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Full Length Research Paper

Polyphenols content, antioxidant and antimicrobial activities of extracts of some wild plants collected from the south of Tunisia

Mohamed Bouaziz[1], Abdelhafidh Dhouib[1]*, Slim Loukil[1], Makki Boukhris[2] and Sami Sayadi[1]

[1]Laboratoire des Bioprocédés, Pôle d'Excellence Régionale AUF, (PER-LBP), Centre de Biotechnologie de Sfax, BP: (1177), 3018 Sfax, Tunisie.
[2]Laboratoire d'Ecologie Végétale, Département des Sciences de la vie, Faculté des Sciences de Sfax, BP: (802), 3018 Sfax, Tunisie.

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25 wild plants were collected from the south of Tunisia. The dried aerial parts were extracted under a continuous reflux set-up in a Soxhlet extractor with hexane, ethyl acetate, methanol and water. The extracts were screened for total phenolic content, antioxidant and antimicrobial activities. Total phenolic contents were determined using a spectrophotometric technique, based on the Folin-Ciocalteau reagent and calculated as pyrogallol equivalents (PyE) per 100 g of dry weight (dw). Total phenolic content ranged from traces to 2225 ± 267 mg PyE/100 g dw in *Periploca angustifolia* hexane extract and *Erodium glaucophyllum* methanol extract, respectively. Total flavonoids and flavonols were measured by a colorimetric assay and expressed as mg of rutin equivalents (RuE) per 100 g dw and mg of quercetin equivalents (QuE) per 100 g dw respectively. The highest amounts of flavonoids of 315 mg RuE/100 g dw and of flavonols of 63 mg QuE/100 g dw was recorded in the ethyl acetate extract of *Acacia radiana*. The antioxidant capacity of all extracts was evaluated with the (1,1-diphenyl-2-picrylhydrazyl, DPPH and 2,2'-azino-bis [3-ethylbenzthiazoline-6-sulphonic acid]) ABTS tests. Several extracts showed antioxidant capacity higher than (butylated hydroxytoluene) BHT. Different trend was observed for each antioxidant system with respect to solvents used. All tested plants showed antimicrobial activity, but this activity depended closely on the nature of the solvent used and of the microorganism tested. *Rhanterium suaveolens* ethyl acetate extract showed the largest spectre of antibacterial activity and *Bassia indica* ethyl acetate extract showed the highest antifungal activity. Interestingly, some of the collected plants had a high phenolic content and powerful antioxidant and antimicrobial activities. These plants may serve as sources of antioxidants with new chemotypes and potential beneficial health properties.

Key words: Plant extract, phenolic compound, antioxidant activity, antimicrobial activity.

INTRODUCTION

Saharan plants are known by their resistance to several stress factors; their high content of natural antioxidants such as phenolic compounds, have attracted a great deal of public and scientific interest because of their health-promoting effects as antioxidants. Over the last decades there has been a substantial amount of research on the effects of mild water stress, nitrogen limitation and light exposure on plants (Tester and Bacic, 2005; Moffat, 2002). Resource availability determines the individual carbon/nutrient balance in a plant species. Under stress, a decline in the acquisition of a resource produces a change in the C/N ratio that corresponds with a change in the balance of chemical compounds. For example, nutrient and water stresses can reduce growth more than photosynthesis as well as excess carbon relative to nutrients is allocated to carbon-based compounds. This

*Corresponding author. E-mail: abdelhfidh.douib@cbs.rnrt.tn. Tel.: +21674874452. Fax: +21674874452.

implies that an accumulation of non-structural carbohydrates and a nutrient dilution would occur in the plant. Phenolics are carbon-based secondary compounds synthesised, at least partially, through the phenylpropanoid pathway (Camm and Towers, 1973). They can act as a carbon sink in the plant. Many studies showed a large number of responses including increase in protective pigment biosynthesis such as tannin and phenolic compounds (Northup et al., 1995; Estiarte et al., 1994). Coley et al. (1985) assumed that a plant species has a range of genetically determined constitutive phenolic defences. Their synthesis is regulated by several abiotic and biotic environmental factors and depends on how these factors affect growth and photosynthesis (Bryant et al., 1983; Waterman et al., 1984). Under extreme climatic conditions, Saharan plants could constitute a reservoir of new natural, safe and effective biomolecules potentially useful as antioxidants. Several Saharan plant species are traditionally used by local inhabitants for the aromatisation and conservation of food. They are the basis of treatment and cure of various diseases from minor infections to serious illnesses as diabetes, asthma, rheumatism, dysentery, skin diseases, wound cicatrisation, bites of viper and stings of scorpion and a horde of other ailments (Le Floch, 1983; Bouhadjera et al., 2005; Stocker et al., 2005). Furthermore, it is evident that in Saharan regions the use of herbal remedies in their natural and crude form to substitute modern drugs, accounts for a lot in the local population especially considering the extension of territory, lack of infrastructure, roads and fast transport. Hence, there is an urgent need for a planned utilization and preservation of our medicinal plants in their natural habitats, which contribute also to increase our reservoir of genetic materials (Hammiche and Maiza, 2006). Recently, plant extracts and phytochemicals with antibacterial or antifungal properties have been investigated actively as alternatives to synthetic pesticides due to their perceived increased level of safety and minimal environmental impact (Rai and Mares, 2003). Highlighting the beneficial properties will increase the value of Saharan vegetation and will improve living conditions in these neglected regions.The purpose of this study is to evaluate 25 medicinal plants from Saharan climate growing on different types of soils (calcic-magnesic, saline sodic, pebble, sandy or calcareous and wind borne sandy soils) and under different stress conditions of drought, saltiness, nitrogen limitation and light exposure. The evaluations will include total phenolics, flavonoids and flavonols contents. In addition, antioxidant and antimicrobial activities were carried out.

MATERIALS AND METHODS

Plant materials

Aerial parts of 25 endemic Saharan plants, representative of different types of soil (saline sodic, gypsum, calcareous and sandy) were collected in November 2006 from different regions of theial parts of 25 endemic Saharan plants, representative of different types of soil (saline sodic, gypsum, calcareous and sandy) were collected in November 2006 from different regions of the south of Tunisia (Table 1). These endemic species were authenticated by Professor Makki Boukhris, Laboratory of Biology, Faculty of Sciences of Sfax, Tunisia. Herbarium voucher specimens were deposited in the Bioprocesses Laboratory, Centre of Biotechnology of Sfax, Tunisia.

Reagents

2,2-diphenyl-1-picrylhydrozyl-(DPPH),-2,6-di-tert-butyl-4-hydro xytoluene (BHT), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and 2,2'-Azinobis (3-ethylbenzothiaz- oline-6-sulfonate) (ABTS) were purchased from Sigma-Aldrich, USA. Pure HPLC solvents were used in all cases.

Extraction procedures

Plants were dried in the shade to ambient temperature until total dehydration. Dried aerial parts of plants were blended into fine powder and stored in the dark at a dry place. The powdered aerial part of plants were extracted in a 1/10 (w/v) of hexane, ethyl acetate, methanol, and water respectively for 12 h under a continuous reflux set-up in a Soxhlet extractor (hot) or at room temperature (cold) for 24 h. The extracts were concentrated by rotary evaporation (Büchi Rotavapor; Büchi Laboratories, Switzerland) at 40°C under vacuum to dryness and the yield of extraction was determined. All the dried extracts were preserved in the refrigerator until further use. Plant extracts were dissolved in ethanol/water (20/80, v/v) at 50 mg/ml before use in the antimicrobial assay.

Determination of total phenolics

The amount of total phenolic was determined using Folin–Ciocalteu reagent, as described by Singleton and Rossi (1965). Briefly, a 50 µl aliquot of the different extracts was assayed with 250 µl of the Folin reagent and 500 µl of sodium carbonate (20%, w/v). The mixture was vortexed and diluted in water to a final volume of 5 ml. After incubation for 30 min at room temperature, the absorbance was read at 765 nm in 1 cm path length quartz cuvette and the total phenolic content in the extract was expressed as pyrogallol equivalents (PyE), using a calibration curve of a freshly prepared pyrogallol solution. 5 determinations were performed on each sample (n = 5). For pyrogallol, the curve of absorbance versus concentration is described by the equation $y = 0.0012 x - 0.0345$ ($R^2 = 0.9997$).

Determination of total flavonoids

Total flavonoids were measured by the colorimetric assay developed by Zhishen et al. (1999). A 1 ml aliquot of appropriately diluted sample or standard solutions of rutin (20, 40, 60, 80 and 100 mg/l) was added to a 10 ml volumetric flask containing 4 ml of double distilled water. At zero time, 0.3 ml (5% $NaNO_2$) was added to the flask. After 5 min, 0.3 ml (10% $AlCl_3$) was added. At 6 min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed again and the absorbance was measured against a blank at 510 nm. Total flavonoids of samples were expressed on a dry weight basis as mg rutin equivalents RuE/100 g. Samples were analyzed in three replications.

Table 1. Ethno-botanical data of the investigated wild vegetal species of the Saharan and sub-Saharan regions of the south of Tunisia.

Plant No.	Latin name	Local name	Region of sampling	Climate	Soil nature	Ethnomedical use	References
P 1	*Astragalus armatus*	gu'tet	Gabes	Sub-Saharan	Gypsum	Anaemia, tonic, stimulant	Monteil, 1953
P 2	*Euphorbia guyoniana*	Lebbina	Douz	Saharan	Sandy	Antitussive and analgesic	Ech-Chahad et al. 2006
P 3	*Erodium glaucophyllum*	Kahkul, Kabshia, Ragma, Dahma, Murrar and Tamir	Gabes	Sub-Saharan	Gypsum	Oxytocic, astringent and antibacterial	Gohar et al. 2003
P 4	*Cleome amblyocarpa*	Mnitna	Gafsa	Sub-Saharan	Calcareous Sandy	Rhume Scabies, rheumatic fever and inflammation	Harraz et al., 1995
P 5	*Nitraria retusa*	Ghardaq	Gafsa	Sub-Saharan	Calcareous Sandy	Inflammation, fever	Shaltout et al., 2003
P 6	*Ziziphus lotus*	Sedra, Nbeg	Gabes	Sub-Saharan	Sandy, Gypsum and Limestone	Demulcent, treatment of throat and broncho-pulmonic irritations, antiinflammatory, analgesic.	Borgi et al., 2007
P 7	*Atractylis serratuloides*	Sirr	Gabes	Sub-Saharan	Gypsum	-	-
P 8	*Acacia radiana*	Thalh	Bouhedma	Sub-Saharan	Calcareous Sandy	Stomach diseases: diarrhea, aches	Hammiche & Maiza, 2006
P 9	*Bassia muricata*	-	Douz	Saharan	Sandy	Skin diseases: dermatosis, pustules, boils and infected wounds, renal and rheumatic diseases	Hammiche & Maiza, 2006, Kamel et al, 2001
P 10	*Tricholaena teneriffae*	-	Bouhedma	Sub-Saharan	Calcareous Sandy	-	-
P 11	*Pituranthos chloranthus*	Guezzeh	Gafsa	Sub-Saharan	Calcareous Sandy	Fever, diabetes, asthma, rheumatism.	Hammiche & Maiza, 2006, Vérité et al., 2004
P 12	*Anabasis oropediorum*	Ajram	Gafsa	Sub-Saharan	Calcareous Sandy	Skin diseases, eczema	Hammiche & Maiza, 2006
P 13	*Traganum nudatum*	Dhamran	Chott El Djerid	Sub-Saharan	Saline Sodic	Constipation	Hammiche & Maiza, 2006
P 14	*Rhanterium suaveolens*	Aarfaj	Douz	Saharan	Sandy	-	Chaieb et Boukhris, 1998
P 15	*Hyparrhenia hirta*	Sibous	Gabes	Sub-Saharan	Gypsum	Diuritic	Chaieb et Boukhris, 1998
P 16	*Diplotaxis harra*	Harra	Matmata	Sub-Saharan	Sandy Clay and Limestone	Anaemia, tonic, stimulant, restorative	Bellakhdar, 1997
P 17	*Henophyton deserti*	Alga	Douz	Saharan	Sandy	Abdominal Diseases and Wound Cicatrisation Treatment and against scorpions bites	Le Floch, 1983, Bouhadjera et al., 2005
P 18	*Moltkioppsis ciliata*	-	Chott El Djerid	Sub-Saharan	Sandy Saline	Gall, wound, bleeding, hemorrhage	Bellakhdar, 1997
P 19	*Echiochilon fruticosum*	-	Gabes	Sub-Saharan	Gypsum	Unidentified symptom, disease	Ichikawa, 1987
P 20	*Periploca angustifolia*	Hallab	Gabes	Sub-Saharan	Gypsum	Rheumatisms, Diabetes, Abortive, hypotensive	Hammiche & Maiza, 2006
P 21	*Lycium shawii*	Sakkoum, Awsag	Gabes	Sub-Saharan	Sandy, Gypsum and Limestone	-	Chaieb et Boukhris, 1998
P 22	*Gymnocarpos decander*	Jrad	Gabes	Sub-Saharan	Gypsum	-	Chaieb et Boukhris, 1998
P 23	*Salsola vermiculata*	Gadham	Gabes	Sub-Saharan	Gypsum	Estomac	Bellakhdar, 1997
P 24	*Bassia indica*	-	Gabes	Sub-Saharan	Sandy, Gypsum	Cardiotonic	Chaieb et Boukhris, 1998
P 25	*Limonium pruinosum*	Fouchfache	Gabes	Sub-Saharan	Gypsum	-	-

Determination of total flavonols

The content of flavonols was determined as described by Miliauskas et al. (2004). The quercetin calibration curve was prepared by mixing 2 ml of (0.5, 0.4, 0.3, 0.2, 0.166, 0.1, 0.05, 0.025, and 0.0166 mg/ml) quercetin ethanolic solution with 2 ml of (20 g/l) aluminium trichloride solution and 6 ml of (50 g/l) sodium acetate solution. The absorption at 440 nm was read after 2.5 h at 20°C. The same procedure was carried out with 2 ml of plant extract (10 g/l) instead of quercetin solution. All determinations were carried out in three replications. The content of flavonols, as quercetin equivalents (QuE) was calculated by the following formula: X = (C V)/m; where X: flavonols content (mg QuE/g plant extract); C: the concentration of quercetin solution (mg/ml), established from the calibration curve; V and m: the volume and the mass of plant extract in (ml) and (g) respectively.

Determination of antioxidant activity using the free radical scavenging activity (DPPH)

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity of the sample extracts was evaluated according to the method employed by Gulluce et al. (2007). 4 ml of methanolic solution of varying concentration samples (25, 50, 100 and 150 µg/ml) were added to 10 ml of methanol solution of DPPH ($1.5\ 10^{-4}$ M). The prepared solutions were mixed and left for 30 min at room temperature. The optical density was measured at 520 nm using a spectrophotometer (UV-1650PC Shimadzu, Japan). Test samples and positive control BHT were tested over the same range of sample concentrations. The antioxidant activity of each test sample and BHT was expressed in terms of concentration required to inhibit 50% DPPH radical formation (IC_{50} µg/ml) and calculated from the graph plotted inhibition percentage against extract concentration.

Determination of antioxidant activity using the free radical scavenging activity (ABTS)

The Trolox equivalent antioxidant capacity (TEAC) assay, which measures the reduction of the ABTS radical cation by antioxidant, was performed as previously described by Re et al. (1999). Briefly, ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 - 24 h before use. For the study of phenolic compounds the $ABTS^{\bullet+}$ solution was diluted in ethanol or water (lipophilic and hydrophilic assay, respectively) to an absorbance of 0.70 ± 0.02 at 734 nm. For the photometric assay 1 ml of the $ABTS^{\bullet+}$ solution and 100 µl of the antioxidant solution were mixed for 45 sec and measured immediately after 5 min at 734 nm (absorbance did not change significantly for up to 10 min). Compounds were assayed at 5 different concentrations determined within the linear range of the dose-response curve. A calibration curve was prepared with different concentrations of Trolox (0-20 µM). Results were expressed in mM of Trolox.

Antimicrobial assay

Authentic pure cultures of *Staphylococus aureus subsp. aureus* CIP 4.83, *Pseudomonas aeruginosa* CIP 82.118, *Escherichia coli* CIP 53.126, *Salmonella enterica* CIP 80.39, *Bacillus subtilis* CIP 52.62, *Candida albicans* CIP 48.72 and *Aspergillus niger* CIP 1431.83 were obtained from the culture collection of Institute Pasteur, France. The bacteria were cultivated in nutrient broth (NB) or nutrient agar (NA) (Difco) at 37 ± 0.2°C for *Staphylococus, Escherichia* and *Salmonella,* and at 30 ± 0.2°C for *Pseudomonas* and *Bacillus.* Fungi and yeasts were cultured on malt extract broth (MEB) or agar (MEA) (Difco) at 28 ± 0.2°C. The cultures of bacteria and fungi were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures. Susceptibility of the tested organism to the extracts was determined by employing the disc-diffusion method (Bauer et al., 1966). The bacterial and yeast cultures in the exponential phase of growth or fungal spore solution adjusted to the appropriate dilution was spread on NA or MEA plates in order to give a population of approximately 10^6 CFU/plate. Sterile filter paper discs of 9 mm diameter impregnated with 40 µl of each test plant extract, were placed on the surface of the inoculated agar plates. Respective solvent ethanol/water (20/80, v/v) without plant extract and Polymyxine B antibiotic biodisc (bioMerieux sa), served as negative and positive controls. The plates were incubated for 24 h or 3 days at 28, 30 or 37°C according to the optimum growth temperature of the microbial strain, under aerobic conditions and the diameter of the inhibition zone around each disc was then measured and recorded. Each experiment was carried out in triplicate.

Statistical analysis

Data, unless otherwise specified, were expressed as means ± standard deviation of triplicate experiments.

RESULTS AND DISCUSSION

Many Saharan plant species have been used in folk medicine by the indigenous population for anti-fever, -diarrhea, -diabetes, -asthma, -rheumatism and -cancer therapies. This paper describes the total phenolic content, the antimicrobial and antioxidant activities of 25 different wild plant species, representative of different soils of the south of Tunisia and commonly used in Tunisian ethnomedicine. A summary of the ethnobotanical data of these plants is given in Table 1. About 100 extracts were achieved from these 25 plants. The method and yield (%, dry weight basis) of extraction by the various solvents are given in Table 2. The results showed that, there is a significant variation in yield of extraction between plant species for the same solvent and between solvents for the same plant species.

Total phenolic content

There was a wide range of total phenolic concentrations in the analyzed plants extracts as shown in Table 3. The values ranged between 3 and 2225 mg PyE/100 g dw as measured by the Folin-Ciocalteau reagent. The highest total phenols amounts of 2225, 969, 874, 671, 625 and 529 mg PyE/100 g dw were found in *E. glaucophyllum* methanol extract, *Astragalus armatus* water extract, *A. armatus* methanol extract, *Echiochilon fruticosum* Methanol extract, *Rhanterium suaveolens* methanol extract and *Salsola vermiculata* methanol extract respectively. Results showed clearly that, methanol gives the highest extraction yields of phenolics, followed by ethyl acetate. Water and hexane are less efficient and irregular extrac-

Table 2. Wild vegetal species of the Saharan and sub-Saharan regions of the south of Tunisia; method of extraction and percentage yield (% of dry weight) of various solvents.

Plant	Dry mass (g)	Hexane extract		Ethyl acetate extract		Methanol extract		Water extract	
		ME	Yied (%)	ME	Yied (%)	ME	EY (%)	ME	Yied (%)
P 1 (thorn and stem)	40	-	-	Hot	5.52 ± 0.39	Hot	34.90 ± 2.44	Cold	4.10 ± 0.16
P 1 (leaves)	43	-	-	Hot	12.09 ± 0.85	Hot	10.56 ± 0.74	Cold	-
P 2 (aerial part)	94.6	Cold	7.50 ± 0.30	Cold	10.26 ± 0.41	Cold	7.40 ± 0.30	Cold	0.74 ± 0.03
P 3 (aerial part)	104	-	-	Cold	5.52 ± 0.22	Cold	33.1 ± 1.32	-	-
P 4 (aerial part)	43	Cold	4.9 ± 0.75	Cold	6.04 ± 0.24	Cold	10.1 ± 1.23	-	-
P 5 (aerial part)	97	-	-	Cold	8.96 ± 0.36	Cold	9.79 ± 0.39	-	-
P 6 (aerial part)	95	Cold	4.00 ± 0.16	Cold	4.74 ± 0.19	Cold	26.30 ± 1.05	Cold	0.31 ± 0.01
P 7 (aerial part)	40	-	-	Cold	5.00 ± 0.20	Cold	5.25 ± 0.21	-	-
P 8 (aerial part)	40	Cold	0.58 ± 0.20	Cold	0.42 ± 0.09	Cold	4.77 ± 1.20	Cold	13.02 ± 2.11
P 9 (aerial part)	40.7	Cold	2.45 ± 0.10	Cold	3.68 ± 0.15	Cold	5.15 ± 0.21	-	-
P 10 (aerial part)	20	-	-	Cold	1 ± 0.20	Cold	2.5 ± 0.65	Cold	2.5 ± 0.95
P 11 (aerial part)	200	Hot	1.20 ± 0.08	Hot	2.50 ± 0.18	Hot	3.10 ± 0.22	Cold	5.00 ± 0.20
P 12 (aerial part)	71	Cold	1.00 ± 0.04	Cold	4.64 ± 0.19	Cold	1.12 ± 0.05	Cold	1.83 ± 0.07
P 13 (aerial part)	40	Cold	1.50 ± 0.06	Cold	3.50 ± 0.14	Cold	2.50 ± 0.10	Cold	10.50 ± 0.42
P 14 (aerial part)	93	Cold	0.86 ± 0.03	Cold	4.51 ± 0.18	Cold	2.68 ± 0.11	Cold	2.58 ± 0.10
P 15 (aerial part)	30	Cold	1.26 ± 0.23	Cold	0.76 ± 0.13	Cold	0.96 ± 0.18	Cold	3.33 ± 1.2
P 16 (aerial part)	30	Cold	1.40 ± 0.43	Cold	1.73 ± 0.40	Cold	4.40 ± 0.75	Cold	9 ± 2
P 17 (leaves)	52.5	Hot	3.10 ± 0.22	Hot	6.52 ± 0.46	Hot	13.00 ± 0.91	-	-
P 17 (seeds)	14.7	Hot	42.20 ± 2.95	Hot	5.95 ± 0.42	Hot	3.00 ± 0.21	-	-
P 18 (aerial part)	18.5	-	-	Cold	5.40 ± 0.22	Cold	2.70 ± 0.11	Cold	4.86 ± 0.19
P 19 (aerial part)	115	-	-	Hot	5.62 ± 0.39	Hot	8.34 ± 0.58	Cold	6.00 ± .024
P 20 (aerial part)	20	Cold	1.25 ± 0.65	Cold	1 ± 0.25	Cold	7.36 ± 0.95	Cold	15.28 ± 3.1
P 21 (aerial part)	70	-	-	Hot	4.28 ± 0.3	Hot	5.85 ± 0.43	-	-
P 22 (aerial part)	95	Cold	0.73 ± 0.03	Cold	4.84 ± 0.19	Cold	2.84 ± 0.11	Cold	9.10 ± 1.10
P 23 (aerial part)	20	-	-	Cold	13 ± 1.43	Cold	23.5 ± 1.25	-	-
P 24 (aerial part)	26.4	Cold	1.13 ± 0.05	Cold	11.74 ± 0.47	Cold	10.36 ± 0.41	Cold	12.50 ± 0.50
P 25 (aerial part)	20	Cold	1 ± 0.52	Cold	0.87 ± 0.12	Cold	10.4 ± 1.03	-	-

ME: Mode of extraction.

widely distributed in the plant kingdom and that they are sometimes present in surprisingly high concentrations (Harborne, 1993). Flavonoids and flavonols as ones of the most diverse and widespread group of natural compounds, are probably the most important natural phenolics (Agrawal, 1989). These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties

(Cushnie and Lamb, 2005; Vinson et al., 1995). Therefore, total flavonoids and flavonols were determined in the extracts (Table 3). Results showed that ethyl acetate solvent had the highest ability of extraction of flavonoids among the studied solvents. Indeed, except the 2 species of *Euphorbia guyoniana* and *Ziziphus lotus* where we recorded traces of flavonoids, the concentration in the other plants, ranged between 8 mg/100 g dw in *Henophyton deserti* and 315 mg/100 g dw in *Acacia radiana*. The levels and types of flavonoids vary significantly, depending on not only plant species but also solvent nature. For example, flavonoids were not detected in *H. deserti* hexane extract whereas they reached 181 mg/100 g dw in its methanol extract. The total flavonols contents varied in narrower range than total phenols and flavonoids which ranged from traces to 63 mg QuE/100 g dw. Indeed, the highest flavonols amounts of 63.85, 53 and 41 mg QuE/100 g dw were obtained in ethyl acetate extract of *A. radiana, Cleome amblyocarpa* and *Anabasis oropediorum* respectively and 56.26, 40.8 and 40 mg QuE/100 g dw in methanol extract of *P. angustifolia, Tricholaena teneriffae* and *E. glaucophyllum*, respectively. However, hexane known as the fat extractant, appeared to be inadequate in flavonols known by their hydrophilic character. Indeed, Table 3 shows that flavonols are traces or not detected in all hexane extracts of the studied plants. The studied Saharan plants, even if they had grown in the same environment and had undergone the same stressful conditions, had different patterns and different capacity of accumulation of total phenolics. For example, *E. guyoniana*, *Bassia muricata* and *R. suaveolens* growing in the same Saharan climate and on the same sandy soil type of the region of Douz, possessed different total phenolic contents in their methanol extract. Among methanol extract of these three plants, *E. guyoniana* exhibited the lowest phenolic level of 280 mg PyE/100 g dw (Table 3) as well as the lowest ABTS radical scavenging activity of 0.50 mM (Table 4). Conversely, *R. suaveolens* exhibited the highest total phenolic content of 625 mg PyE/100 g dw and ABTS radical scavenging activity of 2.12 mM.

Antioxidant activity

Plants contain a large and diverse number of hydrophobic and hydrophilic components that are soluble in neutral organic solvents or water; which are generally called extractives. These extractive phenolic compounds are among simple phenols, flavonoids, flavonols and terpenes and have pharmacologically useful antioxidant properties. The radical scavenging activity of the different plant extracts, determined by DPPH and ABTS radicals are shown in Table 4. The results showed that all studied plants possessed antioxidant activity. The revelation of this activity depended mainly on the extraction method and the nature of the used solvent. DPPH antioxidant activity in the approximately 100 tested extracts was generally low (IC_{50} value higher than 1 µg/ml). Among these 100 extracts, only three of them were endowed with high antioxidant activity (IC_{50} value lower to the one of BHT = 0.91 µg/ml): *E. glaucophyllum* methanol extract (IC_{50} = 0.44 ± 0.08 µg/ml), Z*. lotus* water extract (IC_{50} = 0.69 ± 0.12 µg/mL) and *A. radiana* ethyl acetate extract (IC_{50} = 0.87 ± 0.34 µg/ml). Whereas, the ABTS antioxidant activity showed that 23 extracts possessed significantly higher activity compared with BHT. This is in line with the findings of Samaniego et al. (2007) who reported that the best method for determining the antioxidant capacity of olive oil is ABTS and that gives high reproducibility and more specifies variation coefficients. Ethyl acetate, methanol and water extracts of *A. radiana* possessed the highest antioxidant capacities of 3.88, 2.28, and 2.41 mM, respectively. Indeed, ethyl acetate extract of this plant is very rich in total phenolics (501 mg PyE/100 g) and flavonoids (315.96 mg RuE/100 g) and it contained the highest rate of flavonols (63.85 mg QuE/100 g). Hexane, methanol and water extracts of *P. angustifolia* also possessed high anti-ABTS radical activities of 3.02, 2.56 and 2.22 mM, respectively. In spite of traces of phenolics content in its hexane extract, the high antioxidant activity could be owed to other types of molecules. These results can be explained by the fact that, the global antioxidant property of a plant extract is generally considered as the result of the combined activity of a wide range of compounds included, beside phenolics, peptides, organic acids and other components (Gallardo et al., 2006). The ethyl acetate and methanol extracts of *Atractylis serratuloides* and of *R. suaveolens* and the water extract of *B. indica* were also good ABTS radical scavengers with TEAC values of 2.48, 3.56, 2.31, 2.12, and 3.6 mM, respectively. In general, 7.1% of hexane extracts, 14.8% of ethyl acetate extracts, 37.5% of water extracts and 46.2% of methanol extracts had anti-ABTS radical activities higher than that of BHT. The frequency of extracts exhibiting a DPPH-scavenging activity higher than BHT did not reach 7% in the best of cases with water extracts. It has been reported that usually plant methanol extracts are very efficient free radical scavengers and exhibit the highest antioxidant activity (Al-Fatimi et al., 2007). It should be pointed out that during assay, the ethyl acetate, water and hexane extracts were dissolved in methanol, and this procedure could have some effects on the measurement of the radical scavenging activity so far as the extracts were not fully soluble in methanol.

Antimicrobial activity of extracts

The results of testing of the crude extracts for antimicrobial activities against 5 bacterial and 2 fungal species are summarized in Table 5. Among each of the 27 extracts of each of the 4 groups of hexane, ethyl acetate, methanol and water extracts, 17, 23, 14 and 11

Table 3. Total phenol content of extracts expressed as pyrogallol equivalents (mg PyE/100 g dry weight), Total flavonoid expressed as rutin equivalents (mg RuE/100 g dry weight) and total flavonol expressed as quercetin equivalents (mg QuE/100 g dry weight) in the studied wild plants of the south of Tunisia.

Plant	Total phenols (mg PyE/100 g extract)				Total flavonoid (mg RuE/100 g extract)				Total flavonols (mg QuE/100 g extract)			
	Hexane	Ethyl acetate	Methanol	Water	Hexane	Ethyl acetate	Methanol	Water	Hexane	Ethyl acetate	Methanol	Water
P 1 (thorn and stem)	-	87±10	-	969±116	-	23±2	-	2±0.5	-	16±2	-	ND
P 1 (leaves)	399±48	-	874±105	-	-	16±2	10±1	-	-	4±0.5	2±0.5	-
P 2 (aerial part)	5±0.6	23±3	280±34	27±3	ND	Traces	18±2	9±1	ND	ND	7 ±	10±1
P 3 (aerial part)	-	422±51	2225±267	-	-	14±1	203±20	-	-	11±1	40±4	-
P 4 (aerial part)	ND	510±61	12.43 ±2	-	ND	89±9	2.1±0.23	-	ND	53±6.2	ND	-
P 5 (aerial part)	-	95±11	39±5	-	-	13±1	5±0.9	-	-	12±1	4±0.5	-
P 6 (aerial part)	23±3	113±14	274±33	340±41	ND	Traces	27±3	37±4	ND	ND	12.83±2	25±3
P 7 (aerial part)	-	36±4	197±24	-	-	10±1	15±2	-	-	4±0.5	2±0.5	-
P 8 (aerial part)	-	501±36	215±28	194±39	-	315.96±10	15.64±2.5	13.06±2.24	-	63.85±4.2	6.29±1.08	7.98±1.75
P 9 (aerial part)	141±41	396±48	463±56	-	2±0.6	18±2	18±2	1.2±0.8	Traces	12±1	2±0.5	-
P 10 (aerial part)	-	399±42	405±51	203±42	-	153.06±6.2	65.96±4	19.51±3.1	Traces	38.89±5	40.8±5.5	17.20±2.3
P 11 (aerial part)	175±57	380±46	314±38	372±45	Traces	41±4	19±2	38±4	Traces	34±3	10±1	24±2
P 12 (aerial part)	89±11	174±21	159±19	45±5	7±1	55±6	Traces	4±0.5	ND	41±4	1±0.5	Traces
P 13 (aerial part)	38±5	162±19	153±18	155±19	2±0.5	20±2	Traces	12±1	ND	10±1	2±0.5	Traces
P 14 (aerial part)	190±23	370±44	625±75	198±24	2±0.5	38±4	Traces	19±2	Traces	8±1	11±1	Traces
P 15 (aerial part)	-	281±45	302±32	490±51	-	41.12±4	36.29±5.1	32.74±3.8	Traces	12.14±3	16±2.85	21.47±3.6
P 16 (aerial part)	-	161±13	134±18	85±23	-	68.22±2.1	5±0.92	5.64±1.55	Traces	21.89±2.3	1.78±0.65	1.36±0.85
P 17 (leaves)	-	152±19	275±33	-	ND	46±5	181±20	-	ND	11±1	36±2.5	-
P 17 (seeds)	195.4 ±23.5	20±2	134±16	-	ND	8±1	39±4	-	ND	ND	13±2	-
P 18 (aerial part)	-	123±15	81±10	289±35	5±0.5	37±4	-	21±2	-	33±3	18±2	Traces
P 19 (aerial part)	-	345±41	671±81	40±5	3±0.5	52±5	89±8	29±3	-	38±4	10±1	Traces
P 20 (aerial part)	Traces	192±27	321±65	382±40	-	87.9±3.2	41.45±3.2	54.35±4	Traces	21.25±3.75	56.26±6.5	27.79±4.51
P 21 (aerial part)	-	432±52	320±38	-	-	55±6	45±6	30±3	-	27±3	13±1	-
P 22 (aerial part)	3±0.5	75±9	60±7	76±9	1.6±0.5	19±2	21±3	13±1	Traces	13±1	11±1	Traces
P 23 (aerial part)	-	336±40	529±63	-	-	56±6	8±2	8±1	-	23±2	6±1	-
P 24 (aerial part)	5±0.6	185±22	146±18	72±9	18±2	29±3	48±7	40±4	Traces	23 ±2	31 ±3	2±0.5
P 25 (aerial part)	63± 9	324±17	192±23	-	2±0.76	28.5±3.5	108.5±4.8	-	Traces	9.7±1.5	16±2.5	-

ND: not detected.

plant extracts, respectively, were active on at least one of the 7 tested microorganisms. Apolar extracts were more active than polar ones. Indeed, when the hexane extract is active, its spectre is generally larger than that of other solvents. 9 hexane extracts among 17 (53%) were active on the 5 tested bacteria. The ethyl acetate extracts exhibited the largest antifungal activity. Indeed, 13 ethyl acetate extracts among 27 (48%) were

Table 4. Antioxidant capacities DPPH IC_{50} (µg/ml) and ABTS Trolox equivalent (TEAC) mM of the different extracts of the studied wild plants of the south of Tunisia.

Plant	IC_{50} (µg/ml) of the extract				Trolox equivalent (TEAC) mM of the extract			
	Hexane	Ethyl acetate	Methanol	Water	Hexane	Ethyl acetate	Methanol	Water
P 1 (thorn and stem)	-	1.40 ± 0.24	5.58 ± 0.95	52.97 ± 9.01	-	0.23 ± 0.03	0.89 ± 0.09	0.86 ± 0.11
P 1 (leaves)	-	2.3 ± 0.39	6.46 ± 1.10	-	-	0.21 ± 0.01	0.70 ± 0.09	-
P 2 (aerial part)	2.59 ± 0.44	2.77 ± 0.47	1.85 ± 0.32	2.58 ± 0.43	0.20 ± 0.03	0.25 ± 0.03	0.50 ± 0.07	0.23 ± 0.03
P 3 (aerial part)	-	1.76 ± 0.30	0.44 ± 0.08	-	-	0.60 ± 0.04	3.14 ± 0.32	-
P 4 (aerial part)	-	3.45 ± 0.59	-	-	-	0.95 ± 0.14	-	-
P 5 (aerial part)	-	18.24 ± 3.11	5.31 ± 0.90	-	-	0.67 ± 0.12	0.66 ± 0.06	-
P 6 (aerial part)	2.35 ± 0.40	2.21 ± 0.38	1.1 ± 0.20	0.69 ± 0.12	0.25 ± 0.33	0.38 ± 0.05	1.35 ± 0.18	2.72 ± 0.35
P 7 (aerial part)	-	5.11 ± 0.87	4.11 ± 0.70	-	-	2.48 ± 0.58	3.56 ± 0.90	-
P 8 (aerial part)	2.52 ± 0.25	0.87 ± 0.34	1.40 ± 0.12	1.37 ± 0.21	0.18 ± 0.08	3.88 ± 0.76	2.28 ± 0.51	2.41 ± 0.39
P 9 (aerial part)	7.83 ± 1.33	2.13 ± 0.36	1.76 ± 0.30	-	0.39 ± 0.11	1.20 ± 0.26	1.40 ± 0.34	-
P 10 (aerial part)	-	2.00 ± 0.27	1.74 ± 0.35	2.05 ± 0.41	-	1.79 ± 0.44	2.27 ± 0.63	1.13 ± 0.45
P 11 (aerial part)	2.22 ± 0.38	2.24 ± 0.38	2.01 ± 0.34	4.59 ± 0.78	0.20 ± 0.03	0.36 ± 0.05	0.75 ± 0.10	0.40 ± 0.05
P 12 (aerial part)	4.70 ± 0.80	2.23 ± 0.38	3.72 ± 0.63	1.12 ± 0.19	0.24 ± 0.03	0.86 ± 0.11	1.52 ± 0.20	0.44 ± 0.06
P 13 (aerial part)	15.57 ± 2.65	10.57 ± 0.61	2.05 ± 0.13	4.64 ± 0.79	0.17 ± 0.02	2.10 ± 0.20	1.98 ±.0.20	0.25 ± 0.03
P 14 (aerial part)	13.54 ± 2.30	1.92 ± 0.33	1.09 ± 0.19	28.64 ± 4.37	0.33 ± 0.04	2.31 ± 0.60	2.12 ± 0.55	0.56 ± 0.07
P 15 (aerial part)	7.91 ± 1.33	48.99 ±3.20	1.25 ± 0.27	1.66 ± 0.44	0.50 ± 0.06	1.55 ± 0.23	1.86 ± 0.12	2.48 ± 0.57
P 16 (aerial part)	-	1.24 ± 0.38	2.23 ± 0.35	-	-	0.33 ± 0.05	0.88 ± 0.11	1.13 ± 0.10
P 17 (leaves)	5.95 ± 0.85	1.63 ± 0.25	1.20 ± 0.11	-	0.19 ± 0.03	1.75 ± 0.20	1.91 ± 0.19	-
P 17 (seeds)	3.10 ± 0.57	3.87 ± 0.60	2.05 ± 0.30	-	0.32 ± 0.07	0.73 ± 0.21	1.09 ± 0.09	-
P 18 (aerial part)	-	3.61 ± 0.61	1.32 ± 0.22	2.20 ± 0.37	-	0.15 ± 0.02	1.17 ± 0.15	1.83 ± 0.24
P 19(aerial part)	-	1.94 ± 0.33	1.67 ± 0.28	3.83 ± 0.65	-	1.41 ± 0.37	2.72 ± 0.71	1.17 ± 0.15
P 20 (aerial part)	2.03 ± 0.12	1.60 ± 0.25	1.30 ± 0.31	1.03 ± 0.15	3.02 ± 0.35	1.02 ± 0.10	2.56 ± 0.41	2.22 ± 0.38
P 21 (aerial part)	-	28.99 ± 4.93	2.05 ± 0.25	-	-	1.10 ± 0.11	1.90 ± 0.10	-
P 22 (aerial part)	3.94 ± 0.67	1.43 ± 0.24	1.36 ± 0.22	9.78 ± 1.95	-	0.90 ± 0.01	1.86 ± 0.28	1.44 ± 0.19
P 23 (aerial part)	-	3.77 ± 0.64	2.19 ± 0.37	-	-	0.90 ± 0.08	1.41 ± 0.18	-
P 24 (aerial part)	2.18 ± 0.37	6.47 ± 1.10	1.30 ± 0.22	1.39 ± 0.24	0.17 ± 0.02	0.60 ± 0.08	1.44 ± 0.18	3.60 ± 0.47
P 25 (aerial part)	8.55 ± 0.83	3.77 ± 0.47	11.95 ± 1.37	-	0.58 ± 0.09	0.83 ± 0.05	0.58 ± 0.13	
BHT			0.91 ± 0.15				1.80 ± 0.23	-

active on at least one of the two tested fungi. If the ethyl acetate extracts were active, their anti-bacterial activity was more powerful than that of Polymyxine B (diameter of the inhibition halo ≥ the one of Polymyxine B in 18 cases among 31 (58%).

The production and accumulation of a wide range of organic chemicals is one of the major mechanisms by which plants defend themselves against herbivores and attacks by microbial

Table 5. *In vitro* antimicrobial activity as diameter of clear zone (mm) of the different extracts of the studied wild plants of the south of Tunisia.

Plant	Hexane extract							Ethyl acetate extract							Methanol extract							Water extract						
	Sa	Pa	Ec	Se	Bs	Ca	An	Sa	Pa	Ec	Se	Bs	Ca	An	Sa	Pa	Ec	Se	Bs	Ca	An	Sa	Pa	Ec	Se	Bs	Ca	An
P 1 (thorn and stem)	-	-	-	-	-	-	-	ni	10	ni	10	ni	ni	20	11	14	10	10	10	ni	19	ni	ni	10	ni	ni	ni	18
P 1 (leaves)	-	-	-	-	-	-	-	ni	ni	ni	ni	ni	ni	11	11	10	10	10	11	ni	21	-	-	-	-	-	-	-
P 2 (aerial part)	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	10	ni	ni	ni
P 3 (aerial part)	-	-	-	-	-	-	-	10	11	10	10	10	ni	12	ni	ni	ni	ni	ni	ni	ni	-	-	-	-	-	-	-
P 4 (aerial part)	10	10	10	11	10	ni	ni	ni	ni	ni	ni	ni	ni	13	13	11	12	12	13	ni	ni	-	-	-	-	-	-	-
P 5 (aerial part)	-	-	-	-	-	-	-	ni	ni	ni	ni	ni	ni	20	ni	13	ni	ni	ni	ni	17	-	-	-	-	-	-	-
P 6 (aerial part)	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	11	13	ni	ni	ni	10	10	ni	ni	ni
P 7 (aerial part)	-	-	-	-	-	-	-	ni	14	15	14	ni	ni	10	ni	12	ni	ni	ni	ni	ni	-	-	-	-	-	-	-
P 8 (aerial part)	ni	ni	ni	ni	ni	ni	ni	15	ni	ni	ni	ni	11	ni	ni	ni	ni	ni	ni	13	ni	ni	ni	10	ni	ni	ni	ni
P 9 (aerial part)	ni	11	ni	ni	ni	ni	ni	ni	11	ni	ni	ni	ni	ni	ni	ni	10	ni	ni	ni	11	-	-	-	-	-	-	-
P 10 (aerial part)	-	-	-	-	-	-	-	ni	ni	ni	ni	ni	ni	11	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
P 11 (aerial part)	ni	14	ni	10	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	12	ni	ni	ni	ni
P 12 (aerial part)	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	12	ni	ni	ni	14	ni	ni	12	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
P 13 (aerial part)	ni	ni	ni	ni	ni	ni	ni	ni	22	15	ni	15	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
P 14 (aerial part)	ni	12	13	ni	13	ni	ni	18	20	18	14	18	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
P 15 (aerial part)	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	11	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
P 16 (aerial part)	12	ni	ni	ni	12	ni	ni	ni	ni	ni	ni	ni	12	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
P 17 (leaves)	ni	ni	ni	ni	ni	ni	ni	10	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	13	ni	ni	-	-	-	-	-	-	-
P 17 (seeds)	10	10	ni	ni	11	ni	ni	10	11	11	11	10	11	ni	ni	ni	ni	ni	11	ni	ni	-	-	-	-	-	-	-
P 18 (aerial part)	-	-	-	-	-	-	-	ni	15	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	11	ni	ni	ni	ni
P 19 (aerial part)	-	-	-	-	-	-	-	ni	11	16	12	16	ni	ni	ni	ni	Ni	13	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
P 20 (aerial part)	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	11	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
P 21 (aerial part)	-	-	-	-	-	-	-	ni	ni	17	ni	17	ni	ni	ni	10	12	ni	11	ni	ni	-	-	-	-	-	-	-
P 22 (aerial part)	ni	11	10	ni	10	ni	ni	ni	ni	ni	11	ni	ni	ni	ni	12	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
P 23 (aerial part)	-	-	-	-	-	-	-	ni	ni	ni	ni	ni	ni	ni	11	12	ni	ni	ni	ni	ni	-	-	-	-	-	-	-
P 24 (aerial part)	ni	10	ni	ni	ni	ni	11	ni	10	ni	ni	ni	20	20	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
P 25 (aerial part)	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	14	11	ni	ni	18	ni	ni	-	-	-	-	-	-	-
Polymyxine B	**15**	**13**	**10**	**13**	**14**	ni	ni	**15**	**13**	**10**	**13**	**14**	ni	ni	**15**	**13**	**10**	**13**	**14**	ni	ni	**15**	**13**	**10**	**13**	**14**	ni	ni

Sa: *Staphylococus aureus*, Pa: *Pseudomonas aeruginosa*, Ec: *Escherichia coli,* Se: *Salmonella enterica,* Bs: *Bacillus subtilis,* Ca: *Candida albicans,* An: *Aspergillus niger;* ni: not inhibited, (-) not tested.

pathogens and invertebrate pests. Most of these chemicals are products of secondary metabolism, originally thought to be waste products not needed by plants for primary metabolic functions. It is, however, well known now that their presence in different parts of the plant (root, leaves, bark, etc) deter feeding by insects and vertebrates, as well as attacks by viruses, bacteria and fungi (Wink, 1999). Some secondary metabolites are Known to

exhibit both of these functions. For example, anthocyanins and monoterpenes act as insect attractants in flowers, but may be insecticidal and antimicrobial when present in leaves (Wink, 1999).

Conclusion

The present results therefore offer a scientific basis for traditional use of wild plants of the south of Tunisia as traditional medicine for primary healthcare needs and as natural preservative for food or cosmetic products. The total phenolic, flavonoid and flavonol contents, antioxidant capacity and antimicrobial activity vary significantly among different extracts of the selected plants. The antioxidant and antimicrobial activities could be enhanced if active components are purified and adequate dosage determined for proper administration.

Further studies on these wild plants are necessary and should seek to determine toxicity of active constituents, their side effects, pharmacokinetic properties and diffusion in different body sites. This represents the first preliminary report on the phenolic composition, antioxidant capacity and anti-microbial activity of a large number of wild plants in the south of Tunisia.

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