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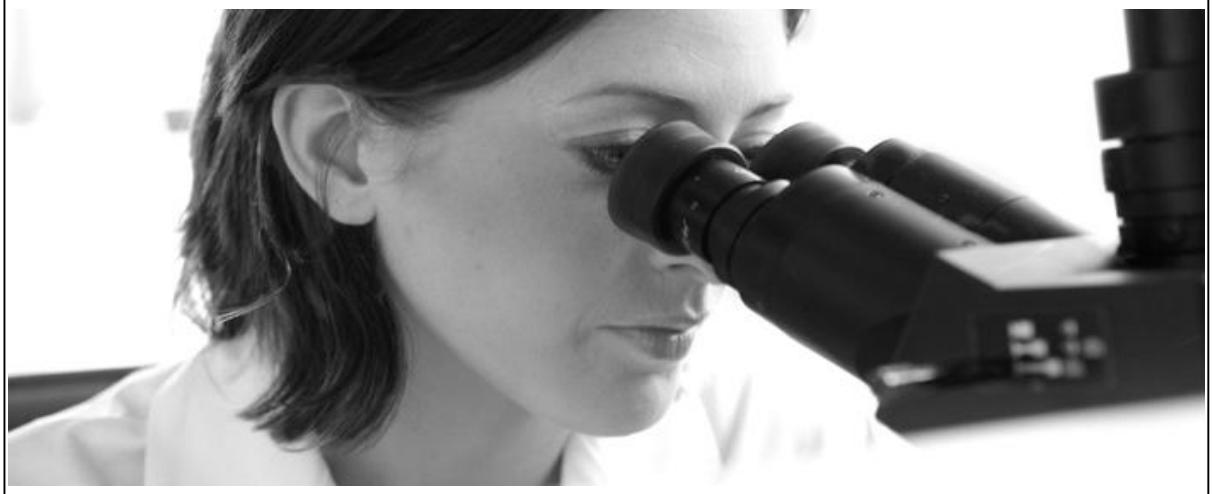

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RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF FLAVONOIDS EXTRACTED FROM SEEDS OF *PIMPINELLA BATTANDIERI CHABERT*

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ABSTRACT

Increasingly, the flavonoids becoming the subject of antioxidant and anti-infective researches, and many groupshave isolated and identified the structures of flavonoids possessing important biological activities. Our study was carry out to elucidate these two aspect of this unknown and rare species. Antioxidant activity was evaluated through the ability of the extract to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals and the reducing power essay, however, the antimicrobial activity was tested with three bacterial strains and three fungi including yeast (*Escherichia coli* ATCC 25922, *Salmonella typhimurium*ATCC 13311, *Staphylococcus aureus* ATCC25923, *Aspergillusniger* 2CA936, *Aspergillusflavius* NRRL3357 and *Candida albicans* ATCC1024). The results indicate a very interesting capacity to trap iron ions and a very strong fungicide against *Aspergillusniger*.

INTRODUCTION

Flavonoids are plant pigments that are synthesized from phenylalanine (Harborne *et al*.1984) .and generally display marvellous colours in the flowering parts of plants (Clifford *et al* 2000). Flavonoids comprise a largegroup of polyphenolic compounds that are characterized bya benzo-y-pyrone structure, which is ubiquitous in vegetables and fruits. Besides their relevance in plants, flavonoidsare important for human health because of their high pharmacological activities as radical scavengers (Cook *et al*.1996). They have been reported to possess a variety of biological activities including antiallergic, antidiabetic, antiinflammatory, antiviral, antiproliferative and anticarcinogenic, hepatoprotective, and antioxidant activities. (Harborne *et al*.1986)(Cowan *et al*1999) (Middleton *et al*.1993). Flavonoids undergo intracellular metabolism, e.g., conjugation with glutathione, and circulating flavonoids are usually *O*-methylated or glucuronidated (Williams *et al*.2004). These structural modifications decrease the ability of flavonoids to donate hydrogen atoms (Rice-Evans *et al* .1997).

PimpinellabattandieriChabert is endemic species described by Alfred Charles Chabert (Roskov *et al*.2000) belong to *Apiaceae* and no researches have been found about this species.

The aims of this study is to develop phytochemical and therapeutic sides of this rare species first and second to find a new molecules able to reduce harmful effects of ROS and/or proliferation of microorganism.

MATERIALS AND METHODS

Plant material

Pimpinella battandieri Chabert was collected from the mountain of Megriss Setif -Algeria in July 2014, determined by Mrs: Nouioua Wafa.

Flavonoids extraction

The dried seeds were milled into coarse powder, then 10 g were defatted three times with petroleum ether (each 40 ml) for 3 hours, at 50 C° (He-Long *et al*.2010). The powder was taking up again three times with 70% ethanol (raw material: solvent ratio was 1: 10) for 90 minutes at 100°C. The extract was pooled and concentrated in vacuum to collect the aqueous residue(10 ml), which was extracted with chloroform, and then acidified with 20% H_2SO_4 (pH = 5)(Chirikova *et al*.2010).and finelyextracted three times with ethyl acetate.

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Determination of total flavonoids contents

The flavonoids contents in the extract was estimated by the Aluminium chloride solution according to the method described by Bahorun *et al* (Bahorun *et al*.1996). Briefly, 1 ml of the methanol solution of the extract was added to 1 ml of 2% $AlCl_3$ in methanol. After 10 minutes, the absorbance was determined at 430 nm. Quercetin was used as a standard. Results were expressed as mg equivalent Quercetin per gram of extract (mg EQ/GE).

DPPH Assay

The donation capacity of extract was measured by bleaching of the purple-colored solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al*. (1998) (Hanato *et al*.1998).

One milliliter of the extract at different concentrations was added to 0.5 ml of a DPPH-methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 minutes in the dark. The absorbance of the resulting solution was then measured at517 nm. The antiradical activity was expressed as IC_{50} (micrograms per milliliter), the antiradical dose required to cause a 50 % inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = [(A_0 - A_1)/ A_0] \times 100$$

Where A_0 is the absorbance of the control at 30 minutes and A_1 is the absorbance of the sample at 30 minutes. BHT was used as a standard and samples were analyzed in triplicate (Bettaieb *et al*.2011).

Reducing power

The reducing power was determined according to the method of Oyaizu (Oyaizu *et al*.1986). The extract (2.5 ml) was mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 10 mg/ml potassium ferricyanide. The mixture was incubated at 50 C° for 20 minutes; after, 2.5 ml of 100 mg/ml trichloroacetic acid were added, the mixture was centrifuged at 200g for 10 minutes. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1ml of 1 mg/ml ferric chloride. The absorbance was measured at 700 nm against a blank.

A higher absorbance indicates a higher reducing power. EC_{50} value (mg extract/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis(Huang *et al*.2006). BHT was used as standard.

Test strains and culture media

Strains of bacteria were obtained from the American Type Culture Collection, three bacterial strains were tested: *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 13311 and *Staphylococcus aureus* ATCC25923. Two fungi: *Aspergillusniger* 2CA936 and *Aspergillusflavus* NRRL 3357; and one yeast: *Candida albicans* ATCC1024.

Muller Hilton agar was used for bacteria culture, the potato dextrose agar for fungi culture and Sabouraud for yeast.

Anti-bacterial Activity

Agar disc diffusion method (NCCLS.1999) (NCCLS.1997). was employed for the determination of antibacterial activities of flavonoids extracted from *Pimpinellabattandieri Chabert*.

Briefly, a suspension of the tested microorganism (0.1 ml 10^8 cells per ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 10 µl of 100 mg/ml of the extract and placed on the inoculated plates. These plates were incubated at 37 C° for 24 hours. Gentamicin (10 µg/disc) was used as standards and dimethylsulfoxide DMSO as a control. The antibacterial activity was determined by measuring of inhibition zone diameters (mm) and was evaluated according the parameters suggested by Alves *et al*. (2000) [19]:

- <9 mm, inactive ;
- 9–12 mm, less active ;
- 13–18 mm, active;
- >18 mm, very active.

Antifungal activity

The antifungal activity was tested by disc diffusion method with modifications (Alves *et al*.2000). The potato dextros agar plates were inoculated with each fungal culture (*Aspergillusniger* 2CA936, *Aspergillusflavus NRRL 3357*), 8 days old by point inoculation.The spore suspension was prepared in an emulsion of 0, 5 % tween 80, adjusted to a concentration of 2-3 × 10^6 spores/ml, corresponding to 0.15 to 0.17 absorption at 530 nm (Yazdani *et al*.2012). However, *Candida albicans* ATCC1024 suspension is obtained from a culture in Sabouraud 24 hours 37 C° adjusted to 10^5CFU / ml.

One hundred microliter of suspension was placed over agar in Petri dishes and dispersed. Then, sterile paper discs (6 mm diameter) were placed on agar to load 10 µl of each sample at 100 mg/ml.

Nystatin 100µg, clotrimazon 50 µg and amphotericin 100 µg were used as standards and dimethylsulfoxide DMSO as control. Inhibition zones were determined after incubation at 27 C° for 48 hours.

Statistical analysis

Results were expressed as the mean ± standard deviation. Data was statistically analyzed using t test of Student with the criterion of P values < 0.05 to determine any significant differences between flavonoids of *Pimpinellabattandieri Chabert*seeds and standards, using Graph pad prism 5 Demo Software.

RESULTS AND DISCUSSION

Flavonoids extracted from the seeds of *Pimpinellabattandieri Chabert* reach the yield of 1, 86% with 670, 46±19,70mg EQ/GE of pure flavonoids.

The results of radical scavenging activities using DPPH stable free radicals are presented. Figure 1:

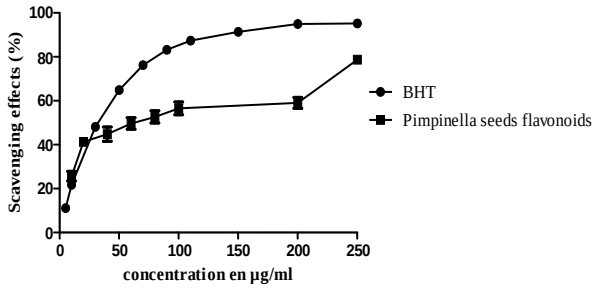

Figure 1 scavenging activity on the DPPH radical of flavonoids extracted from seeds of PimpinellabattandieriChabert.

Results revealed that flavonoids have a high free radical scavenging potential (78, 72 %) with $IC_{50}$of 109, 08±0, 20µg/ml*** against 96, 58% of free radical scavenging potential and $IC_{50}$of 34, 01±1,1µg/ml for BHT.

The role of antioxidants in the inhibition of antioxidant processes occurring in living organisms consists of: scavenging free radicals and quenching singlet oxygen, disconnection of radical reactions, chelate metals which catalyze the oxidation process, inhibition of certain enzymes (eg, oxidases). Flavonoids are active in all these processes (Nijveldt *et al*. 2001). Effectiveness offlavonoids in DPPH radical scavenging depends largely on their structure, hydrophobicity, biological and oxidative activity. The ability and disconnection of radical chain reactions byflavonoids is mainly dependent on the presence in Bring of at least two *o*-hydroxyl groups. It enables the formation of intramolecular hydrogen bond between hydroxyl groups, which increases the stability of the phenoxyl radicals (Majewska *et al*.2011). However the weakness of our extract due to the presence of other molecules which affect the ability of scavenging in terms of hydrogen donating ability.

The reducing capacity of a compound may be considered as an important indicator of its antioxidant activity (Hsu *et al*.2006). Figure 2 shows the dose response curve for the reducing power of the extract:

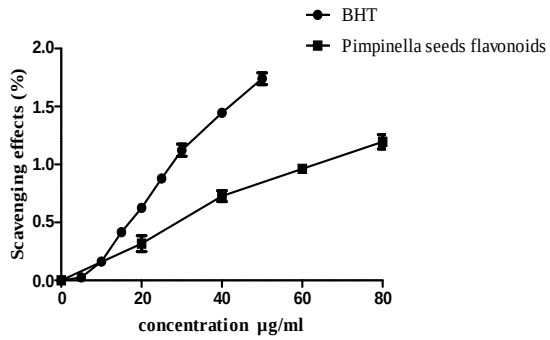

Figure 2 reducing power of flavonoids extracted from seeds of PimpinellabattandieriChabert

The results show that there was an increase in reducing power of the plant extract as the extract concentration increases.EC_{50} of extract (24, 80±1, 24 µg/ml***) was very important in spite the significant difference with BHT (16, 06±0,18µg/ml).

High reducing power of flavonoids suggested their remarkable potency to donate electrons to reactive free radicals, thus converting them into morestable non-reactive species and finally terminate the free radical chain reaction (Zha *et al*.2009). It was confirmed that the hydroxyl groups at C-3' and C-4' of the B-ring to be more active in reducing iron concentration (Moran *et al*.1997).

The antimicrobial activity results appear be negative against all bacterial and fungi strains except *Aspergellusniger* which stop the mycelia growth and spores, still in germination step (table 1).

Table 2 Inhibition zones in millimetre of the antifungal activity of flavonoids, standard and control.

	Aspergillus flavius NRRL3357	***Aspergillus niger 2CA936***	***Candida albicans ATCC1024***
Nystatin	15,53±0,79	9,40±0,22[a]	9,29±0,19
Clotrimazon	23,86±1,15	15,85±0,32[b]	44,28±0,49
Amphotericin	16,20±1,19	17,55±0,14[c]	15,58±0,12
Flavonoids	–	*100% inhibition of spores formation* [a,b,c]	–
Control	*No inhibition*	*No inhibition*	*No inhibition*

The similar letters in different column indicate a very significant difference.

The antimicrobial action of flavonoids may be relatedto their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and so forth. Lipophilicflavonoids may also disrupt microbial membranes (Cowan *et al*.1999) (Mishra *et al*.2009).

CONCLUSION

For the best of our knowledge, this is the first time which flavonoids extracted from seeds of *PimpinellabattandieriChabert* were studied for their antioxidant and antimicrobial activities.

The results demonstrate an important capacity of chelating iron and a powerful action against *Aspergellusniger*. Further investigations were needed to determine the molecular composition of our extract and to reveal other capacities of this rare endemic species.

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