INTRODUCTION

Medicinal plants have continued to receive a lot of attention from researchers due to their pharmacological effects such as antioxidant, anti-inflammatory and antibacterial properties. The resistance to antibiotics by pathogenic microorganisms in a public health problem in the world. Indeed, many bacterial strains show resistance to several groups of antibiotics [1]. However, in the beginning of the 20th century, scientists have begun to search other molecules with antibacterial activity. Medicinal plants are natural and safer sources of anti-free radical compounds. Reactive oxygen species (ROS) provoke oxidative damage which plays a role in...
the pathogenesis of diseases such as cancer, atherosclerosis and Alzheimer’s [2,3].

Brassicaceae is a large family made up of vegetables (Brassica crops), medicinal plants (Capsella bursa-pastoris), oil-rich plants (Brassica napus) and the model species of plant science (Arabidopsis thaliana). Among these plants, the seeds of Lepidium sativum (garden cress) are widely used in Arabic countries for their medicinal properties. They have various nutritional and medicinal attributes, and are recommended for anti-diarrheal [4], cardiotonic, hypotensive, antimicrobial, bronchodilator and hypoglycemic applications [5]. These activities can be attributed to bioactive compounds as flavonoids present in this plant. Flavonoids (C6-C3-C6) can be classified into different sub-classes (flavones, flavanones, flavonols, isoflavones, flavanols, chalcones and anthocyanins) and they are commonly found conjugated to sugars in the form of O-glycosides or C-glycosides forms [6].

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EXPERIMENTAL

Plant seed collection

Seeds of L. Sativum were collected from an area in the Northwest of Algeria in May 2012. The seeds were identified by Dr H. Hadj-Arab of the laboratory of Eco-genetic and Biosystematics, Houari Boumedienn, according to the flora of Algeria. The identification was done in the herbarium of Botany Department, Ecole National Supérieure Agronomique d’Alger (ENSA), and a voucher specimen no. P12 was assigned.

Extract preparation

Twenty grams of the seeds were extracted three times with 70 % methanol for 48 h. The extract was then filtered through Whatman No.2 paper. Two more 20g batches were similarly extracted, and the combined extract was concentrated in vacuum at 40 °C. It was extracted with different solvents (50 mL each) in order: hexan, ethyl ether, ethyl-acetate and n-butanol. Each solvent extract was evaporated and taken up in methanol. Only the n-butanol fraction was used for the identification of phytochemical composition, as well as for antimicrobial and antioxidant assays.

LC–MS analysis of flavonoids

The extract was injected into the ESI source using a Waters Acquity I-Class separation module with an Acquity PDA UV detector. Separation was achieved on an Uptide C18 ODB, 100 x 2.1 mm. The flow rate was 0.4 ml/min, with elution gradient of 0.1 % acetic acid (A) and 0.5 % acetic acid (B) in acetonitrile, in the order 0 – 2 min 5 % A, 4min 10 % A, 17min 40 % A, 21–23min 100 % B, 23 – 28min 5 % A. The components were identified and quantified at positive and negative ESI modes using triple quadrupole mass spectrometer (Xevo TQ-S, Waters) at full scan. The operating conditions used were: capillary and extraction voltages of 2.80kVand 3V, respectively; with gas desolvation and source block temperatures fixed at 150 and 300 °C, respectively. Desolvation and nebulisation were facilitated using nitrogen gas at flow rates of 200 and 1000L/h, respectively.

Determination of total phenolic contents

The levels of total phenolic compounds in the extract were quantified colorimetrically as gallic acid equivalents (GAE) with Folin-Ciocalteu reagent as described earlier [8]. The results were expressed as GAE/gram dry weight.

Determination of minimum inhibitory concentration (MIC) of extract

Antimicrobial test was carried out on five reference strains: S. aureus (ATCC 25923, meticillin-resistant), S. aureus (ATCC 43300 meticillin-sensitive), E. feacalis (ATCC 29212),
E. coli (ATCC 25922), and P. aeruginosa (ATCC27852). The MIC and IC_{50} values for the extract were determined using the serial broth dilutions method in sterile tubes [1,9,10]. The ranges of final extract concentrations used were 0.05–32mg/mL. Each tube was inoculated with a standardized inoculum of 10^6 cells. This inoculum was obtained by diluting 2-3 well-isolated colonies on nutrient agar in 10 mL of nutrient broth. Following homogenization, the diluted colonies were placed in an incubator for 3 h at 37 °C. Thereafter, one milliliter of each culture medium was introduced into 10mL of nutrient broth in a tube containing the extract. The tubes were assessed for turbidity as evidence of bacterial growth. After incubation at 37 °C for 24 h, And MIC was calculated in terms of the lowest extract concentration that produce 100% growth suppression. Unexposed organisms were used as control.

**Determination of in vitro antioxidant activity**

**DPPH method**

The method of Brand-Williams et al [11] was used. To 1mL of extract was added 2mL of 0.4 mM DPPH in methanol. The mixture was incubated in the dark for 30min, and the absorbance was read at 517nm. All determinations were performed in triplicate. The DPPH scavenging activity (D) was calculated using Eq 1.

\[
D(\%) = \left(1 - \frac{A_t}{A_s}\right)\times 100
\]

\(A_t\) = absorbance of the test sample, and \(A_s\) = absorbance of the standard control (BHT)

**Reducing power (FRAP) method**

Reducing power (FRAP) was evaluated using the potassium ferricyanide colorimetric method as described by Oyaizu [12].

**β-Carotene/linoleic acid method**

β-Carotene/linoleic acid spectrophotometric method was used to estimate the antioxidant activities of the extract [13]. The principle of this procedure is that the substrate β-carotene undergoes color change as a result of linoleic acid oxidation. The decrease in absorbance is measured after 120min at 470nm. In the assay, an aliquot of the extract solution (0.2mL) was mixed thoroughly with 4.8mL of linoleic acid emulsion and the absorbance was read at 470nm, with butylated hydroxyl toluene (BHT) as standard. The % inhibition of β-carotene bleaching (B) was calculated as relative antioxidant activity (RAA) [14] using Eq 2.

\[
B(\%) = \left(1 - \frac{A_t}{A_{BHT}}\right)\times 100
\]

\(A_t\) = absorbance of the test sample, and \(A_{BHT}\) = absorbance of the standard control (BHT)

**Statistical analysis**

The data are presented as mean ± SD and were analyzed by one-way analysis of variance (ANOVA) using Statistica software, followed by Tukey’s multiple range tests. P < 0.05 was considered statistically significant.

**RESULTS**

Compounds identified in *Lepidium sativum* seed extract

Seventeen Compounds were identified from the seed extract of *Lepidium sativum* by LC-MS, as shown in Table 1.

These compounds included glucotropaeoline (glucosinolate), sinapoyl malate, sinapic acid, sinapine, sinapoyl diglucose and ferulic acid., Glucotropaeoline was the most abundant component (67.5 %) followed by sinapine (13.43 %). In addition, *Lepidium sativum* contained relatively high concentrations of flavonoids. From the results obtained, quercetin and kaempferol were the main flavonols present, with varying degrees of glycosylation (mono, di and tri-glycoside). Many isomers of hexose rhamnose and other derivatives were also present. All the glycosides were attached to a rhamnose.

Four components (heterosides) were identified for the first time. According to their HPLC retention times and MS profiles, these heterosides were identified as two isomers of kaempferol hexose rhamnose (ion at m/z 595 [M-H]^-) and three derivatives of kaempferol rhamnose_hexose (benzoyl-hexose) (ion at 861 m/z [M-H]^-). The other compounds have been previously identified [15].

Kaempferol di-hexose rhamnose and quercetin di-hexoside rhamnose were the most important heterosides present in *L. sativum* (at high levels 3.77 and 2.07 %, respectively).

**Antioxidant activity and total phenolic contents of the extract**

The results are presented in Table 2. The IC_{50} of the n-butanol extract in the DPPH radical scavenging assay was 67.1±0.3 µg/mL. Reducing power (FRAP) and % inhibition of β-carotene bleaching were 100±0.05 µg.mL^{-1} and 54.91%, respectively. The antioxidant activity of the butanolic extract was < the standards (ascorbic acid, quercetin and α-tocopherol).
The activity of n-butanol extract from *L. sativum* has exhibited appreciable antibacterial activity against all the bacteria strains tested irrespective of Gram strain. This is in agreement with the results of antimicrobial studies on *L. sativum* seeds [16,17]. This is also in agreement with the results available in the literature regarding the phytochemical compositions of leaves of *L. sativum* and leaves of other Brassicaceae plants indicate the presence of three flavonols viz quercetin, kaempferol and isorhamnetin [18]. Thus, it seems that isorhamnetin is absent in the seeds. However, while the same acylation was present in the Algerian variety, quercetin was replaced by a kaempferol. The results available in the literature regarding the phytochemical compositions of leaves of *L. sativum* and leaves of other Brassicaceae plants indicate the presence of three flavonols viz quercetin, kaempferol, and isorhamnetin [18]. Thus, it seems that isorhamnetin is absent in the seeds.

**DISCUSSION**

The main flavonols present in many Brassicaceae species are quercetin, kaempferol and their derivatives (mono-, di-, tri-glycosides) [16,17]. This is in agreement with the results obtained in the present study. A previously unknown quercetin-3-O-(6-O-benzoyl)-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside-7-O-α-L rhamnopyranoside was identified by Fan et al. [7]. However, while the same acylation was present in the Algerian variety, quercetin was replaced by a kaempferol. The results available in the literature regarding the phytochemical compositions of leaves of *L. sativum* and leaves of other Brassicaceae plants indicate the presence of three flavonols viz quercetin, kaempferol, and isorhamnetin [18]. Thus, it seems that isorhamnetin is absent in the seeds. Results from various studies indicate that all flavonoids glycosides possess strong antimicrobial activity against strains of *P. aeruginosa* and *S. aureus* but exhibit a very low activity against *E. coli* [19]. This is also in agreement with the results of antimicrobial activity of the n-butanol extract used in this study. Studies on methanol and chloroform extract of *L. sativum* have also shown low antibacterial activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* [20]. The antibacterial activity of

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**Table 1**: Quantitative and qualitative characterization of phytochemical compounds from *L. sativum*

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT</th>
<th>Molecular ion MH-</th>
<th>Molecular ion MH+</th>
<th>Proportion crude extract (%)</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucopaoline</td>
<td>-3.43</td>
<td>408, 410</td>
<td>/</td>
<td>187.12</td>
<td>67.507</td>
</tr>
<tr>
<td>Sinapoyl malate</td>
<td>-7.73</td>
<td>339, 223</td>
<td>207</td>
<td>4.62</td>
<td>1.67</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>-7.95</td>
<td>193</td>
<td>195</td>
<td>0.38</td>
<td>0.137</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>-8.15</td>
<td>223</td>
<td>225</td>
<td>1.53</td>
<td>0.552</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>-12.9</td>
<td>573, 223</td>
<td>575</td>
<td>7.41</td>
<td>2.675</td>
</tr>
<tr>
<td>Sinapine</td>
<td>+5.97</td>
<td>/</td>
<td>310</td>
<td>37.2</td>
<td>13.43</td>
</tr>
<tr>
<td>Q di-hexose rhamnose</td>
<td>+5.99</td>
<td>771</td>
<td>773</td>
<td>5.73</td>
<td>2.07</td>
</tr>
<tr>
<td>K di-hexose rhamnose</td>
<td>+6.4</td>
<td>755</td>
<td>757</td>
<td>10.46</td>
<td>3.77</td>
</tr>
<tr>
<td>K hexose rhamnose 1</td>
<td>+7.15</td>
<td>/</td>
<td>595</td>
<td>4.68</td>
<td>1.69</td>
</tr>
<tr>
<td>Q hexose rhamnose</td>
<td>+7.35</td>
<td>609</td>
<td>611</td>
<td>0.17</td>
<td>0.06</td>
</tr>
<tr>
<td>K hexose rhamnose 2</td>
<td>+7.75</td>
<td>593</td>
<td>595</td>
<td>0.26</td>
<td>0.837</td>
</tr>
<tr>
<td>K rhamnose (benzo) di-hexose 1</td>
<td>+8.84</td>
<td>859</td>
<td>861</td>
<td>2.96</td>
<td>1.867</td>
</tr>
<tr>
<td>K rhamnose (benzo) di-hexose 2</td>
<td>+9.25</td>
<td>859</td>
<td>861</td>
<td>0.31</td>
<td>0.11</td>
</tr>
<tr>
<td>K hexose rhamnose 3</td>
<td>+10.19</td>
<td>/</td>
<td>595</td>
<td>1.57</td>
<td>0.566</td>
</tr>
<tr>
<td>Q rhamnose</td>
<td>+10.94</td>
<td>447</td>
<td>449</td>
<td>0.51</td>
<td>0.322</td>
</tr>
<tr>
<td>K rhamnose (benzo) di-hexose 3</td>
<td>+11.01</td>
<td>859</td>
<td>861</td>
<td>0.98</td>
<td>0.35</td>
</tr>
<tr>
<td>K rhamnose</td>
<td>+12.45</td>
<td>431</td>
<td>433</td>
<td>0.29</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Q = quercetin, K = kaempferol

**Table 2**: Total phenolic content expressed as mg/g gallic acid equivalent (EAG), and antioxidant activity of n-butanol extract *L. sativum* seeds

<table>
<thead>
<tr>
<th>Material</th>
<th>Total polyphenols mg/gEAG</th>
<th>DPPH (IC50[µg.mL⁻¹])</th>
<th>Antioxidant activity</th>
<th>β–Caroten(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>1.629±0.13</td>
<td>67.1±0.3*</td>
<td>100±0.051</td>
<td>54.91±0.116*</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>-</td>
<td>4.04±0.13*</td>
<td>7.53±0.104*</td>
<td>30.55±0.117*</td>
</tr>
<tr>
<td>Quercetin</td>
<td>-</td>
<td>6.72±0.158*</td>
<td>20±0.01*</td>
<td>45.37±0.12**</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>98.55±0.248*</td>
</tr>
</tbody>
</table>

Values (mean ± SD, n = 3); *significant difference (p < 0.05)

**Table 3**: Antibacterial activity of n-butanol extract *L. sativum* seeds

<table>
<thead>
<tr>
<th>Parameter</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. aureus 1</th>
<th>S. aureus 2</th>
<th>E. faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50</td>
<td>0.41</td>
<td>0.42</td>
<td>0.47</td>
<td>0.42</td>
<td>0.4</td>
</tr>
<tr>
<td>MIC</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**Antibacterial activity**

The results of antibacterial activity of *Lepidium sativum* which was evaluated in vitro against five bacterial strains Table 2. The calculated MIC and IC50 are presented in the Table 3. The n-butanol fraction has exhibited appreciable antibacterial activity against all the bacteria strains tested irrespective of Gram strain.
flavonol glycosides towards Gram-positive bacteria may be related to the cell membrane constituents and structures of these organisms. The flavonols may also be inhibitory to the activities of cellular enzymes. It has been also reported that flavonol glycosides chelate metals, an effect which can inhibit some enzymes [21]. It is important to mention that the permeability of the bacterial cells to the tested compounds is one of the factors that determine their antibacterial effects.

The flavonols have the ability to donate hydrogen ions to synthetic free radical compound (DPPH) [22], and to reduce potassium ferricyanide (Fe⁴⁺) to potassium ferrocyanide (Fe²⁺) by donating an electron [23]. These antioxidants can stop the conversion of linoleic hydro-peroxide or eliminate this peroxide [24]. The differential activities of flavonoids could depend on the solvent used for extraction and on the position of substituents in the flavonoid nucleus. These differences in substituents give rise to the major bioactive compounds such as quercetine, kampferol and their glycosides which are present in Lepidium sativum seeds [7,15-17].

CONCLUSION

The results of this study indicate that n-butanol extract of L. sativum seed possesses significant antioxidant properties, and significant antibacterial activities against selected Gram-positive and Gram-negative pathogens. Further investigations are needed to elucidate the structures of the identified compounds in detail using nuclear magnetic resonance (NMR). It would also be helpful to carry out in vivo tests on these compounds in their isolated and purified forms.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES


