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# Dual amylase/glucosidase inhibition, antilipolytic and antiproliferative potential of the aerial parts of *Pistacia atlantica, Pistacia lentiscus* and *Pistacia terebinthus* on a obesity related-colorectal cancer cell line panel

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**ABSTRACT**

*Pistacia species* (*P. spp*) have been used as a treatment for various diseases, including diabetes and inflammation. This study aimed to identify the main components of flavonoids in *Pistacia species* and evaluate the effect of aqueous extracts of *P. spp* on pancreatic enzymes and on cancer cells associated with obesity in colon and rectum. HPLC was used to identify the major components of flavonoids. The potent inhibitory effect of *Pistacia species* against pancreatic α-amylase, α-glucosidase and lipase was examined. The antiproliferative efficacy of the plant extract against several colorectal cancer cell lines were then measured**.** The main flavonoids component found in *Pistacia species* are quercetin-3-β-D-glucoside, rutin, kaempferol and vitexin. The starch blockade $IC_{50}$ (μg/mL) of *Pistacia species* in a descending order were: *P. lentiscus* leaves: 1.09±0.01; *P. atlantica* leaves: 0.96±0.09 and *P. atlantica* fruits: 0.48±0.02. *Pistacia species* exerted promising inhibition effect for pancreatic lipase (PL). Besides the aglycones of *P. atlantica* leaves, all the tested aqueous extracts exerted appreciably novel antiproliferative activity against the tested colorectal cancer cell lines. This study provides useful indication for the Pistacia species as a potential novel therapeutic agent against diabesity and cancer.

## INTRODUCTION

Medicinal herbs have been used in folk and traditional medicine for centuries throughout the world, including Jordan. These uses include treating diabetes, cancer and many other diseases [1,2]. Among the twenty species of the genus *P.*, the five most common are *P. vera*, *P. atlantica*, *P. terebinthus*, *P. thinjuk* and *P. lentiscus* [3]. Specimens of *P. lentiscus* L, *P. atlantica Desf*, *P. terbinthus* L, *P. vera* L and *P. thinjuk* can be found in the fringes of the Mediterranean basin and in Central Asia [4,5]. Genus P. vera is the only species that is considered commercially farmed, while the rest are mostly considered as suitable only as root stock [6].

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The plant is used in traditional medicine for various purposes, among others, as an antiseptic, hypotensive and digestive [7]. Pharmaceutical studies have demonstrated the activity of this plant as antioxidants, antimicrobials and antivirals agents [7-9]. *P. lentiscus* is known as the 'mastic' tree, especially the aerial part, which is used in the treatment of high blood pressure due to its diuretic effect. It also contains active components such as anthocyanins, essential oils (EO), flavanols, glycosides, gallic acid and resin [10]. Oxidized hydrocarbons and monoterpenes are the main chemical components of EO in several plants, including *P. terebinthus*, *P. lentiscus* and *P. atlantica*, where α-pinene was mentioned as a major compound [11,12]. Smaller quantities of Sesquiterpenes were isolated when compared with the quantity

of monoterpenes. β-caryophyllene and Germacrene-D have been identified in the leaves of *P. terebinthus* and *P. lentiscus* [13,14]. Several triterpenoids have been isolated from the resins of *P. terebinthus* and *P. lentiscus*, including tirucallol [15]. Flavonoids were also detected in different parts of these species, and 3-quercetin glucoside was reported as the most abundant and obtainable for the aerial parts of *P. atlantica* and *P. lentiscus* [16].

Obesity is one of the main causes of coronary disorders and death [17]. The extent to which natural sources are used as a treatments for obesity and diabetes stems from their ability to inhibit digestive enzymes such as intestinal α-glucosidase, pancreatic α-amylase and triacyglycerol lipase (LP) [2,18]. It is noticeable that when the AE of *P. atlantica*, *P. lentiscus* and *P. terebinthus* are used at high concentrations, it shows dual inhibition of α-amylase and α-glucosidase *in vitro* [10].

This current study is aimed to identify the main components of flavonoids in *P. spp*., as well as to investigate the *in vitro* inhibitory effects on extra-pancreatic digestive enzymes and to examine the cytotoxicity of extracts of the three species of Algerian *Pistacia* against a number of cancer cell lines associated with obesity, especially colorectal cancer cells, as compared to normal fibroblasts. In the course of the study, the results are also evaluated in comparison with standard medications.

## MATERIAL AND METHODS

### Plant collection

*P. spp*. were gathered from diverse areas during the early period of flowering in Autumn 2020, in Algeria. Voucher of samples were kept at the Institute of Agronomy (INA), The University of Algiers. All plants were cleaned of extraneous impurities and dried only in the shade and at room temperature.

### Preparation of all *P. spp*. extracts

Aqueous extracts (AEs) were made by refluxing 10 g of plant extract with 100 mL of distilled water for 15 min at room temperature, the extracts were kept overnight and filtered twice with filter paper until the volume of the filtered solution (crude aqueous solutions) was 100 mL and at a concentration of 100 mg/mL [19]. The stock of crude extracts was then sonicated, and the test concentrations were monitored prior to investigations. For the PL trials, water was evaporated using a rotary evaporator, and solid residues were compiled and stored in dry conditions while pending analysis. To study the cytotoxicity, the dried and coarsely powdered plant material (10 g) was re-flushed for half an hour by 70% ethanol, then kept overnight. This action was followed by filtering and, afterwards, the solvent was evaporated. Extracts of 100 mg were dissolved in 10 ml DMSO as a stock solution.

### Extraction of flavonoids aglycones

Air dried aerial parts (fruits and leaves) were first finely powdered. The sample material was then extracted with 320 mL of 2N hydrochloric acid at 40°C for 40 mn in a water bath in order to break the C-O-C connections of the heterosides. Subsequently, two successive extractions with diethyl ether (100 mL) and n-butanol (100 mL) were carried-out [20]. As a result of this action, the ethered phase contained the free aglycones and the butanolic phase contained the C-glycosides. The two organic phases were then evaporated to dryness under vacuum at 40°C, the residues were afterwards dissolved in methanol (5 mL).

### Extraction of flavonoids heterosides

The powder sample was homogenized in 400 mL cold aqueous ethanol 70% for 2 days. After removing alcohol under vacuum at 40°C, boiling water (100 mL) was added and heterosides were removed by an extraction with n-butanol (100 mL) [21]. Evaporation of the organic phase for occurrence of dryness under vacuum at 40°C was then conducted. The residues were liquefied by using 5 mL methanol.

### Analytical high-performance liquid chromatography (HPLC)

HPLC analytical reagent (Agilent HP 1100) was used to analyze the natural components such as the phenolic compounds (chromophores). The system was fully managed by using advanced software (Chromeleon® version 6.30) which has spectral libraries built as reference compounds when automatically searching for the respective compounds in the study. The conditions of analytical *HPLC* are: Flow rate: 0.8 ml/mn, the column type and dimensions were RP 18 and 250×4.6 mm, respectively; 5 µm and UV detection wavelengths: 260 nm ≤ λ ≤ 380 nm.

### Analysis of free aglycones

Free aglycones were analyzed in elution gradient mode at wavelength λ=365 nm, the injected volume was 20 µl. Two solvents A and B (2% acetic acid and methanol, respectively) were used as mobile phase [22-25]. The elution program for the separation is shown in Table 1.

***Table 1.*** Elution program for the separation

| Time (mn) | Solvent B (%) | Solvent A (%) |
|---|---|---|
| 0 | 5 | 95 |
| 30 | 70 | 30 |
| 40 | 70 | 30 |
| 44 | 5 | 95 |
| +44 | 5 | 95 |

### Analysis of C – glycosides and heterosides

The separation of C-glycosides and heterosides was accomplished through using the exact procedure and device for both, being λ=380 nm. In this part of the research, solvent A consisted of a 98: 2 ratio of water/acetic acid, whereas solvent B was of 2:19:79 ratio of acetic acid/water /acetonitril. The program of separation by elution is shown in Table 2 [22,26].

### *In vitro* PL activity assay for *P. spp.* AEs and orlistat

All AEs examined preliminarily were dissolved in 2.5 mM Tris-HCl buffer (Promega, USA), pH 7.4 and contained 2.5 mM NaCl to produce 6.25, 12.5, 25, 50 and 100 mg/ml as stock solutions. A 20 µL aliquot of each stock solution was then added to each reaction mixture, giving 125, 250, 500, 1000

***Table 2.*** Elution program for the separation

| Time (min) | Solvent B (%) | Solvent A (%) |
|---|---|---|
| 0 | 12 | 88 |
| 20 | 17 | 83 |
| 30 | 22 | 78 |
| 45 | 37 | 63 |
| 60 | 40 | 60 |
| 65 | 70 | 30 |
| 68 | 70 | 30 |
| 73 | 12 | 88 |
| 83 | 12 | 88 |

and 2000 µg/mL as final concentrations. The extracts were treated by preparing them via traditional methods, where all the organic solvents were neutralized as DMSO or others even on the scale of the minimum concentrations of these solvents [27]. Moreover, six different stock solutions that ranged between 0.625 and 20 µg/mL and containing 1 mg/mL in DMSO as reference drug (orlistat) were used [2,19]. Afterwards, 20 µl of every stock solution in the reaction mixture was separately applied to make an ultimate concentration ranging from 0.0125 to 0.4 µg/mL.

### Spectrophotometric quantification of PL inhibition by *P. spp*. AEs and orlistat

The *in vitro* activity of PL enzyme was assessed as previously described [28]. The tested extracts, essential oil compounds and orlistat were compared to the control after determining the 50% inhibition ($IC_{50}$) of PL.

### *In vitro* enzymatic starch digestion assay

*In vitro* enzymatic digestion of starch was determined using acarbose, as a control drug [2,29]. The breakdown extent of polysaccharide into glucose by plant aqueous extract was estimated by employing 1-100 mg/mL as plant aqueous extract range. The impacts of 1000 µg/mL concentrations of acarbose were also established. Tap water samples containing no acarbose or plant extract served as control.

### *In vitro* antiproliferative assay

Colorectal obese HT29, SW620, SW480 and HCT116 cancer cells were cultured in high concentrations of glucose DMEM containing 10% FCS (obtained from Bio Whittaker, Verviers, Pelgium). Caco2 cell line was cultured in RPMI 1640 consisting of 10% FBS, 10 mM HEPES Buffer, 2 mM L-glutamine, 50 µg/ml gentamicin, 100 units/ml penicillin and 100 mg/m/ streptomycin sulfate. Sulforhodamine B (SRB) was employed to measure cytotoxicity by colorimetric assay and to assess how cell viability was reduced [30]. Briefly, the respective cells were cultured at 5000/well on 96 flat-bottom culture plates and left to adhere overnight before intended treatment in the particular wells for 72 h at 37°C in an incubator of 5% $CO_2$ with humid air. Absorbance measurements were taken at 570 nm via a microplate reader. A primary cell culture was used for establishing selectivity of cytotoxicity verification against periodontal fibroblasts (PDL) through obtaining the IC50 value of minimal antiproliferation capacity. Cisplatin and doxorobocin were utilized as positive controls [31-33].

### Statistical analysis

Data are results of minimally three independent replicates (n≥3) and represented as mean ± SEM. The difference between tests and control groups were calculated by way of ANOVA (one-way analysis of variance). Dunnett test was applied when needed (version 3.02 for windows; GraphPad Software, San Diego, CA, USA). When data results led to $P<0.05$, $P<0.01$ and $P<0.001$, they were considered statistically significant.

## RESULTS

### Analysis of flavonoids of *pistacia spp* by HPLC

Detected area (%) and retention time (RT) of flavonoids of *pistacia spp* extracts are listed in Table 3.

***Table 3.*** Detected area (%) and retention time (RT) of the major flavonoids in the leaves and fruits extracts of *Pistacia spp*.

| Plant | Extracts | Type of Flavonoids Compounds | RT (min) | Area% |
|---|---|---|---|---|
| *P. lentiscus* (leaves) | Aglycones | Kaempferol<br>Rhamnetin | 42.183<br>71.170 | 2.7464<br>1.2493 |
| | C-glycosides | Vitexin<br>Luteolin-7-glucoside | 36.419<br>41.149 | 8.0680<br>12.0825 |
| | Heterosides | Vitexin<br>Quercetin-3-β-D-glucoside<br>Apigenin-7-glucoside | 36.440<br>42.045<br>46.294 | 8.4617<br>24.4546<br>4.3159 |
| *P. lentiscus* (fruits) | Aglycones | Apigenin<br>Rhamnetin | 69.542<br>71.536 | 1.0271<br>3.450 |
| | C-glycosides | Vitexin<br>Luteolin-7-glucoside<br>Quercetin-3-β-D-glucoside | 36.467<br>41.086<br>42.912 | 6.5170<br>7.3561<br>12.5479 |
| | Heterosides | Vitexin<br>Luteolin-7-glucoside<br>Quercetin-3-β-D-glucoside<br>Apigenin-7-glucoside | 36.441<br>41.078<br>42.862<br>46.313 | 10.2864<br>15.1944<br>30.0939<br>16.2043 |
| *P. atlantica* (fruits) | Aglycones | Luteolin | 36.030 | 12.21 |
| | C-glycosides | Coumarin | 70.181 | 0.6531 |
| | Heterosides | nd | - | - |
| *P. atlantica* (leaves) | Aglycones | Myrecetin<br>Quercetin<br>Kaempferol | 22.592<br>25.379<br>28.264 | 1.7261<br>0.9261<br>0.4481 |
| | C-glycosides | nd | - | - |
| | Heterosides | Vitexin-2-O-rhamnoside<br>Vitexin<br>Rutin<br>Quercetin-3-β-D-glucoside | 35.007<br>36.467<br>40.505<br>42.312 | 1.32<br>2.61<br>1.13<br>2.55 |
| *P. terebinthus* (leaves) | Aglycones | Luteolin | 36.641 | 0.33 |
| | C-glycosides | nd | - | - |
| | Hetherosides | Rutin | 40.392 | 11.4322 |
| *P. terebinthus* (fruits) | Aglycones | Kaempferol | 26.879 | 29.5094 |
| | C-glycosides | Apigenin-7-glucoside | 46.861 | 2.7023 |
| | Hetherosides | Vitexin<br>Quercetin-3-β-D-glucoside<br>Apigenin-7-glucoside | 36.691<br>42.054<br>46.849 | 6.7779<br>2.2049<br>3.0179 |

RT – Retention time (minutes), area – peak area relative, nd – no detection

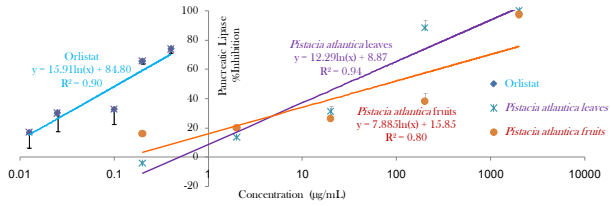
### *In vitro* PL inhibitory effects of *P. spp*. AEs and reference drugs

The anti-lipase activity profiles of pancreatic triacylglycerol of the three Algerian *P. spp*. AEs gradients are shown in Figs. 1A-C. The value of Orlistat's PL-IC50 (0.114±0.01 µg/mL), is equivalent to 0.2±0.0 µM, which is comparable to what was reported previously for PL-IC50 values (Table 4). In parallel to the rendering of orlistat, a concentration dependent manner for PL inhibition trend was obtained for the investigated extracts. Minimally, three independent measurements for PL-IC50 values were obtained (Table 4).

***Table 4.*** $IC_{50}$ values of *in vitro* starch digesting enzymes and PL (mg/mL – µg/mL) as a function of *P. spp.* AEs, acarbose and orlistat concentrations

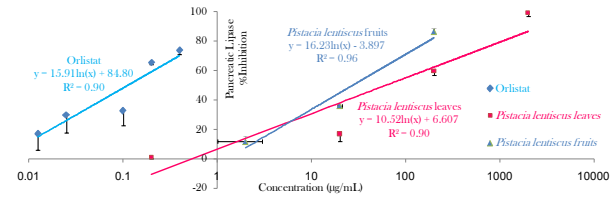
| *Pistacia spp.* | Pancreatic Triacylglycerol Lipase IC50 (µg/mL) | Enzymatic Starch Digestion IC50 (mg/mL) |
|---|---|---|
| *P. atlantica* fruits | 73.45±2.37 | 0.48±0.02 |
| *P. lentiscus* fruits | 28.80±0.64 | 7.01±0.63 |
| *P. terebinthus* fruits | 13.06±0.39 | 7.52±0.42 |
| *P. atlantica* leaves | 30.32±1.41 | 0.96±0.09 |
| *P. lentiscus* leaves | 63.12±0.91 | 1.09±0.01 |
| *P. terebinthus* leaves | 35.20±2.33 | 9.15±0.15 |
| Reference | Orlistat | Acarbose |
| Drug | 0.114±0.01 | 0.2±0.02 (µg/mL) |

Data are results of three independent replicates (n=3) and indicated by mean of ± SEM



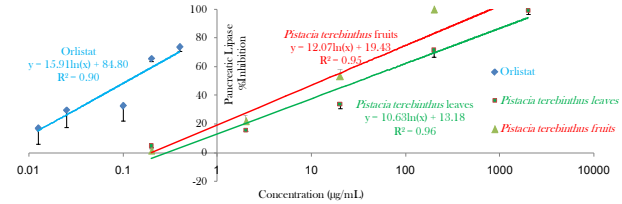

Data are results of three independent replicates (n=3) and indicated by mean of ± SEM

***Figure 1A.*** Inhibitory effects for *in vitro* of AEs of *P. atlantica* leaves and fruits and orlistat on the activity of PL




Data are results of three independent replicates (n=3) and indicated by mean of ± SEM

***Figure 1B.*** Inhibitory effects for *in vitro* of AEs of *P. lentiscus* leaves and fruits and orlistat on the activity of PL

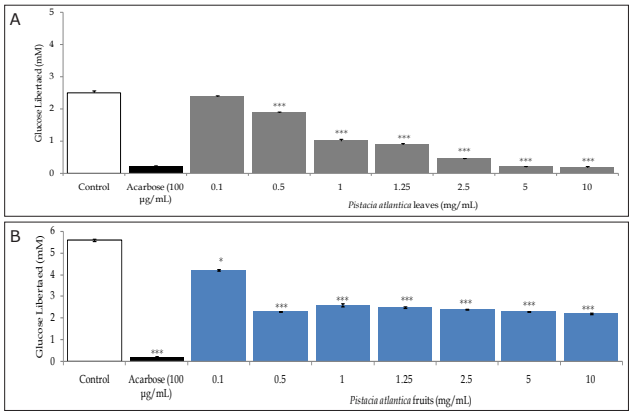



Data are results of three independent replicates (n=3) and indicated by mean of ± SEM

***Figure 1C.*** Inhibitory effects for *in vitro* of AEs of *P. terebinthus* leaves and fruits and orlistat on the activity of PL

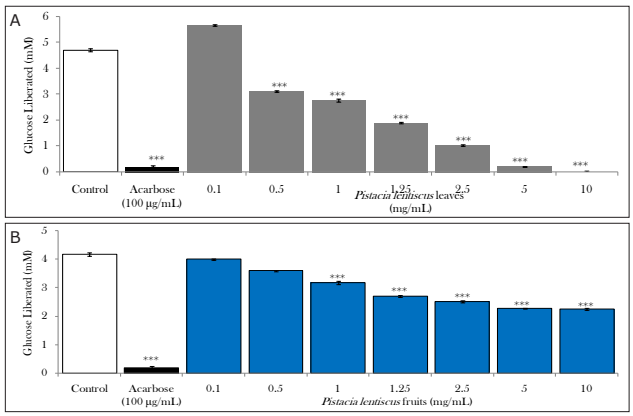
## *In vitro* inhibitory effects of *P. spp.* AEs on enzymatic starch digestion

It is thought that inhibition of carbohydrate hydrolysis enzymes in the digestive tract, as with the starch blocker, acarbose, can lead to a prolongation of digestion of the total carbohydrate and thus a reduction in postprandial blood glucose and hyperinsulinemia. Glucose release from starch using 0.1 mg/ml acarbose as the control drug was inhibited by 97.6% versus drug-free control incubations, n=3 (p<0.001), (Figures 2A-B, 3A-B, and 4a-b). For each *P spp* AEs at concentrations 0.1-10 mg/mL, a substantial dose-related reductions in aldohexose release from culinary polymeric cornstarch (p<0.001 *vs.* plant-free control determinations, n=3) was shown (Figures 2A-B, 3A-B and 4A-B). Each of the three Algerian *P. spp* decreases the starch hydrolysis enzymes using a gradient of dosage between 0.1 and



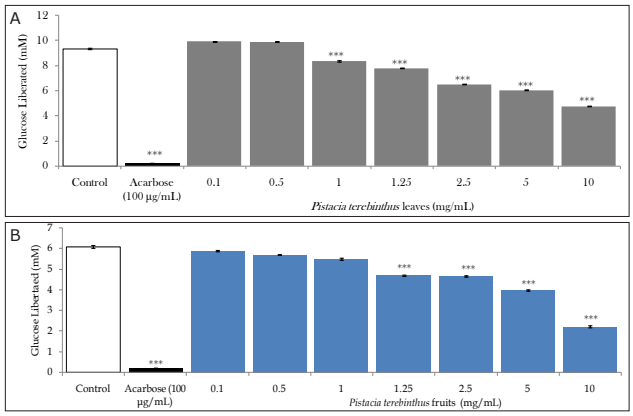

Data are results of three independent replicates (n=3) and indicated by mean of ± SEM. ***The difference between tests and control was statistically significant (p<0.001)

***Figure 2A-B.*** Inhibitory effects for *in vitro* AEs of Algerian *P. atlanticas* leaves on the activity of starch digesting enzymes




Data are results of three independent replicates (n=3) and indicated by mean of ± SEM. ***The difference between tests and control was statistically significant (p<0.001)

***Figure 3A-B.*** Inhibitory effects for *in vitro* AEs of Algerian *P. lentiscus* leaves and fruits on the activity of starch digesting enzymes




Data are results of three independent replicates (n=3) and indicated by mean of ± SEM. ***The difference between tests and control was statistically significant (p <0.001)

***Figure 4A-B.*** Inhibitory effects for *in vitro* AEs of Algerian *P. terebinthus* leaves and fruits on the activity of starch digesting enzymes

10 mg/mL. Table 4 shows the decrease in enzymatic starch hydrolysis as dependent upon on the $IC_{50}$ (mg/mL) values for the high-importance dose by each of the three Algerian pistachio species (0.1-10 mg/mL).

### Antiproliferative activity of selected *P. spp.* extracts against obesity related colorectal cell lines

NCI nominates a medicinal herb for its therapeutic or prophylactic effects if its crude extract antiproliferative IC50 value is less than 30 µg/mL [34]. Table 5 shows the results regarding the anti-proliferative efficiencies of using cisplatin and doxorubicin against the tested colorectal cancers. The table reveals that the extracts lacked anti-proliferative activity with the exception of leaf extracts of both *P. atlantica* and *P. terebinthus* against HCT116. Exceptionally, cytotoxicity against SW480 was demonstrated by the leaves and fruits of *P. atlantica* as well, while *P. terebinthus* fruits proved substantially potent over 72h incubations. Nevertheless, *P. atlantica* and *P. terebinthus* lacked selective cytotoxicity in PDL fibroblasts wells (Table 5). However, *P. lentiscus* were the only antineoplastic extracts that show selective cytotoxicity.

***Table 5.*** Antiproliferative effect for *in vitro* crude extracts of *P. spp*. on four cancergenic cell lines of colorectal and PDL fibroblasts

| Treatment | Cytotoxicity (%Control) $IC_{50}$ value: mean ± SEM (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | HT29 | HCT116 | SW620 | CACO2 | SW480 | PDL Fibroblasts |
| Cisplatin | 1.13 ±0.01 | 3.5 ±0.08 | 2.6 ±0.02 | 4.3 ±0.05 | 2.4 ±0.3 | 1.01 ±0.02 |
| Doxorobocin | 0.19 ±0.06 | 0.10 ±0.02 | 0.09 ±0.02 | 1.15 ±0.00 | 0.05 ±.008 | 0.014 ±0.001 |
| *P. atlantica* (leaves) Aglycones | 78.3 ±3.8 | 64.3 ±4.7 | 51.1 ±2.8 | NI | **23.2 ±1.3** | 34.7 ±5.02 |
| *P. atlantica* (leaves) C-glycosides | 139.5 ±4.2 | 63.3 ±3.2 | 92.7 ±7.5 | NI | 32.7 ±4.6 | 46.5 ±3.6 |
| *P. atlantica* (leaves) Heterosides | 39.2 ±1.2 | **29.9 ±0.5** | 40.2 ±5 | NI | **17.6 ±1.5** | 22.1 ±1.6 |
| *P. atlantica* (fruits) Aglycones | NI | NI | NI | NI | 60.6 ±0.79 | NI |
| *P. atlantica* (fruits) C-glycosides | 85.7 ±0.9 | 160.3 ±14.5 | 128.7 ±9 | NI | **27.3 ±1.9** | 69.2 ±3.4 |
| *P. atlantica* (fruits) Heterosides | 52.2 ±2.9 | 59.4 ±7.1 | 66.4 ±2.3 | NI | NI | 25.3 ±3 |
| *P. terebinthus* (leaves) Aglycones | 40.1 ±3.2 | **13.2 ±2.5** | 82.3 ±4.7 | 101.8 ±1.6 | 50.7 ±3.8 | 49.7 ±3.0 |
| *P. terebinthus* (leaves) C-glycosides | **21.9 ±1.7** | **20.1 ±2.3** | 30.3 ±3.2 | 57.3 ±7.1 | 37.8 ±3.0 | 28.3 ±1.7 |
| *P. terebinthus* (leaves) Heterosides | 121.4 ±5.4 | 67.8 ±2.5 | 61.4 ±7.3 | 127.1 ±45.9 | 154.7 ±9.4 | 79.1 ±3.9 |
| *P. terebinthus* (fruits) Aglycones | 130.5 ±3.1 | 269.5 ±40.1 | 219.2 ±16.9 | NI | NI | 72.6 ±6.5 |
| *P. terebinthus* (fruits) C-glycosides | 82.6 ±2.4 | 110.3 ±9.7 | 94.8 ±11.7 | NI | 76.7 ±4.75 | 69.2 ±2.3 |
| *P. terebinthus* (fruits) Heterosides | 78.3 ±3.8 | 60.5 ±8.6 | 56 ±6 | NI | **16.7 ±2.3** | 31.3 ±3.1 |
| *P. lentiscus* (leaves) Aglycones | NI | NI | NI | NI | 179.3 ±18.5 | NI |
| *P. lentiscus* (leaves) C-glycosides | NI | NI | NI | NI | 27 ±2 | NI |
| *P. lentiscus* (leaves) Heterosides | 99.5 ±4.7 | NI | NI | NI | 55.6 ±4.9 | NI |
| *P. lentiscus* (fruits) Aglycones | 173.5 ±1.7 | NI | NI | NI | 224.5 ±1.5 | NI |
| *P. lentiscus* (fruits) C-glycosides | NI | NI | NI | NI | 45.1 ±4.3 | NI |
| *P. lentiscus* (fruits) Heterosides | NI | NI | NI | **43 ±2.6** | 216.3 ±28.9 | NI |

Results are mean ± SD (n = 3-4 independent replicates). $IC_{50}$ values (concentration at which 50% inhibition of cell proliferation took place in comparison to non-induced basal 72 h incubations). NI is non inhibitory

## DISCUSSION

Flavonoids are secondary metabolites considered as being among the compounds of the polyphenol group [35]. Flavonoids are important in normal plant development and growth in addition to their role in plant protection [36]. Flavonoids are a diverse class of low molecular weight compounds commonly present in the leaves, bark, flowers and seeds of plants. These compounds mainly protect plants from pathogens and herbivores and provide protection against ultraviolet radiation [37]. Anthocyanins give the distinctive red and blue color to vegetables, berries and wine [38]. Polyphenols have important health benefits such as reducing the risk of heart disease and protecting against cancer [39].

In this study, an attempt was made to extend this pharmacological strategy by identifying the main flavonoid components in *P. spp* and to assess their AEs for their anti-obesity activity by exploring their inhibitory ability for intestinal carbohydrates, fat digestion and absorption enzymes, in comparison with acarbose and orlistat as standard drugs [40].

Inhibitors of hydrolase enzymes that break down carbohydrates can have significant benefits in dealing with obesity and type 2 diabetes. Interestingly, among the constituents of plant phenolics, flavonoids have an inhibitory activity against the α-amylase enzyme [41]. Our results show that the most common flavonoid components found in *P. atlantica*, *P. lentiscus* and *P. terebinthus* are apigenin, luteolin, quercetin-3-β-D-glucoside, rutin, kaempferol and vitexin. These could have inhibitory potential against pancreatic α-amylase, α-glucosidase and lipase.

In parallel with our findings, several studies considered flavonoids such as apigenin, luteolin, quercetin and kaempferol as pancreatic α-amylase and α-glucosidase inhibitors [42-44]. Furthermore, it was reported that these flavonoids possess an inhibitory effect against pancreatic α-amylase and α-glucosidase [45]. It was suggested that the unsaturated rings and the number of hydroxyl group correlate positively with the inhibitory activity against α-amylase and α-glucosidase [45]. Interestingly, apigenin, luteolin, quercetin-3-β-D-glucoside, rutin, kaempferol and vitexin have unsaturated rings and high numbers of hydroxyl group.

Our results show that *P. atlantica*, *P. lentiscus* and *P. terebinthus* have inhibitory effect against PL. Different studies have reported as well that plant extracts rich in flavonoids also demonstrate inhibitory activity on PL [46-48]. It has been suggested that the inhibitory activity against PL of flavonoids is related to the position and number of hydroxyl groups [46]. Similar criteria were found in flavonoids present in *P. atlantica*, *P. lentiscus* and *P. erebinthus* (which have been found responsible for the anti-lipase activity shown in our study).

Different suggested mechanisms have linked obesity with colorectal cancer [49], including that of enhancing the proliferation and angiogenesis of colorectal cancer cells under the influence of Leptin [50]. In contrast, adiponectin act as growth inhibitor in colorectal cancer [51]. In this study, the antiproliferative efficacies of *P. atlantica, P. lentiscus* and *P. terebinthus* extract against different obesity related colorectal cell lines were displayed. Corresponding to our result, the major flavonoids constituents in *P. spp* which include apigenin, luteolin, quercetin-3-β-D-glucoside, rutin, kaempferol and vitexin could be the reason for inducing cytotoxity in different colorectal cancer cells. *In vitro* and *in vivo* studies report that the flavonoids have cytotoxic effect on human and animal cancer by targeting different signaling pathways [52-54].

## CONCLUSIONS

*P. atlantica*, *P. lentiscu*s, and *P. terebinthus* show a dose-dependent role against the activity of pancreatic amylase, glucosidase and PL *in vitro*. The results indicate that these plants can significantly improve glucose balance by delaying the digestion of carbohydrates. Therefore, they can be used as a useful food additives for managing diabetes and thus be potential sources for discovering new and effective oral diabetes treatment. The extracts of the three *P. spp* plants were also monitored for their anti-cancer activity using colorectal cancer cells. The results indicated that these extracts have selective activity against the proliferation of these cells, which suggest that *P. spp* contains bioactive materials that can act against cancer and diabetes.

## CONFLICT OF INTEREST

There is no conflict of interest to declare.

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