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Antioxidant and antimicrobial activities of selected medicinal plants from Algeria

Krimat Soumia[1], Dob Tahar[1*], Lamari Lynda[2], Boumeridja Saida[3], Chelghoum Chabane[4], Metidji Hafidha[1]

[1]*Laboratoire de Molécules Bio-Actives et Valorisation de la Biomasse, ENS kouba, Algeria*

[2]*Laboratoire de Biologie des Systèmes Microbiens, ENS, Kouba, Algeria*

[3]*Departement of Natural sciences, ENS, Kouba, Algeria*

[4]*Laboratoire de chromatographie, faculté de chimie, USTHB, Algiers, Algeria*

PEER REVIEW

Peer reviewer

Elekofehinti Olusola Olalekan, Ph.D, Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.
Tel: +2348034450611
E-mail: sola_eleko@yahoo.com, olusola.elekofehinti@aaua.edu.ng

Comments

The is a good study in which the authors demonstrated the antimicrobial and radical scavenging properties of 20 medicinal plants from Algeria.
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ABSTRACT

Objective: To evaluate the antioxidant and antimicrobial activity of methanolic extract extracts of selected Algerian medicinal plants.

Methods: Antioxidant activity of extracts was evaluated in terms of radical scavenging potential (2,2-diphenyl-1-picrylhydrazyl) and β-carotene bleaching assay. Total phenolic contents and flavonoid contents were also measured. Antimicrobial activity of these plants was examined against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.

Results: The values of IC_{50} ranged from 4.30 μg/mL to 486.6 μg/mL for the DPPH method, while total antioxidant activity using β-carotene/linoleic acid bleaching assay ranged from 17.03% to 86.13%. It was found that *Pistacia lentiscus* showed the highest antioxidant capacities using DPPH assay (IC_{50}=4.30 μg/mL), while *Populus trimula*, *Origanum glandulosum*, *Centaurea calcitrapa*, *Sysimbrium officinalis* and *Rhamnus alaternus* showed the highest percent of total antioxidant activity in β-carotene/linoleic acid bleaching assay. Total phenolic and flavonoid contents ranged from 3.96 to 259.65 mg GAE/g extract and from 1.13 to 26.84 mg QE/g extract, respectively. The most interesting antimicrobial activity was obtained from *Sysimbrium officinalis*, *Rhamnus alaternus*, *Origanum glandulosum*, *Cupressus sempervirens*, *Pinus halipensis* and *Centaurea calcitrapa*.

Conclusions: The results indicated that the plants tested may be potential sources for isolation of natural antioxidant and antimicrobial compounds.

KEYWORDS

Antioxidant activity, DPPH, Antimicrobial activity, Disc diffusion method, Total phenolic content, Total flavonoid content

1. Introduction

Since ancient times, medicinal plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternatives remedies for the treatment of many infectious diseases the preservation of food. Plant products are rich sources of a variety of biologically active compounds, mainly phenolics, and these phytochemicals have been found to possess various biological properties like antioxidant and antimicrobial potentials[1].

Natural antioxidants such as polyphenols, flavonoids and related compounds are very important for inhibition of oxidative damage caused by reactive oxygen species and

*Corresponding author: Dob Tahar, Laboratoire de Molécules Bio-Actives et Valorisation de la Biomasse, ENS kouba, Algeria.
Tel: +213 555 631 955
E-mail: dob_tahar@yahoo.fr
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reactive nitrogen species, which are produced in biological systems and foods. They play an essential role in prevention of human diseases such as cardiovascular, cancer, diabetes and degenerative disorders including Parkinston and Alzeimer's diseases[2].

In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in treatment of infectious diseases. This situation forced scientists to search for new antimicrobial substances from various sources like medicinal plants which are good sources of novel antimicrobial agents[3].

The main aim of this research project was to screen some plant species used in Algerian traditional medicine, with respect to their antioxidant and antimicrobial activity, total phenolic contents and total flavonoids contents, as potential sources of natural antioxidants and antimicrobial agents.

2. Materials and methods

2.1. Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl), butylated hydroxytoluene (BHT), gallic acid, linoleic acid, ascorbic acid, Folin-Ciocalteu's reagent, β-carotene, anhydrous sodium sulfate, sodium carbonate and aluminium chloride hexahydrate were obtained from Sigma-Aldrich (Steinhein-Germany), Fluka (Steinhein-Germany) or Merk (Darmstadt, Germany). Solvents were purchased from Sigma-Aldrich. All solvents used for extraction were of analytical grade.

2.2. Plant material

All plant materials were collected in April 2010 from Setif (at Babor mountain) (Table 1). The botanical identification of the collected plants was authenticated by Agronomic National Institute of Alger and voucher specimen of the plant have been deposited (Table 1). Fresh plant samples were cleaned, air-dried in the shade and then powdered by laboratory mill.

2.3. Extraction procedure

One gram of the dried plant material is extracted for 48 h with 10 mL of 70% (v/v) aqueous methanol at room temperature. This procedure was repeated successively three times with fresh solvent each time, followed by filtration. Filtered extracts were mixed and concentrated to dryness under reduced pressure at 40 °C using vaccum rotary evaporator and the residue obtained was redissolved in methanol. All extracts obtained were kept in the dark at 4 °C prior to use.

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging activity assay

The method of Braca *et al.*[4] was used for determination of scavenging activity of DPPH free radical. Different methanolic dilutions of extracts (5 μg/mL to 1 000 μg/mL) were mixed with equal volumes of freshly prepared DPPH methanol solution (0.004% w/v). The reaction mixture was vortexed thoroughly and then left to stand at room temperature in the dark for 30 min, the absorbance was read at λ=517 nm using a blank containing the same concentration of extracts without DPPH. Ascorbic acid and BHT were taken as standards. Percentage of inhibition was calculated using the following equation:

$$\% \text{ inhibition}=[(Ac-As)/Ac]\times 100$$

Where Ac is the absorbance of control reaction (which contain equal volumes of DPPH solution and methanol without any test compound), and As is the absorbance of the sample. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of scavenging effect percentage against extract concentration.

2.4.2. β-carotene/linoleic acid bleaching assay

This test was carried out according to a described procedure[5], based on the aptitude of various extracts to decrease the oxidative discoloration of β-carotene in an emulsion. A total of 2 mg of β-carotene was dissolved in 10 mL of chloroform. A total of 1 mL of this solution was pipetted into a round-bottom flask containing 20 mg of linoleic acid and 200 mg of Tween-80. Chloroform was completely evaporated using a vacuum evaporator. Then, 50 mL of distilled water was added slowly to the residue and the mixture was vigorously agitated to form a stable emulsion. A total of 4.8 mL of the obtained emulsion were transferred into different test tubes containing 0.2 mL of individual extracts (2 mg/mL). The mixture was then gently mixed and placed in a water bath at 50 °C for 120 min. Absorbance at 470 nm was measured every 30 min for 120 min. Blank solution was prepared in a similar way except that addition of β-carotene was omitted. Ascorbic acid and BHT were used as standards. The bleaching rate (R) of β-carotene was calculated according to first-order kinetics, as described by Al-Saikhan *et al.*[6]:

$$R=\ln(A_{t=0}/A_{t=t})/t$$

Where, ln=natural log, t is the time in minutes, $A_{t=0}$ is the initial absorbance of the emulsion immediately after sample preparation (t=0 min) and $A_{t=t}$ is the absorbance at time t (30, 60, 90, and 120 min). The percentage of antioxidant activity (AA) was calculated using the following equation:

$$AA=[(R_{control}-R_{sample})/R_{control}]\times 100$$

Where, $R_{control}$ and R_{sample} are average bleaching rates of the negative control and the antioxidant (plant extract, ascorbic acid or BHT), respectively.

2.5. Total phenolic contents

The total phenolic content of each extract was determined spectrophotometrically, using the Folin-Ciocalteu assay[7]. Briefly, an aqueous aliquot (0.25 mL) of the extract was added to 3.75 mL of distilled water in a test tube, followed by 0.25 mL of Folin-Ciovalteu's reagent. After 3 min, 0.75 mL of 20% sodium carbonate was added. Tube contents were vortexed and heated at 40 °C for 40 min. The blue coloration

was read at 760 nm. The concentrations of total phenolic compounds, expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g extract), were calculated according to the following equation that was obtained from the standard gallic acid graph:

Absorbance=0.103 5 gallic acid (μg/mL)+0.104 6 (R^2=0.98)

2.6. Flavonoid contents

Flavonoid contents in the extracts were determined by a colorimetric method described by Ramful *et al.*[8]. A total of 1.5 mL of 2% $AlCl_3 \cdot 6H_2O$ dissolved in methanol was added to equal volumes of the diluted extract. The mixture was shaken and the absorbance was read at 440 nm after 10 min incubation at room temperature. The concentrations of flavonoid compounds determined as mg of quercetin equivalents per gram of extract (mg QE/g extract) were calculated according to the following equation that was obtained from the standard quercetin graph:

Absorbance=0.282 9 quercetin (μg/mL)-0.115 5 (R^2=0.99)

2.7. Antimicrobial activity

2.7.1. Tested microorganisms

Antimicrobial activity was tested against a panel of microorganisms: Gram-positive bacteria [*Bacillus subtilis* ATCC 6633 (*B. subtilis*), *Staphylococcus aureus* CIP 7625 (*S. aureus*)], Gram-negative bacteria [*Escherichia coli* CIP 54.8 (*E. coli*), *Pseudomonas aeruginosa* CIP A22 (*P. aeruginosa*)] and one yeast [*Candida albicans* (IPA 200) (*C. albicans*)]. All microorganisms were graciously supplied from stock cultures of the Microbiology Laboratory of the Department of Biology, Ecole Normale Supérieure, Alger, Algeria. Bacterial strains were cultured in Muller-Hinton agar and yeasts were cultured in Sabouraud Dextrose agar. All microbial strains were incubated for 24 h at 37 °C.

2.7.2. Detection of antimicrobial activity

The antimicrobial activity was achieved by the agar diffusion method. The microbial cultures were harvested and then suspended in sterile saline (0.9% NaCl) and the cell density was adjusted to 0.5 McFarland. Sterile 5.5 mm paper discs, impregnated with 10 μL of the extracts solutions (100 mg/mL), were placed on the inoculated surface. Before incubation, all Petri dishes were stored in the dark at 4 °C for 1 h, to allow the diffusion of the extracts from disc to medium without microbial growth. At the end of incubation time (18-24 h at 37 °C), the diameter of the zones of inhibition around each disc (in millimeters, diameter of the disc included) were used as a measure of antimicrobial activity. Levofloxacin (10 μg/disc) was used as positive control for bacteria and nystatin (10 μg/disc) for yeast.

2.8. Statistical analysis

All experiments were carried out in triplicate. Data were expressed as mean±SD. Differences were evaluated by One-way analysis of variance (ANOVA) test completed by a student's *t*-test. The correlations between antioxidant activities and total phenolic and/or flavonoid contents were determined using analysis of variance (ANOVA) and quantified in terms of the correlation factor. Differences were considered significant at $P<0.05$.

3. Results

3.1. Antioxidant activity

3.1.1. DPPH radical scavenging activity

The hydromethanolic crude extracts of 20 plant species were tested for antioxidant activity using the DPPH assay. The results were expressed as IC_{50}, which is defined as the concentration of substrate at 50% inhibition. As shown in Table 1, there is a wide range of free radical scavenging activity between the plant species analyzed. The values of IC_{50} ranged from 4.30 μg/mL and 486.6 μg/mL. IC_{50} values were found to be in the following order: ascorbic acid>*Pistacia lentiscus* (*P. lentiscus*)>*Origanum glandulosum* (*O. glandulosum*)>*Salix alba* (*S. alba*)>*Anacyclus clavatus* (*A. clavatus*)>*Calycotome spinosa* (*C. spinosa*)=*Cupressus sempervirens* (*C. sempervirens*)>*Teucrium polium* (*T. polium*)>*Globularia alypum* (*G. alypum*)>*Helychrisum stoechas*>*Plantago major* (*P. major*)>*Rhamnus alaternus* (*R. alaternus*)>*Ulmus campestris* (*U. campestris*)>BHT>*Marrubium vulgare* (*M. vulgare*)>*Populus trimula* (*P. trimula*)>*Ficus carica* (*F. carica*)>*Pinus halipensis* (*P. halipensis*)>*Sysimbrium officinalis* (*S. officinalis*)>*Centaurea calcitrapa* (*C. calcitrapa*)>*Herniaria glabra* (*H. glabra*)>*Ajuga iva* (*A. iva*). The DPPH radical scavenging effect of ascorbic acid was higher (IC_{50}=4.1 μg/mL) than all the plant species studied, except *P. lentiscus* which showed no significant difference with ascorbic acid ($P>0.05$).

3.1.2. β-carotene/linoleic acid bleaching assay

ANOVA showed a significant difference ($P>0.05$) in antioxidant activity among hydromethanolic extracts of the plant species analyzed. As shown in Table 1, the values ranged from 17.03% to 86.13%. *P. trimula*, *O. glandulosum*, *C. calcitrapa*, *S. officinalis* and *R. alaternus* showed the highest percent of total antioxidant activity among the plants. The lowest levels of antioxidant activity were obtained from the hydromethanolic extracts of *A. iva* (19.35%) and *M. vulgare* (17.03%). The antioxidant activity of all extract was significantly lower than that of BHT (96.92%).

3.2. Total phenolic and flavonoid contents

The amount of total phenolic, measured by Folin-Ciocalteu method, varied widely in herb materials and ranged from 3.96 to 259.65 mg GAE/g extract (Table 1). The highest total phenolic content was detected in *S. alba* with 259.65 mg GAE/g extract, while *A. iva* was the lowest one (3.96 mg GAE/g extract).

Table 1

Antioxidant capacity, total phenolic contents and flavonoid contents of 20 Algerian medicinal plants.

Scientific name	Plant part	Voucher specimen number	Total phenolic contents (mg GAE/g extract)	Total flavonoid contents (mg QE/g extract)	DPPH (IC_{50})	β-carotene/linoleic acid (%)
A. iva	Aerial parts	LA4/10	3.96±0.87	1.16±0.02	486.60±2.90	19.35±2.00
A. clavatus	Aerial parts	AA1/10	71.09±3.84	3.60±0.25	27.20±0.80	30.81±3.97
C. spinosa	Leaves	FC1/10	228.42±8.86	4.87±0.12	29.20±0.80	62.74±3.54
C. calcitrapa	Aerial parts	AC2/10	57.50±1.55	3.28±0.08	231.70±2.90	84.74±1.41
C. sempervirens	Leaves	CC1/10	143.55±4.47	3.09±0.10	29.20±0.80	26.86±5.77
F. carica	Leaves	MF1/10	23.70±2.75	3.75±0.07	113.30±1.50	31.63±2.82
G. alypum	Leaves	PG1/10	25.38±1.09	3.76±0.03	39.30±1.50	54.20±3.47
Helichrysum stoechas	Aerial parts	AH3/10	15.43±1.21	4.36±0.17	46.30±1.50	54.39±3.91
H. glabra	Aerial parts	CAH1/10	34.48±3.97	4.90±0.64	332.50±10.60	57.05±4.72
M. vulgare	Aerial parts	LM5/10	47.58±2.81	2.01±0.09	84.20±0.30	17.03±1.68
Origanum glandulosum	Aerial parts	LO3/10	96.36±5.82	7.56±0.10	12.80±0.20	85.76±1.61
P. major	Aerial parts	PP1/10	106.70±4.77	1.54±0.02	48.00±1.00	66.53±4.08
P. halipensis	Leaves	PIP1/10	108.66±5.91	2.80±0.10	115.50±2.10	47.22±2.33
P. lentiscus	Leaves	ANP1/10	205.22±9.32	8.21±0.09	4.30±0.10	71.57±4.64
P. trimula	Leaves	SP1/10	116.60±5.20	3.98±0.14	88.70±1.10	86.13±1.20
R. alaternus	Leaves	RR1/10	107.95±6.58	26.84±1.18	54.16±0.80	82.77±3.51
S. alba	Cortex	SS2/10	259.65±11.53	1.13±0.09	15.50±0.50	70.71±5.40
S. officinalis	Flowers	BS1/10	48.87±2.99	4.86±0.31	145.00±5.00	83.94±3.31
T. polium	Aerial parts	LT6/10	134.00±5.25	3.44±0.14	30.20±1.60	26.52±3.26
U. campestris	Leaves	UU1/10	24.21±1.12	3.60±0.09	61.50±0.70	62.14±5.68
Ascorbic acid	–		–	–	4.00±0.10	11.05±1.43
BHT	–		–	–	72.16±0.10	96.92±0.51

The range for total flavonoid content was from 1.13 to 26.84 mg QE/g extract (Table 1). *R. alaternus* showed the highest flavonoid content while *S. alba* showed the lowest one with 26.84 and 1.13 mg QE/g extract, respectively.

A moderate correlation between the total phenolic contents and antiradical properties tested by DPPH assay was observed (R^2=0.50). However, there was no notable correlation between the content of total phenolic and antioxidant activity in β-carotene/linoleic acid bleaching assay (R^2=0.25).

3.3. Antimicrobial activity

The results of the antimicrobial screening of 20 species against four bacteria species and one yeast are summarized in Table 2 (inhibition zones in the agar diffusion assay). All the tested extracts revealed antimicrobial activity showing different selectivity for each microorganism. Gram positive bacteria were the most sensitive. Concerning Gram negative bacteria, only the extract of *S. officinalis* was able to inhibit the growth of *E. coli*, while seven plant extracts were found to be active against *P. aeruginosa* with diameter of inhibition zones ranging from 10.0 to 20.5 mm.

Twelve plant extracts showed activity against *B. subtilis*, and the most interesting activity was obtained from *O. glandulosum*. On the other hand, 12 plant extracts were found to be active against *S. aureus* (inhibition zone was ranged from 7.0 to 14.0 mm). Additionally, no inhibitory effect on the tested bacteria was observed for *A. clavatus*, *H. glabra*, *M. vulgare* and *U. campestris* extracts. In the case of *C. albicans*, results from agar diffusion method indicate that *O. glandulosum* showed the highest activity compared to other plant species.

Table 2

In vitro antimicrobial activity of selected plants from Algeria.

Selected plants	Inhibition zone (mm) against				
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
A. iva	–	9.0	–	–	7.0
A. clavatus	–	–	–	–	9.0
C. spinosa	7.0	10.0	–	–	7.0
C. calcitrapa	11.0	13.0	–	15.0	13.0
C. sempervirens	8.0	7.0	–	20.5	8.0
F. carica	–	11.0	–	–	7.0
G. alypum	9.0	8.0	–	–	11.0
Helichrysum stoechas	7.0	9.0	–	–	9.0
H. glabra	–	–	–	–	–
M. vulgare	–	–	–	–	9.0
O. glandulosum	14.5	–	–	–	21.0
P. halipensis	7.0	8.0	–	18.0	7.5
P. lentiscus	–	–	–	10.0	–
P. major	7.0	–	–	–	–
P. trimula	7.0	8.0	–	–	7.0
R. alaternus	11.0	14.0	–	19.5	13.0
S. alba	7.0	–	–	–	–
S. officinalis	13.0	13.0	9.0	–	11.0
T. polium	–	9.0	–	11.0	–
U. campestris	–	–	–	–	7.0
Levofloxacin	29.0	32.0	29.0	24.0	–
Nystatin	–	–	–	–	33.0

4. Discussion

4.1. Antioxidant activity

The antioxidant activity can be measured using several methods. Among them DPPH free radical scavenging assay (DPPH) and β-carotene bleaching assay were used in this

study.

The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants[2,9].

It was observed that the radical scavenging activity in hydromethanolic extracts of most of the studied plants was high and varied significantly. Hydromethanolic extract of *P. lentiscus* had the hightest DPPH radical scavenging activity, which was similar than of ascorbic acid and higher than of synthetic antioxidant BHT. Other workers also reported that antioxidant activity of plants is higher than that of synthetic antioxidant[10,11]. The result is in accordance with the previous published data showing the high antioxidant activity of *P. lentiscus*[12].

In recent years, various plant species have been tested as botanical antioxidants using DPPH assay[10-19]. These species exhibited a broad range of antioxidant activities. The highest antioxidant activity has been reported for *Cynomorium coccineum* and *Myrtus nivellei*, (IC_{50}=4.09 and 4.9 μg/mL, respectively)[13], whereas the lowest antioxidant was reported for *Thelesperma megapotamicam* (IC_{50}=2000 μg/mL)[16].

In the β-carotene/linoleic acid bleaching assay, oxidation of linoleic acid produces hydroperoxides which attack β-carotene molecules and cause a rapid discoloration of the solution whereas antioxidants prevent β-carotene bleaching[20]. In this study, *P. trimula*, *O. glandulosum*, *C. calcitrapa*, *S. officinalis*, *R. alaternus* show high antioxidant activity, largely preventing the bleaching of β-carotene which indicates a good capacity to reduce the rate of β-carotene degradation, and this is an important issue in food preservation, especially in the prevention of lipid peroxidation that causes food spoilage[20].

4.2. Total phenolic and flavonoid contents

Plant phenols are of interest because they are an important group of natural antioxidants and some of them are potent antimicrobial compounds. Therefore, it is necessary to determine the total amount of phenols and flavonoids in the plant extracts chosen for the study.

As shown above, the content of the total phenolics and flavonoids in the hydromethanolic extracts of the studied plants varied significantly. The twenty analyzed plant species have the same geographic origin and grow in the same natural condition, nevertheless the plants belong to different families. It is well known that the amount of phenolic compounds vary with respect to families and varieties[10-18].

4.3. Correlation between antioxidant and phenolic content

Results obtained from experimental data revealed a weak to moderate correlations between the total phenolic contents and the antioxidant activity. Some authors demonstrated that antioxidant was not solely dependent on phenolic content but it may be due to other phytoconstituents as triterpenoids or combine effect of them[11,21]. Different types of phenolic compounds have different antioxidant activity, which mainly depends on their chemical structure[22].

4.4. Antimicrobial activity

Plant has long been a very important source of drug and many plants have been screened for compounds with potential therapeutic activity. Therefore, it is vital to evaluate the antimicrobial activity of selected medicinal plants.

In this study, the antimicrobial activity of 20 Algerian medicinal plants was evaluated by using disk diffusion method. The microorganisms as they are important pathogens and also due to rapidly developed antibiotic resistance as antibiotic use increases.

The results show that the mean zone of inhibition produced by positive controls (levofloxacin and nystatin), was larger than those produced by all hydromethanolic extracts. This may be attributed to the fact that the plant extracts being in crude form contain smaller concentrations of bioactive compounds[3].

In general, the Gram positive bacteria were found to have more susceptibility as compared to Gram negative bacteria species. This is in line with earlier studies which attribute the observed differences to the variation in chemical composition and structure of cell wall of both types of microorganisms[23-26].

The extracts of *S. officinalis*, *R. alaternus*, *O. glandulosum*, *C. sempervirens*, *P. halipensis* and *C. calcitrapa* showed the highest antimicrobial activity compared to other extracts. This difference is probably due the phytochemical differences between plant species[27].

In conclusion, the results of these screening investigations confirm the great potential of plants tested for production of bioactive compounds and are useful for rationalizing the use of medicinal plants in primary health care. Further study will be aimed at isolating and identifying the substances responsible for the biological activity of these plant extracts, which may be further exploited in herbal formulations.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The background of this research is good. Evaluation of IC_{50} of various plants reveals the ones with potent phytochemicals for further studies.

Research frontiers

The current research evaluate the radical scavenging and antimicrobial activities of 20 Algerian medicinal plants.

Related reports

Total phenol and alkaloids in plants have been shown to be good scavengers of free radical. Free radicals have been implicated in a variety of human diseases.

Innovations and breakthroughs

In this study, the authors have demonstrated the various plants with the specific antimicrobial and radical scavenging properties paving way for further studies into isolation and characterization of these compounds in the plants.

Applications

The antiradical and antimicrobial properties of the plants studied (especially the most potent plants), will make the plants a candidate in the management of oxidative stress related diseases and as antibiotics.

Peer review

The is a good study in which the authors demonstrated the antimicrobial and radical scavenging properties of 20 medicinal plants from Algeria.

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