2 \times 10^5$  cells/well into a 96-well plate one day before infection. The following day, the growth media were withdrawn, and the cells were rinsed with phosphate-buffered saline.

The virus's infectivity was measured by the crystal violet technique, which monitored CPE and allowed

for calculating the proportion of viable cells. A 0.1 mL diluted virus suspension containing 50% of the virus stock's CCID50 (cell culture infective dose) was introduced to mammalian cells. Three days after infection, this dose was chosen to produce the necessary CPEs. The 0.01 mL of media containing the required compound concentration was used to treat the cells. Then, the antiviral activity of each test sample was tested using a tenfold diluted concentration range of 0.1–10,000 g/mL. The cell controls (nondrug-treated, non-infected cells) and the viral controls (nondrug-treated, virus-infected cells). Culture plates were incubated at 37 °C in 5% CO<sub>2</sub> for 72 h. Light microscopy was used to monitor the development of the cytopathic effect. After washing with PBS, the monolayers of cells were fixed and stained with a 0.03% crystal violet solution in 10% formalin and 2% ethanol. After washing and drying, individual wells' optical densities were evaluated spectrophotometrically at 570/630 nm. This equation determined the proportion of antiviral activity of the test compounds: antiviral activity = [(mean optical density of cell controls - mean optical density of virus controls) / (optical density of the test - mean optical density of virus controls) times 100%. Based on these findings, the 50% CPE inhibitory dosage (IC<sub>50</sub>) was established. We performed a cytotoxicity assay before this assay. Cells were seeded at  $2 \times 10^4$  cells/well density in a 96-well plate. After that, the cells were reintroduced to a culture medium containing serially diluted samples and cultured for 72 h. Then, the media were withdrawn, and the cells were rinsed with PBS. The following procedure was performed identically to those reported before for the antiviral activity assay.

### SCP extract against leukemia (THP1) and lymphoma (U937) Cell culture

Nawah Scientific Inc. provided THP1: acute monocytic leukemia (AML) and U937: human lymphoma (Mokatam, Cairo, Egypt). At 37 °C, cells were cultured in RPMI medium containing 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, and 100 mg/mL streptomycin.

#### *Cytotoxicity assay*

The WST-1 test was used to determine the viability of cells using an Abcam® kit (ab155902 WST-1 Cell Proliferation Reagent). Aliquots of 50 L cell suspension ( $3 \times 10^3$  cells) were seeded in 96-well plates and cultured for 24 h in complete media. Cells were then treated with another portion of 50 µL media with serial doses of drugs. After 48 h of drug exposure, cells were treated with a 10 µL WST-1 reagent, and the absorbance at 450 nm was determined following 1 h using a BMG

LABTECH®-FLUOstar Omega microplate reader (Allmendgrün, Ortenberg).

### Statistical analyses

The chemical assays were performed in triplicate, and the results were demonstrated as the mean and standard deviation. One-way ANOVA was used to carry out the statistical analysis. Furthermore, the LSD test was used to examine the statistical significance of mean differences, with a p-value equal to 0.05. GraphPad Prism was used to conduct the statistical analysis.

## Results and discussion

### Phytochemical analysis

The presence of therapeutically active phytochemicals alkaloids, flavonoids, glycosides, tannins, phenolic, terpenes, and the absence of saponins was discovered during a phytochemical screening of SCP, as shown in Table 1.

These phytoconstituents (flavonoids, alkaloids, glycosides, tannins, and phenolic substances) may be responsible for the health benefits of the extract of SCP. The alkaloids are employed as analgesics, hallucinogens, stimulants, anesthetics, and antibacterial agents, and glycosides are said to have potent antibacterial properties [27]. Tannins are plant metabolites with antibacterial capabilities, and phenolics have an antioxidant capacity, which may help the body fight pathology-induced free radical production [28].

The existence of phenolic compounds in peas' seed coats was verified by Duenas et al. [29]. Pea was analyzed for their phenolic profiles and antioxidant capacities by Troszynska and Ciska [11]. Free and esterified phenolic acids were detected in pea, with the latter having greater quantities in the seed coat. The seed coat contained condensed tannins, which have been demonstrated to have extremely significant antioxidant activity [30].

### Total bioactive compounds

Total proteins, carbohydrates, flavonoids, and phenols were evaluated to assess total bioactive content.

**Table 1** Phytochemical analysis of SCP extract

Phytochemical	Results
Alkaloids	+
Flavonoid	+
Glycosides	+
Tannins	+
Saponins	—
Terpenes	—
phenolic	+

— Absent, + Present

The SCP extract contained a substantial amount of proteins ( $0.24 \pm 0.02$  mg/mL extract), Carbohydrates ( $29.53 \pm 4.23$  mg/g), flavonoids ( $15.89 \pm 0.28$  mg QE/g extract), and phenolic ( $22.37 \pm 0.59$  mg GAE / g extract) as shown in (Table 2).

To our knowledge, there is little or no available data in the literature on the total proteins, carbohydrates, flavonoids, and phenolics of the studied extract. Comparing our findings to Oomah et al. [31] revealed a total phenolic content of yellow pea hulls ranging between 2.6 and 9.1 mg catechin/g material, considering the extraction solvent. The phenolic content of black soybean seed coats from 60 categories ranged between 0.512 and 60.58 mg GAE/g [32]. The total phenolic content of lentil seed coats from various types ranged from 24.63 to 87.16 mg of catechin equivalents per gram of dry weight [31]. As a result, the total phenolic content values of the seed coats of the peas studied are equivalent to those of other widely farmed pulse crops such as lentils or soybeans. Several biological properties of phenolics have been reported, especially the significant antioxidant activity mentioned by Cao et al. [33] correlating with this study's findings. The extract showed a high percentage of protein, which increased its nutritional value.

### GC–MS analysis of the extract

Sixteen compounds in the SCP extract were identified using GC–MS, as shown in Table 3. The major constituents are (-)-Spathulenol (14.55%), Hexadecanoic acid, 2,3-Dihydroxypropyl Ester (10.86%), 12-Methyl-E, E-2,13-octadecadienoic-1-ol (6.57%), 11,14-Eicosadienoic acid, methyl ester (6.61%), cis-13-Eicosenoic acid (5.78%) (Table 3, Fig. 1). The components' fragmentation is shown in (Figs. 2 and 3).

These compounds have all been shown to have anti-cancer, anti-inflammatory, antioxidant, antimicrobial, antihypertensive, hypercholesterolemic, dermatitogenic, anti-tumor, antispasmodic, antiviral, immunoinhibitory, apoptosis-inducing, hemolytic, antihistamine, analgesic, anesthetic, allergic, anticonvulsant, anti-salmonella, antiseptic, and antiarthritic activity. Antioxidant activity is shown by Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate, 12-Methyl-E, E-2,13-octadecadienoic-1-ol, and Dotriacontane. Antimicrobial activity is demonstrated by

**Table 2** Total bioactive compounds in SCP extract

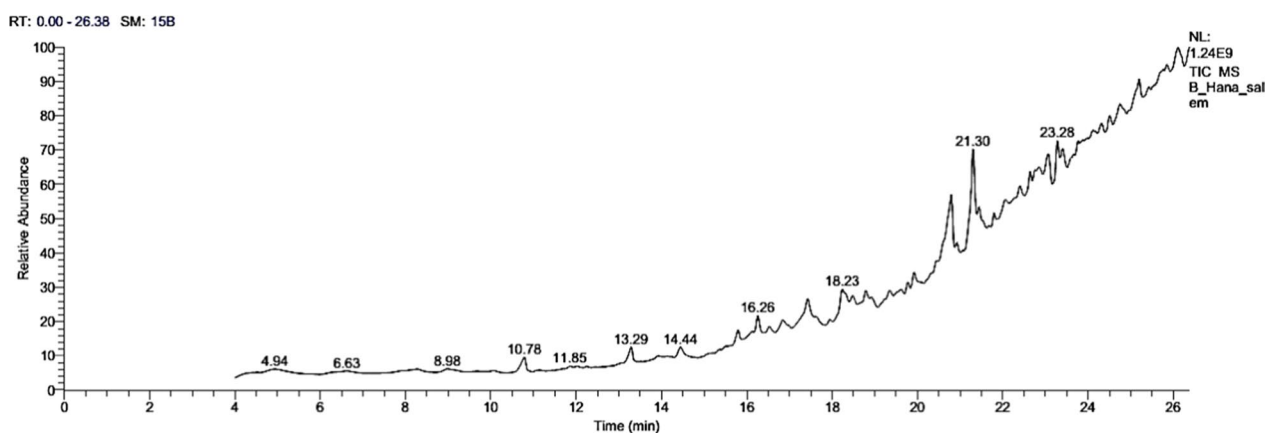
	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (mg QE/g extract)	Total Protein Content (mg/g extract)	Total soluble carbohydrate (mg/g extract)
SCP extract	$27.04 \pm 0.94$	$17.19 \pm 0.48$	$0.24 \pm 0.02$	$29.53 \pm 4.23$

All results are expressed as Mean  $\pm$  SD (n = 6)

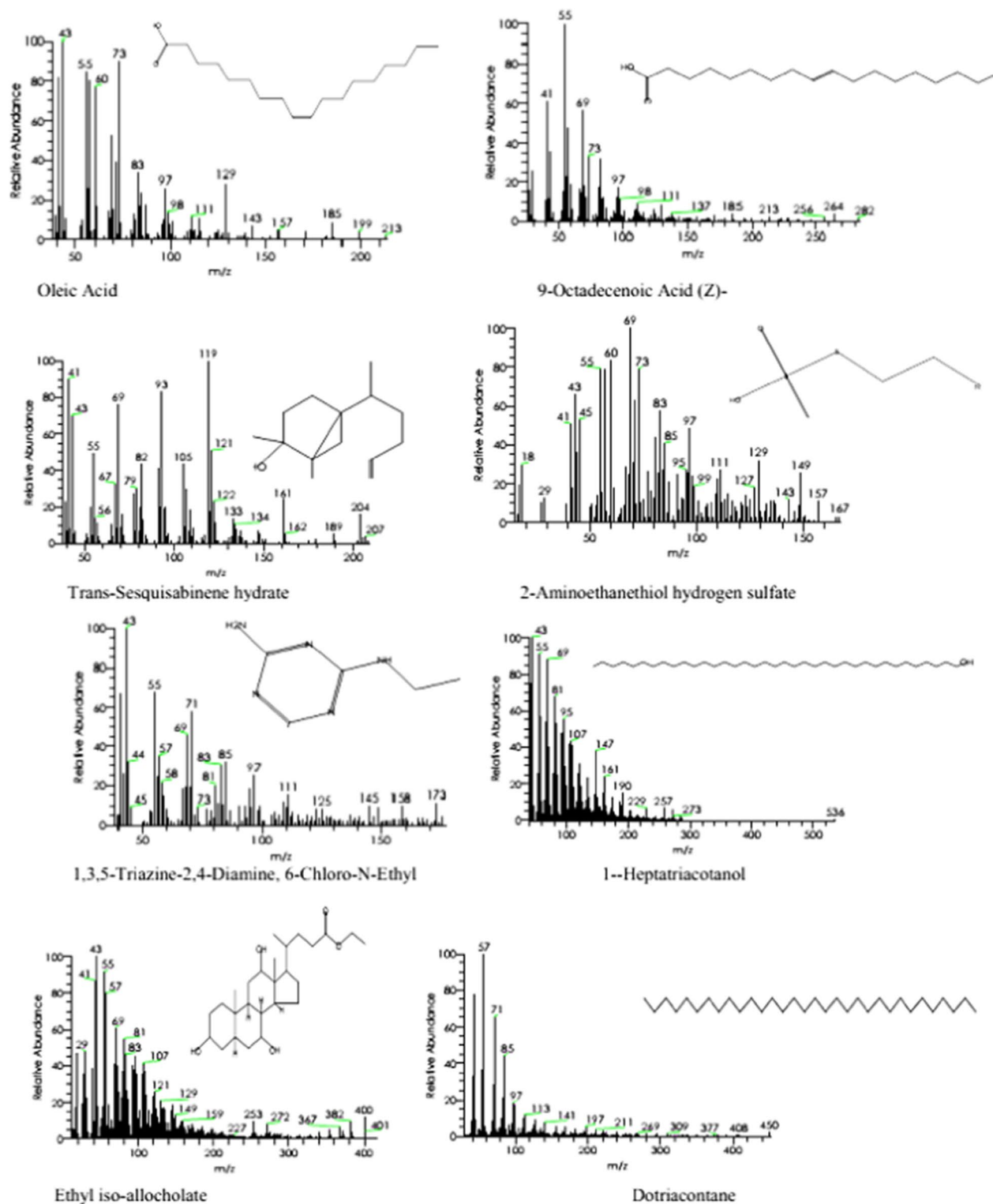
**Table 3** GC–MS analysis of SCP extract

No.	RT	Name of the compound	MF	MW	Peak area (%)	Compound nature	Activity
1	10.79	9-Octadecenoic Acid (Z)-	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	2.61	Fatty acids	Antihypertensive increases HDL, and decreases LDL [35]
2	13.29	Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	2.72	Fatty acids	Antimicrobial [36], hypercholesterolemic [29], dermatitogenic [37], anti-inflammatory, and anti-tumor activity [38]
3	15.8	2-Aminoethanethiol Hydrogen Sulfate	C <sub>2</sub> H <sub>7</sub> NO <sub>3</sub> S <sub>2</sub>	157	2.25	Ester	No activity reported
4	16.25	Trans-Sesquisabinene hydrate	C <sub>15</sub> H <sub>26</sub> O	222	2.93	Terpenoids	Anti-cancer [39]
5	17.42	1-Heptatriacotanol	C <sub>37</sub> H <sub>76</sub> O	536	3.41	Alcohol	Antimicrobial activity [40]
6	18.23	1,3,5-Triazine-2,4-Diamine, 6-Chloro-N-Ethyl	C <sub>5</sub> H <sub>8</sub> ClN <sub>5</sub>	173	5.25	1,3,5-triazine-2,4-diamines	No activity reported
7	18.79	Dotriacontane	C <sub>32</sub> H <sub>66</sub>	450	2.48	Alkanes	Antimicrobial, antioxidant, antispasmodic, antibacterial, and antiviral [41]
8	19.92	Ethyl iso-allocholate	C <sub>26</sub> H <sub>44</sub> O <sub>5</sub>	436	3.03	Steroid	Antimicrobial activity, and anti-inflammatory [42]
9	20.8	Hexadecanoic Acid, 2,3-Dihydroxypropyl Ester	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330	10.86	Ester	Antimicrobial activity, and anticancer activity on cancer cell lines [43]
10	21.3	(-)-Spathulenol	C <sub>15</sub> H <sub>24</sub> O	220	14.55	Terpenes	Immunoinhibitory, anti-inflammatory, anticancer, and apoptosis inducer [44]
11	21.45	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	C <sub>16</sub> H <sub>28</sub> O <sub>3</sub>	268	1.54	Terpenes	Antioxidant, and hemolytic [45]
12	21.79	E-8-Methyl-9-tetradecen-1-ol acetate	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	268	1.76	Acetate	No activity reported)
13	23.28	12-Methyl-E,E-2,13-octadecadien-1-ol	C <sub>19</sub> H <sub>36</sub> O	280	6.57	Alcohol	Antihistamine, antioxidant, analgesic, anesthetic, allergic, antibacterial, anticonvulsant, anti-salmonella, and antiseptic [46]
14	24.5	17-Pentatriacontene	C <sub>35</sub> H <sub>70</sub>	490	2.29	Unsaturated aliphatic hydrocarbons	Anti-inflammatory, anticancer, antibacterial, and antiarthritic [47]
15	25.19	Cis-13-Eicosenoic acid	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310	5.78	Fatty acids	Anti-inflammatory activity [48]
16	26.11	11,14-Eicosadienoic acid, methyl ester	C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>	322	6.61	Esters	Antimicrobial activity [49]

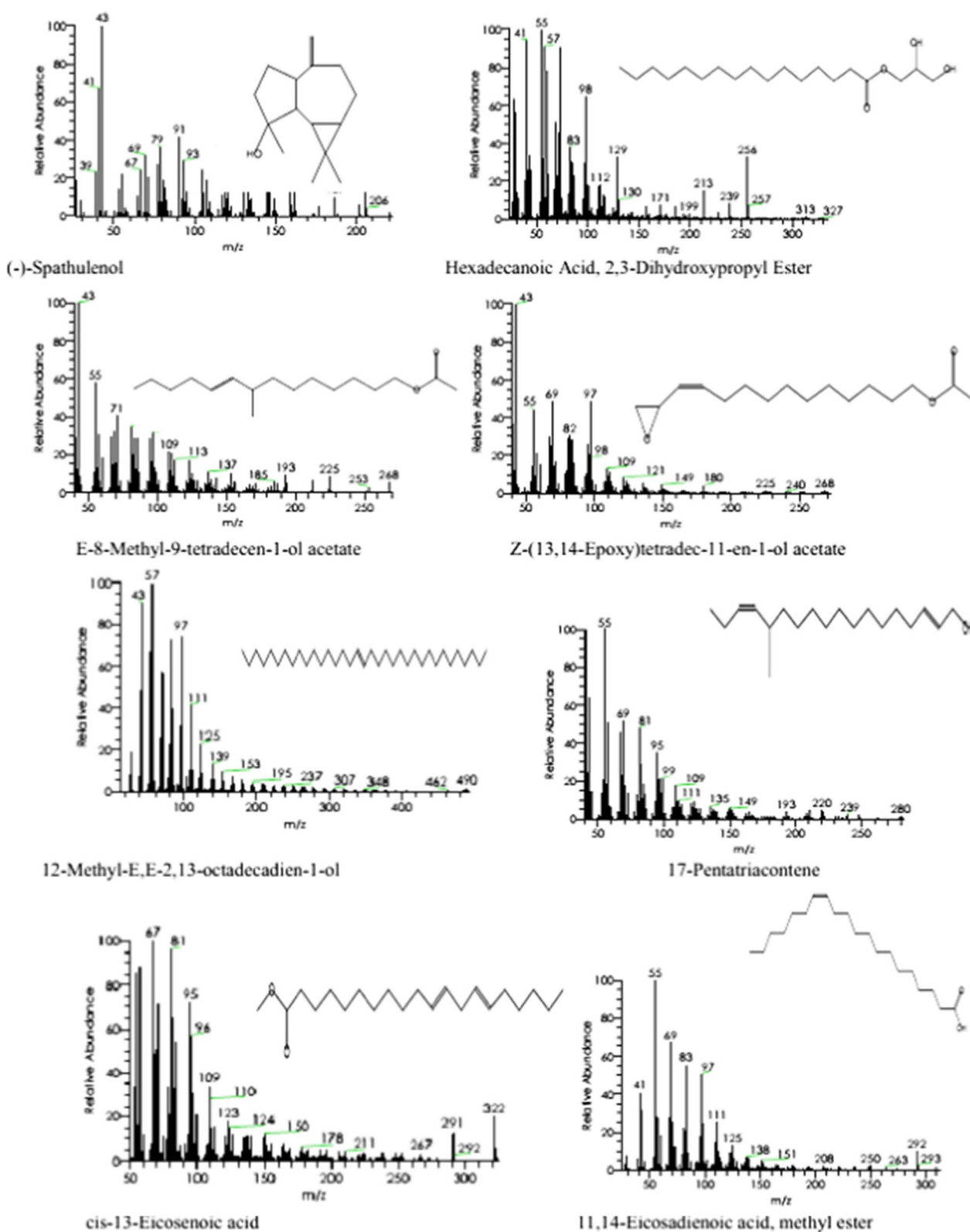
RT, relation time; MF, molecular formula; MW, molecular weight

**Fig. 1** GC–MS of Chromatogram of SCP extract





**Fig. 2** Mass spectrums molecular structures of components



**Fig. 3** Mass spectrums molecular structures of components

12-Methyl-E, E-2,13-octadecadienoic-1-ol; 1-Heptatriacotanol; Dotriacontane; Ethyliso-allocholate; Hexadecanoic acid, 2,3-Dihydroxypropyl ester; 11,14-Eicosadienoic acid, methyl ester; 17-Pentatriacontene and Oleic acid. Anticancer activity is demonstrated by Trans-sesquisabinene hydrate, Oleic acid; 17-Pentatriacontene; (-)-Spathulenol; and Hexadecanoic acid, 2,3-Dihydroxypropyl ester. Anti-inflammatory activity is demonstrated by Oleic acid; cis-13-Eicosenoic acid; 17-Pentatriacontene, and (-)-Spathulenol. Correlations between phytochemicals and their biological activities are becoming more prevalent [34].

We describe the presence of biological activity of several significant components identified using GC–MS analysis. Thus, this form of GC–MS analysis is the first step toward elucidating the nature of the active components in peas. This type of study will aid in developing a more detailed study.

#### Antioxidant activity

Antioxidants are bioactive compounds with an inhibitory effect that reduces the damage produced by reactive free radicals. Compared to the standard Trolox, the

outcomes of the antioxidant property evaluated by the three assessment methods, ABTS radical cation scavenging, DPPH radical scavenging, and ORAC tests, are shown in Table 4. According to the results, SCP extract showed higher antioxidant activity. In the ORAC test, the extract caused the fluorescence signal to decay (Fig. 4).

Polyphenols found in vegetables are responsible for various biological reactions, including antioxidant activity [50]. According to Troszynska and Ciska [11], legume antioxidant compounds were primarily found in the seed coat. These findings are consistent with earlier research [51]. The seed coat, in general, plays a significant part in the chemical and physical defense mechanism of seeds exposed to oxidative damage, UV light, and other environmental conditions. As a result, the seed coat contains various bioactive substances, including polyphenols, which protect against oxidative stress [52].

#### Antimicrobial activities

Infectious diseases are one of the world's most significant challenges, with about 57 million people dying yearly [53]. Pharmacological firms have developed several novel antibiotics in the last three decades. However, their harmful effects and the widespread growth of multi-drug resistance (MDR) microorganisms reduce their effectiveness [54]. Therefore, the MDR efflux pump is crucial to finding novel and innovative treatments.

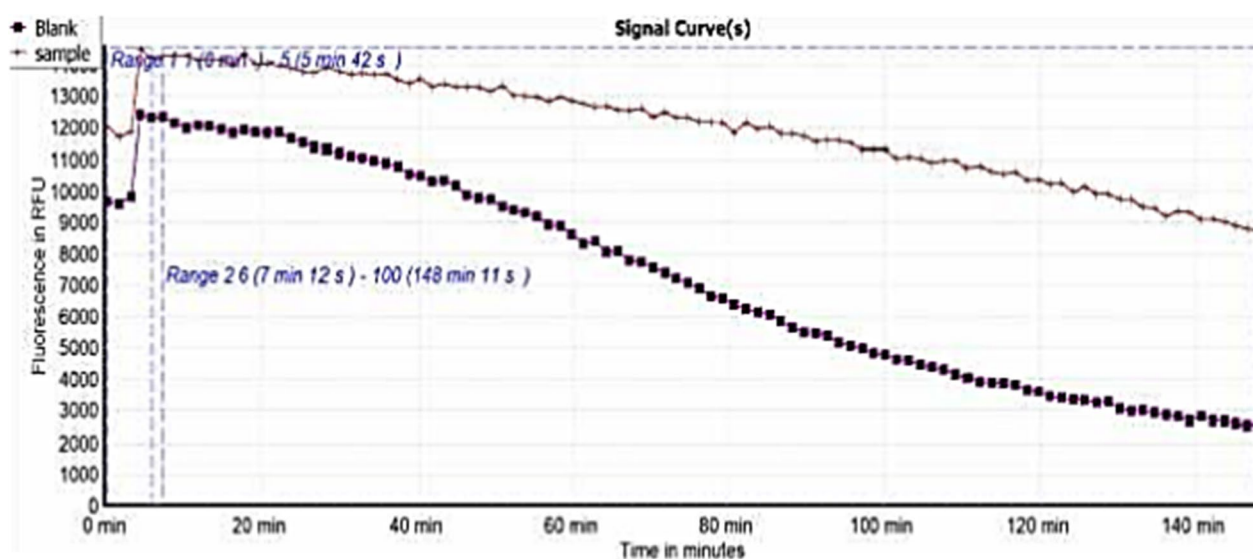
Medicinal plants have improved patients' lives for thousands of years because they contain many chemical components that have a specific physiological effect on the human body. Alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolics represent the most prominent

**Table 4** Antioxidant activity of SCP extract

	DPPH (IC <sub>50</sub> µg/mL)	ABTS (µM TE/mg extract)	ORAC (µM TE/mg extract)
SCP extract	79.16 <sup>a</sup> ± 1.77	61.22 <sup>a</sup> ± 4.81	67.40 <sup>a</sup> ± 5.20
Trolox	24.00 ± 0.16	35.30 ± 0.30	31.71 ± 0.01

All results are expressed as mean SD (n = 6)

<sup>a</sup> Significantly different from Trolox (TE) at (p ≤ 0.05)

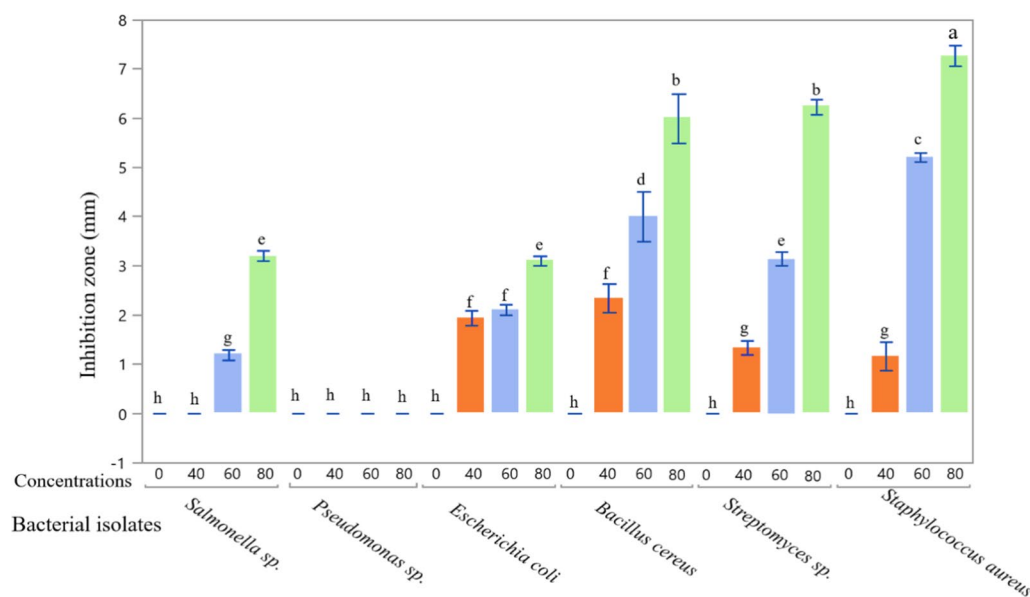


**Fig. 4** Signal curves indicating the decay of fluorescein upon applying the extract

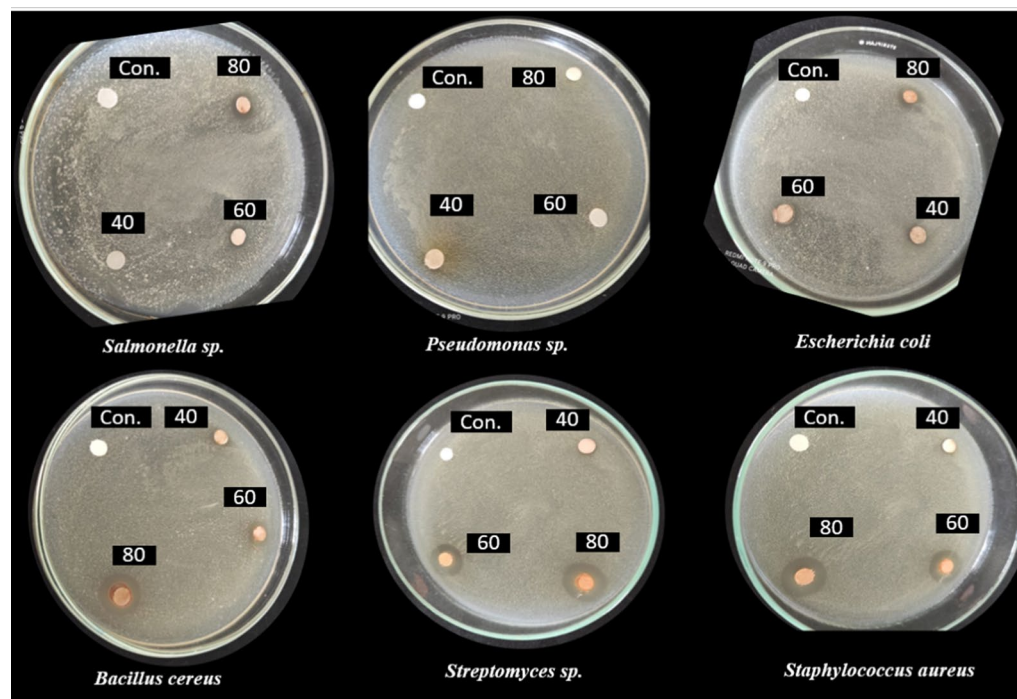


chemicals. They have provoked the interest of pharmacists due to their medicinal efficacy and minimal toxicity [55]. SCP extract had various degrees of antibacterial activity (Figs. 5 and 6). SCP extract significantly affected all tested bacterial strains except for *Pseudomonas* sp.

See Figs. 5 and 6. The effect of the three concentrations varied among the strains. In general, 80% SCP extract concentration has shown the highest inhibition zones compared with 40 and 60% control. Also, the obtained data revealed that the most affected bacterial strains



**Fig. 5** The impact of SCP extract at different concentrations on six bacterial strains. The data values were expressed as mean  $\pm$  SD in mm of the inhibition zone demonstrated. All values with the same alphabetic superscript indicate no statistically significant differences, while those with different alphabetic superscripts indicate statistically significant differences according to Tukey's post-hoc analysis of variance (ANOVA)



**Fig. 6** The interaction between SCP extract at different concentrations and six bacterial strains

belonged to gram-negative bacteria (*E. coli*, *Salmonella* sp., and *Pseudomonas* sp.). *Staphylococcus aureus* was the most sensitive strain to SCP extract, demonstrating a 7.27 mm inhibition zone at 80% concentration. *Streptomyces* sp. and *Bacillus cereus* at the same concentration of 6.23 and 6.0 mm, respectively.

Due to active chemicals that can reduce bacterial growth without necessarily getting into the bacterial cell, the extract could inhibit the above-listed gram-negative bacteria [56]. We discovered alkaloids and flavonoids in the extract have antibacterial properties. As a result, we conclude that these metabolites are responsible for their antibacterial properties. The results are consistent with Jaberian et al. [57] findings that plant extracts exhibit antibacterial properties, which they attribute to powerful chemicals such as flavonoids, alkaloids, tannins, and other substances. Tannins are potent detoxifying agents

that prevent protein development by precipitating the protein components.

#### Antiviral activity

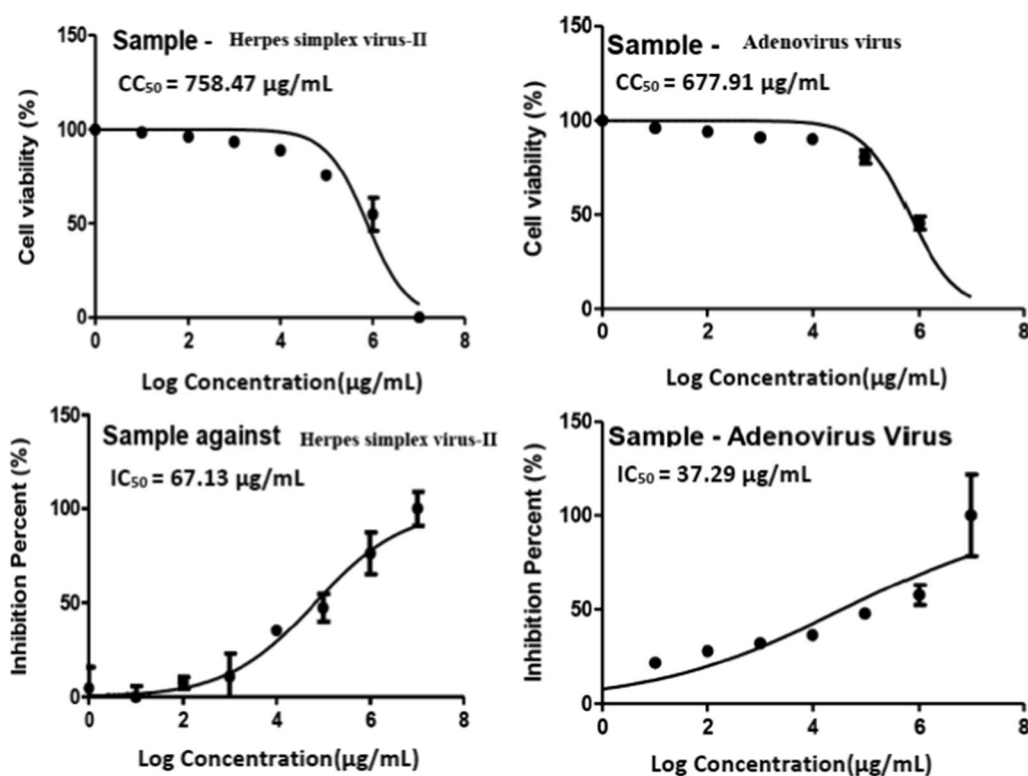
Antiviral activity was tested against herpes simplex II and adenovirus viruses. The CPE-inhibition assay will identify potential antivirals against human Herpes simplex virus type II. The dose–response assay was designed to determine the range of efficacy for the chosen antiviral, i.e., the 50% inhibitory concentration ( $IC_{50}$ ) and the range of cytotoxicity ( $CC_{50}$ ). This assay is critical for determining antiviral efficacy in cell culture systems. The results of the 50% cytotoxic concentrations ( $CC_{50}$ ) and the 50% inhibitory concentration ( $IC_{50}$ ) were determined using GraphPad PRISM software (Graph-Pad Software, San Diego, USA). Table 5 shows that the SCP extract had strong antiviral activity against adenovirus, with a selectivity index of 18.2. Figure 7 shows the dose–response curves. The results in Table 5 show that the tested sample showed moderate antiviral activity against Herpes virus type-2 with a selective index = estimated  $CC_{50}$ /estimated  $IC_{50}$  = 11.3. Consequently, the tested sample is a good candidate for further experiments as anti-Herpes viruses.

The extract's antiviral impact seems mostly attributable to interacting with viral uptake, enzyme activity, replication, potentiating the host immune system, and

**Table 5** Antiviral effect of SCP extract

	Virus	$CC_{50}$ ( $\mu\text{g/mL}$ )	$IC_{50}$ ( $\mu\text{g/mL}$ )	SI
SCP extract	Adenovirus	677.91	37.29	18.20
	Herpes simplex virus-II	758.47	67.13	11.30

SI, selectivity index =  $CC_{50}/IC_{50}$ ;  $IC_{50}$ , half-maximal inhibitory concentration;  $CC_{50}$ , half-maximal cytotoxic concentration



**Fig. 7** Cytotoxicity concentration 50 ( $CC_{50}$ ) and the 50% inhibitory concentration ( $IC_{50}$ ) (on Vero cells and HSV-2

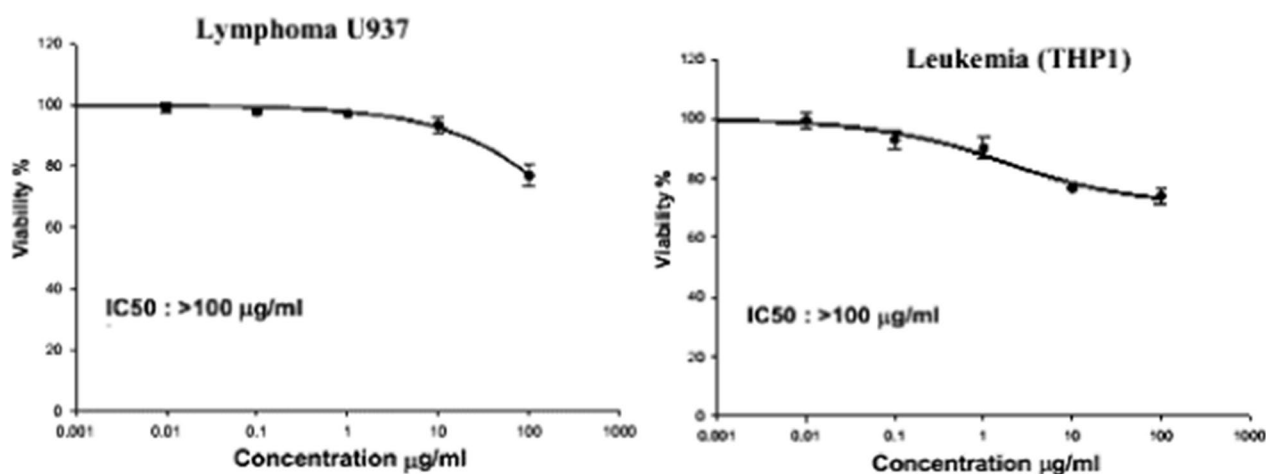
functional peptides [58]. The anti-HIV action of the extract of SCP could be due to ubiquitin-like protein and lectin suppression of the reverse transcriptase enzyme, respectively [59]. Flavonoids treat HIV-1 protease and HIV-1 integrase enzymes in HIV replication [60].

#### Leukemia and lymphoma cell lines

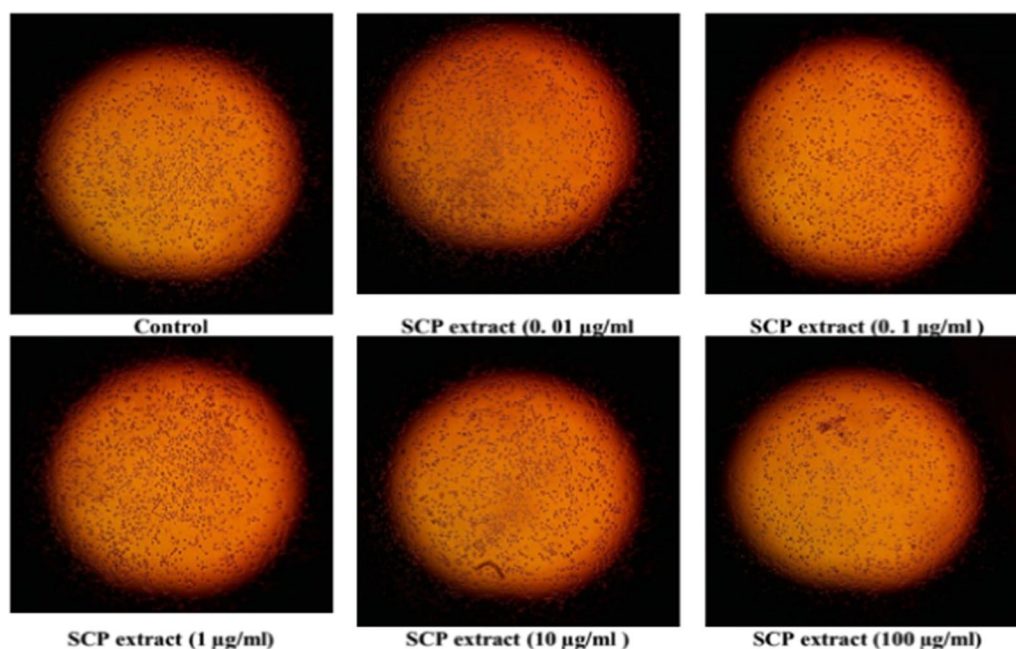
The WST-1 assay was used to assess the cytotoxicity of plant extract on cell lines from patients with mononuclear acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL) after 24-h incubation. The findings

showed that the 100% cell proliferation of the leukemic THP1 cells was significantly reduced in a dose-dependent manner to 97.25%, 88.33%, 86.49%, 76.69%, 72.06% upon treatment with 0.01, 0.1, 1, 10, and 100 µg/mL of SCP extract respectively. Accordingly, the obtained  $IC_{50}$  value was  $> 100$  µg/mL, as shown in Figs. 8 and 9. The SCP extract was tested against lymphoma (U937) cells. The SCP extract reduced the viability of lymphoma U937 cells to 72.99%. See Figs. 8 and 10.

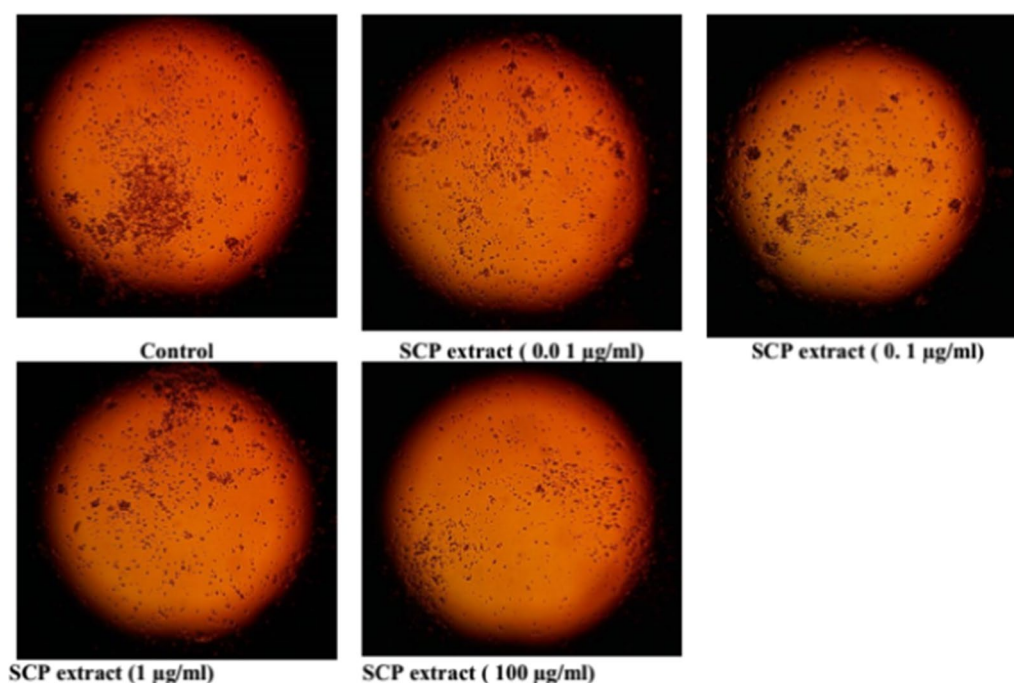
Acute lymphoblastic leukemia (ALL) malignancy causes lymphoid blast cells to accumulate in



**Fig. 8** Cytotoxic effect of the SCP extract against lymphoma U937 and leukemic cells (THP1)



**Fig. 9** Photomicrographs of lymphoma U937 cell lines for treatment with extract of SCP



**Fig. 10** Photomicrographs of acute monocytic leukemia (THP1) cell lines for treatment with extract of SCP

hematopoietic organs, particularly the bone marrow. The overproduction of damaged white blood cells causes leukemia. Although tyrosine kinase inhibitors are widely used, responses are phase-dependent, and recurrences are common. This research aimed to investigate the anti-cancer effect of the entire SCP extract against the THP1 leukemia cell line, which is extremely undifferentiated, and see if any apoptotic mechanisms are altered in vitro. Because synergetic effects could make combinatorial acts more noticeable, we decided to test the whole extract in leukemia cells rather than individual elements.

It is well-established that most phenolic compounds, glycosides, and tannins dissolve in ethanol and water solutions [61]. As a result, these chemical groups may include the most active compounds for leukemia [62]. Cell-to-cell communication is widely recognized as the most effective cell-type distinction in development and established tissue organization patterns [63]. At virtually every stage of development, cells send and receive signals from neighboring cells, which are essential for proper differentiation and function [64]. Numerous epidemiological studies have indicated an inverse correlation between a vegetable and fruit-rich diet and humans' risk of developing cancer [65]. Flavonoids and other phenolics are particularly intriguing candidates for preventing cancer [61]. There is significant evidence of the potential inhibitory effects of certain plant phenolic extracts on carcinogenesis and mutagenesis [63]. Due to the apparent

diversity of dietary phenolics, including flavonoids, and the numerous possible pathways reported, scientists assumed that a single substance was not responsible for all the associations between cancer prevention and plant food intake [62]. El-Shemy et al. [62] suggested that phytochemicals in vegetables and fruits contributed significantly to their anticancer properties.

## Conclusions

The SCP extract contains a vast spectrum of bioactive substances. The antiviral activity of SCP extract against herpes simplex virus type II and adenovirus type 7 seems promising. The current study recommends this by-product for pharmaceutical applications and its antiviral, anticancer, and antioxidant properties. As a result, this extract should be advertised to the public as a health supplement due to its high concentration of antioxidants.

## Acknowledgements

Not applicable.

## Author contributions

HSSG conceived the project. HSSG and MOAO designed and performed the experiments. HSSG analyzed the data. HSSG and MOAO, and MEM wrote the manuscript. HSSG and MOAO, and MEM provided critical discussion, editing, and final approval of the manuscript. All authors contributed to the preparation of the manuscript. All authors read and approved the final manuscript.



## Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

## Data availability

The datasets utilized and analyzed during this investigation are available upon reasonable request from the corresponding author.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that there are no conflicts of interest concerning the work described in this manuscript.

Received: 8 July 2022 Accepted: 23 January 2023

Published online: 01 February 2023

## References

- Aarestrup FM. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. *Basic Clin Pharmacol Toxicol*. 2005;96:271–81.
- Griffin DW. The quest for extraterrestrial life: what about the viruses? *Astrobiology*. 2013;13:774–83.
- World Health Organisation (WHO). Progress Report on HIV, Viral Hepatitis and Sexually Transmitted Infections. Accountability for the global health sector strategies, 2016–2021. Geneva: WHO; 2019.
- Forsythe SS, McGreevey W, Whiteside A, Shah M, Cohen J, Hecht R, Bollinger LA, Kinghorn A. Twenty years of antiretroviral therapy for people living with HIV: global costs, health achievements, economic benefits. *Health Aff*. 2019;38:1163–72.
- Ehwarieme R, Agarwal AN, Alkhateb R, Bowling JE, Anstead GM. A surprising cause of liver abscesses in a post chemotherapy patient: Herpes Simplex virus. *Cureus*. 2021. <https://doi.org/10.7759/cureus.17744>.
- Nabavi SF, Di Lorenzo A, Izadi M, Sobarzo-Sánchez E, Daglia M, Nabavi SM. Antibacterial effects of cinnamon: from farm to food, cosmetic and pharmaceutical industries. *Nutrients*. 2015;7:729–48.
- Marcus R, Sweetenham JW, Williams ME. Lymphoma: pathology, diagnosis and treatment. Cambridge: Cambridge University Press; 2007.
- Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K, et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature*. 2008;456:66–72.
- Rosilio C, Sahra IB, Frédéric B, Peyron JF. Metformin: a metabolic disruptor and anti-diabetic drug to target human leukemia. *Cancer Lett*. 2014;346(2):188–96.
- Renata N, Marta O, Natalia N. Plant polyphenols as chemopreventive agents. In: Watson RR, Preedy V, Zibadi S, editors. *Polyphenols in human health and disease*. 1st ed. Cambridge: Academic Press; 2014. p. 1289–307.
- Troszynska A, Ciska E. Phenolic compounds of seed coats of white and coloured varieties of pea (*Pisum sativum* L.) and their total antioxidant activity. *Czech J Food Sci*. 2002;20:15–22.
- Kumari T, Deka SC. Potential health benefits of garden pea seeds and pods: a review. *Legume Sci*. 2021;3:e82. <https://doi.org/10.1002/leg3.82>.
- Fahim JR, Attia EZ, Kamel MS. The phenolic profile of pea (*Pisum sativum*): a phytochemical and pharmacological overview. *Phytochem Rev*. 2019;18:173–98.
- Stanisavljević N, Ilić M, Jovanović Ž, Cupić T, Dabić DM, Natić M, Tesić Z, Radović S. Identification of seed coat phenolic compounds from differently colored pea varieties and characterization of their antioxidant activity. *Arch Biol Sci*. 2015;67:829–40.
- Maniyan A, John R, Mathew A. Evaluation of fruit peels for some selected nutritional and anti-nutritional factors. *Emer Life Sci Res*. 2015;1:113–9.
- Jamil M, Mirza B, Yasmeen A, Khan MA. Pharmacological activities of selected plant species and their phytochemical analysis. *J Med Plants Res*. 2012;6:5013–22.
- Gerhardt P, Murray RGE, Wood WA, Krieg NR. *Methods for general and molecular bacteriology*. Washington, DC: American Society for Microbiology; 1994. p. 607–54.
- Haq I, Ullah N, Bibi G, Kanwal S, Ahmad MS, Mirza B. Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. *Iran J Pharm Res*. 2012;11:241–9.
- Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal*. 2002;10:178–82.
- Gazwi HSS, Shoeib NA, Mahmoud ME, Soltan OIA, Hamed MM, Ragab AE. Phytochemical profile of the ethanol extract of *Malvaviscus arboreus* red flower and investigation of the antioxidant, antimicrobial, and cytotoxic activities. *Antibiotics*. 2022;11(11):1652. <https://doi.org/10.3390/antibiotics11111652>.
- Lu C, Li H, Li C, Chen B, Shen Y. Chemical composition and radical scavenging activity of *Amygdalus pedunculata* pall leaves' essential oil. *Food Chem Toxicol*. 2018;119:368–74.
- Hao J, Zhang W, Wang H, Ziya N, Luo Y, Jia P, Zhang G, Ng T. Purification and properties of a laccase from the mushroom *Agaricus sinodeliciosus*. *Biotechnol Appl Biochem*. 2020;68:297–306.
- Liang Z, Cheng L, Zhong GY, Liu RH. Antioxidant and antiproliferative activities of twenty-four *Vitis vinifera* grapes. *PLoS ONE*. 2014;9(8):e105146.
- Manoj K, Agarwal RC, Sanjay D, Ral VK, Benito J. Antimicrobial activity of extract of *Terminalia chebula* Retz. on gram positive and gram negative. *Int J Curr Pharm Res*. 2009;1:56–60.
- Allen ON. *Experiments in soil bacteriology*. 3rd ed. Minnea Polis: Burgess Publishing Co; 1959. p. 117.
- Donalisio M, Nana HM, Ngane RAN, Gatsing D, Tchinda AT, Rovito RV, Cagno C, Cagliero FF, Boyom Rubiolo P, et al. In vitro anti-Herpes simplex virus activity of crude extract of the roots of *Nauclea latifolia* Smith Rubiaceae. *BMC Complement Altern Med*. 2013;13:266.
- Dangoggo SM, Faruq UZ, Hassan LG. Preliminary chemical and antibacterial activity of *Pergularia tomentosa*. *Sokoto J Vet Sci*. 2002;4:8–11.
- Al-Humaid AL, Mousa HM, El-Mergawi RA, Abdel-Salam AM. Chemical composition and antioxidant activity of dates and dates camel—milk mixtures as a protective meal against lipid peroxidation in rats. *Am J Food Technol*. 2010;5:22–30.
- Duenas M, Estrella I, Hernandez T. Occurrence of phenolic compounds in the seed coat and the cotyledon of peas (*Pisum sativum* L.). *Eur Food Res Tech*. 2004;219:116–23.
- Hagerman AE, Riedl KM, Jones A, et al. High molecular weight plant polyphenolics (tannins) as antioxidants. *J Agric Food Chem*. 1998;46:1887–92.
- Oomah BD, Caspar F, Malcolmson LJ, Bellido AS. Phenolics and antioxidant activity of lentil and pea hulls. *Food Res Int*. 2011;44:436–41.
- Zhang RF, Zhang FX, Zhang MW, Wei ZC, Yang CY, Zhang Y, Tang XJ, Deng YY, Chi JW. Phenolic composition and antioxidant activity in seed coats of 60 Chinese black soybean *Glycine max* L. Merr. varieties. *J Agric Food Chem*. 2011;59:5935–44.
- Cao G, Sofic E, Prior RL. Antioxidant and pro-oxidant behavior of flavonoids: structure activity relationship. *Free Radic Biol Med*. 1997;22:749–60.
- Stanisavljević NS, Ilić M, Jovanović ŽS, Cupić T, Dabić DC, Natić MM, et al. Identification of seed coat phenolic compounds from differently colored pea varieties and characterization of their antioxidant activity. *Arch Biol Sci*. 2015;67:829–40.
- Arora S, Kumar G, Meena S. Screening and evaluation of bioactive components of *Cenchrus ciliaris* L. by GC–Ms analysis. *Int Res J Pharm*. 2017. <https://doi.org/10.7897/2230-8407.08699>.
- Novak AF, Solar JM, Mod RR, Magne FC, Skau EL. Antimicrobial activity of some N-substituted amides of long-chain fatty acids. *Appl Microbiol*. 1969;18:1050–6.
- Natali A, Toschi E, Baldeweg S, Ciociaro D, Favilla S, Sacca L, Ferrannini E. Clustering of insulin resistance with vascular dysfunction and low-grade inflammation in type 2 diabetes. *Diabetes*. 2006;55:1133–40.
- Newmark HL. *Cancer epidemiology*. Bio mark Prev. 1997;6:1101–3.



39. Yunfeng Z, Dong W, Siyuan G, Xuewu Z, Mingfu W, Chen F. Chemical components and antioxidant activity of the volatile oil from *Cassia tora* L. seed prepared by supercritical fluid extraction. *J Food Lipids*. 2007;14:411–23.
40. Kalaiarasan A, Kumar P, John SA. GC/MS determination of Bioactive components of *Bulbophyllum kaitense*. Reichib Leaves Estern Ghats in India. *New York Sci J*. 2011;4(10):29–31.
41. Nithya P, Kaleeswari S, Poonkothai M. Antimicrobial activity and phytochemical analysis of fruit extracts of *Terminalia bellerica*. *Int J Pharm Pharmaceut Sci*. 2014;6:639–42.
42. Hameed IH, Ibraheem IA, Kadhim HJ. Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus officinalis* leaves. *J Pharmacogn Phytother*. 2015;7:790–106.
43. Klavina L, Springe G, Nikolajeva V, Martsinkevich I, Nakurte I, et al. Chemical composition analysis antimicrobial activity and cytotoxicity screening of moss extracts moss phytochemistry. *Mol*. 2015;20:17221–43.
44. Martins A, Hajdú Z, Vasas A, Csopor-Löffler B, Molnár J, Hohmann J. Spathulenol inhibit the human ABCB1 efflux pump. *Plant Med*. 2010;76:608.
45. Vijisara ED, Arumugam S. C-MS analysis of bioactive constituents of *Indigofera suffruticosa* leaves. *J Chem Pharm Res*. 2014;6:294–300.
46. Lakshmi CNDM, Prabhakara JPR, Saritha K. Phytoconstituents profile of *Clitoria ternatea* by GC-MS and its age-related anticholinergic activity against aluminum and restraint stress. *Int Res J Pharm*. 2018. <https://doi.org/10.7897/2230-8407.09224>.
47. Dinesh KG, Karthik M, Rajakumar R. GC-MS analysis of bioactive compounds from ethanolic leaves extract of *Eichhornia crassipes Mart Solms*. and their pharmacological activities. *Pharma Innov J*. 2018;7:459–62.
48. Sosa AA, Bagi SH, Hameed HI. Analysis of bioactive chemical compounds of *Euphorbia lathyris* using gas chromatography-mass spectrometry and Fourier—transform infrared spectroscopy. *J Pharmacogn Phytother*. 2016;8:109–26.
49. Kiliç T, Dirmenci T, Gören AC. Fatty acids composition of seeds of some species of *Nepeta* L. *Nat Prod Res*. 2007;21:465–8.
50. Phang CW, Malek SN, Ibrahim H. Antioxidant potential cytotoxic activity and total phenolic content of *Alpinia Pahangensis Rhizomes*. *BMC Complement Altern Med*. 2013;13:243.
51. Zia-Ul-Haq M, Amarowicz R, Ahmad S, Riaz M. Antioxidant potential of some *pea Pisum sativum* L. cultivars commonly consumed in Pakistan. *Oxid Commun*. 2013;36:1046–57.
52. Osawa T, Ramarathnam N, Kawakishi S, Namiki M, Tashiro T. Antioxidative defense systems in rice hull against damage caused by oxygen radicals. *Agric Biol Chem*. 1985;49:3085–7.
53. Fauci AS, Touchette NA, Folkers GK. Emerging infectious diseases: a 10-year perspective from the National Institute of Allergy and Infectious Diseases. *Emerg Infect Dis*. 2005;11:519–25.
54. Hancock EW. Mechanisms of action of newer antibiotics for gram-positive pathogens. *Lancet Infect Dis*. 2005;5:209–18.
55. Inayatullah S, Prenzler PD, Obied HK, Rehman AU, Mirza B. Bioprospecting traditional Pakistani medicinal plants for potent antioxidants. *Food Chem*. 2012;132:222–9.
56. Mulaudzi RB, Ndhala AR, Kulkarni MG, Finnie JF, Staden JV. Antimicrobial properties and phenolic contents of medicinal plants used by the Venda people for conditions related to venereal diseases. *J Ethnopharmacol*. 2011;135:330–7.
57. Jaberian H, Piri K, Nazari J. Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. *Food Chem*. 2013;136:237–44.
58. Patel DK, Dutta SD, Ganguly K, Cho SJ, Lim KT. Mushroom-derived bioactive molecules as immunotherapeutic agents: a review. *Molecules*. 2021;26(5):1359.
59. Wang CR, Ng TB, Li L, Fang JC, Jiang Y, Wen TY, Qiao WT, Li N, Liu F. Isolation of a polysaccharide with antiproliferative hypoglycemic antioxidant and HIV-1 reverse transcriptase inhibitory activities from the fruiting bodies of the abalone mushroom *Pleurotus abalonus*. *J Pharm Pharmacol*. 2011. <https://doi.org/10.1111/j.2042-7158.2011.01274.x>.
60. Ngaski MM. Phytochemical screening and proximate analysis of *Cassia siamea* leaves. M.Sc. Dissertation Unpublished Submitted to postgraduate School, Usmanu Danfodiyo University, Sokoto; 2006.
61. Bravo L. Polyphenols: chemistry dietary sources metabolism and nutritional significance. *Nutr Rev*. 1998;56:317–33.
62. El-Shemy HA, Aboul-Enein AM, Aboul-Enein KM, Fujita K. Willow leaves' extracts contain anti-tumor agents effective against three cell types. *PLoS ONE*. 2007;2:e178.
63. Freeman M, Gurdon JB. Regulatory principles on developmental signaling. *Ann Rev Cell Dev Biol*. 2002;18:515–39.
64. Riboli E, Norat T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am J Clin Nutr*. 2003;78:559S–569S.
65. Meyers KJ, Watkins CB, Pritts MP, Liu RH. Antioxidant and antiproliferative activities of strawberries. *J Agric Food Chem*. 2003;51:6887–92.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Ready to submit your research? Choose BMC and benefit from:**

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

**At BMC, research is always in progress.**

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

