A COMPARATIVE STUDY OF THE ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF THE PHENOLIC EXTRACTS FROM PALM POLLEN GROWING IN OUED SOUF (ALGERIA)

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Abstract. The present study is quantitative in nature where the researcher highlighted the importance of quality of effective active compounds (polyphenols) that are present in palm pollen, which, plays an effective role in the treatment of many diseases. In this study, we were able to identify these compounds by detection and extraction. We quantify the phenolic compounds using the device UV-Visible. The results revealed that the total amount of phenolic compounds were higher in the phenolic extract of the Oued El Alanda region estimated at (494.496 mgEAG/g), wheres total flavonoids were higher in the phenolic extract of the Hamraia region estimated at (229.59 mgER /g). In the next step, we quantified and qualitatively estimated using the HPLC device, found that the phenolic extracts contained most of the reference phenolic compounds in varying proportions. Then the antioxidant efficacy has also been tested for phenolic compounds by the cyclic voltammetry technique, The highest total antioxidant effect was estimated in the extract of the region Hamraia (6.92 mgEAG/g). Finally, we performed another test on the antibacterial efficacy of these extracts in three types of bacteria. However, the result was positive only in one type of bacteria, known as the bacteria *Listeria monocytogenes ATCC 19115*, where we recorded a very effective inhibitory potency with Oued El Alanda extract estimated at 18 mm a maximum concentration that is greater than the inhibitory diameters in industrial antibiotic (Amoxicillin, Ampicillin*).*

Keywords: Palm pollen; active compounds (polyphenols); biological efficacy.

INTRODUCTION

The palm (*Phoenix dactylifera* L.) is a dioecious, monocotyledon species belonging to the family Arecaceae, with male female and flowers occurring on separate plants [42].

Several palm products, such date fruits and palm juice, palm pollen have traditionally been used in folk medicine for the treatment of various health diseases and disorders, including memory disturbances, fever, inflammation, paralysis, and loss of consciousness [2].

The palm pollen is among the natural active compounds of medicinal plants, palm pollen is a fine powder material produced by male flowering date palm plants. Plants are pollinated through the transfer of pollen from the stamen of a flower to the stigma of another. Fresh pollen consists of water (5–36%) and solids (64–95%). It contains mineral salts, vitamins, sugars, lipids, growth factors, certain antibacterial activities and over 100 kinds of enzymes and co-factors [20], pollen and pollen products have a long history of use in traditional herbal medicine and have been reported to display a wide range of antimicrobial [5], anti-oxidative [14], anti-toxicant [27], and hepato-protective [17] activities.

Several previous studies on palm pollen showed the presence of estrone, a-amirin, triterpenoidal saponins, flavonoids and a crude gonadotrophic substance [32, 37]. More recently, palm pollen has been noted to contain estrone, estradiol and estriol, in addition to five flavonoid compounds [38], due to the renewed interest in bioactive plant-derived medicinal compounds, pollen-derived products have gained increasing popularity as dietary supplements in various parts of the world. A variety of pollen-based food products and formulations, such as candy and chocolate bars, are currently marketed and commercialized throughout the world. Free radicals have previously been reported to be implicated in the human pathogenesis of at least 50 diseases. Accordingly, there has been a growing interest in plant-based dietary components to counteract oxidative stress-induced disease since it is involved in various diseases and may exacerbate their symptoms [1].

Nowadays, much attention has been paid to health promotion related to the activity of photochemical, and increasing attention has been given to the isolation of novel bioactive compounds from medicinal plant as an effective strategy for the treatment of different diseases [18].

Despite this large flow of data on the promising effects of palm pollen, no studies have so far been performed to explore the antioxidant and antimicrobial prosperities of palm pollen in various extracts. Accordingly, the present study was undertaken to evaluate and compare, for the first time, the phenolic and flavonoid compounds as well as the antioxidant (DPPH) and antimicrobial activities.

In this work, we conducted a comparative study of the palm pollen for two areas of Oued Souf (Hamraia and Oued El-Alanda). This study was conducted to study the natural active compounds found in the pollen through the extraction of these active compounds naturals and the study of antioxidant and anti-bacterial activity [4, 11].

MATERIAL AND METHODS

Chemicals and reagents

Methanol, chloroform, n-butanol, petroleum ether and ethyl acetate were purchased from VWR Merk (France), folin-ciocalteu reagent, Gallic acid, Rutin, were procured from Sigma–Aldrich Inc (Paris, France). The reagents for the microbial activity were Nutrient agar and sabouraud dextrose agar.

Preparation of extracts

100g of the of palm pollen material were macerated three times in a hydro alcoholic mixture (methanol /water; 70/30; V/V) with renewed solvent every time for 24 hours. After filtration, the extract underwent successive liquid-liquid extractions using increasing polarity solvents starting with petroleum ether then ethyl acetate and finally methanol [11, 29].

HPLC Analysis

The Phenolic compounds have been separated and identified by liquid chromatography system high-performance reverse phase mark (SHIMADZU, Japan) equipped with a UV diode array detector (DAD) and a chromatographic column filled with a grafted silica gel, octadecyl type RP-HPLC- C18 (25cm x 46 mm). The detector (DAD) was adjusted to a scan from 200 to 400 nm, whereby the column temperature was maintained at 25 °C. The volume injected was 20 µL and the mobile phase used was made up of two solvents A and B: Solvent A (acetonitrile), Solvent B (acetic acid 0.2%). The separation method adopted was the gradient elution with a speed set at 1 mL/min.

Identification of phenols and flavonoids were performed by comparing the retention times with authentic compounds injected in the same chromatographic conditions [19].

Determination of total phenolic content (TPC)

Total phenolics content of each extract was determined using the Folin–Ciocalteu's reagent (FCR). Briefly, a dilute solution of Gallic acid in methanol (0.3-0.03 mg/mL) was mixed with 0.5 mL of folin-ciocalteu reagent, followed by 0.8 mL of Na_2CO_3 (7.5 %). The reaction mixture was incubated for 30 min in a dark room. The absorbance of the mixture obtained is directly measured by UV-visible spectrophotometer at 765 nm. The concentration of total phenolics in the extracts was expressed as mg of Gallic acid equivalent (GAE) per g of dry weight. The obtained correlation coefficient of the calibration curve was $R^2 = 0.999$. All results presented are means (±SEM) and were analyzed in three replications [28, 30, 36].

Total flavonoids content (TFC)

This method is based on the oxidation of the flavonoids by sodium nitrite solutions ($NaNO_2$, 5%) and aluminium chloride ($AlCl_3$, 10%) leading to the formation of a brownish complex having a maximum absorbance of 510 nm. The observed OD (optical density measures the intensity lost, when light passes through an optical component) was compared to that obtained by a known concentration of Rutin used as standard. In each of test tubes, we add 500 µL from Rutin solutions in methanol (between 0.3-0.03 mg/mL) at different concentrations. Then, we put successively 75 µL of a solution of $NaNO_2$ (5%). After 5 minutes we add 125 µL of $AlCl_3$ (10%) and after 6 minutes we add 500 µL of NaOH (1N) and 500 µL of distilled water. the reaction mixture was incubated for 30 min in a dark room. A calibration curve is prepared at different concentrations with standard solutions of Rutin. The absorbance of the mixture obtained is directly measured by UV-visible spectrophotometer at 420 nm and the results are expressed in mg Rutin equivalent /g of dry matter (RE /g DM). The data were analyzed in three separate experiments. The obtained correlation coefficient of the calibration curve was $R^2 = 0.899$. The obtained results are presented as mean (± SEM). The same technique was applied with the plant extracts [11, 15, 16, 24, 26].

Antioxidant activity (Total antioxidant assay by Cyclic voltammetry)

The measurement of antioxidant potential was carried out by determining the products resulting from the oxidation or by assessing the ability to trap radicals. The first mode requires prior knowledge of the compounds from oxidation. The second mode links a number of trapped radicals of used antioxidant. Evaluation of the ability of the compound (extract) to trap free radicals is therefore to measure its ability to scavenge free radicals and slow or inhibit the creation of free radicals. The method used to evaluate antioxidant activity in this article is cyclic voltammetry method [9, 28, 36].

Electrochemical measurements have advantages for the determination of antioxidant activity such as their use as a rapid proof of the antioxidant capacity of a lot of organics. The oxidation potentials measured by cyclic voltammetry, have been used to compare the antioxidant strength of compounds such as phenolic acids, flavonoids, etc. [3, 6, 25, 39]. Cyclic voltammetry has been successfully applied to analyze antioxidants present in plant extracts, phenolic standards [34].

Antimicrobial activity assays

Microorganisms

In total three bacterial cells were used in this study which was composed of one gram-positive bacteria cell and two gram-negative bacteria cells. The bacterial cells assayed included one gram-positive that was *Listeria monocytogenes ATCC 19115*. The gram-negative two bacterial strains were used; *Escherichia coli ATCC 25922, Pseudomonas aerigunosa ATCC 27853*. All strains were obtained from the Laboratory of Microbiology Hospital Djilali Ben Amer EL OUED 39000, Algeria.

Incubation conditions

Nutrient agar was used culture medium for bacteria which was incubated for 24 h at the temperature of 37 °C and yeasts were cultured in sabouraud dextrose agar (SDA; 4% dextrose, 2% neopeptone and 1.7% agar) for 24-48 hours at the temperature of 30°C [10, 41].

Disc diffusion assay

Butanol extracts of palm pollen were dissolved in methanol-water 50% for a final concentrations (10, 1, 0.1 mg/mL). The antimicrobial activity was estimated by the method of disc diffusion, 100 µl of suspension for each microorganism 108 colony-forming units (CFU)/mL containing 20 mL of nutrient agar for bacteria, after was placed in the Petri sterilized filter paper disc (7 mm in diameter).

We saturated the disks with different concentrations of phenolic extracts inside Petri dish with reference disk saturated with methanol 50%. We let it in an inverted position in the incubator under the temperature 37°C for 24 hours. This operation replicated with an industrial antibiotic. The diameter of the inhibition zone around each disc was measured for three replicates. Different bacterial strains are treated in a diffusion method.

We obtain extracts of phenolic solutions in three concentrations from each extract, then saturate the tablets with the extract in a petri dish. The antibacterial activity of phenolic extracts has been studied on the following bacterial species; (*Listeria monocytogenes ATCC 19115*, *Pseudomonas aeruginosa ATCC 27853*, *Escherichia coli ATCC 25922*). After incubation for 24 hours, we measure the diameters of the inhibitors of the extracts.

Statistical analysis

Data were analyzed using statistical tests whereby the obtained results were presented in mean values, and standard deviations (SD). All measurements were repeated for each trial three times. Statistical calculations were carried out by MS Excel 2007 software, correlations were obtained by Pearson correlation coefficient using bivariate correlations test. P value was set at 0.05. Therefore, the obtained value less P value (0.05) was regarded as a statistically significant and P values < 0.01 was regarded extremely statistically significant.

RESULTS

Extract yield

The methanol is one of the best solvents for phenolic compounds compared to other solvents and recently used in several studies. Mass yield obtained by phenolic extract for each palm pollen, are mentioned in Table 1.

Identification by HPLC

The identification of compounds phenolic extracts the majority of phenolic extract for palm pollen in the region Hamraya and Oued Alanda, HPLC were carried out on the basis of the comparison of their retention times with those obtained for the same standard compounds.

As show in Figure 1 and Table 2, polyphenols and Flavonoids rates are determined in plant extracts according to the calibration curve (peak areas as a function of the concentration of the standards).

Meaning of symbols:

- **PPH_{ph}:** phenolic extract for palm pollen in Hamraya region;
- **PPA_{ph}:** phenolic extract for palm pollen in Oued Alanda region.

1- High performance liquid chromatography was used for the quantification of the phenols in the extract **PPH_{ph}** and the Figure 2 shows the result from the studied.

- Through the resulting color drawing, we can know the types of phenolic compounds present in the palm pollen grains of the Hamraya region. The results are shown in the Table 3.

- Through the above Table 3, (7) phenolic compounds were identified in the phenolic extract of the sample PPH_{ph}. This is done by comparing their retention time with the retention time of the reference phenolic compounds.

Table 1. Mass yield obtained by phenolic extract for palm pollen

Phenolic extract palm pollen	Dry weight extract g / 100 g of plants powder
in the region Hamraya	14.35 ± 0.07
in the region Oued El Alanda	17.47± 0.17

Note: results are expressed as the mean and ±standard deviation of three independent experiments.

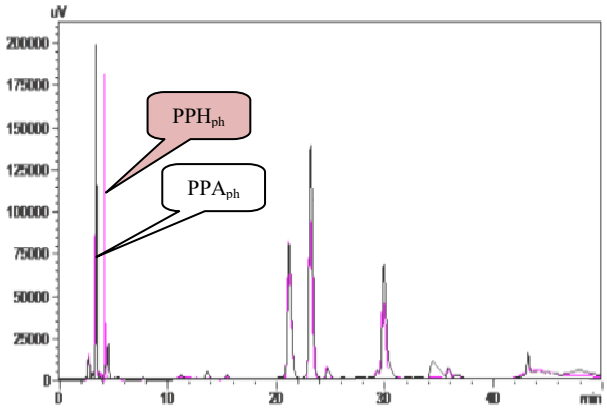

Figure 1. Chromatographic profile of the extract Recorded in UV at 254 nm

Table 2. Quantification of phenolic and flavonoids compounds identified in a Methanoic extract from palm pollen

Phenolic compounds	$t_{r(R)}$ (min)	PPH_{ph} (mg/g)	PPA_{ph} (mg/g)
Gallic acid	5.29	ND	1.80
Chlorogenic acid	13.392	0.075	0.932
Vanillic acid	15.531	1.12	0.187
Caffeic acid	16.277	ND	0.049
Vanillin	21.46	**6.92**	**3.35**
p-Coumaric acid	23.817	**8.90**	**13.35**
Rutin	28.37	0.77	0.157
Naringenin	34.788	0.53	**6.085**
Quercetin	45.047	**4.51**	0.32

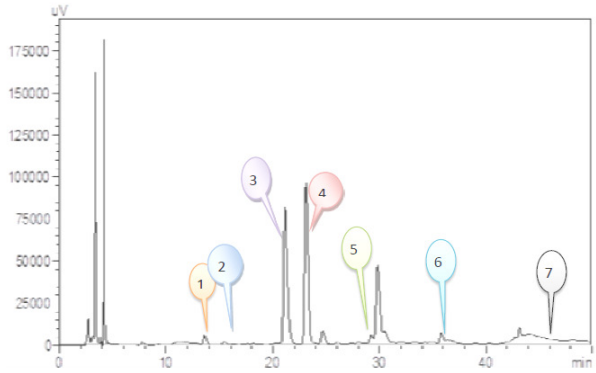

Figure 2. Chromatographic profile of the extract **PPH_{ph}** Recorded in UV at 254 nm

Table 3. Quantification of phenolic and flavonoids compounds **PPH_{ph}** identified in a Methanoic extract from palm pollen of the Hamraya region

Peak number	Phenolic compounds	$t_{r(R)}$ (min)	PPH_{ph} (mg/g)
1	Chlorogenic acid	13.392	0.075
2	Vanillic acid	15.531	1.12
3	Vanillin	21.46	**6.92**
4	p-Coumaric acid	23.817	**8.90**
5	Rutin	28.37	0.77
6	Naringenin	34.788	0.53
7	Quercetin	45.047	**4.51**

2- High performance liquid chromatography was used for the quantification of the phenols in the extract **PPA_{ph}** and the Figure 3 shows the result from the studied .

- Through the resulting color drawing, we can know the types of phenolic compounds present in the palm pollen grains of the Oued Alanda region. The results are shown in the Table 4.

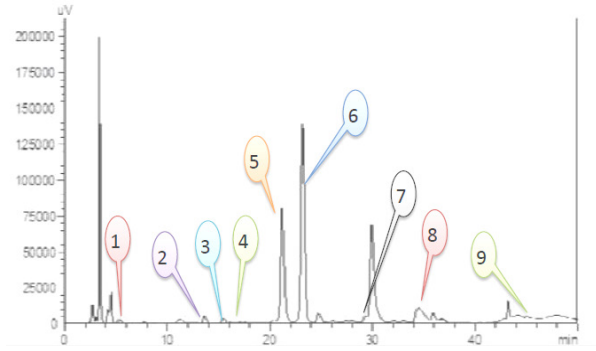

Figure 3. Chromatographic profile of the extract **PPA_{ph}** Recorded in UV at 254 nm

Table 4. Quantification of phenolic and flavonoids compounds **PPA_{ph}** identified in a Methanoic extract from palm pollen of the Oued Alanda region

Peak number	Phenolic compounds	$t_{r(R)}$ (min)	PPA_{ph} (mg/g)
1	Gallic acid	5.29	1.80
2	Chlorogenic acid	13.392	0.932
3	Vanillic acid	15.531	0.187
4	Caffeic acid	16.277	0.049
5	Vanillin	21.46	**3.35**
6	p-Coumaric acid	23.817	**13.35**
7	Rutin	28.37	0.157
8	Naringenin	34.788	**6.085**
9	Quercetin	45.047	0.32

- We note from Table 4 that the sample **PPA_{ph}** contains many phenolic compounds, as (9) phenolic compounds were identified by comparing their retention time with the retention time of the reference phenolic compounds.

Total phenolic and flavonoids

The results of the quantitative analyses of polyphenols and flavonoids in the extracts of PPH_{ph} and PPA_{ph} , are reported in Table 5.

Table 5. Total Phenolic and flavonoids content of PPH_{ph} and PPA_{ph}

Methanoic extract from palm pollen	TPC (mg GAE/g)	TFC (mg ER/g)
PPH_{ph}	229.59	46.90
PPA_{ph}	494.496	33.11

Estimation of antioxidant activity of the palm pollen extracts by Cyclic voltammetry

- The standard curve for gallic acid

A known concentration of gallic acid was prepared, 1mg/mL, and with this concentration we were prepared Other different concentrations of gallic acid (900 µg / mL - 400 µg/mL) and we also prepare the electrochemical cell, then we plot the cyclic voltmeter curves according to the increasing concentrations of gallic acid in the cell, and the following curves were obtained as follows: Shown in the Figure 4.

- After drawing the toroidal voltmeter curves of the concentrations, we find the current density at the peak of the rising wave for each curve, until we obtain a standard curve for gallic acid representing the current density electric in terms of concentration I = F (C) as shown in the following Figure 5.

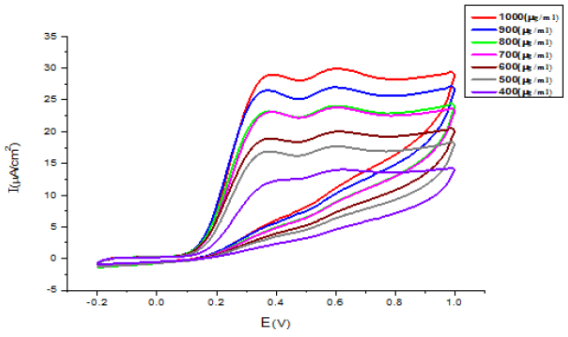

Figure 4. Volt-metric toroidal curves of gallic acid

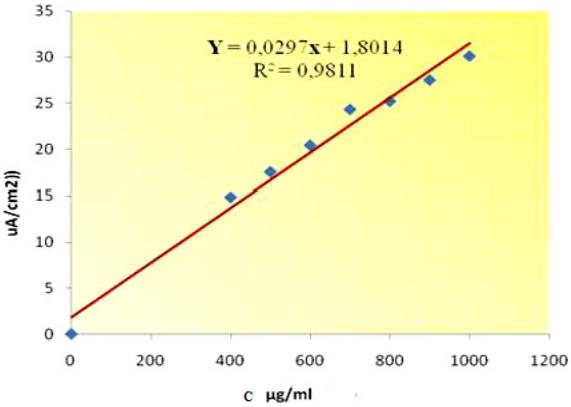

Figure 5. Standard curve for gallic acid according to the cyclic voltmeter method

- Through the following curves show in Figure 6. We project the peaks of the curves for each extract phenolic to the y-axis. We find the value of the current intensity, and through the relationship of the current density in terms of concentration of the standard compound of gallic acid.

I = 0.0297C + 1.8014. In the same way prepared for gallic acid, we plot the annular voltage curve of the studied samples with the known concentration. From Standard curve for gallic acid Figure 5 we deduce the intensity of the anode current to obtain the equivalent concentration of gallic acid in the following shown in the Table 6.

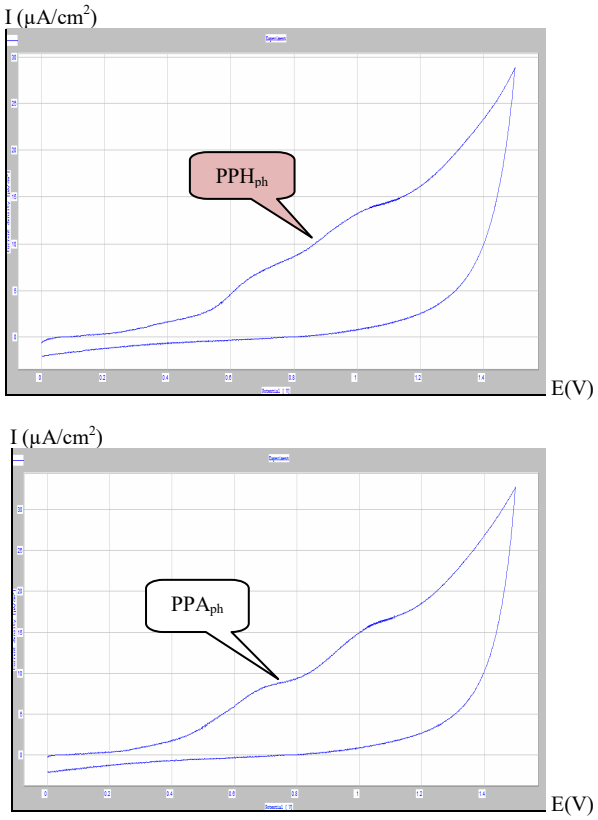

Figure 6. Cyclic voltammetry of two extracts of palm pollen (pH = 7)

Table 6. Total antioxidant capacity (TAC) of the palm pollen extracts

–	I (µA/cm²)	C (µg/mL)	TAC (mg EAG /g)
PPA_{ph}	7.522	192.61	5.77
PPH_{ph}	8.648	230.52	6.92

Table 7. Diameters bacteria's inhibition resulting from different concentrations of antibiotics and extracts

Microorganisms Bacteria	Diameter of zone inhibition (mm)			
	PPH_{ph}	PPA_{ph}	Amo	Amp
Listeria monocytogenes ATCC 19115	17	18	14	12
Escherichia coli ATCC 25922	9	7	10	12
Pseudomonas aeruginosa ATCC 27853	ND	ND	ND	19

Note: ND – no detected; Amo – Amoxicillin; Amp – Ampicillin.

Antimicrobial activity

After incubation for 24 hours, we measure the diameters of the inhibitors of the extracts and antibacterial activity of extracts and antibiotics on The three bacterial strains , resulting from different concentrations of antibiotics and extracts are shown in Table 7.

DISCUSSION

Extract yield

From Table 1, which shows the extraction yield (g/100 g dry weight), the mass yield obtained phenolic extract for palm pollen in the Hamraya region is 14.35 % and phenolic extract for palm pollen in the Oued El Alanda region is 17.47 %. Where we can suggest that the reason for this difference in yield percentages is due to the difference in soil quality from one region to another one, which affected the yield of the product.

HPLC analysis

From Figure 1 and Table 2, the phenolic compounds for extract palm pollen in the regions of Hamraya and Oued Alanda by HPLC confirm the presence in PPH_{ph} of majority Vanillin with a retention time 21.46 min, and *p*- Coumaric acid with a retention time 23.817 min, and Quercetin with a retention time 45.047 min, with respectively 6.92 mg/g of extract and 8.9 mg/g and 4.51 mg/g, and confirm the presence in PPA_{ph} of majority Vanillin, and *p*-Coumaric acid, and Naringenin with a retention time 34.788 min, with rates respectively 3.35 mg/g of extract and 13.35 mg/g and 6.085 mg/g.

This diversity can be attributed to many biological factors, including genetic and agricultural differences, as well as other environmental factors, such as stages of ripeness, salinity, temperature, water pressure and conditions of light intensity. HPLC analysis provides insight into phenolic compounds in palm pollen and change of phenol and flavonoids.

The results presented in this study are similar to previous studies [14], and are almost similar in terms of the type of phenolic compounds that make up them, and the difference is only relatively in quantities.

This result can be explained by the physiological role of every compound during the different stages of growth, for gallic acid was been helped to adaptation the plans with the climatic conditions [40]. The Caffeic acid has been accelerated to aging of plants The vanillin does accelerate the maturity of fruits by modifying the taste and flavor of the fruit [35] and it has the role of anti-abiotic stress. As for ρ-Coumaric acid has a role in the reduction of the vegetative growth of the plants and it is a good stimulated for antioxidant activity [13, 35].

Total phenolic and flavonoids

In Table 5, The technique methods of estimation of total phenolic. The result regarding the first PPH_{ph}. The total phenolic content of different extraction techniques

ranges from 229.59 mg EAG /g, the content of flavonoids in Rutin equivalent varies from 46.90 mg ER/g. Regarding to the second PPA_{ph}, with same method the results range from 494.496 mg EAG /g, the content of flavonoids in Rutin equivalent varies from 33.11 mg ER/g Ms.

From the results of PPAph extracts generally exhibited higher polyphenolic contents than the PPHph extracts, this difference is due to the geographical variations, and climatic changes. Overall, the findings indicated that both PPAph and PPHph are rich in phenolic and flavonoid contents, which could be the major contributor to their anti-oxidative properties. These result suggest that both varieties of palm pollen offer promising sources of beneficial bioactive compounds for human health and nutrition.

These results were much better than some researchers have found in this field [14, 20-22, 29] this difference is due to the harsh desert nature in which it grows in the well-known Oued Souf region, which is among the hottest regions in the world.

Overall, the results indicate that PPAph can be considered a promising source of new natural antioxidants and antimicrobial agents for use in various products, pharmaceuticals, and medicines.

Total antioxidant assay by Cyclic voltammetry

The amount of antioxidant compounds was estimated by the cyclic voltmetery method using the gallic acid curve in Figure 6 and Table 6, as the amount reached 5.77mg EAG/g of the extract PPAph it is considered a large amount of antioxidants, and the amount reached by extraction from PPHph 6.92 mg EAG /g, is a very acceptable amount of antioxidants. This study by cyclic voltmetery, it is considered a new and modern study.

Some researchers have used this method to evaluate antioxidants on different plant species [8, 12, 33], but no one has previously used them to evaluate antioxidants in a palm pollen.

Antimicrobial activities

In Table 7, we note that the phenolic extract gave the best inhibitory diameter against the bacteria *Listeria monocytogenes ATCC 19115*.

The highest inhibition value was recorded by the phenolic extract of the Oued El Alanda region of 18 mm which exceeded the estimated inhibition diameter of antibiotics.

From the study of antibacterial activity of extracts and antibiotics on The three bacterial strains showed that the ability of the extracts to inhibit *Listeria monocytogenes ATCC 19115* bacteria was better than antibiotics.

Whereas against *Escherichia coli ATCC25922* bacteria, the highest inhibition value was recorded by the 9 mm diameter phenolic extract in the Hamraya region which did not exceed the antibiotic inhibition diameter.

While against *Pseudomonas aeruginosa ATCC 27853* bacteria, we did not record any inhibiting diameter with phenolic extract.

Grampositive bacteria should be more susceptible because they only contain an external peptidoglycan, which is not an effective permeable barrier and may facilitate the penetration of hydrophobic compounds [7]. According to other researchers [23], palm pollen from various extracts exhibited antimicrobial activity against several strains, with *Listeria monocytogenes ATCC 19115*, this was also found by Tunisian research [14].

The low to moderate antimicrobial activity observed in other extracts can be explained by a weaker concentration than the resulting simple polar compounds such as phenolic compounds, which have been reported to have good antimicrobial activity [31].

To the authors'knowledge, this study is the first to report on the antioxidant new method by Cyclic voltammetry analysis of palm pollen extracts from different Algerian cultivars (PPH and PPA). The findings clearly indicated that all the tested extracts exhibited significant phenolic and flavonoid contents and displayed good antioxidant activities.

The antioxidant efficacy of phenolic extract for the region of El-Hamraia (PPH_{ph}) was better than El-Alanda region (PPH_{ph}) where it was estimated at (6.92 mg EAG / g) it can be argued that the high of flavonoids in the phenolic extract of palm pollen of region of Hamraia is responsible for this activity.

The result of the study of the effectiveness of anti-bacteria, where we recorded a very effective inhibitory ability at the $\mathbf{PPA_{ph}}$ extract for the Oued El Alanda region estimated at 18 mm at the largest concentration agains the bacteria *Listeria monocytogenes ATCC 19115*, even larger than the impact of industrial antibiotics (Amoxiciliin Ampicillin), and this because it contains a larger amount of polyphenols.

The results of this study offer a scientific basis that can be further enriched so as to support the efficacy traditionally associated with palm pollen extracts.

This extract might open promising opportunities for the development of more efficient, safe, and cost-effective food preservatives against food deterioration, antioxidant agents.

We recommend that future studies on Palm pollen extract should focus on the examination of the *in vivo* antioxidant activity.

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Received: December 25, 2019
Accepted: October 10, 2020
Published Online: October 14, 2020
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House