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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* (Saliacea), *Teucrium fruticans* (lamiaceae), *Dittrichia graveolens* (Asteraceae) 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:

$$AA\% = (AE_{48h} / AC_{48h}) *100$$

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 ºC. 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

Plant	AA%
C. myrtifolia	73.74 ± 1.35
T. fruticans	53.57 ± 3.07
P. alba	57.01 ± 4.05
D. graveolens	43.98 ± 4.35
BHT	100

Table 2. Antibacterial activity of plant extracts

	Inhibition zone diameter [a] (mm)					
	E. coli ATCC 25922	*E. coli*	*Salmonella. spp*	*Staphylococcus spp*	*B. Subtilis ATCC 6633*	*M. luteus ATCC 1445*
C. myrtifolia	11.2 ± 0.20	10.9 ± 0.33	11.2 ± 0.37	13.9 ± 0.23	13.4 ± 0.21	16.0 ± 0.20
T. fruticans	-	-	-	6.5 ± 1.00	7.7 ± 0.50	-
P. alba	-	-	-	8.2 ± 0.25	8.7 ± 0.88	13.5 ± 0.50
D. graveolens	-	-	-	-	-	-
Chloramphenicol	19.0 ± 0.57	19.5±0.20	20.0 ± 1.00	ND	33,7±1,45	36.7 ± 0.33
Penicillin	ND	ND	ND	24.5 ± 0,50	ND	ND
DMSO	-	-	-	-	-	-

(-): 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.

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References

1- Tadeg H., Mohammad E., Asres K., Gebre-Mariam T., Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. J Ethnopharmacol. (2005); 100: 168-175.

2- Al-Hussaini R a Mahasneh, A.M. Microbial growth and quorum sensing antagonist activities of herbal plants extracts. Molecules 2009, 14, 3425-3435.

3- Camarda, I., 1990. Ricerche etnobotaniche nel comune di Dorgali, Sardegna centro orientale. Bollettino della Societ`a Sarda di Scienze Naturali 27, 147–204.

4- Al-Hussaini R and Adel M. Mahasneh, Antibacterial and Antifungal Activity of Ethanol Extract of Different Parts of Medicinal Plants in Jordan. Jordan Journal of Pharmaceutical Sciences, Volume 4, No. 1, 2011

5- Gülhan Vardar-Ünlü, Sibel Silici and Mehmet Ünlü,Composition and in vitro antimicrobial activity of Populus buds and poplar-type propolis. World Journal of Microbiology and Biotechnology . Volume 24, Number 7, 1011-1017, 2007

6- Kartal, N., M. Sokmen, B. Tepe, D. Daferera, M. Polissiou and A. Sokmen, 2007. Investigation of the antioxidant properties of Ferula orientalis L. using a suitable extraction procedure. Food Chem., 100: 584-589.

7- Tepe, B.; Daferera, D., Sokmen. A.; Sokmen, M.; Polissiou, M. Antimicrobial and antioxidant activities of the essential oil and various extracts of salvia tomentosa Miller (Lamiaceae). Food Chemistry 2005, 90 , 333-340.

8- Boudkhili M., Greche H., Bousta D., Farah A., El Ouali Lalami A., Aarab L. Antioxidant Activities of Some Moroccan's Plants. International Review of Chemical Engineering. 2011; 3(5); 537-541.

9- H. Hanato, T. Kagawa, J. Yasuhara, T. Okuda, Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effect. Chemical and Pharmaceutical Bul 36: (1988) 1090–1097.

10- L. L Zhang, L. Yi-ming, Tannins from Canarium album with potent antioxidant activity, Zhejiang University, Science B (2008): 407-415.

11- 11 H.SQALLI, A. EL OUARTI, A.ENNABILI , S.IBNSOUDA, A.FARAH , A. HAGGOUD, A.HOUARI , M.IRAQUI. Evaluation de l'effet antimycobactérien de plantes du centre-nord du maroc. *Bull. Soc. Pharm. Bordeaux*, 2007, 146, 271-288

12- 12 Wamidh H. Talib and Adel M. Mahasneh, Antimicrobial, Cytotoxicity and Phytochemical Screening of Jordanian Plants Used in Traditional Medicine, 2010, 15, 1811-1824; doi:10.3390/molecules1503 1811.

13- 13 G.H. Shahidi bonjar, S.Aghighi and A.Karimi Nick. Antibacterial and antifungal survey in plants used in indigenous herbal-Medicine of South Regions of Iran. J of Bio Scie (2004) ; 4 (3) : 405-412.

14- 14 Vardar-Unlu, G., Silici, S., & Unlu, M. (2008). Composition and in vitro antimicrobial activity of Populus buds and poplar-type propolis. World of Journal of Microbiology Biotechnology, 24, 1011–1017.

15- 15 Samec.D., Gruz.J., Strnad.M., Kosalec.I., Jurisic Grubesic.R., Karlovic.K, Lucic.A., Piljac.Zegarac.J. Antioxidant and antimicrobial properties of Teucrium arduini L(Lamiaceaea), flower and leaf infusions (Teucrium arduini L antioxidant capacity).Food and Chemical toxicology, volume 48, issue1, Januray 2010, pages 113-119.

16- 16 Tsuchiya, H., M. Sato, T. Miyazaki, S. Fujiwara and S. Tanigaki et al., 1996. Comaprative study on the antibacterial activity of phytochemical flavones against methicillin-resistant Staphylococcus aureus. Ethenopharmacology, 50: 27-34.

17- 17Mason, T.L. and Wasserman, B.P.. Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds. Phytochemistry. 26: 2197-2202. 1987.

18- 18 Ya, C., Gaffney, S.H., Lilley, T.H. and Haslam, E. Carbohydrate-polyphenol complexation. p. 553. In: Hemingway, R.W. and Karchesy, J.J. (ed.), Chemistry and significance of condensed tannins. Plenum Press; New York. 1988

19- 19Goren, N., Woerdenbag, H. and Bozok-Johansson, C. Cytotoxic and antibacterial activities of sesquiterpene lactones isolated from Tanacetum praeteritum subsp. praeteritum. Planta Medica. 62: 419-422. 1996.

20- 20Bin Shan a, Yi-Zhong Cai a, John D. Brooks b, Harold Corke, The in vitro antibacterial activity of dietary spice and

medicinal herb extracts. International Journal of Food Microbiology 117 (2007) 112–119
21- 21MAHAMAT B.Contribution à l'étude des Combretaceae du Sénégal. Comparaison de l'activitéanti-bactérienne de trois espèces. Thèse de Doct. d'Etat en Pharmacie, 1990, n°44.
22- 22BASSENE E., MAHAMAT B., LO M., BOYE C.S, FAYE B. Comparaison de l'activité antibactérienne de trois Combretaceae : C. micranthum, Guiera senegalensis et Terminalia avicennioides. Fitoterapia, 1995, 66, (1), 86-87.
23- 23KOLODZIE J., KAYSER O., LATTE KP., FERREIRA D. Evaluation of the antimicrobial potency of tannins and related compounds using the microdilution both method. Planta medica, 1999, 65, (5), p.444-446.
24- Smith-Palmer, A., Stewart, J., Fyfe, L. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Letters in Applied Microbiology(1998) 26, 118–122.
25- Zaika, L.L., 1988. Spices and herbs — their antimicrobial activity and its determination. Journal of Food Safety 9, 97–118.
26- Ceylan, E., Fung, D.Y.C.. Antimicrobial activity of spices. Journal of Rapid Methods and Automation in Microbiology (2004)12, 1–55.
27- Lopez, P., Sanchez, C., Batlle, R., Nerin, C., 2005. Solid- and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. Journal of Agricultural and Food Chemistry 53, 6939–6946.

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