In vitro antioxidant and antimicrobial properties of some Moroccan’s Medicinal plants.

M. Boudkhili¹*, H. Greche¹, S. Bouhdid¹, F. Zerargui², and L. Aarab³

¹National Institute of Medicinal and Aromatic Plants, University of Sidi Mohamed Ben Abdellah, BP 8857, 30100 -Atlas- Fès, Morocco.
²Laboratory of biochemistry, Faculty of Natural Science de la nature and Earth, University Ferhat Abbas, Algeria.
³Laboratory of the Molecules Bioactives (LMBSF), Technology and, University Sidi Mohamed Ben Abdellah, B.P. 2202 –Route of Imouzzer, Fes, Morocco.

*Corres. author: boudkhillimeryem@yahoo.fr
Tel. 00212662347176, Fax 00212535689500

Abstract: Antioxidant activity of leaves extracts of some Moroccan’s plants were evaluated spectrophotometrically by coupled oxidation of beta-carotene and linoleic acid. Disc diffusion technique was used for in vitro antibacterial screening. Zones of inhibition were observed in disc diffusion for antimicrobial investigation against tree Gram-positive and tree Gram- negative pathogenic bacteria. These plants species have very interesting antioxidant activities, with that of Coriaria myrtifolia being the most noteworthy among all plants tested. Furthermore, these extracts showed average zone of inhibition ranged from 8-16 mm, and weak to moderate activity was recorded. A large zone of inhibition was observed 16mm against Micrococcus luteus inhibited by C.myrtifolia.

Keywords: Antioxidant, Antimicrobial, Bacteria, Morocco, Plant extracts.

INTRODUCTION

Medicinal plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines in preventive, promotive and curative applications. In recent years, the growing demand for natural source of plants in some biological activities has been searched in the world [1]. Such knowledge is important for the development of health-promoting functional foods containing both antioxidant source and eventually probiotic bacteria. In this study, we are interesting to antioxidant and antibacterial activities of some Moroccan’s plants.

These plants are belonging to Salicaceae (Populus alba), lamiaceae (Teucrium fruticans) Asteraceae (Dittrichia graveolens) and Coriariaceae (Coriaria myrtifolia). Some of these plants have been used in traditional medicine.

In the folk medicinal traditions, P.alba is used in the treatment as depurative, and calming of tooth decay [2]. Some Teucrium species are used for their...
antibacterial, anti-inflammatory and antipyretic activities [3]. Antibacterial activities studies are available in the literature on P.alba from different sites Jordan’s species [4], Turkey’s plant [5] and Japanese P.alba. Although, few study of antibacterial and antioxidant activities have been reported of the extracts of T.fruticans and D.graveolens. To our knowledge, there is no published report on the antimicrobial activities of extract of C.myrtifolia. The purpose of this work is to investigate the antibacterial effect of these plants on Escherichia coli, Staphylococcus spp, Salmonella spp, Bacillus subtilis, Micrococcus luteus, and also to evaluate the antioxidant activity by β-carotene assay.

MATERIALS AND METHODS

Plant material
All plants were collected from the North of Morocco in April 2010, were collected in April 2010 from the from the North of Morocco and identified by Professor A.Ennabili from the National Institute of Medicinal and Aromatic Plants, where a specimen of this species is conserved in the herbarium of botanic department (herborization Code).

Chemicals
All the compounds were purchased from Sigma (St Louis, MO, USA). The solvents were analytical grade.

Preparation of extracts
The air-dried and finely ground samples were extracted in Soxhlet apparatus, first with hexane and then with methanol 85%. The methanol was removed by a rotary evaporator at temperature not higher than 40°C. Then the concentrated samples were dried in a lyophiliser. The prepared extract was stored at -20°C until further examination.

Preparation of test microorganisms
Six strains were used in this assay; three references strains (Micrococcus luteus ATCC 14452, Bacillus subtilis ATCC 6633 and Escherichia coli ATCC 25922) and three isolates of the Regional Laboratory of Epidemiological Diagnostic and Environmental Hygiene, Fes, Morocco (Escherichia coli, Staphylococcus spp and Salmonella spp). All the microbial strains were transferred on nutrient agar slants and transferred in to nutrient broth and stored at -40°C until required for the study.

β-Carotene–linoleic acid assay
The measurement of the antioxidant activity of this extract 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 linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml distilled water saturated with oxygen was added with vigorous shaking. Thus, 350 μl of the extract, or BHT prepared in methanol at 2 mg/ml were added and the emulsion system was incubated for up to 48 h at room temperature. After this incubation period, absorbance of the mixtures was followed in 490 nm in regular intervals of time during 48 h. Antioxidative capacities of the extracts were compared with BHT and blank consisting of only 350 μl methanol at the same concentration. The relative antioxidant activity was calculated according to the formula: AAR% = (At:48h (sample) / A t:48h( BHT) ) *100

Where At:48h (sample) is the absorbance of the test compound after 48h, and A t:48h( BHT) is the absorbance of synthetic antioxidant reagent BHT used as the positive control. The tests were carried out in triplicate.

Antimicrobial effects against bacteria
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 (106 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 plate. Pure DMSO was used as negative control while penicillin (10 μg) and chloramphenicol (30 μg) were used as positive control for comparative purposes. All tests were performed in triplicates. Plates were then incubated for 24 h at 37 °C and size of inhibition zone diameters surrounding filter paper disc was measured.

Statistical analysis
Data were expressed as the mean ± SEM. Comparisons of means were performed by using the t-test of Student. The level of statistical significance was set at p < 0.05.

RESULTS AND DISCUSSIONS
The aim of this study is to investigate in vitro antioxidant activity and evaluate the antibacterial activity of methanolic extracts of some Moroccan’s plants. The antioxidant activity of different extracts was evaluated by spectrophotometer following the discoloration of β-carotene at 490 nm. The results of
the percentage of antioxidant activity according to the time are shown in Figure 1.

As seen in table 1, all the extracts studied inhibited the oxidation of β-carotene very significantly compared to the control, and a very important way. This effect is due either to the inhibition of peroxidation of linoleic acid is the radical scavenging hydroperoxides formed during the peroxidation of linoleic acid (scavenger effect) [7].

The C.myrtifolia extract had the highest value of the percentage of antioxidant activity at 24 hours (57%, p<0.05) among the tested extracts. The Teucrium fruticans showed the next highest value (53% p<0.001), followed by P.alba (49%, p<0.05) and D.graveolen (45%; p<0.01).

**Antibacterial effect**

The results of the antibacterial activities of studied extracts are given in Table 2. Among the used extracts, C.myrtifolia showed the highest antibacterial activity while the no activity was observed for D.Graveolens extract.

The methanolic extract of P.alba revealed some antibacterial activity against gram positive bacteria (diameter of inhibition ranging from 8 to 13mm; including the diameter of the disc—6 mm ). The T.fruticans extract was poorly active against Staphylococcus spp and B.subtilis and inactive against M.luteus. The Gram negative strains tested were resistant to the effect of T.fruticans and P.alba extracts. As shown in Table 2, at the concentration of 10 mg/ml, the diameters of growth inhibition zone of C.myrtifolia ranged from 11 to 16mm with the highest inhibition zone values observed against M.luteus. So, the methanolic extract of C.myrtifolia was more effective against the Gram positive bacteria .

Furthermore all bacteria E.coli, E.ssp, S.ssp, Stap, B.Subtilis, and M.luteus were quite resistant to D.graveolens.

In general, the extract of C.myrtifolia showed better activity than others extracts, followed by P.alba which inhibited the M.luteus (13,5mm).

**Table 1: antioxidant activity of plants**

<table>
<thead>
<tr>
<th>specimens</th>
<th>AA%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.myrtifolia</td>
<td>56.83*</td>
</tr>
<tr>
<td>T.fruticans</td>
<td>53.13***</td>
</tr>
<tr>
<td>P.alba</td>
<td>49.86*</td>
</tr>
<tr>
<td>D.graveolen</td>
<td>44.96**</td>
</tr>
<tr>
<td>BHT</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 2. Antimicrobial activity of methanol extracts (10 mg/ml) of plants selected

<table>
<thead>
<tr>
<th></th>
<th>E.Coli</th>
<th>E.coli</th>
<th>Salmonelle.spp</th>
<th>S.spp</th>
<th>B.Subtilis</th>
<th>M. luteus</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.myrtifolia</td>
<td>11,2±0,2</td>
<td>10,9±0,33</td>
<td>11,2±0,37</td>
<td>13,9±0,233</td>
<td>13,4±0,21</td>
<td>16±0,2</td>
</tr>
<tr>
<td>T.Fruticans</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6,5±1</td>
<td>7,66±0,5</td>
<td>6</td>
</tr>
<tr>
<td>P.alba</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>8,25±0,25</td>
<td>8,66±0,88</td>
<td>13,5±0,5</td>
</tr>
<tr>
<td>D.Graceolens</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>19±0,57</td>
<td>19,5±0,2</td>
<td>20±1</td>
<td>ND</td>
<td>33,66±1,45</td>
<td>36,66±0,33</td>
</tr>
<tr>
<td>Penicilline</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>24,5±0,5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DMSO control</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

(-): no inhibition; a: diameter of zone of inhibition (mm) including disc diameter (6mm); ND: Not determined. The results are means ± SD of three measurements.

The extract of C.myrtifolia shows also moderately antibacterial activity that can be related to the polar phenolic compounds [10]. Several studies proved that the important phytoconstituents like flavanoids [11], polyphenols [12], tannins [13], sesquiterpenes [14], etc., are effective antimicrobial substances against a wide range of microorganisms. Thus, there is a correlation closely associated with the antibacterial activity of the extracts, and their phenolic constituents [15]. Also the researches of MAHAMAT [16], BASSENE [17], KOLODZIE [18] proved this correlation with tannins. This could explain some of the differences in the antibacterial activity between the Coriaria extracts and others extracts, if we assume that the extract of C.myrtifolia is richer in total phenolic, flavonoids and tannins compounds (data published).

Results obtained for D.graceolens did not show antibacterial activity against bacterial studied and no published data are available for comparison. Although Populus alba is a potential inhibitor of growth of mycobacteria [19], but it showed very weak activity against bacteria [4]. Wamadih [20], Shahidi [21], demonstrated that no antibacterial activity in P.alba against E.coli, Staphilococcus, baccilus, and salmonella. In this context, Vardar [22] reported that the Populus were found to not inhibit Gram-negative bacteria; and the ethanolic extract of this plant demonstrated weak antiquorom sensing activity [2]. Further, this study is clearly in agreement with these findings.

The Genus of Teucrium is known for its poor antibacterial effect against S.aureus et B.subtillis [23]. Coll [24] reported that one of the most potent of the T.fruticans derived neoclerodanes showed very low antifeedant activity. These results are in agreement with those of this study that exhibited the weak activity against S.aureus et B.subtillis. Several studies investigated that gram-positive bacteria are generally more susceptible to non polar phenolic compounds than gram-positive microorganisms [25]-[26]-[27]-[28]. Our results suggested that Gram-positive bacteria are generally more sensitive to the spice and herb extracts than Gram-negative bacteria. However, the extract of C.myrtifolia tested in this study did not in agreement with this trend described above. Although the moderately inhibitory activities of these plants against negative bacteria can be explained probably that this plant contents some particular anti Gram negative substances [15].

**CONCLUSION**

In conclusion, this study contributes to the knowledge of the antioxidant and antimicrobial potential in vitro. The data reported showed that the extracts of plants studied exerted good antioxidant activity. The extracts of the tested medicinal plants exhibited varying degrees, weak to moderate, antibacterial activity against an array of Gram-positive, Gram-negative bacteria. Diameter of inhibition zones of C.myrtifolia extract showed superior activities although for Microccus luteus. This activity for this extract is reported for the first time. However further studies are needed to focus on isolation of the bioactive, biological and chemical characterization of this important plant.

The next study will focus on the separation and the purification of molecules responsible for the antioxidant activity from C.myrtifolia and evaluate the safety of this plant.

**Acknowledgement**

The authors acknowledge the support of the University of Algeria for his collaboration. We would like to thank Prof A.Ennabili for the identification of plants.
REFERENCES


Journal of Microbiology Biotechnology, 24, 1011–1017.


*****