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# Antioxidant and in *vitro* anti-inflammatory activities of the crude extracts of *Bunium pachypodum* P.W. Ball (Apiales Apiaceae) tubers from Algeria

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**ABSTRACT** The objective of present study is to valorize different extracts (aqueous, ethanol and acetone) of the tubers of *Bunium pachypodum* P.W. Ball (Apiales Apiaceae) plant, by carrying out a phytochemical screening, and determine total phenolic and flavonoid contents, then evaluate the antioxidant activity by DPPH method (2, 2-diphenyl 1-1picrylhydrazyl) and FRAP (Ferric reducing antioxidant power), as well as the anti-inflammatory activity by stabilization method of human red blood cells and inhibition human albumin protein denaturation method. Phytochemical analysis of extracts showed presence for the presence of flavonoids, quinones, reducing sugars and saponins. Comparison between the extracts showed that aqueous extract exhibited the highest total polyphenols and flavonoids contents, followed by ethanol extract. The antioxidant activity evaluated through the DPPH assay showed that acetone extract was the more active, followed by aqueous extract, but lower active than the control (ascorbic acid). In contrast, it is the aqueous extract that has shown the most important reducing power. However, no significant difference was observed between the three extracts. At 1000 µg/ml, aqueous extract showed the most significant anti-hemolytic activity with 54%, and at the concentration of 500µg/ml, it showed 70.19% inhibition of albumin denaturation, statistically similar ($P > 0.05$) to that of the reference anti-inflammatory drug.

**KEY WORDS** Antioxidant; anti-inflammatory; *Bunium pachypodum*; crude extract.

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## INTRODUCTION

The tubers (rhizomes) of this plant are widely used in traditional medicine, in the form of dry powder, as astringent, anti-diarrheal, anti-inflammatory but especially for the treatment of thyroid disorders (Bousetla et al., 2015; Taïbi et al., 2021).

Is known that several diseases such as cancer (Raafat et al., 2011), diabetes (Bandeira et al., 2012), asthma (Salam et al., 2012), those cardiovascular (Meagher & Rader, 2001) and inflammatory (Sequeira et al., 2012) are the result of damage induced by free radicals formed as a result of oxidative stress.

Oxidative stress is defined as an imbalance between the reactive oxygen species (ROS) production, and the removal of these species by the

antioxidant defense mechanism (Sies, 1991; Sarr et al., 2015). This imbalance can have various causes, such as nutritional deficiency in antioxidants, environmental exposure to pro-oxidative factors, or endogenous overproduction of inflammatory origin (Favier, 1997, Favier, 2003).

Indeed, there is a correlation between chronic inflammation and oxidative stress (Ercan et al., 2006, Sequeira et al., 2012). According to Moreno-Macias & Romieu (2014) oxidative stress causes a disruption of cell signaling and alters arachidonic acid metabolism (Moreno-Macias & Romieu, 2014). Oxidative stress has also been shown to increase inflammation associated with cytokine production by TH1 and TH2 cells (King et al., 2006). Prolonged use of anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), to reduce swelling and pain of inflammation carries risks of gastro-intestinal, cardiovascular and other toxicities (Abramson & Weaver, 2005). Consequently, the use of natural bioactive molecules present in plants may be an alternative with lesser side effects.

Medicinal plants are considered today as a valuable material for the development of food and pharmaceutical products capable of serving as preventive agents for several types of diseases related to oxidative stress (Rao, 2012). In fact, it has been shown that ROS can be scavenged or neutralized by antioxidant substances naturally present in medicinal plants (Mouhoubi-Tafinine et al., 2016) and are usually the products of their metabolism (Gurib-Fakim, 2006) such as polyphenols. The properties of polyphenols are widely studied in the medical field where they are known to have antiviral, anti-tumor, anti-inflammatory, anti-allergic and anti-cancer activities and positive actions on obesity, diabetes, Alzheimer's and Parkinson's diseases (Manach et al., 2004; Pan et al., 2015).

The aim of this work is the phytochemical study and evaluation of the antioxidant and anti-inflammatory activity of the *Bunium pachypodum* tuber extracts from our region, knowing that this plant is little studied scientifically.

## MATERIAL AND METHODS

### *Chemicals*

All chemicals were purchased from Prochima - Sigma (represented by Algerian Chemical Society, Tlemcen, Algeria).

### *Plant material*

*Bunium pachypodum* were harvested in the region of Ain Temouchent (North West of Algeria) during the period March–April (2021). The taxonomical classification was performed by botanists from the University of Ain Temouchent (Algeria). The tubers of *Bunium pachypodum* were washed in tap water and peeled. Then they were cut and dried in for 10 days at room temperature, in shade. The dried plant materials were powdered with a laboratory mill. The obtained powder was stored in glass jars, sealed and stored away from light.

### *Preparation of extracts*

Each ten grams of dried powder were extracted with three different solvents (100 ml): distilled water (Aq), ethanol-water (Eth) (70:30, v/v) and acetone-water (Ace) (70:30, v/v) at room temperature, on shaker for 24h. After filtration, the extracts were concentrated using a rotary evaporator (Stuart) under vacuum at 40 °C, they were completely dried at 40 °C in the oven. The extracts were weighed to calculate the yield percentage using the following equation: Yield % = (weight of dry extract/weight of dry tubers before extraction) × 100%. The extracts were stored at 4 °C until further analysis.

### *Phytochemical screening of the plant extract*

A small portion of the dry extracts was used for the phytochemical tests in accordance with several methods (Trease & Evans, 1987; Rizk et al., 1982; Sofowora, 1993; Bruneton, 1999) with little modifications. The following methods were applied:

Saponins: 10 ml of distilled water was added to 5 ml of the extract (aqueous, hydro-ethanolic, hydro-acetonic). After stirring for 2 min, the appearance of a persistent froth after 15 min or an emulsion formed after addition of olive oil, revealed presence of saponins.

Triterpenes and sterols: for each 2.5 ml of extract, 0.12 ml of acetic anhydride, 0.12 ml chloroform and 0.5 ml of sulfuric acid were added. The positive test is revealed by the formation of brownish-red or purple ring on the surface (presence of

sterols) or the appearance of a greenish purple coloration (presence of triterpenes).

Tannins: 5 ml of distilled water and 2 drops of $FeCl_3$ was added to 0.5 ml of extract. The appearance of a greenish brown color indicates the presence of condensed tannins. If the coloring is blue-black, it indicates the presence of hydrolyzable tannins.

Flavonoids: 5 ml of each extract was treated with 5 ml with 5 ml of a diluted solution mixture ($NH_3$+$H_2SO_4$). The presence of flavonoids was indicated by a yellow coloration which disappears after a while.

Alkaloids: 5 ml of 1% aqueous HCL was added to 0.2 ml of extracts. The mixture is stirred in a water bath for 15 min and filtered. Then a few drops of Wagner's reagent are added to 1 ml of filtrate. Precipitation or turbidity indicates the presence of alkaloids.

Anthraquinones: 5 ml of KOH (10%) was added to 5 ml of extracts. After shaking, the occurrence of a red coloration was indicative of the anthraquinones.

### *Determination of total phenol*

The amount of total phenol in the three extracts was determined with Folin-Ciocalteu reagent using the method of Wang & Weller (2006).

Of the samples (3 replicates), 0.1 ml of each was mixed with 2.5 ml of 10% Folin ciocalteu. After stirring, the mixture was incubated for 5 min at room temperature. Then 2.5 ml of 1% $Na_2CO_3$ solution was added. The resulting mixture was incubated at ambiant temperature for 15 min. The absorbance of the samples was measured at 725 nm using UV/visible light.

A calibration curve is performed under the same operating conditions using gallic acid as a positive control at different concentrations (0.018, 0.037, 0.075, 0.15, 0.31, 0.625, 1.25 mg/ml). The total phenolic content was expressed as mg equivalents of gallic acid per gram of dry plant matter (mg GAE/gDM).

### *Determination of flavonoïds*

Aluminum chloride colorimetric method was used for flavonoïds determination, using the protocol established by Dewanto et al. (2002).

Of the samples (3 replicates), 500 µl of each was mixed with 2 ml of distilled water. Then, 150 µl of $NaNO_2$ (7.5%) was added. After 6 minutes, 150 µl of $AlCl_3$ (5%) was added. At 6 min, 2 ml of NaOH (4%) was added to the mixture. The total volume is completed with addition of 5 ml of distilled water and remains at room temperature for 15 minutes. The absorbance of the reaction mixture was measured at 510 nm with UV visible spectrophotometer.

The content of flavonoids was determined from extrapolation of calibration curve which was made by preparing catechin solution at different concentrations (0.0045, 0.018, 0.037, 0.075, 0.15, 0.31, 0.625, 1.25 mg/ml). The concentration of flavonoids was expressed as mg of catechin equivalent (CE) per g of dry weight of plant material (mg CE/g DW).

### *Determination of the antioxidant activity*

The stable radical 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH), was used for determination of free radical scavenging activity of the extracts (Sánchez-Moreno et al., 1998).

To prepare the DPPH solution, 2.5 mg of DPPH powder is solubilized in 100 ml of ethanol. 50 µl of different concentrations of each extract and the ascorbic acid standard were added to 1950 µl of ethanolic solution of DPPH. The negative control was prepared by mixing 50 µl of ethanol with 1950 µl of DPPH ethanolic solution. After 30 min at room temperature, the absorbance was measured 515 nm using a spectrophotometer.

The scavenging ability of the extracts was expressed as the inhibition percentage and was calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Where Abs control is the absorbance of DPPH + ethanol; Abs sample is the absorbance of DPPH radical + sample (extract or standard).

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC50 value.

The reducing power of the extract was determined according to the method of (Karagözler et

al., 2008). 1 ml of samples at different concentrations (0.075, 0.15, 0.31, 0.62, 1.25, 2.5, 5.10 mg/ml) diluted in distilled water was mixed with 2.5 ml of a phosphate buffer solution (0.2M; pH 6.6) and 2.5 ml of potassium ferricyanid [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50 °C for 20 min, followed by the addition of 2.5 ml of trichloro-acetic acid (10%).

The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 ml), mixed with distilled water (2.5 ml) and 0.5 ml of iron chloride solution ($FeCl_3$, $6H_2O$) (0.1%). The absorbance was recorded at 700 nm and compared to ascorbic acid standard. The ferric reducing power capacities of the plant extract and standard were expressed graphically by plotting absorbance versus concentration.

### *In vitro anti-inflammatory activity*

The human red blood cell membrane stabilization method (HRBC method) was used for the estimation of anti-inflammatory activity *in vitro* of plant extract, after induction of hemolysis by hypotonic solution associated with high temperature, according to the protocol of Gandhidasan et al. (1991) and Mongelli et al. (1997) with some modifications.

Blood was collected from a healthy human volunteer who had not taken any NSAIDs in the 2 weeks prior to the experiment, and centrifuged at 3000 rpm. The supernatant part was pipetted out. The packed cells were washed with equal volume of isosaline solution and centrifuged again. The process was repeated until the supernatant was clear. A 10% HRBC suspension was then prepared with normal saline and used immediately.

Various concentrations of extracts were prepared (50, 100, 300, 500 and 1000 µg/ml) using normal saline and to each concentration, 2 ml hyposaline, 1 ml of phosphate buffer (0.15 M, pH 7.4) and 0.5 ml of HRBC suspension were added. The mixture was incubated at 56 °C for 30 min, then were cooled under running water for 20 min and centrifuged at 3000 rpm. The supernatants were separated and this absorbance was estimated spectrophotometrically at 560 nm. Salicylic acid (50, 100, 300, 500 and 1000 µg/ml) was used as reference standard and control was prepared by omitting the extracts. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula:

$$\% \text{ Protection} = 100 - (\text{Abs sample}/\text{Abs control}) \times 100$$

This method consists of preparing four solutions (Kar et al., 2012). Test solution (0.5 ml) is composed of 0.45 ml the aqueous solution of human albumin (5%) and 0.05 ml of different concentrations (100, 300, 500µg/ml) of extracts.

Test control solution (0.5 ml) consists of 0.45 ml the aqueous solution of human albumin (5%) and 0.05 ml of distilled water. Product control solution (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of extracts with (100, 300, 500µg/ml).

Standard solution (0.5 ml) consists of 0.45 ml of the aqueous solution of human albumin (5%) and 0.05 ml of salicylic acid (100, 300, 500µg/ml).

The samples were incubated at 37 °C for 20 min, and then the temperature was increased to 57 °C for 3 min. After cooling, 2.5 ml of phosphate buffer saline (pH 6.3) was added to the above solutions.

The absorbance (Abs) was measured at 416 nm and the percentage inhibition of protein denaturation was calculated as follows, the control represents 100% protein denaturation:

$$\text{Percentage inhibition} = 100 - [(\text{Abs test solution} - \text{Abs product control})/(\text{Abs test control})] \times 100$$

### *Statistical analysis*

Experiments were conducted in three parallel measurements and results were expressed as mean ± standard deviation (SD). Student's *t*-test was used to analyze level of statistical significance between two groups (*P<*0.05*). Analysis of variance was performed by ANOVA procedure with one factor followed by Tukey's test in the software XLSTAT. Differences were considered to be significant at *P< 0.05.

## RESULTS AND DISCUSSION

### *Extraction Yield*

As it shown in Table 1, the percentage yield of water-ethanol extract was important than water-acetone and aqueous extracts. According Do et al. (2014), the combined use of water and organic solvent may facilitate the extraction of chemicals that

are soluble in water and/or organic solvent like ethanol, acetone and methanol.

Ethanol is known as a good solvent for polyphenols extraction and is non-polluting and non-toxic to health compared to other solvents such as methanol (Jokić et al., 2010). However, methanol has been found to be more efficient in extraction of lower molecular weight polyphenols, while aqueous acetone is better for extraction of higher molecular weight flavanols (Dai & Mumper, 2010; Jokić et al., 2010).

### *Phytochemical screening*

The presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent (Turkmen et al., 2006). Polar solvents are frequently used for recovering polyphenols from plant matrices (Do et al., 2014). The preliminary phytochemical test (Table 2) showed the strong presence of saponins, flavonoids and reducing sugars in all extracts. Sterols and antraquinones are reported present only in ethanol extract and acetone extract respectively, whereas triterpens, tannins and alkaloids tested negative in all extracts. The result is in agreement with those of Dehimi et al. (2021), concerning the presence of flavonoids in the aqueous and acetone extract of *B. pachypodum* tubers. However, it remains different regarding the absence of saponins and the presence of tannins in their extracts.

Flavonoids represent the large class of polyphenolic compounds present in plants that have beneficial effects on health. They are known to have pharmacological activity, particularly anti-allergic, anti-inflammatory, anti-viral or anti-carcinogenic effects (Huck et al., 2002). The plant extracts revealed to contain saponins as well, known to produce inhibitory effect on inflammation (Just et al., 1998) and are major ingredients in traditional Chinese medicine which are responsible for most of the observed biological effects (Liu & Henkel, 2002).

In addition, a good percentage of reducing sugars was discovered, which justifies the use of *B. pachypodum* in food in the past years in Algeria.

### *Total phenol and flavonoid contents in* B. pachypodum

The amounts of total phenols and flavonoids in *B. pachypodum* tubers are reported in Table 3. Aqueous extract showed the highest amounts of total phenols and flavonoids, followed by ethanol extract and acetone extract. However, statistically no significative difference (P> 0.05) was observed between the extracts about flavonoids contents. In contrast, aqueous extract is significantly higher (*P < 0.05) than that of the ethanol extract.

The obtained total phenol and flavonoids contents in our study were lower than that reported by

| Extract | Yield (%) |
|---|---|
| Aq | 6.65 |
| Eth | 11.5 |
| Ace | 6.9 |

Table 1. Extraction yield of *B. pachypodum* extracts. Aq: Aqueous, Et: Ethanol-water, Ac: Acetone-water;

| Phytochemical compounds | Aq | Eth | Ace |
|---|---|---|---|
| Saponins | +++ | ++ | ++ |
| Triterpens | − | − | − |
| Sterols | − | +++ | − |
| Tanins | − | − | − |
| Flavonoids | +++ | +++ | +++ |
| Alkaloids | − | − | − |
| Anthraquinones | − | − | + |

Table 2. Phytochemical screening of crude extracts of *B. pachypodum*. Aq: Aqueous, Et: Ethanol-water, Ac: Acetone-water; +++: appreciable amount (positive within 5 mins); ++: moderate amount (positive after 5 mins); +: trace amount (positive after 10 min); −: completely absent.

| Extracts | Phenolic contents (mgGAE/gDM) | Flavonoid contents (mg CAE/gDM) |
|---|---|---|
| Aq | 2.30 ±0.07 | 1.15 ±0.15 |
| Eth | 2.09±0.24 | 0.70±0.09 |
| Ace | 1.49±0.05 | 0.65±0.08 |

Table 3. Phenolic and flavonoid contents in *B. pachypodum* tubers extracts. Aq: Aqueous, Et: Ethanol-water, Ac: Acetone-water, GAE: gallic acid equivalents, CAE: cathechin equivalents, DM: dry matter. Results are expressed as mean of three experiments ± SD. Mean values are not statistically significant by student's test (P > 0.05).

Dehimi et al. (2021). Furthermore, these authors showed high total phenols and flavonoids contents in acetone extract followed by methanol extract.

Such differences of quantifications with our study may be due to the effect of several factors such as origin, climatic conditions (Amaral et al., 2010), as well as many parameters related to the extraction method as temperature, time contact, solvent type, etc.) (Pinelo et al., 2005).

### *Antioxidant activity assays*

For evaluating the antioxidant capacities of the crude extracts of *B. pachypodum*, two complementary test systems, DPPH and the reducing power, were applied. According to Nuutila et al. (2003), two or more methods should always be used to evaluate the antioxidant activities of plant extracts. As displayed in Fig. 1, the antiradical activity values for investigated extracts and ascorbic acid increases with the increase in the concentrations and exceeded 60% for acetone and ethanol extracts and 90% for ascorbic acid.

The three extracts are statistically similar ($P > 0.05$) but significantly lower ($^*P < 0.05$) than that of ascorbique acid at 1.25 mg/ml (Fig. 2). It appears from these results that DPPH radical scavenging by extracts is classified according to the following order: Acetone > Aqueous > Ethanol. This difference of antioxidant potential is strongly dependent on the extraction solvent.

The IC50 calculated are reported in the Table 2. Low IC50 associates to a strong inhibitory capacity of DPPH radical. The values showed that the IC50 of acetone extract is lower followed by aqueous and ethanol extracts. However, they remain superior than standard. This confirms the results presented in Fig. 5. Our results are different from those obtained by Dehimi et al. (2021) on tubers of *B. pachypodum*, who noted a low scavenger effect equal to 24.65 mg/ml and 21.18 mg/ml for the acetone and methanolic extracts respectively. According to their results, the aqueous extract recorded no activity.

| | Extract | IC 50 (mg/ml) |
|---|---|---|
| *B. pachypodum* | Eth | 2.52 ± 0.52 |
| | Aq | 1.50 ± 0.32 |
| | Ace | 0.46 ± 0.09 |
| *Standard* | Asc ac | 0.0008 ± 0.0001 |

Table 4. IC50 of the of the DPPH radical scavenging of *B. pachypodum* and standard Aq: Aqueous, Et: Ethanol-water, Ac: Acetone-water, Asc ac.: ascorbique acid.

The reduction of DPPH radical by the plant extracts has been attributed by several authors, at the presence of phenolic compounds which yield easily to reduce protons (Li et al., 2009). However, we noticed that the acetone extract that showed the best DPPH radical scavenging activity had lower amounts of phenols. According to Kwee & Niemeyer (2011), the antioxidant activity of extracts is approximately connected to their phenolic composition and strongly depends upon their phenolic structures.

Since the antioxidant activity of a substance is usually correlated to its reducing capacity, the FRAP assay provides a reliable method to study the antioxidant activity of various compounds (Kwee & Niemeyer, 2011). As shown in Fig. 3, the reducing power of the extracts and of ascorbic acid increases with the increase of the concentration. Indeed, an increase in absorbance means an increase in the reducing power of the tested extracts (Öztürk et al., 2007).

The analysis of the reducing power of aqueous, ethanol and acetone extract to the concentration 2.5 mg/ml resulted in absorbance between 0.50 and 0.80 (Fig. 4). As can be observed, there is no significant ($P > 0.05$) difference between the three extracts. According to the polarity of each extract, they were classified as following: Aqueous> Ethanol>Acetone. However, Dehimi et al. (2021) have shown that the high reducing power have been reported for acetone extract and is related to his phenolic compounds.

### In vitro *anti-inflammatory activity*

Inflammation is a complex process, which involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration (Grabowski and Tortora, 2000). The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may as well stabilize lysosomal membranes; by limiting the inflammatory response.

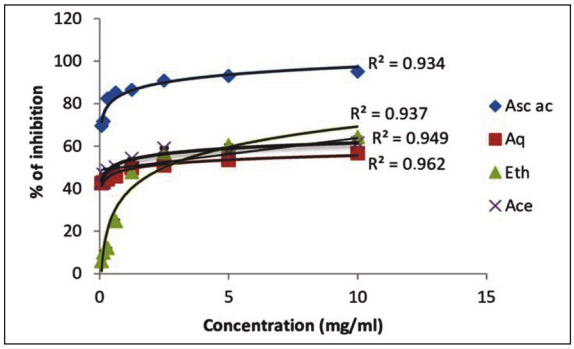


Figure 1. Antiradical (DPPH) activity of *B. pachypodum* extracts.

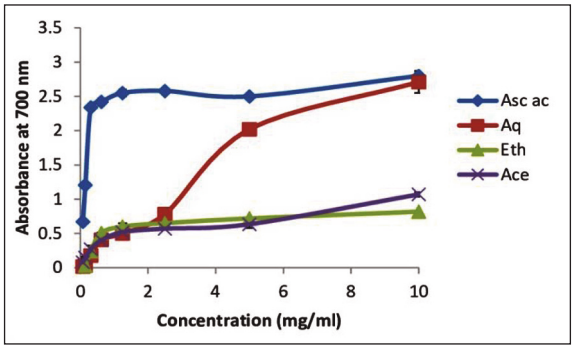


Figure 3. Antioxidant Activity Using the Ferric Reducing (FRAP) of *B. pachypodum* extracts.

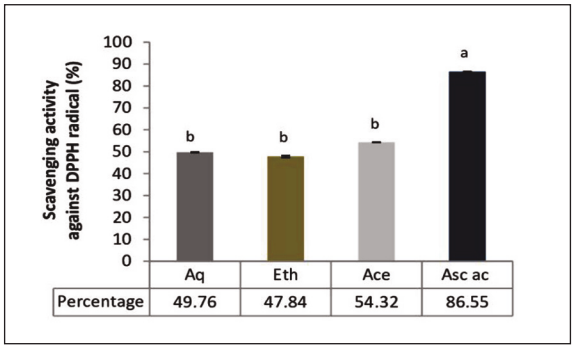


Figure 2. Means antioxidant activity against DPPH radical of aqueous, ethanol, acetone extracts and standard at 1.25 mg/ml. Values bearing the same letter showed no significant difference (P > 0.05). The results are sorted in decreasing order: a > b (*P < 0.05)

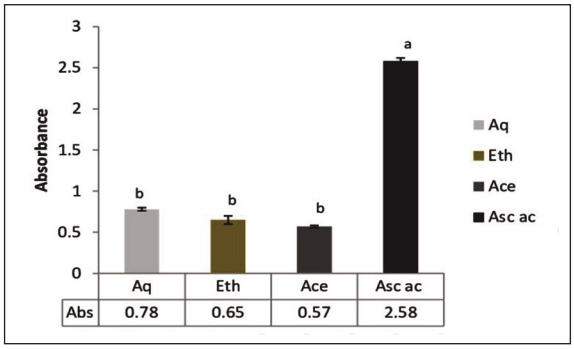


Figure 4. Reducing power of aqueous, ethanol, acetone extracts and standard at 2.5 mg/ml. Values bearing the same letter showed no significant difference (P > 0.05). The results are sorted in decreasing order: a > b (*P < 0.05).

The results obtained by HRBC method (Table 3) show that the extracts were concentration dependent. With increasing concentration, the anti-inflammatory activity also increases. At concentrations ranging from 50 to 1000 µg/ml, the three extracts showed anti-hemolytic activity, with percentages of protection up to 54%. Those of standard salicylic acid reached 64%.

Results of Anova analysis (Fig. 5) indicated that aqueous and acetone extracts are statistically similar (P > 0.05) than standard but significantly superior (*P< 0.05) than that of ethanol extract.

In questioning the literature on the anti-inflammatory effect of the *B. pachypodum* tubers, we found only the study of El Kolli et al. (2017), performed on the hydro-methanolic extract of *B. pachypodum* from the Eastern region of Algeria (Setif), who showed the maximum inhibition ≈ 90 % at 2000 µg/ml. According some authors, the activities difference of plants may be due to the geographical origin, locality, climate conditions, and the harvest time of the collected plant material (Oke et al., 2009, Hossain & Nagooru, 2011).

Denaturation of tissue proteins is another cause of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo* (Kar et al., 2012). So, several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation (Rahman et al., 2015).

According to our results (table 4), salicylic acid showed a maximum inhibition between 60 and 80% observed in the different concentrations (100, 300, 500 µg/ml). The aqueous extract inhibits albumin denaturation in a range of 50–70%; while for the ethanolic extract, the inhibition rate is between 50–60%, and for the acetone extract between 30 and 40%.

At the concentration of 500µg/ml, the most potent inhibitory effect was obtained with the aqueous extract (70.19%). This inhibition is statistically similar (P>0.05) to that of the reference anti-inflammatory drug (salicylic acid) (Fig. 6). El Kolli et al. (2017) have reported that denaturation inhibitory percentage of methanolic extract of *B. pachypodum* exceeded 50%. Also, these authors revealed that the inhibition of protein denaturation is concentration dependent (El Kolli et al., 2017).

Many studies on the antioxidant and anti-inflammatory activities of plants showed that these activities are related to the chemical composition. According to the phytochemical screening performed on the studied extracts of *B. pachypodum*, we have reported the presence of a variety of phytochemical constituents like saponins, presents in aqueous extract, reducing sugars, flavonoids, and anthraquinones.

Some works have reported that plants extracts which contain saponins, are known to produce inhibitory effect on inflammation (Just et al., 1998, Cheeke et al., 2006).

The results are also related to the flavonoid content of the extracts, since several authors have shown the role of flavonoids in conferring antioxidant and anti-inflammatory activity (Xu et al., 2019), and have been known to inhibit protein kinase C and L-arginine/NO pathways (Meotti et al., 2006).

In addition, the aqueous extract which is rich in total phenols and flavonoids showed the best anti-oxidant activity by the FRAP method and

| Conc. (µg/ml) | Percentage of protection (%) | | | |
|---|---|---|---|---|
| | Aq | Eth | Ace | Sal Ac |
| 50 | 30.70 ± 1.57 | 5.26 ± 0.76 | 8.77 ± 1.92 | 43.86 ± 2.79 |
| 100 | 38.60 ± 0.60 | 7.02 ± 0.70 | 15.79 ± 2.55 | 59.65 ± 2.18 |
| 300 | 39.47 ± 0.92 | 29.82 ± 1.12 | 47.37 ± 2.54 | 60.53 ± 0.57 |
| 500 | 41.23 ± 2.87 | 32.48 ±0.67 | 49.12 ± 1.40 | 61.40 ± 0.71 |
| 1000 | 54.39 ± 2.50 | 47.37 ± 0.94 | 50.88 ± 1.43 | 64.91 ± 1.41 |

Table 5. Effect of extracts of *B. pachypodum* on HRBC membrane stabilization. Sal Ac: Salicylic Acid.

| Treatment | Concentration (µg/ml) | Percentage denaturation (%) | Percentage of inhibition (%) |
|---|---|---|---|
| Control | - | 100 | 0 |
| Aq | 100 | 41.51 ± 0.80 | 58.89 ± 0.42 |
| | 300 | 32.08 ± 1.41 | 67.92 ± 1.34 |
| | 500 | 29.81 ± 0.92 | 70.19 ± 1.42 |
| Eth | 100 | 47.17 ± 1.12 | 52.83 ± 1.47 |
| | 300 | 42.30 ± 2.01 | 57.70 ± 1.41 |
| | 500 | 38.46 ± 2.18 | 61.54 ± 2.23 |
| Ace | 100 | 63.46 ± 1.68 | 36.54 ±2.00 |
| | 300 | 61.53 ± 1.80 | 38.46 ± 1.89 |
| | 500 | 57.69 ± 2.54 | 42.31 ± 2.05 |
| Sal Ac | 100 | 30.77 ± 1.21 | 69.23 ± 1.44 |
| | 300 | 26.92 ± 0.57 | 73.08 ± 1.40 |
| | 500 | 19.23 ± 1.08 | 80.77 ± 2.37 |

Table 6. Effect of extracts of *B. pachypodum* in-vitro anti-inflammatory activity on human protein denaturation method.

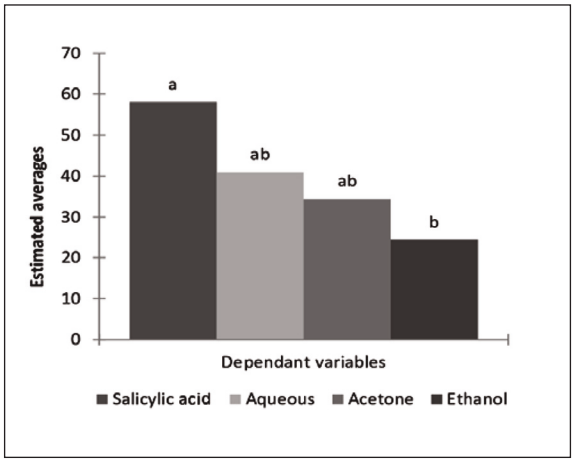


Figure 5. Synthesis (Estimated averages) - Concentration (µg/ml) by Anova test in HRBC membrane stabilization method. The results are sorted in decreasing order: a > ab> b (*P<0.05).

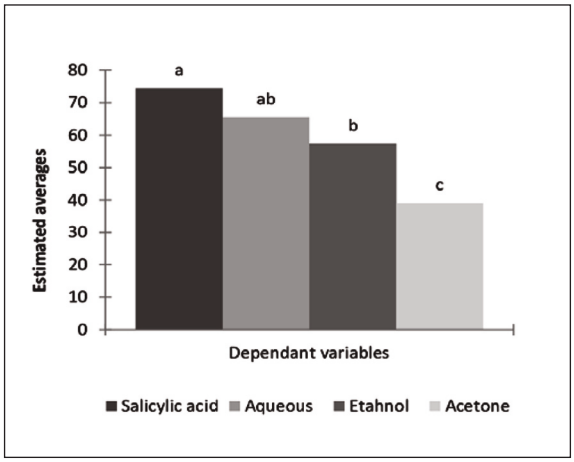


Figure 6. Synthesis (Estimated averages) - Concentration (µg/ml) by Anova test in protein denaturation method. The results are sorted in decreasing order: a > ab > b >c (*P < 0.05)

anti-inflammatory activity. Therefore, the anti-inflammatory activity of the extract is due to the phenolic compounds in it (Govindappa et al., 2011).

We made no characterize the phytochemical constituents in crude extracts in the present study. However, researchers have reported that the anti-inflammatory properties could be attributed to the presence of quercetin which is known to decrease inflammation NF-kβ, thereby reducing inflammation (Das et al., 2012). Moreover, it has been demonstrated that natural anthraquinones possess a broad range of bioactivities such as anticancer, anti-inflammatory, immunosuppressive, antimicrobial and antioxidant activities (Locatelli, 2011, Reynolds, 2004, Duval et al., 2016).

On the other hand, the tubers of *B. pachypodum* are rich in polysaccharides, Wang et al. (2011) indicated the pharmacological role of polysaccharides for prevention of inflammation and atherosclerosis. Also, work of Madhu et al., that tested the efficacy of *Curcuma longa* containing polysaccharides in osteoarthritis patients, indicates the involvement of polysaccharides against inflammation (Madhu et al., 2013).

It was found that the richest extract in phenolic compounds (aqueous extract) is the most active extract, having a significant anti-inflammatory potential. Indeed, plant phenolic extracts are generally mixtures of different classes of phenolic compounds which are soluble in the used solvent (Naczk & Shahidi, 2004).

## CONCLUSIONS

In conclusion, the present study demonstrated an important antioxidant and anti-inflammatory activity of tubers of *B. pachypodum*, mainly the aqueous extract, due to its bioactive components. It can provide scientific basis to justify it traditional use. It would be interesting to widen the panel of biological activities, especially the antifungal, antibacterial and antitumor activities, and to isolate, purify and identify the molecules responsible for these pharmacological properties, since it is a very little studied plant, especially in our region, despite its use by the population.

## REFERENCES

Abramson S.B. & Weaver A.L., 2005. Current state of therapy for pain and inflammation. Arthritis Research & Therapy, 7: 1–6.

Amaral J.S., Valentão P., Andrade P.B., Martins R.C. & Seabra R.M., 2010. Phenolic composition of hazelnut leaves: Influence of cultivar, geographical origin and ripening stage. Scientia horticulturae, 126: 306–313.

Bandeira S.D.M., Guedes G.D.S., Fonseca L.J.S.D., Pires A.S., Gelain D.P., Moreira J.C.F., Rabelo L.A., Vasconcelos S.M.L. & Goulart M.O.F., 2012. Characterization of blood oxidative stress in type 2 diabetes mellitus patients: increase in lipid peroxidation and SOD activity. Oxidative medicine and cellular longevity: 819310. https://doi.org/10.1155/2012/819310.

Bousetla A., Zellagui A., Derouiche K. & Rhouati S., 2015. Chemical constituents of the roots of Algerian *Bunium incrassatum* and evaluation of its antimicrobial activity. Arabian Journal of Chemistry, 8: 313–316.

Bruneton J., 1999. Pharmacognosie, phytochimie, plantes médicinales, 3ème éd. Lavoisier, Paris, 1120.

Cheeke P., Piacente S. & Oleszek W., 2006. Anti-inflammatory and anti-arthritic effects of *Yucca schidigera*: a review. Journal of Inflammation, 3: 1–7.

Chou C.T., 1997. The Antiinflammatory Effect of an Extract of *Tripterygium wilfordii* Hook F on Adjuvant-induced Paw Oedema in Rats and Inflammatory Mediators Release. Phytotherapy Research: An International Journal Devoted to Medical and Scientific Research on Plants and Plant Products, 11: 152–154.

Dai J. & Mumper R.J., 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 15: 7313–7352.

Das N., Sikder K., Ghosh S., Fromenty B. & Dey S., 2012. *Moringa oleifera* Lam. leaf extract prevents early liver injury and restores antioxidant status in mice fed with high-fat diet. Indian Journal of Experimental Biology, 50: 404–412.

Dewanto V., Wu X., Adom K.K. & Liu R.H., 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry, 50: 3010–3014.

Do Q.D., Angkawijaya A.E., Tran-Nguyen P.L., Huynh L.H., Soetaredjo F.E., Ismadji S. & Ju Y.-H., 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of Food and Drug Analysis, 22: 296–302.

Duval J., Pecher V., Poujol M. & Lesellier E., 2016. Research advances for the extraction, analysis and uses of anthraquinones: A review. Industrial Crops and Products, 94: 812–833.

El Kolli H., Laouer H. & El Kolli M., 2017. Chemical composition and biological activities of the essential oils and the methanolic extracts of *Bunium incrassatum* and *Bunium alpinum* from Algeria. Journal of the Chilean Chemical Society, 62: 3335-3341.

Ercan H., Birben E., Dizdar E.A., Keskin O., Karaaslan C., Soyer O.U., Dut R., Sackesen C., Besler T. & Kalayci O., 2006. Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma. Journal of Allergy and Clinical Immunology, 118: 1097–1104.

Favier A., 1997. The oxidative stress: Interest of its monitoring in clinical chemistry and problems of the choice of an appropriate parameter. Annales de Biologie Clinique, 55: 9–16.

Favier A., 2003. Le stress oxydant. L'actualité chimique, 108: 863–832.

Gandhidasan R., Thamaraichelvan A. & Baburaj S., 1991. Anti inflammatory action of Lannea coromandelica by HRBC membrane stabilization. Fitoterapia, 62: 81–83.

Govindappa M., Naga S.S., Poojashri, M. & Rappa C., 2011. Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. Journal of Pharmacognosy and Phytotherapy, 3: 43–51.

Grabowski S.R. & Tortora G.J., 2000. Principles of anatomy and physiology. Wiley New York/Chichester, 1288 pp.

Gurib-Fakim A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine, 27: 1–93.

Hossain M.A. & Nagooru M.R., 2011. Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Corydyline terminalis* L. Kunth. Pharmacognosy Journal, 3: 25–30.

Huck C., Buchmeiser M. & Bonn G., 2002. Fast analysis of flavonoids in plant extracts by liquid chromatography–ultraviolet absorbance detection on poly (carboxylic acid)-coated silica and electrospray ionization tandem mass spectrometric detection. Journal of Chromatography A, 943: 33–38.

Jokić S., Velić D., Bilić M., Bucić-Kojić A., Planinić M. & Tomas S., 2010. Modelling of solid-liquid extraction process of total polyphenols from soybeans. Czech Journal of Food Sciences, 28: 206–212.

Just M.J., Recio M.C., Giner R.M., Cuéllar M.J., Màñez S., Bilia A.R. & Rìos J.-L., 1998. Anti-inflammatory activity of unusual lupane saponins from *Bupleurum fruticescens*. Planta medica, 64: 404–407.

Kar B., Kumar R.S., Karmakar I., Dola N., Bala A., Mazumder U.K. & Hadar P.K., 2012. Antioxidant and in vitro anti-inflammatory activities of *Mimusops elengi* leaves. Asian Pacific Journal of Tropical Biomedicine, 2: S976–S980. https://doi.org/10.1016/S2221-1691(12)60346-3

Karagözler A.A., Erdağ, B., Emek Y.Ç. & Uygun D.A., 2008. Antioxidant activity and proline content of leaf extracts from *Dorystoechas hastata*. Food Chemistry, 111: 400–407.

King M.R., Ismail A.S., Davis L.S. & Karp D.R., 2006. Oxidative stress promotes polarization of human T cell differentiation toward a T helper 2 phenotype. The Journal of Immunology, 176: 2765–2772.

Kwee E.M. & Niemeyer E.D., 2011. Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars. Food Chemistry, 128: 1044–1050.

Li H., Wang X., Li Y., Li P. & Wang H., 2009. Polyphe-

nolic compounds and antioxidant properties of selected China wines. Food chemistry, 112: 454–460.

Liu J. & Henkel T., 2002. Traditional Chinese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities? Current Medicinal Chemistry, 9: 1483–1485.

Locatelli M., 2011. Anthraquinones: Analytical techniques as a novel tool to investigate on the triggering of biological targets. Current Drug Targets, 12: 366–380.

Madhu K., Chanda K. & Saji M., 2013. Safety and efficacy of Curcuma longa extract in the treatment of painful knee osteoarthritis: a randomized placebo-controlled trial. Inflammopharmacology, 21: 129–136.

Manach C., Scalbert A., Morand C., Rémésy C. & Jiménéz L., 2004. Polyphenols: food sources and bioavailability. The American journal of clinical nutrition, 79: 727–747.

Meagher E. & Rader D.J., 2001. Antioxidant therapy and atherosclerosis: animal and human studies. Trends in cardiovascular medicine, 11: 162–165.

Meotti F.C., Luiz A.P., Pizzolatti M.G., Kassuya C.N.A., Calixto J.B. & Santos A.R., 2006. Analysis of the antinociceptive effect of the flavonoid myricitrin: evidence for a role of the L-arginine-nitric oxide and protein kinase C pathways. Journal of Pharmacology and Experimental Therapeutics, 316<. 789-796.

Mongelli E., Desmarchelier C., Coussio J. & Ciccia G., 1997. Biological studies of *Bolax gummifera*, a plant of the Falkland Islands used as a treatment of wounds. Journal of ethnopharmacology, 56: 117–121.

Moreno-Macias H. & Romieu I., 2014. Effects of antioxidant supplements and nutrients on patients with asthma and allergies. Journal of allergy and clinical immunology, 133: 1237–1244.

Mouhoubi-Tafinine Z., Ouchemoukh S. & Tamendjari A., 2016. Antioxydant activity of some algerian honey and propolis. Industrial Crops and Products, 88: 85–90.

Naczk M. & Shahidi F., 2004. Extraction and analysis of phenolics in food. Journal of chromatography A, 1054: 95–111.

Ohba H. & Amirouche R., 2003. Observation of the Flora of Tadmait and Tidikelt, Central Sahara, Algeria. Journal of Japanese Botany, 78: 104–111.

Oke F., Aslim B., Ozturk S. & Altundag S., 2009. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chemistry, 112: 874–879.

Öztürk, M., Aydoĝmus- Öztürk, F., Duru M.E. & Topcu G., 2007. Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): An edible medicinal plant. Food chemistry, 103: 623–630. https://doi.org/10.1016/j.foodchem.2006.09.005

Pan H., She X., Wu H., Ma J., Ren D. & Lu J., 2015. Long-term regulation of the local renin–angiotensin system in the myocardium of spontaneously hypertensive rats by feeding bioactive peptides derived from Spirulina platensis. Journal of agricultural and food chemistry, 63: 7765–7774.

Pinelo M., Rubilar M., Jerez M., Sineiro J. & Núñez M.J., 2005. Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. Journal of agricultural and food chemistry, 53: 2111–2117.

Quezel P. & Santa S., 1963. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Éditions du Centre National de la Recherche Scientifique, 2 vol., 1170 pp.

Raafat Y., Eman N., Omama El S., Nadia F. & Maha G., 2011. Evaluation of anti-oxidant status and radioprotective activity of a novel anti-cancer drug in mice. Journal of Cancer Therapy, 2: 1–13. https://doi.org/10.4236/jct.2011.25083.

Rahman H., Eswaraiah M.C. & Dutta A., 2015. In-vitro anti-inflammatory and anti-arthritic activity of *Oryza sativa* Var. *joha* rice (an aromatic indigenous rice of Assam). American-Eurasian Journal of Agricultural & Environmental Sciences, 15: 115–121. https://doi.org/10.5829/idosi.aejaes.2015.115.121

Rao V., 2012. Phytochemicals: a global perspective of their role in nutrition and health. BoD–Books on Demand, 550 pp.

Reynolds T., 2004. Aloes: the genus *Aloe*. CRC Press, Boca Raton, FL, 386 pp.

Rizk A., Am R., Al Nagdy S. & El Missiry M., 1982. Constituents of plant growing in Qatar. Fitoterapia, 52 : 35–42.

Salam M.T., Zhang G.Y. & Begum K., 2012. Epigenetics and childhood asthma: current evidence and future research directions. Epigenomics, 4 : 415–429.

Sánchez-Moreno C., Larrauri J.A. & Saura-Calixto F., 1998. A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture, 76: 270–276.

Sarr S.O., Fall A.D., Gueye R., Diop A., Diatta K., Diop N., Ndiaye B. & Diop Y.M., 2015. Etude de l'activité antioxydante des extraits des feuilles de *Vitex doniana* (Verbenacea). International Journal of Biological and Chemical Sciences, 9: 1263–1269.

Sequeira S., Rao A.V. & Rao A., 2012. Increased oxidative stress and altered antioxidants status in patients with chronic allergic rhinitis. Advances in Bioscience and Biotechnology, 3: 951–956. https://doi.org/10.4236/abb.2012.327117

Shahsavari N., Barzegar M., Sahari M.A. & Naghdibadi H., 2008. Antioxidant activity and chemical charac-

terization of essential oil of *Bunium persicum*. Plant foods for human nutrition, 63: 183–188.

Sies H., 1991. Oxidative stress: from basic research to clinical application. The American journal of medicine, 91: S31–S38.

Sofowora A., 1993. Recent trends in research into African medicinal plants. Journal of ethnopharmacology, 38: 197–208.

Taïbi K., Abderrahim L.A., Helal F. & Hadji K., 2021. Ethnopharmacological study of herbal remedies used for the management of thyroid disorders in Algeria. Saudi Pharmaceutical Journal, 29: 43–52. https://doi.org/10.1016/j.jsps.2020.12.004

Trease G. & Evans W., 1987. A text book of pharmacognosy. ELSB Baillere Tindal. Oxford, 811 pp.

Turkmen N., Sari F. & Velioglu Y.S., 2006. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. Food chemistry, 99: 835–841.

Wang L. & Weller C.L., 2006. Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology, 17: 300–312.

Xu Y.-B., Chen G.-L. & Guo M.-Q., 2019. Antioxidant and anti-inflammatory activities of the crude extracts of *Moringa oleifera* from Kenya and their correlations with flavonoids. Antioxidants, 8: 296. https://doi.org/10.3390/antiox8080296