INTRODUCTION

Plants have been used in the treatment of many diseases and they have formed an important resource for medicinal preparations. The family Brassicaceae (Cruciferae) contains over 330 genera and about 3,700 species including mustard which importance has increased recently. There are three species of mustard: White mustard (Sinapis alba L.), brown mustard (Sinapis nigra L.) and oriental (Sinapis juncea L.).

Sinapis alba L. is a widespread plant in the Mediterranean region and has great economic and medical importance due to its anti-tumor and insecticidal properties. The major compounds of Sinapis alba L. are glucosinolates, fatty oil, proteins and phenyl propane derivatives. It has been used in the treatment of common cold, bronchitis, rheumatism and in the treatment of inflammation of the respiratory tract and the gastrointestinal tract in homeopathy, but it should be avoided in children under 6 years, gastrointestinal ulcers and inflammatory kidney diseases. Mustard has high amounts of antioxidants, vitamins (B complex) and minerals (Calcium, magnesium, iron, potassium and selenium).

Sinapis alba L. seeds contain of fatty acid (41.3% Erucic acid), glucosinolates, and phenolic acids (P-Hydroxy benzoic acid, trans-Sinapic, trans- Caffeic, and trans- Ferulic). Kaempferol is the main flavonoid in the leaves extract of the plant, and there are isorhamntin and quercetin. Phenols and flavonoids have antioxidant activity, these natural antioxidants have anti-cancer properties, inhibit apoptosis and reactive oxygen species (ROS) generation and have free radical scavenging activity. Mustard leaves extract decreases the damage of oxidative stress by reducing the level of oxygen radicals.

White mustard has anti proliferative, proapoptotic, antioxidant and antimicrobial properties. It can be used as a food preservative because mustard glucosinolates reduce bacterial growth in salad packages, which leads to increasing vegetables' shelf life. In addition, it can be regarded as a part in anticancer therapy.
because of its low toxicity\textsuperscript{10}. The plant has antibacterial activity against \textit{Staphylococcus aureus} and no activity against \textit{Escherichia coli} and \textit{Pseudomonas aeruginosa} because Gram-negative bacteria are more resistant than Gram-positive bacteria\textsuperscript{11}.

The aim of this study was to compare between leaves, flowers and fruits of \textit{Sinapis alba} L. which is widespread in Syria and estimate the phenolic and flavonoid contents in the extracts of parts of the plant as well as to determine the \textit{2,2-diphenyl-1-picryl hydrazyl} (DPPH) scavenging activity and antibacterial activity against some selected pathogenic bacteria.

\section*{MATERIALS AND METHODS}

\subsection*{Plant material}

The plant was collected between January and April 2019 between 8 and 9 AM from different areas of Damascus and its countryside. It was identified by Dr. Imad Kadi, Department of Plant Biology, Faculty of Science, Damascus University.

The plant parts (leaves, flowers, and fruits) were separated from each other and dried at room temperature for 14 days in the shade and were powdered in an electric grinder (Multimaxy, GR-1000, Taiwan) to prepare the plant extracts.

\subsection*{Preparation of plant extracts}

Plant parts powder (25 g) was placed in the Soxhlet device, and the extraction was performed by adding 600 ml of solvent (methanol 70\% and chloroform), at 60\°C, for 6 hours, the extract was filtered with filter paper (Zelpa, Belgium). Solvent was removed using a rotatory evaporator under vacuum at 60\°C (RV 10 digital IKA, Germany) and then put in the shaking incubator (JSSI-100C –JSR, India) at 40\°C with stirring until the weight was relatively constant. The crude extracts were stored at 6\°C in airtight containers. The process was repeated three times for each sample and yields were calculated. The extraction yield was calculated by the following equation:

\[
Yield\% = \left( \frac{\text{weight of dry extract}}{\text{weight of dry powdered plant material}} \right) \times 100.
\]

\section*{Determination of total phenolic contents in the plant extracts}

The method published by Abdeltaif S \textit{et al.} was applied to determine total phenolic contents in samples\textsuperscript{12}. 20 \textmu l of extract was mixed with 1.58 ml of distilled water and 300 \textmu l of 20\% sodium carbonate and 100 \textmu l of Folin-Ciocalteu reagent (Merck KGaA Darmstadt, Germany). The blank was concomitantly prepared, containing 2 ml ethanol. The samples were mixed and then left in a dark place at room temperature for 45 min, the absorbance was measured at 765 nm (UV-VIS Spectrophotometer (T80+), PG Instruments, United Kingdom). The total phenols were identified by a calibration curve of gallic acid in ethanol within the concentration range 0-500 mg/l (Fig.1). Means were calculated from three parallel analyses as gallic acid equivalents in mg/g of dry plant.

\section*{Determination of total flavonoid contents in the plant extracts}

Aluminum chloride colorimetric method was used to determine total flavonoid contents in samples as mentioned by Kitaz A, 2017\textsuperscript{13}. One ml of extract was mixed with 1 ml of aluminum trichloride 2\% (Riedel-de Haen, Germany). The blank was concomitantly prepared, containing 2 ml methanol. The samples were mixed and then left in a dark place at room temperature for 30 min, the absorbance was measured at 464 nm (UV-VIS Spectrophotometer (T80+), PG Instruments, United Kingdom). The calibration curve was produced within the concentration range 0-40 mg/l of quercetin (Fig.2). Means were
calculated from three parallel analyses as quercetin equivalents in mg/g of dry plant.

**Evaluation of DPPH scavenging activity**

The free radical scavenging capacity of *Sinapis alba* L. extracts against DPPH (Tokyo Chemical Industry, Japan) was measured based on Alhajali O *et al.*, 2021 method\(^1\). 300 µl of each concentration of extract was mixed with 3 ml of 45 µg/ml ethanolic solution containing DPPH radicals. The samples were mixed and then left in the dark for 30 min, and the absorbance was measured at 518 nm (UV-VIS Spectrophotometer (T80+), PG Instruments, United Kingdom). The calibration curve was produced within the concentration range of 9-175 µg/ml of ascorbic acid (Fig.3). Results were calculated by preparing a series of concentrations (0.5-1-2-3-4-5 mg/ml) from each extract and showed as IC\(_{50}\) (the inhibitory concentration of half of free radicals). The percentage of scavenging activity was calculated using the following equation:

\[
\text{DPPH scavenging activity} (\%) = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100.
\]

Where:

- \(A_{\text{control}}\): The absorbance of control (Ethanol + DPPH)
- \(A_{\text{sample}}\): The absorbance of sample (Extract + DPPH).

**Statistical analysis**

SPSS software program, version 26 was used for statistical analysis.

Two-Way Analysis of Variance (ANOVA) test was used to define the significance of differences between *Sinapis alba* L. parts in the determination of total phenols and flavonoids contents and free radical scavenging capacity.

T-Test was used to define the significance of differences between *Sinapis alba* L. parts and ascorbic acid in the determination of free radical scavenging capacity.
RESULTS AND DISCUSSION

RESULTS

Yield of extraction

Yield of extraction ranged from 41.31% in the 70% methanolic flower extract to 4.39% in the chloroform extract of the fruits (Table 1).

Table 1: Phenol, flavonoid contents, and IC<sub>50</sub> value of Sinapis alba L. parts and positive control.

| Solvent   | Sinapis alba L. part | Yield % | Phenols (mg gallic acid equivalents/ g dry plant) | Flavonoids (mg quercetin equivalents/ g dry plant) | IC<sub>50</sub> (mg/ml) |
|-----------|----------------------|---------|-------------------------------------------------|--------------------------------------------------|-------------------------|
| Methanol 70% | Leaves  | 32.15 ± 0.85 | 19.282 ± 0.222 | 2.068 ± 0.008 | 3.963 ± 0.001 |
|           | Flowers | 41.31 ± 2.11 | 28.302 ± 0.467 | 2.072 ± 0.014 | 2.872 ± 0.001 |
|           | Fruits  | 33.42 ± 0.10 | 22.007 ± 0.393 | 1.354 ± 0.012 | 2.836 ± 0.001 |
| Chloroform | Leaves  | 6.52 ± 0.24  | 2.179 ± 0.023  | 1.425 ± 0.002 | 7.374 ± 0.002  |
|           | Flowers | 7.52 ± 0.39  | 1.811 ± 0.012  | 0.458 ± 0.003 | 17.853 ± 0.002 |
|           | Fruits  | 4.39 ± 0.09  | 1.596 ± 0.007  | 0.446 ± 0.003 | 10.972 ± 0.090 |

Ascorbic acid 0.09 ± 0.000

Values are mean ± standard deviation
DISCUSSION

Yield of extraction

Yields of methanolic extracts were higher than chloroform extract yields, this may be due to the plant material, which contains elevated levels of polar compounds that dissolve in methanol because of its higher polarity than chloroform.17

Determination of total phenolic and flavonoid contents

The importance of these calibrations is to determine the differences between Sinapis alba L. grown in Syria and global Sinapis alba L. Results of total phenolic contents agree with the content of Vergun O et al., 2019 who studied ethanol leaf extract of white mustard that contains total phenols content 73.58 mg gallic acid/ g dry extract.18 Nevertheless, Zhang D et al., 2019 estimated total phenols content of ethanol seeds extract to be 53.2 mg gallic acid/ g dry extract.19 Lower than the previous results.18 Total flavonoid contents of this study were lower than flavonoids found by Vergun O et al., 2019 which were measured as 62.91 mg quercetin/ g dry extract, this may be caused by different plant environment or different calibration method.18

Anti-Oxidant activity of Sinapis alba L. parts

Methanolic fruit extract had the highest free radical scavenging activity, this may be due to the high content of antioxidants in seeds which are in the fruits (like vitamin E, sterols, phosphatides and omega 3)20.

Free radicals scavenging activity results are compatible with Ronak F, 2016 and Mayengbam S et al., 2014. The percentage of inhibition of free radicals in white mustard seed methanol extract was calculated by Mayengbam S et al., 2014, it was 39% at concentration 2 mg/ml. However, the percentage of inhibition of white mustard powder ranged from 15 to 36% at a concentration of 1 mg/ml according to Ronak F, 2016.21

The statistically significance level value of Two-Way ANOVA Test was (P= 0.000< 0.05), which indicates that there was a statistically significant difference between methanolic and chloroform extracts.

The statistically significance level value of T-Test was (P> 0.05) in methanolic extracts, which indicates that there was not a statistically significant difference between methanolic extracts and ascorbic acid.

Table 2: Pearson’s correlation between extract concentration, total phenolic content, total flavonoid content and DPPH scavenging activity

|                        | Extract concentration | Total phenolic content | Total flavonoid content | DPPH scavenging activity |
|------------------------|-----------------------|------------------------|-------------------------|--------------------------|
| Extract concentration  | 1                     | 1.000**                | 1.000**                 | 0.793**                  |
| Total phenolic content | 1.000**               | 1                      | 1.000**                 | 0.793**                  |
| Total flavonoid content| 1.000**               | 1.000**                | 1                       | 0.793**                  |
| DPPH scavenging activity| 0.793**               | 0.793**                | 0.793**                 | 1                        |

**Correlation is significant at the 0.01 level
Table 3: The zone of inhibition of Sinapis alba L. extracts and controls against selected bacteria

| Sinapis alba L. part | Concentration | Diameter of inhibition zone (mm) |
|---------------------|--------------|----------------------------------|
|                     |              | Escherichia coli | Staphylococcus aureus | Pseudomonas aeruginosa |
| Leaves              | 200 mg/ml    | ND                | 14.0 ±0.0             | 7.5 ±0.0             |
|                     | 300 mg/ml    | ND                | 15.0 ±0.0             | 8.0 ±0.0             |
|                     | 500 mg/ml    | ND                | 16.0 ±0.0             | 9.0 ±0.0             |
|                     | 700 mg/ml    | ND                | 17.2 ±0.2             | 10.0 ±0.0            |
| Flowers             | 200 mg/ml    | ND                | 12.2 ±0.2             | 7.5 ±0.0             |
|                     | 300 mg/ml    | ND                | 13.0 ±0.0             | 8.0 ±0.0             |
|                     | 500 mg/ml    | ND                | 14.0 ±0.0             | 9.0 ±0.0             |
|                     | 700 mg/ml    | ND                | 15.0 ±0.0             | 10.0 ±0.0            |
| Fruits              | 200 mg/ml    | ND                | 13.2 ±0.2             | 7.5 ±0.0             |
|                     | 300 mg/ml    | ND                | 15.0 ±0.0             | 8.0 ±0.0             |
|                     | 500 mg/ml    | ND                | 16.0 ±0.0             | 9.0 ±0.0             |
|                     | 700 mg/ml    | ND                | 17.2 ±0.2             | 10.0 ±0.0            |

Cefaclor 30 µg  
Levofloxacin 5 µg  
Tobramycin 10 µg  
Gentamicin 10 µg  
DMSO -

Values are mean inhibition zone (mm) ± standard deviation.  
ND: Not Detected. R: Resistance. I: Intermediate. S: Sensitive

The statistically significance level value of T-Test was (P < 0.05) in chloroform extracts, which indicates that there was a statistically significant difference between chloroform extracts and ascorbic acid.

Extract concentration, total phenolic content, total flavonoid content and DPPH scavenging activity in each plant part showed significantly positive correlation. This means that concentration of extract; total phenols and flavonoids have an important role in DPPH scavenging activity of Sinapis alba L. because poly phenols have a reducing role due to its hydroxy groups, which gives it hydrogen-donating property.

Anti-Bacterial activity of Sinapis alba L. parts

The zones of inhibition with diameter less than 12 mm were considered as having no antimicrobial activity, diameters between 12 and 16 mm were considered moderately active and with 16 mm were considered highly active according to Sujatha A et al., 20135. These mean no antimicrobial activity against Escherichia coli and Pseudomonas aeruginosa.

All extracts have moderate activity against Staphylococcus aureus except leaves and fruits extracts, which at 700 mg/ml concentration, have high activity.

Gram-negative bacteria (Like Escherichia coli and Pseudomonas aeruginosa) have an envelope which is made of three layers (Outer membrane, peptidoglycan cell wall and inner membrane). This envelope gives bacteria impermeable and resistant properties.

These results are similar to Camacho C et al., 2019 and Ronak F, 2016, but Weldu H et al., 2019 displayed higher activity against Staphylococcus aureus and Escherichia coli11&21&25.

According to previous studies the increasing of extract concentrations have a direct relation to give a high antimicrobial activity due to the increase in phenols, flavonoids and glucosinolates contents.
The statistically significance level value of One-Way ANOVA Test was (P= 0.151 > 0.05), which indicates that there was not a statistically significant difference between the extracts of plant parts, the reason could be that all parts have similar concentrations of the active compounds which are given the antimicrobial activity.

Conclusion

This study concluded that the methanolic extracts of Sinapis alba L. have good phenolic and flavonoid contents, good free radical scavenging activity and have anti-bacterial activity against Staphylococcus aureus but not against Escherichia coli or Pseudomonas aeruginosa. There was no statistically significant difference between the plant parts studied, so any part of them can be used in the food and pharmaceutical industries.

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النشاط المضاد للتآكس والمضاد للجراثيم في أوراق وأزهار وثمار نبات الخردل الأبيض النامي في سوريا

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يهدف البحث إلى المقارنة بين أجزاء الخردل الأبيض الذي ينمو في سوريا ، وتحديد المحتوى الفينولي والفلاقونئيدي في الخلاصات بالإضافة إلى حساب النشاط الكامبيسيي للجذور الحرة والمضادات للجراثيم. بلغ أعلى محتوى فينولي 8,63 مل ج/ملل. من حمض الغاليك/ح نبات جاف ، وأعلى محتوى فلافونئيدي 72,07 مل ج/ملل. من كيرستين/ح نبات جاف في خلاصة الأزهار الميثانولية، بينما بلغ أقل محتوى فينولي 1,59 مل ج/ملل. من حمض الغاليك/ح نبات جاف ، وأقل محتوى فلافونئيدي 44,01 مل ج/ملل. من كيرستين/ح نبات جاف في خلاصة الثمار الكلورورومية.

في التفاعل مع مركب دي فينيل بيكريل هيدرازيل تراوحت التركيزات المثبطة للأكسدة بين 2,83 و 17,69 مل/ملل. حيث أثبتت الخلاصات الميثانولية هالات تثبيط لجراثيم العقوديات المذهبة قطرها 17,2 مم في خلاصة الأوراق والثمار، و15 مم في خلاصة الأزهار عند تركيز 700 مل/ملل. أظهرت الدراسة أن خلاصات نبات الخردل الأبيض الميثانولية تمثلت محتوى فينوليا وفلاقونئيديا جيداً، ونشاط كاسحاً للجذور الحرة، وفعالية مضادة لجراثيم العقوديات المذهبة.