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Impact of geographic variation on the chemical composition and antioxidant activity of Algerian propolis

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Abstract:

The main purpose of this research is to assess the antioxidant properties of methanolic extracts of propolis from the provinces of Tipaza (RT), Tebessa (RTb), El-Oued (RE), and Constantine (RC) in Algeria, and the values will be associated with total amounts of polyphenolic compounds. In this study, Spectrophotometry was used to calculate the total phenolic and flavonoid contents of a methanolic extract of propolis. Further, the antioxidant properties of the extracts were calculated using the reducing power (FRAP and RP) and radical scavenging (DPPH assays) and determined antioxidant total by (phosphomolybdate assay). As a result, the total polyphenolic content was 384.7± 18.0, 353.2±13.9, 105.17±2.77, and 42.12±1.42 mg gallic acid equivalent/g extract in methanolic extracts of propolis from RT, RTb, RC, and RE, respectively. RT, RTb, RC and RE propolis had IC50 values of 0.38488, 0.4340, 1.2807 and 0.8645 mg/mL, respectively, for scavenging DPPH radicals. Finally, our findings support the use of a natural source of antioxidant compounds that may be used in the prevention of free radical-related diseases. Also, our findings indicate that geographic origin matters, with sunshine hours and temperature being the most important determinants of Propolis phenolic accumulation and antioxidant properties. Tipaza propolis, on the other hand, had the highest antioxidant activity values.

Keywords: Algerian propolis, Polyphenol, Flavonoid, Geographic Origin, Antioxidant.

1. Introduction

Propolis is a natural material made up of over 160 components (Papotti et al., 2012) that bees use to fill holes in their hive (Bankova et al.,2000) . It has been used for a variety of purposes throughout history, most notably as medicine. Propolis is increasingly popular as a health drink, and it is widely used in food to improve health and prevent disease (Groot,2013).

Several studies have documented a wide range of biological activities, including anticancer (Szliszka et al.,2009) . Antineoplasic (Silva et al.,2019) , antioxidative (Kumazawa et al., 2004; Lima et al., 2009; Silva et al., 2011) , antimicrobial (Koo et al.,2000) , anti-inflammatory (Hori et al., 2013 ; Paulino et al.; 2003; Paulino et al., 2008), antiviral (Amoros et al., 1994) , as antibiotic, antifungal (Majiene et al., 2007) . These activities are related to the phenolic constituents, especially phenolic acids and flavonoids (de Mendonca et al., 2007; Kumazawa et al., 2004) . Food flavonoid content may be a significant dietary factor causing this effect (Amoros et al., 1994; Choi et al., 2006) .

For this reason, for the past 30 years, propolis has been the focus of extensive pharmacological and chemical research (Bankova, 2005) . As a result, much valuable information has been gathered. Fortunately, it is important to note that in the last decade, the paradigm concerning propolis chemistry dramatically changed. (Bankova, 2005) . In the 1960s, propolis was thought to have complex chemistry. (Silva-Carvalho et al., 2015) , The chemical properties and health compounds of propolis are well understood to be highly dependent on several ecological factors, including geographic region, plant source, weather, and harvest process. (Valencia et al., 2012; Shi et al., 2012).

The goal of this study was to see how effective Algerian propolis is in vitro at reducing free radicals. Total phenol content (TPC) was calculated by using Folin-Ciocalteau Reagent. Total flavonoid content (TFC) was calculated by using aluminum chloride process. Thereafter, assess propolis' antioxidant activity, calculated by using the reducing power (FRAP and RP) and radical scavenging (DPPH assays) and determined antioxidant total by (phosphomolybdate assay).

2. Materials and Methods

2.1. Materials

2.1.1. Plant material

During the year 2019 (between October-December), four raw propolis

samples were collected in different geographical regions of Algeria (Tipaza (RT), Tébessa (RTb), El-Oued (RE), and Constantine (RC)). Propolis samples were kept at 4°C (for a period not exceeding 72 hours) in the dark before they were used. All of the chemicals used in this analysis are of high purity and super quality.

2.1.2. Chemicals

Methanol (99%), Ethanol (70%), Folin Ciocalteu reagent was all purchased from biochem chemopharma Co (Canada). Sodium Carbonate (Na_2CO_3) (7.5%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Aluminum chloride ($AlCl_3$) (2%), 2,4,6-tripyridyl-Striazine (TPTZ), Ferric chloride hexahydrate ($FeCl_3$- $6H_2O$) (0.1%), and potassium ferricyanide ($K_3Fe(CN)_6$) (1%), trichloroacetic acid (10%), were purchased from Sigma–Aldrich Inc. (Steinheim, Germany).

2.2. Methods

2.2.1. Instrument

UV-Visible spectrophotometer (PRIM Advanced SCHOTT Instruments Gmbh), centrifuge Machine (SLW centryge, Ultra-8TL), rotary evaporator (IKA Evaporator RV 06-ML).

2.2.2. Preparation of samples

Firstly, the total phenolic compounds in propolis were extracted using 70% ethanol as a solvent because it yields a higher extraction yield and prevents the extraction of mixed wax (Hemmami et al., 2020 , Ribéreau-Gayon et al., 1968) . The propolis from each region is then cut into small pieces and smoothed before being macerated in a solvent (10 g propolis in 100 ml ethanol 70 %) to remove the water-soluble and alcohol-soluble components (Touzani et al., 2018) . Magnetic stirring at a low temperature is often used to ensure that the propolis has been impregnated with the solvent. The mixture is filtered after a 24-hour incubation period in the dark. The residue from the first filtration is treated with 100 ml of 70% ethanol for the second maceration. The mixture is filtered after 48 hours of incubation (Escriche et al., 2018) . The hydro-alcoholic extracts are then mixed and chilled in the refrigerator for 1 hour before being centrifuged for 10 minutes at 3000 rpm (Touzani et al., 2018), followed by evaporation of the solvent at 45°C (Badria et al., 2018) . Finally, the extracts were stored in tightly sealed bottles at 4°C and held in the dark to be used in future experiments.

2.3. Phytochemical investigation

2.3.1. Total phenolic content (TPC)

Mix 500 µl of each propolis extract with 0.25 ml of folic-Ciocalteu reagent diluted 10 times. After 3 min, we add 800 µl of sodium carbonate solution (Na_2CO_3) (7.5 %). The tubes are stirred and

incubated for 30 minutes at room temperature, with light protection. A visible UV spectrophotometer is used to calculate the absorbance against a blank at 765 nm (Hemmami et al., 2020) .

2.3.2. Total flavonoid content (TFC)

The wavelength spectrophotometer (420 nm) is used to measure flavonoids quantitatively. The process entails adding 1.5 mL of different propolis extracts with 1.5 mL of $AlCl_3$ solution (2%) and the tube was thoroughly shaken before being left in the dark for an hour before the color turned yellow (Chelalba et al., 2020) .

2.4. Antioxidant activity

The DPPH radical, RP, and FRAP tests were used to assess the antioxidant activity of various propolis extracts.

2.4.1. DPPH assay

The DPPH test was performed in compliance with the protocol Chouikh et al. (2016). 0.5 ml of each extract solution is added to 1 ml of a methanolic solution of DPPH (1 mM) at the same time. The absorbance was measured at 517 nm after 30 minutes of incubation at room temperature in the dark (Atef et al.,) .

A normal antioxidant solution: ascorbic acid and -α-tocopherol, whose absorbance was determined under the same conditions as the samples and for each concentration, acted as a positive control. The following equation measures the inhibition of the DPPH free radical by percentage I % (Khelef et al., 2019) :

$$\boldsymbol{I}\,\% = [(A_{blanc} - A_{sample}) / Ablanc] \times 100 \qquad (1)$$

Where: A_{blanc} is the absorbance of the control; A_{sample} is the absorbance of the sample.

2.4.2. Ferric Reducing Antioxidant Power test (RP)

The RP test was carried out using the Mohammed Adel M et al (2018) process with some modifications. The FRAP solution is prepared by mixing in a 10: 1: 1 volume ratio of the following three solutions: sodium acetate buffer solution ($300\,mM$), TPTZ solution ($10\,mM$) and $FeCl_3$- $6H_2O$ solution ($20\,mM$) respectively. The FRAP solution is placed in a thermostatically regulated bath at $37\,°C$. The test consists of the reaction of $100\,\mu l$ of each extract with $3\,ml$ of FRAP solution in glass hemolysis tubes. The absorbance of the mixture was estimated at $593\,nm$ after $30\,min$ of incubation at room temperature (Mesbahi et al., 2019).

2.4.3. Ferric reducing antioxidant power (FRAP)

The RP test was carried out using the Abdelkerim R et al (2014) process with some modifications. To $500\,\mu l$ of the sample at various concentrations; $1.25\,ml$

of $pH = 6.6$ $(0.2\,M)$ phosphate buffer solution and $1.25\,ml$ of $K_3Fe\,(CN)_6$ (1 %) potassium ferricyanide solution is added. The mixture is incubated at $50°C$ for $20\,min$, 1.25 ml of trichloroacetic acid (10 %) is applied to stop the reaction and the tubes are centrifuged at 3000 rpm/10 min. 1.25 ml of supernatant is added to 1.25 ml of distilled water and 250 µl of iron trichloride ($FeCl_3$, $6H_2O$) solution (0.1%). The absorbance is read against a blank at 700 nm using a visible UV spectrophotometer (Rebiai et al., 2014) .

2.5. Statistical analysis

To interpret the results obtained from the experiments carried out, the MINITAB program (version 13) is used to express them in the form of a mean ± mean standard deviation, n=3. The curves and histograms are plotted by using Microsoft EXCEL.

3. Results and discussion

3.1. Extraction yield, total polyphenols, and flavonoids Content

3.1.1. Extraction yield

The extraction yield (mg/g dry weight) is shown in Figure 1. In general, the propolis of RT recorded the highest yield (37%) while the propolis of R_E provided the lowest yield (14%). Propolis RTb and R_C both had intermediate values. The difference in yield between samples may be attributed to its high content of soluble polysaccharides, which could be precipitated by using methanol (Lee et al., 2007).

Tipaza propolis gives the best yields because this region is located on the coast, which is a wetland hence, a high yield.

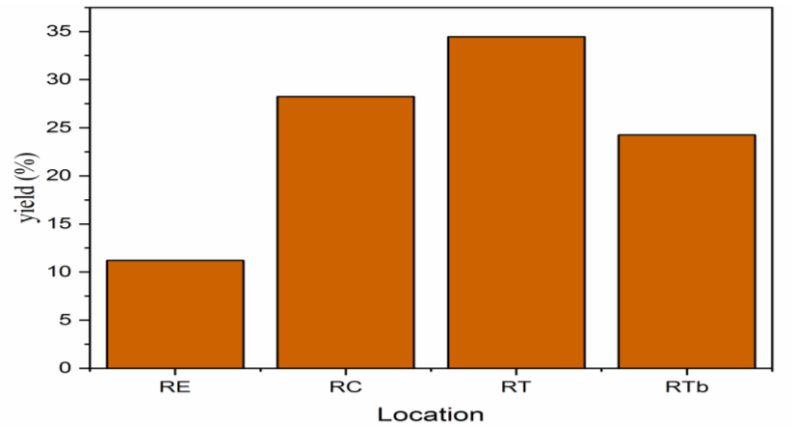

Fig.1: Yield (%) of different extract of propolis

3.1.2. Total polyphenols and flavonoids Content

The total polyphenol and flavonoid content of propolis samples is shown in

Table 1. Complete polyphenol content in methanolic extracts of propolis (RT, RC, and RTb, RE) as determined by the Folin-ciocalteu Reagent method shows 384.7 ±18.0 mg as the highest value (RT) and 42.12±1.42 mg as the lowest value (RE) Gallic acid equivalent per 100 mg of propolis powder.

TPC is found in the following order in propolis extracts: RT>RTb>RC>RE. These findings revealed that Algerian propolis contains more phenolics than those mentioned by Da Silva et al (da Silva et al., 2006) and Potkonjak et al (Potkonjak et al., 2012) . In comparison to Kumazawa et al (Kumazawa et al., 2004) . and Choi et al (Choi et al., 2006) , it has a lower phenolic material.

TFC levels varied significantly between propolis samples, ranging from 37.27±1.86 mg RE/100 g to 2.152±0.546 mg RE/100 g, with the following rating order: R_T>R_{Tb}>R_E>R_C. It contains more flavonoids than those found by Da Silva et al (da Silva et al., 2006) and Shiva Mohammad Zadeh et al (Mohammadzadeh et al., 2007) . The content of propolis is determined by the local vegetation and the season in which it is obtained (Kumazawa et al., 2004) .

Table 1. The total polyphenol and flavonoid contents of propolis samples

Compound (concentration)	(TPC)(mg/ g)	(TFC) (mg/ g)
RT	384.7± 18.0	37.27±1.86
RC	105.17±2.77	2.152±0.546
RTb	353.2±13.9	35.674±0.833
RE	42.12±1.42	29.42 ±3.57

3.2. Antioxidant activities

3.2.1. Scavenging effects on DPPH radical

DPPH is a free radical compound that has been widely used to determine the ability of various samples to scavenge free radicals (Hatano et al., 1997) . Antioxidants' capacity to scavenge DPPH free radicals is believed to be due to their hydrogen-donating ability (Tang et al. 2002) . DPPH inhibition was investigated to establish the scavenging effect of DPPH on methanol extract of propolis, and the results are shown as relative activities toward regulation. The activities of propolis samples and ascorbic acid as free

radical scavengers increased as concentration was increased, as shown in Table 3. The radical scavenging process of all of the extracts was concentration base.

Table 2. DPPH radical scavenging activities (%) of ascorbic acid, α-tocophérol and methanol propolis extract obtained from (RT), (RC), (RTb), and (RE) (RE).

Table 2: DPPH scavenging activity (%) and IC_{50} of different

Echantillon	IC_{50} ±Ecart type (En mg /ml)
R_T	0.38488±0.00960
R_C	1.2807±0.0589
R_{Tb}	0.4340±0.0195
R_E	0.8645±0.0245
Acid ascorbic	0.028401±0.158
α-tocophérol	0.26836±0.00415

In general, the IC_{50} values (the concentration required to inhibit radical formation by 50 percent and was obtained from interpolation from linear regression analysis) of different propolis were used to compare their DPPH radical scavenging activity. RT had the highest activity, followed by RTb, RE, and RC had the lowest activity. This may be due to the extracts' higher polyphenol content (RT, RTb) (RT, RTb). The radical scavenging behavior of the extracts of different propolis increases with increasing concentration and follows the given orders, according to the analysis (ARP) of (Table 2.) Acide ascorbique >α-tocophérol >RT > RTb > RE > RC.

According to the current findings, when the DPPH radical reacts with hydrogen donors in antioxidant principles, the extracts of Algerian propolis reduce the DPPH radical to the corresponding hydrazine.

3.4.3. Ferric Reducing Antioxidant Power test (RP)

The reducing power of a compound serves as a significant indicator of its future antioxidant activity. In general, an antioxidant exerts its activity by breaking the free radical chain via the donation of hydrogen atoms (Meir et al., 1995) .Table. 3 shows the reducing power of different sample extracts compared to the Vit C standard.

The tested samples were unable to reduce the Fe^{3+}/ferricyanide complex to the ferrous form and the synthetic antioxidant (Vit C) had the better reducing ability (26.411 µmol Fe(II)/mg extract) than the different samples.

The test findings show that the samples include a large number of electron donors capable of reducing oxidized intermediates in lipid peroxidation processes. In the reducing assay, the yellow hue of the solution changes to various colors of blue and green depending on the compound's ability to decrease free radicals (Re et al., 1999). The conversion of the Fe^{3+}/ ferricyanide complex employed in this technique to the ferrous form is the core principle of the test. As a result, the presence or absence of ferrous ions in solution causes the color change.

Propolis samples' reducing power differed substantially. The decreasing power was highest in the RT and lowest in the RC for all of the samples.

It's been claimed that the antioxidant activity and reducing power of chemical components in specific dietary products are directly related (Sahreen et al.,2011).

Table 3: The potential antioxidants of propolis extract and positive control

Sample	Vit C	RE	RTb	RT	RC
µmol Fe(II)/mg extract	26.411±0.150	1.323±0.130	1.474±0.122	2.406 ±0.155	0.5863±0.0982

3.4.4. Ferric reducing antioxidant power (FRAP)

The FRAP assay is dependent on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ producing an extreme blue Fe^{2+}-TPTZ complex with maximal absorption at 593 nm. Therefore, the FRAP assay is also used to test the ability of an antioxidant to donate an electron. The ferric reducing capacity of tested methanolic extracts in terms of mg Trolox equivalents is shown in Fig. 2. In general, the trend was almost the same as that of the DPPH radical.

When compared to other assays that evaluate free radical inhibition, the FRAP test is the only one that directly analyzes antioxidants (or reductants) in a sample (Halvorsen et al., 2002). The FRAP values reflect the equivalent concentration of electron-donating antioxidants with the reduction of ferric iron (Fe^{3+}) to ferrous ion

(Fe^{2+}) (Halvorsen et al., 2002). Because the only chemicals that FRAP does not react with are thiols, it is regarded an acceptable evaluation for total antioxidants in plants ingested by people. Humans can only absorb a little quantity of glutathione from food, and there are practically no additional antioxidant thiols in dietary plants (Halvorsen et al., 2002).

Propolis samples converted the Fe III -TPTZ complex most effectively to ferrous (Fe II).

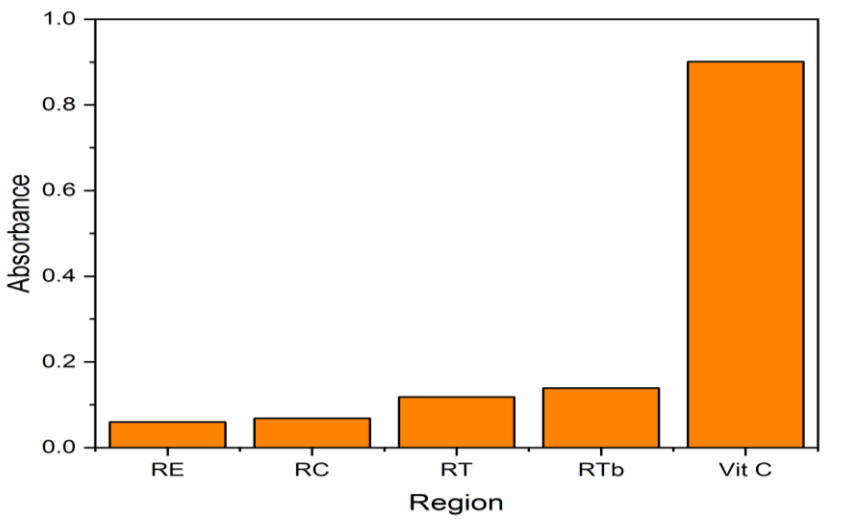

Figure 2: Reducing power of extracts and ascorbic acid

Collected together, the comparative study of the antioxidant activity of propolis samples by using 4 in vitro complementary assays proved that RT demonstrated the highest antioxidant activity, whilst the RC showed the lowest. These results indicate that the antioxidant activity was due to particular phenolic components. In this sense, the decrease of the antioxidant potential in the RC may be due, partially at least, to the composition of their phenolic compounds in particular the inclusion of sugar moieties (glycosylated phenolics) recognized for their poor antioxidant activity (Procházková et al., 2011; Saija et al., 1995) . Taking into account the complexity of the antioxidant mechanism which includes four principal mechanisms: I simple hydrogen atom transfer from the antioxidant to the radical (Mayer et al.,

2004) , (ii) single-electron transfer from the antioxidant to the radical leading indirect H-abstraction (Rojano et al., 2008) , (iii) sequential proton-lossN electron transfer (Klein et al., 2007) and (iv) metal chelating (Gülçin et al., 2010) , it is difficult to get a deeper insight into the antioxidant mechanism of propolis samples based on the abovementioned assays and further tests are urgently needed.

4. Conclusion

Our results backed the hypothesis that geographic location has a significant effect on the phenolic composition and antioxidant activities of propolis extracts, with the magnitude of antioxidant activities being largely determined by a few key phenolic components in the extracts. The Tipaza field RT had the highest antioxidant activity in vitro, while the Constantine region RC had the lowest antioxidant activity. The findings also revealed that there was a significant variation in the antioxidant activities of propolis phenolics in vivo amongst different sites, with the best impact coming from the RT position. The findings indicated that propolis has significant health benefits for humans and that it could be used as an antioxidant source.

The importance of growing atmosphere selection for better use of this product in the pharmaceutical and food industries was suggested by these findings. Isolation, purification, and bioactivity analysis of propolis phenolics should be conducted in the future to determine the specific compounds responsible for antioxidant activities.

Conflict of interest

The authors declare no conflict of interest.

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