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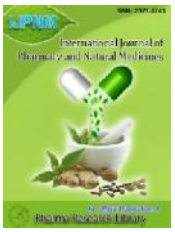

Research Article **Open Access**

Evaluation of antioxidant and antimicrobial activity of tannins extracted from the branch of *Pimpinella battandieri Chabert.*

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A B S T R A C T

The evaluation of antioxidant and antimicrobial capacities of tannins extracted from the branch of *Pimpinella battandieri* Chabert were studied. The antioxidant investigation has been carried out by two methods: radical scavenging activity (DPPH) and the reducing power. 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, *Aspergillus niger* 2CA936, *Aspergillus flavius* NRRL3357 and *Candida albicans* ATCC1024). Our results exhibit a low antioxidant and antibacterial activities but a very strong antifungal capacities.

Keywords: Antioxidant, Antifungal, *Pimpinella battandieri* Chabert, Tannins

A R T I C L E I N F O

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1. Introduction

Free radicals, chemical reactions, and several redox reactions of various compounds may cause protein oxidation, DNA damage, and lipid peroxidation in living cells [1]. Increased consumption of whole grains, fruits, and

vegetables is related to a reduced risk of chronic diseases [2]. Application of synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxytanisole (BHA), propyl gallate and citric acid in food processing has led to the appearance of remarkable side effects. For example, these substances can exhibit carcinogenic effects in living organism and enlarge the liver size and increase microsomal enzyme activity [3] [4]. Due to these limitations, there is an increasing interest in finding naturally and biologically produced antioxidants capable of inhibiting free radical reactions, retarding oxidative rancidity of lipids and protecting the human body from diseases [5] [6]. Nowadays, multiple drug resistance has developed due to the indiscriminutes ate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases, a situation that forced scientists to search for new antimicrobial substances [7] It is reported [8] [9] [10] [11] [12] [13] [14] [15] [16] [17] [18] [19] that phenolic compounds from herbs and spices are active against many bacteria and fungi and have antioxidant activity. Recently, there has been considerable interest in the use of such antioxidants and antimicrobial compounds from natural sources, not only for the preservation of foods and improving the shelf life of food products but also for increasing the stability of fats and oils and to control the human and plant diseases of microbial origin [20] [21] [22] [23] [17] [19]. Tannins is defined as "Any phenolic compound of sufficiently high molecular weight containing sufficient hydroxyls and other suitable groups (i.e. carboxyls) to form effectively strong complexes with protein and other macromolecules under the particular environmental conditions being studied [24]. This study aimed the identification of the antioxidant and the antimicrobial activities of the total tannins extracted from the branch of *Pimpinella battandieri* Chabert.

2. Materials and method

Plant material: *Pimpinella battandieri* Chabert is endemic species described by Alfred Charles Chabert [25], belong to *Apiaceae* was collected from the mountain of Megriss Setif -Algeria in May 2014. Determined and air-dried, then tested for their antioxidant and antimicrobial capacities.

Tannins extraction

The method of Mohamad Ibrahim *et al.* (2005), was adopted [26]. Briefly, powdered plant material (20 g) was macerated in 200 mL of diluted acetone (70 % v/v) for 24 hours; the supernatant was separated from the residue by filtration using Whatman #1 filter paper, the fraction was concentrated and dried to a constant weight in a vacuum oven at 45°C and the residues obtained was stored at 18 °C.

Determination of total tannins contents

Tannins content was evaluated using the haemoglobin precipitation assay. An aliquot of 0.5 mL of the extract is mixed with 0,5 mL of haemolysis bovine blood to reach a final concentration of 1mg/mL, the mixture was centrifuged at 480 g for 20 minutes and the absorbance was measured at 578 nm [27]. In same time, tannic acid at various concentrations (100 – 600 µg / mL) was used in the same manner as the standard curve. Result was expressed as mg equivalent of tannic acid / gram of extract (ETA/GE).

DPPH assay

The donation capacity of extracts were measured by bleaching of the purple-coloured solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al*. (1998) [28]. One millilitre 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 laboratory temperature for 30 minutes in the dark. The absorbance of the resulting solution was then measured at 517 nm. The antiradical activity was expressed as IC_{50} (micrograms per millilitre), the ability to scavenge the DPPH radical was calculated using the following equation:

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

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 analysed in triplicate [29].

Reducing power

The reducing power was determined according to the method of Oyaizu (1986), [30]. The extract (0.5 –10 mg/mL) in methanol (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 cooling, 2.5 mL of 100 mg/mL trichloroacetic acid were added and the mixture was centrifuged at 200g for 10 minutes. The upper layer (5 mL) was mixed with 5mL of deionized water and 1 mL of 1 mg/mL ferric chloride, and the absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher reducing power. IC_{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. Ascorbic acid was used as standard [31].

Antimicrobial activity:

Test strains and culture media

Three bacterial strains were tested: *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 13311 and *Staphylococcus aureus* ATCC25923. Two fungi: *Aspergillus niger* 2CA936 and *Aspergillus flavus* NRRL 3357; and one yeast: *Candida albicans* ATCC1024. Muller Hinton agar was used for bacteria culture, the potato dextrose agar for fungi culture and Sabouraud for yeast.

Anti-bacterial activity

Agar disc diffusion method was employed for the determination of antibacterial activities of tannins extract from the branch of *Pimpinella battandieri* Chabert [32] [33]. 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 different concentration 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 a standards and dimethylsulfoxide DMSO as a control. The antibacterial activity was determined by measuring the inhibition zone diameters (mm) and evaluated according the parameters suggested by Alves *et al*. (2000) [34].

- < 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 [32]. The potato dextrose agar plates were inoculated with each fungal culture (*Aspergillus niger* 2CA936, *Aspergillus flavus NRRL 3357*), 8 days old by point inoculation. The spore suspensions were 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 [35].

However, *Candida albicans* ATCC1024 suspension is obtained from a culture in Sabouraud 24 hours at 37 °C adjusted to 10^5 CFU / 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 the sample at different concentrations. Clotrimazon 50 µg was used as a standards and dimethylsulfoxide DMSO as a control. Inhibition zones were determined after incubation at 27 °C for 48 hours.

Statistical analysis

Results were expressed as the mean ± standard deviation (experiments were done in triplicate). Data was statistically analysed using t test of Student with the criterion of P values < 0.05 to determine whether there were any significant differences between tannins extract of *Pimpinella battandieri* Chabert and standards, using Graphpad prism 5 Demo Software.

3. Results and Discussion

The yield of tannins gave 25,5 %, however, tannins quantification touch 158,83±2,40 mg EAT/GE. No data are available in the literature regarding the antioxidant activity of tannins extracted from the branch of *Pimpinella battandieri* Chabert. Acetone extract gave a very important yield of total tannins. The recovery of polyphenols from plant materials is influenced by their solubility in the extraction solvent, the type of solvent, the degree of polymerization of phenols, the interaction of phenols with other plant constituents and the formation of insoluble complexes [36]. Furthermore, solvent polarity plays a key role in increasing phenolic solubility [37], which explains the highest quantity of extract. In the DPPH assay, the radical scavenging ability of tannins and also the standard was measured spectrophotometrically (figure 1). In general, the tannins were able to reduce the stable radical DPPH to the yellow colour DPPH-H with IC_{50} value of 127,82±2,14 µg/mL*** showed weak activity in comparison with BHT with IC_{50} values of 8,76±0,69 µg/mL.

DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol. The reduction of DPPH• is monitored by decrease of the absorbance of its radical at 517 nm. It is reduced in the presence of an antioxidant molecules resulting in uncoloured solutions. The DPPH assay is an easy and rapid way to evaluate antioxidant activity [38]. However, research has shown tannins to be natural antioxidant [39]. The results are shown as the relative activity against the standard, they were absolutely weak in comparison with the standard. Szymusiak *et al*. (1999) [40], reported that antioxidant polyphenols can also act as oxidants. A similar phenomenon was observed by Sahu and Gray (1997) [41], who noticed that hydroxyl radicals derived from these compounds may initiate peroxidation of oxidized nuclear membrane lipids.

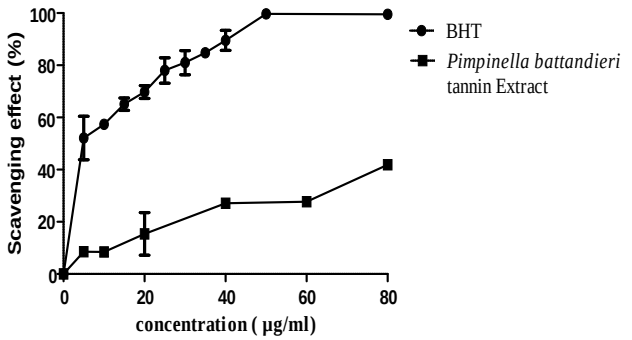

Figure 1: Scavenging effect of tannins extracted from the branch of *Pimpinella battandieri* Chabert**.**

Rice-Evans *et al*. (1996) [42], noticed that an antioxidant response of phenols depends on the relative positions of the hydroxyl groups in the ring. However, little is known about pro-oxidant effect of the polyphenols, and it is likely that different groups of tannins have different properties [43], and that may explain the weak antioxidant activity of our extract.

Reducing power of the samples is also presented in figure 2; as can be seen, the reducing power of tannins extracted from the branch of *Pimpinella battandieri Chabert* increases by increasing the concentration of tannins with low power electron donation of reactive free radicals. The reducing power of the extract was 207,48±3,61 µg/ mL*** to give an optical density of 0.5, which remained significantly lower than ascorbic acid 8,46±0,09 µg/ mL to give the same colour intensity.

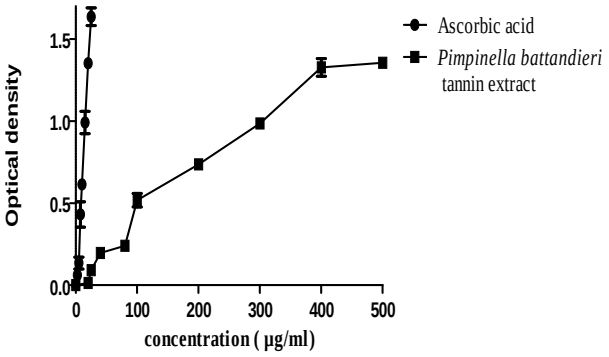

Figure 2: Reducing power of tannins extracted from the branch of *Pimpinella battandieri Chabert.*

Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [44]. Polyphenols may also have the possibility of chelating metal ions and preventing iron and copper-catalysed formation of initiating radical species [45] [46].

Glahn *et al.* (2002) [47], observed that Gallic acid, similar to tannic acid, can bind Fe. They concluded that inhibition of Fe absorption by phenolic compounds is likely to be due to the binding of Fe, thus making the iron unavailable for absorption. In accordance with findings from the DPPH assay, weakness of reducing power and DPPH values of tannins corresponded to structural heterogeneity of tannins. The high antioxidant or pro-oxidant activities of high-molecular-weight tannins is in part a result of the increased number of phenolic hydroxyl groups present per molecule as molecular weight increases [48] [49] .

Figure 3: Inhibition zone of the extract on *Aspergillus flavus NRRL 3357*

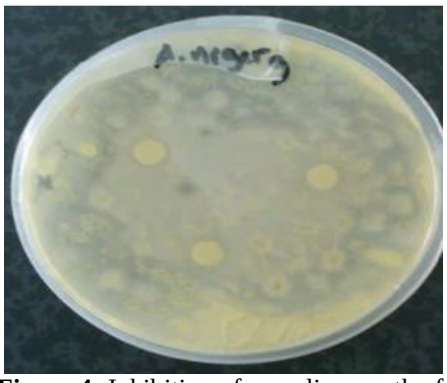

Figure 4: Inhibition of mycelia growth of *Aspergillus niger 2CA936*

Statistical treatment of results shows a very significant difference between tannins used against *Aspergillus niger 2CA936* and standards which mean that the extract is very strong. However in case of *Aspergillus flavus NRRL 3357* there is also a very significant difference between the extract and the standards but this time tannins extracted from the branch of *Pimpinella battandieri* Chabert are less effective than the standards used. The antimicrobial activity of tannins has been well documented. Filamentous fungi such as *Aspergillus niger, Botrytis cinerea, Chaetomium cupreum, Colletotrichum graminutes icola, Coniophora olivacea, Coriolus versicolor, Crinipellis perniciosa, Fomes annosus, Gloeophyllum trabeum, Merulius lacrymans, Penicillium species, Poria monticola, Trametes hirsuta and Trichoderma viride* were inhibited by tannins from different preparations [50] [51]. The antimicrobial activity of tannins could be due to their complexion with enzymes or substrates [50]. Many microbial enzymes, cellulase, pectinase, xylanase, peroxidase, laccase, and glycosyl transferase were inhibited when mixed with tannins [50]. Tannin toxicity may also be related to their action on the membranes of the microorganisms. Inhibition of the electron transport system [50].

Table 1: *Inhibition zones in millimetre for tannins extracted from Pimpinella battandieri Chabert*

Fungus and bacteria strains	***Tannins 100 mg/mL***	***standard***	***control***
Escherichia coli ATCC 25922	*No inhibition*	*18,50±0,41*	*No inhibition*
Salmonella typhimurium ATCC 13311	*No inhibition*	*19,17±0,24*	*No inhibition*
Staphylococcus aureus ATCC25923	*No inhibition*	*27,67±0,47*	*No inhibition*
Aspergillus niger 2CA936	*44,97±0,05* ***	*15,85±0,32*	*No inhibition*
Aspergillus flavus NRRL 3357	*11,63±0,71* ***	*23,86±1,15*	*No inhibition*
Candida albicans ATCC1024	*No inhibition*	*44,28±0,49*	*No inhibition*

****: highly significant difference; ** very significant difference; * significant difference with* $P < 0{,}001$

4. Conclusion

The present study demonstrates that tannins extracted from the branch of *Pimpinella battandieri Chabert* content a low antioxidant and the antibacterial activities*;* but a very interesting antifungal activity against *Aspergillus niger* by stopping the mycelia development. These results are promising, therefore, further investigations should be targeted on such important issues as activity in real agriculture systems relative to commercially used antifungal extracts, toxicology, processing (highest possible activity and absence of residues in environment) and economic feasibility of practical applications.

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