In vitro antioxidant and antibacterial properties of some Moroccan Medicinal Plants

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Abstract: Antioxidant and antibacterial activities of leaves extracts of Populus alba, Teucrium fruticans, Dittrichia graveolens and Coriaria myrtifolia were investigated. The antioxidant activity was evaluated using beta-carotene-linoleic acid system. All the extracts protected beta-carotene against oxidative discolouration. C. myrtifolia extract was the most effective. The antibacterial effect was assessed by disc diffusion technique against Gram-negative and Gram-positive bacteria. The recorded diameters of inhibition zones showed that among the tested extracts, C. myrtifolia had the highest antibacterial activity (11 to 16 mm) while no activity was observed for D. graveolens extract. Micrococcus luteus was the most sensitive strain.

Keywords: Antioxidant, antibacterial, Moroccan plant extracts, Coriaria myrtifolia.

Introduction

The preservation of food quality and security involves mainly the control of lipid oxidation and growth of spoilage and pathogenic microorganisms. Indeed, food may contain microorganisms that may lead to spoilage and eventually to food-borne illness. In addition, lipid peroxidation constitutes the major cause of fatty foods rancidity. Several antimicrobial and antioxidant molecules are incorporated in food to extend their shelf life. In recent years, there has been a growing demand for food additives from natural source due to increasing consumer concern over artificial ingredients.[1]. Medicinal plants constitute a powerless source of bioactive molecules. Several investigations are
conducted aiming to evaluate the possibility of using plant extracts as functional ingredients in food and drinks. Morocco offers a considerable vegetable biodiversity and is characterized by a rich and varied spontaneous aromatic flora with high levels of endemism. Some of these plants are used by local population for medicinal purposes. In this study, three medicinal plants have been chosen to evaluate their possible antibacterial and antioxidant activities; *Populus alba* (Salicaceae), *Teucrium fruticans* (Lamiaceae) and *Coriaria myrtifolia* (Coriariaceae). *P. alba* is used as depurative and in treating tooth decay [2]. Some Teucrium species are used for their antibacterial, anti-inflammatory and antipyretic activities [3]. Previous studies have reported the antibacterial activity of the extracts of *P. alba* growing in Jordan [4], Turkey [5] and Japan. While few data are available on the antibacterial and antioxidant activities of the extracts of *T. fruticans* and *D. graveolens* and to the best of our knowledge, there are no published reports on the antimicrobial activities of *C. myrtifolia* extracts. The aim of this work was to investigate the antibacterial and the antioxidant effects of *P. alba*, *T. fruticans*, *D. graveolens* and *C. myrtifolia* extracts. The antibacterial activity was evaluated using disc diffusion assay and the antioxidant effect was measured by β-carotene bleaching test.

**Experimental**

**Plant material**

All plants were collected from the North of Morocco in April 2010. The taxonomic identification of plant material was confirmed by A. Ennabili (from The National Institute of Medicinal and Aromatic Plants (NIMAP), Sidi Mohamed Ben Abdellah University). The voucher specimens have been deposited at the Herbarium of the NIMAP.

**Preparation of extracts**

The air-dried and finely ground leaves were extracted using a Soxhlet apparatus, first with hexane and then with methanol 85%. The solvent was removed using a rotary evaporator at temperature not higher than 40°C. Then the concentrated samples were lyophilized. The prepared extract was stored at 4°C until further examination.

**β-Carotene–linoleic acid assay**

The antioxidant activity of the plant extracts was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [6]. A solution of β-carotene/linoleic acid mixture was prepared as following: 0.5 mg of β-carotene was dissolved in 1 ml of chloroform (HPLC grade), 25 μl of linoleic acid and 200 mg of Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml of distilled water saturated with oxygen was added with vigorous shaking. Thus, 350 μl of the extract prepared in methanol at 2 mg/ml were added to 2.5 ml of the emulsion. The reaction mixture was incubated for 48 h at room temperature. The absorbance (490 nm) of the mixture was measured at regular time intervals. BHT was used as positive control. The tests were carried out in triplicate. The relative antioxidant activity was calculated according to the following formula:

\[
\text{AA}\% = \left( \frac{\text{AE}_{48h}}{\text{AC}_{48h}} \right) \times 100
\]

Where AE_{48h} is the absorbance of the extract after 48h, and AC_{48h} is the absorbance of BHT used as the positive control.

**Preparation of test microorganisms**

Six bacterial strains were used in this assay; three reference strains (*Micrococcus luteus* ATCC 14452, *Bacillus subtilis* ATCC 6633 and *Escherichia coli* ATCC 25922) and three strains isolated in the Laboratory of Epidemiological Diagnostic and Environmental Hygiene, Fes, Morocco (*Escherichia coli*, *Staphylococcus* spp and *Salmonella* spp). Bacterial strains were maintained in LB agar slant at 4°C.

**Disc diffusion assay**

The disc diffusion method was employed to determine the antibacterial activities of the extracts against bacterial strains. LB Agar plates were seeded with 1 ml of a diluted culture (10^6 CFU/ml) of the bacterial strain. Sterile 6 mm diameter filter paper discs were impregnated with 10 μl of the extract and placed onto the inoculated plates. Pure DMSO was used as negative control while penicillin (10 μg) and chloramphenicol (30 μg) were used as positive control for comparative purposes. Plates were then incubated for 24 h at 37 °C. The size of the inhibition zones was measured and the antibacterial activity was expressed in terms of the average of inhibition zone diameters. Each extract was tested three times.
Results and Discussions

Antioxidant activity

The antioxidant activity of different extracts was evaluated by following the discoloration of β-carotene at 490 nm. As seen in table 1, all the extracts studied inhibited the oxidation of β-carotene. This effect is due to either; the inhibition of linoleic acid peroxidation or the radical scavenging hydroperoxides formed during the peroxidation of linoleic acid (scavenger effect) [7]. C. myrtifolia extract showed the highest antioxidant activity (73.74 %) followed by that of P. alba (57.01 %), T. fruticans (53.57 %), and D. graveolens (43.98 %).

These results are in accordance with that obtained using DPPH assay and reducing power test (8). However, the antioxidant activity of C. myrtifolia extract was significantly lower than that of BHT in this system. In a previous study, we have found that C. myrtyfolia extract was significantly more effective than BHT in DPPH and reducing power assay (8). This discordance may be explained by the fact that the components responsible for the antioxidant activity of C. myrtifolia extract are more actives in hydrophilic system.

The observed antioxidant activity should be attributed to the high quantity of polyphenols, tannins and flavonoids in the extracts (9). Previous reports have shown that the antioxidant activity of plant extracts increases with total polyphenols, flavonoids and tannins content (10).

Antibacterial effect

The results of the antibacterial activities of the studied extracts are given in Table 2. Among the used extracts, C. myrtifolia showed the highest antibacterial activity (11 to 16 mm) while no activity was observed for D. graveolens extract.

The extract of C.myrtiflia shozs also moderately antibacterial activity that can be related to the polar phenolic compounds [10].

Table 1. Antioxidant activity of plant extracts

<table>
<thead>
<tr>
<th>Plant</th>
<th>AA%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. myrtifolia</td>
<td>73.74 ± 1.35</td>
</tr>
<tr>
<td>T. fruticans</td>
<td>53.57 ± 3.07</td>
</tr>
<tr>
<td>P. alba</td>
<td>57.01 ± 4.05</td>
</tr>
<tr>
<td>D. graveolens</td>
<td>43.98 ± 4.35</td>
</tr>
<tr>
<td>BHT</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 2. Antibacterial activity of plant extracts

<table>
<thead>
<tr>
<th>Plant</th>
<th>E. coli ATCC 25922</th>
<th>E. coli Salmonella spp</th>
<th>Staphylococcus spp</th>
<th>B. Subtilis ATCC 6633</th>
<th>M. luteus ATCC 1445</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. myrtifolia</td>
<td>11.2 ± 0.20</td>
<td>10.9 ± 0.33</td>
<td>11.2 ± 0.37</td>
<td>13.4 ± 0.21</td>
<td>16.0 ± 0.20</td>
</tr>
<tr>
<td>T. fruticans</td>
<td>-</td>
<td>-</td>
<td>6.5 ± 1.00</td>
<td>7.7 ± 0.50</td>
<td>-</td>
</tr>
<tr>
<td>P. alba</td>
<td>-</td>
<td>-</td>
<td>8.2 ± 0.25</td>
<td>8.7 ± 0.88</td>
<td>13.5 ± 0.50</td>
</tr>
<tr>
<td>D. graveolens</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>19.0 ± 0.57</td>
<td>19.5±0.2</td>
<td>20.0 ± 1.00</td>
<td>ND</td>
<td>33.7±1,45</td>
</tr>
<tr>
<td>Penicillin</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>24.5 ± 0.50</td>
<td>ND</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(-): no inhibition; a: Inhibition zone diameter (mm) including disc diameter (6 mm); ND: Not determined.
The results are means ± SD of three measurements. Extract concentration: 10 mg/ml

The methanolic extract of P. alba revealed some antibacterial activity against gram positive bacteria (8 to 13 mm). Previous studies showed that P. alba is a potential inhibitor of mycobacteria [11], but it showed very weak activity against bacteria [4] [12] [13] [14]. Moreover, the ethanolic extract of this plant demonstrated weak antiquorom sensing activity [2].

T. fruticans extract was poorly active against Staphylococcus spp and B. subtilis and inactive against M. luteus and all Gram negative strains tested. Samec et al. have found that T. arduini (another Teucrium specie) was poorly active against S. aureus and B. subtillis [15].

Several studies proved that phytoconstituents like flavonoids [16], polyphenols [17], tannins [18] and sesquiterpenes [19] are effective antimicrobial substances against a wide range of microorganisms. Moreover, a correlation has been established between the antibacterial activity of plant extracts and their phenolic constituents [20] [21] [22] [23]. In a previous study we have found that the extract of C. myrtifolia is richer in total phenolics and flavonoids compared to the other extracts (8). This could explain the differences in antibacterial activity between the extract C. myrtifolia and those of the other plants tested.

Several studies reported that Gram-positive bacteria are generally more susceptible to non polar phenolic compounds than Gram-positive ones [24]-[25]-[26]-[27]. However, the results obtained with C. myrtifolia extract are not in agreement with this finding. This can be explained by the fact that C. myrtifolia contains some particular anti-Gram negative substances.

Conclusion

In conclusion, this study contributes to the knowledge of the in vitro antioxidant and antibacterial effects of four Moroccan plants. The results reported showed that the extracts of plants studied exerted interesting antioxidant activity. Overall, the extract of C. myrtifolia had the highest antibacterial activity while no activity was observed for D. graveolens extract. This study represents the first report of the antibacterial activity of C. myrtifolia leaf extract.

Acknowledgement

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