International Journal of Natural and Engineering Sciences
E-ISSN: 2146-0086 16(3): 137-154, 2022
Research Article

PANCREATIC LIPASE INHIBITORY- AND ANTIPROLIFERATIVE EFFECTS OF *OLEA EUROPAEA* L., *PISTACIA LENTISCUS* L. AND *MARRUBIUM VULGARE* ON OBESITY-RELATED HUMAN COLORECTAL CANCER CELL LINES

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(Received 7th September 2022; Accepted 5th December 2022)

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ABSTRACT. Phytochemical screening and anti-obesity activity of methanol extracts of *Olea europaea* L., *Pistacia lentiscus* L. and *Marrubium vulgare* were analyzed, and since obesity is suggested to be associated with colorectal cancer, the antiproliferative activity of the selected plants against five obesity-related colorectal cancer cell lines was also assessed. Inhibition of pancreatic lipase was measured spectrophotometrically whereas the antiproliferative activity was assessed using the SRB assay. Phytochemical analysis was carried out by TLC. Qualitative analysis of the tested extracts showed the occurrence of luteolin, hyperoside, rutin, and vitexin in both, *P. lentiscus* and *O. europaea*. Extracts of *P. lentiscus* and *O. europaea* showed a very potent anti-lipase activity with IC50 values of 2.8 ± 0.2 µg/mL and 9.7 ± 1.3 µg/mL, respectively, whereas *M. vulgare* extract exerted a moderate inhibition of lipase activity (IC50 = 50.2 ± 1.3 µg/mL). Moreover, evaluation of cytotoxicity of the extracts on obesity-related colorectal cancer cell lines revealed an interesting antiproliferative effect on CACO2 cell line with IC50 values of 11.8 ± 1.2 µg/mL, 18.0 ± 1.2 µg/mL, and 36.6 ± 4.3 µg/mL for *O. europaea*, *P. lentiscus*, and *M. vulgare*, respectively. HT29, SW480, and HCT116 cell lines were also sensitive to *P. lentiscus* treatment (IC50 values of 33.5 ± 1.4 µg/mL, 43 ± 4.7 µg/mL, and 47.8±7.2 µg/mL, respectively). The extracts were safe on human periodontal ligament fibroblasts used as non-cancerous control, unlike the reference antineoplastic drug (cisplatin) whose toxicity has not precluded normal cells.

This study provides pharmacological evidence for the anti-lipase and antiproliferative activities of the selected plants, suggesting that they might be potential candidates for obesity management and cancer prevention and treatment.

Key words: *obesity, colorectal cancer, anti-lipase, antiproliferative.*

INTRODUCTION

Obesity is a syndrome produced as a result of deranged lipid metabolism which presents a great problem for public health. In fact, majorly obesity is prevailing at an alarming frequency in developing countries because of lifestyle changes, fast food intake, and the decrease in physical activity thus generating a panel of chronic and degenerative diseases such as diabetes, cardiovascular diseases, and cancer. Despite the great increase in the incidence of obesity over the last decades, advance in the discovery and development of new anti-obesity remedies is rather limited. In fact, until 2012 orlistat was the only anti-obesity drug approved by the Food and Drug Administration (FDA), and only five anti-obesity drugs (orlistat, lorcaserin, phentermine/ topiramate, naltrexone/ bupropion, and liraglutide) are currently FDA-approved for long-term use. These substances act *via* different mechanisms such as inhibition of lipid digestion and absorption and appetite suppression, but they are, unfortunately, associated with certain undesirable gastrointestinal but also systemic side effects (steatorrhea, flatulence, nausea, vomiting, constipation, diarrhea, dry mouth, paresthesia, headache, dizziness, insomnia, hypoglycaemia, etc.)[1]. On the other side, numerous epidemiological studies suggest that being overweight or obese is associated with a 30-70% increased risk of colorectal cancer (CRC) in men [2]. This latter is the third most common cancer and the fourth leading cause of cancer related death worldwide [3].

Pancreatic acinar cells secrete pancreatic lipase (PL) (triacylglycerol acyl hydrolase), an important enzyme of pancreatic juice, responsible for the digestion of dietary triglycerides (about 50–70% of total dietary lipids) in the small intestine, and essential for their intestinal absorption and simulation. This enzyme is considered a primary modulator of lipid metabolism and thus can be the most potent target for the development of anti-obesity agents. Previously, several studies on plant extracts have been carried out to identify various potent inhibitors of PL [4]. Effectively, some flavonoid-rich diets have been associated with low obesity rates, which could be related to their potential to inhibit PL activity at a molecular level and *via* a sequestering mechanism by forming aggregates in aqueous media [5]. Yet, research on PL inhibitors from Algerian medicinal plants is insufficient, although traditional medicine is considered a common practice in this country to maintain health. Hence, some of these medicinal plants could be a source of PL inhibitors for use as anti-obesity agents.

Knowing that obesity is associated with different colorectal cancers, the inhibition of PL would play a key role in preventing obesity-related CRC. Medicinal plants are promising anticancer agents which provide countless phytochemical substances gifted with diverse biological activities [6]. Thus, the prevention or treatment of CRC by dietary phytochemicals with anti-obesity action as LP inhibition, offers beyond doubt a direction for the development of potential nutraceutical molecules of great therapeutic interest and low secondary effects.

This study was designed to investigate the PL-inhibitory and cytotoxic activities of three medicinal plants widely used in Algerian folk medicine, viz. *Olea europaea* L. (Oleaceae), *Pistacia lentiscus* L. (Anacardiaceae), and *Marrubium vulgare* L. (Lamiaceae), selected on the basis of the ethnomedical information. These three plants occupy an important part of herbal formulations in the treatment of different diseases and disorders in Algerian folk medicine. *O. europaea*, commonly called olive tree in English, olivier in French, and zeytoun in Arabic and in the local dialect, have been

indicated to reduce blood sugar, cholesterol, and uric acid. It has also been used to treat diabetes, hypertension, inflammation, diarrhea, respiratory and urinary tract infections, stomach and intestinal diseases, asthma, hemorrhoids, rheumatism, as laxative, mouth cleanser, and as a vasodilator [7].

M. vulgare L. is commonly called white horehound in English, Marrube blanc in French, farasioun abyad in Arabic, and merriwet in the local dialect. It has been used to fight diabetes, cellulite and obesity. It is also widely known as an emmenagogue, blood purifier, detoxifier, febrifuge, appetizer, gastroprotective, pectoral, antiseptic, cardiotonic, and diuretic [8,9]. *P. lentiscus* L. is commonly called lentisk-mastic tree in English, lentisque in French, and darw in Arabic and in local dialect. Its leaves have been traditionally used in the treatment of arterial hypertension due to their diuretic properties and for treating various diseases such as asthma, ulcer, diarrhea, inflammation, eczema, throat infections and diabetes. They are also provided with antibacterial, antifungal, antipyretic, astringent, hepatoprotective, expectorant, stimulant, and antiproliferative activities [9,10].

Hence, the present study aimed to assess the possible utilization of these plants as a novel source of PL inhibitors for obesity treatment, and to evaluate their antiproliferative effect on five different obesity-related colorectal cancer cell lines, viz. HT29, HCT116, SW480, SW620 and CACO2. Furthermore, some of the bioactive constituents that may be responsible for the bioactivities will be identified using thin-layer chromatography (TLC).

MATERIALS AND METHODS

Chemicals and biochemicals

Dulbecco Modified Eagle Medium (DMEM) containing 25 mM glucose was obtained from Invitrogen (USA). Unless stated otherwise, all reagents, drugs and chemicals were from Sigma (Dorset, UK). All of the chemicals and solvents used in the current study were purchased as the analytical grade from Sigma-Aldrich (St. Luis, MO, USA). For phytochemical analysis, analytical thin-layer chromatography (TLC) plates were obtained from Merk, USA. For spectrophotometric determinations, a UV-VIS spectrophotometer from SpectroScan 80D (UK) was used.

Plant material and extract preparation

The selected plants (*O. europaea*, *P. lentiscus* and *M. vulgare*) were collected from Jijel (Algeria) during spring 2013 (Fig. 1). Plant material was identified by Dr. Hanane Khennouf from the Department of Environment and Agronomy Sciences of the University of Jijel (Algeria). Fresh leaves of the selected plants were shade dried and ground into a fine powder (100 μm) using an electric grinder. The extracts were prepared by soaking 10 g of the dried powder of each plant material with 100 mL of 80% methanol for 72 h under continuous stirring. The solvents were then filtered, centrifuged, de-fatted by hexane and evaporated under vacuum at 40 °C using a rotary evaporator (Heidolph, LABOROT 4003). The obtained solid residues were stored in dry cold conditions until use.

Fig. 1. *Geographical location of the study area (Jjiel region)*

Pancreatic lipase (PL) inhibition assay

Inhibition of PL activity of the methanol extracts of the selected plants was assessed according to the method described by Foddai et al. [11] with slight modifications. Briefly, stock solutions (100 mg/mL) of each plant extract were prepared in 2.5 mM Tris-HCl buffer containing 2.5 mM NaCl (pH 7.4). The reference drug orlistat (dissolved in DMSO at 1 mg/mL) was used as positive control. To initiate the assay, 20 µL of plant extract (final concentrations 0.2 - 2000 µg/mL) or orlistat (final concentrations 0.01-0.4 µg/ mL) were suspended in 875 µL of tris buffer (2.5 mM, pH 7.4 with 0.1 M NaCl) and preincubated with 100 µL of 0.05% (w/v) pancreatic lipase (disolved in tris buffer 2.5 mM, pH 7.4 with 0.1 M NaCl) for at least 1 min prior to addition of the substrate. The reaction was then started by adding of 5 µL of the enzyme substrate (para-nitrophenyl butyrate dissolved at 5% (v/v) in DMSO) all in a final volume of 1000 µL. After shaking the reaction mixture for 30 seconds, the *p*-nitrophenol released during the reaction was measured at 410 nm one minute after adding the substrate then with 0.5 min intervals for 4 minutes. DMSO was used as negative control and its activity was also measured. The slope of the linear segment of the absorbance versus time profiles was used to quantify the inhibitory activity of PL according to the following formula:

$$\text{Inhibitory activity } \% = \frac{\text{Slope}_{\text{control}}^{-} - \text{Slope}_{\text{test}}}{\text{Slope}_{\text{control}}^{-}} \text{ x } 100$$

The IC_{50} (the concentration required for PL 50% inhibition) values were then calculated for the tested extracts and orlistat.

In vitro assay for antiproliferative activity

Cell lines and culture conditions

Colorectal cell lines used were HT29, HCT116, SW480, SW620 and CACO2. Human periodontal ligament fibroblasts (PDL) were also used. Cells were cultured in Dulbecco Modified Eagle Medium (DMEM) containing 25 mM glucose and supplemented with 10% heat inactivated bovine serum, 1% of 2 mmol/L L-glutamine, 50 IU/mL penicillin, and 50 µg/mL streptomycin. Culture medium was renewed once needed until cells reached 80 – 90% confluence.

Antiproliferative assay

Cells were seeded in 96-well plates with a density of 5×10^3 cells/well and incubated at 37 °C in a 95% humidified atmosphere containing 5% CO_2. After 24 h, the cells were treated with the extracts (initially dissolved in DMSO and further diluted in medium to produce 6 concentrations ranging from 5 to 200 µg/mL) and cisplatin (positive control used at concentrations ranging from 5 to 200 µg/mL) and maintained at the same conditions mentioned above for 72 h. At the end of the exposure time, cell growth was analyzed using the sulphorhodamine B (SRB) assay which estimates cell number indirectly by staining total cellular protein with the SRB stain.

Sulphorhodamine B (SRB) assay

Each well was coated with 200 µL of ice-cold 40% trichloro-acetic acid to fix the cells. The plates were incubated at 4 ºC for 1 h, then washed five times with cold water and left to dry in the air. After that, 50 µL of SRB stain 0.4 % w/v (prepared in 1% acetic acid) was added to each well and left in contact with the cells for 30 min at room temperature. Wells were then washed four times with 1% acetic acid until only the stain fixed by cells was left. The plates were allowed to dry, and then 100 µL of 10 mM Tris base (pH 10.5) was added to each well to solubilize the stain. The plates were shaken gently for 20 min on a plate shaker and the absorbance of each well was read on an ELISA reader at 570 nm. Control wells containing non-treated cells receiving DMSO instead of test compounds were also prepared in the same conditions. The percentage of cell survival was calculated as (OD test/OD control) × 100% and the antiproliferative activity of each compound was determined as (100 - the percentage of cell survival). IC_{50} values (concentration needed to reduce cell proliferation by 50% after 72 h of incubation compared to control wells) were calculated for the tested extracts and cisplatin [12].

Thin-layer chromatography analysis

For the TLC analysis aliquots of 1% methanol solutions of tested extracts and standards were applied on 10 × 12 cm plates precoated with silica gel 60 F_{254}. The elution was carried out using a polar and a weak polar solvent systems. The first one-dimensional TLC analysis was performed with ethyl acetate/formic acid/acetic acid/water (100/11/11/25) as a polar mobile phase whereas the second analysis was performed with toluene/diethyl ether/glacial acetic acid (50:50:5) as a weak polar mobile phase.

As standards rutin, vitexin, hyperoside, luteolin, kampferol, apigenin, quercetin, cafeic acid, ferulic acid, chlorogenic and acid hydroxyl-coumarin were used.

As spraying reagents 5% (v/v) ethanol/ diphenyl boric acid 2-aminoethyl ester followed by 5% (v/v) ethanol/ polyethylene glycol 4000 (PEG 4000) was used. Spots were visualized under UV light at 365 nm and the chromatographic profiles of the tested extracts were compared to phytochemical standards.

Statistical analysis

The values are presented as means ± S.D. (Standard Deviation) of 3-4 independent experiments. Statistical comparisons of the results were determined by ANOVA followed by Tukey's or Dunnett's multiple comparisons tests using Graphpad Prism version 7.00 for windows; GraphPad Software, La Jolla California USA. Results were considered significantly different when $p<0.05$.

RESULTS AND DISCUSSION

In vitro inhibitory effect of pancreatic lipase activity by the tested plant extracts

Pancreatic lipase (PL) plays a key role in the efficient digestion of triglycerides (TG) since it is responsible for the hydrolysis of 50 – 70% of total dietary fats. Fatty acids and monoglycerides generated from TG hydrolysis by different lipases, mainly PL, are absorbed into enterocytes and reused for TG synthesis. These newly synthetized TG are finally stored in adipocytes as the major source of energy [4]. Accumulation of adipocytes in various parts of the body leads to obesity. Therefore, the inhibition of PL constitutes one of the most widely studied mechanisms to determine the potential efficacy of natural products as obesity modulating agents. In fact, anti-lipase agents from natural plants have received a potent attention since they are safe and have fewer unpleasant side-effects [13-15].

In our current study, three methanolic extracts prepared from natural plants usually found in the Mediterranean basin and collected from Algeria (*O. europaea*, *P. lentiscus*, and *M. vulgare*) were examined for their anti-lipase activity at different concentrations (0.2 - 2000 µg/ml). Orlistat, a well-known anti-lipase drug, was used as a positive control at concentrations varying from 0.01 µg/ mL to 0.4 µg/ mL. Anti-lipase activity is expressed in terms of the percentage of inhibition (%) of PL (fig. 2) and IC_{50} (µg/mL) which represents the half maximal concentration of tested compounds to inhibit PL activity (Table 1). Thus, a lower IC_{50} value corresponds to a larger anti-lipase activity.

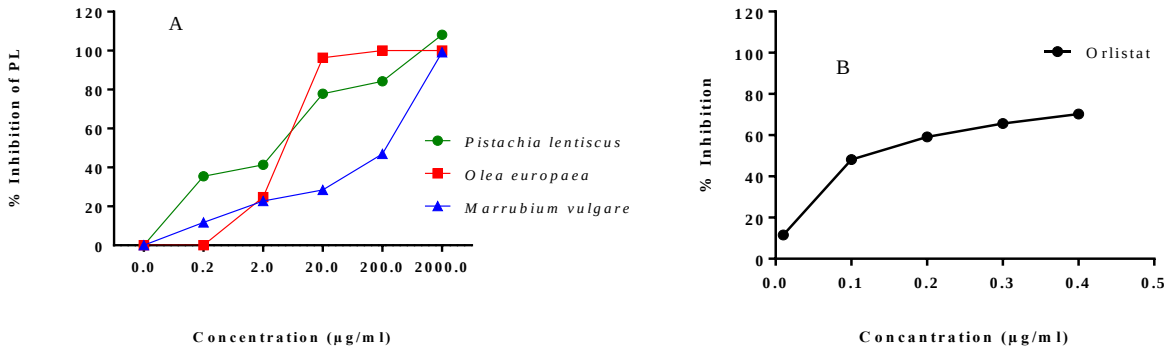

Fig. 2. *Percentage of pancreatic lipase inhibition (%) by Olea europaea, Marrubium vulgare and Pistacia lentiscus methanolic extracts (A) and orlistat (B).*

Table 1. *Pancreatic lipase IC_{50} values (µg/mL) of Olea europaea, Marrubium vulgare and Pistacia lentiscus methanolic extracts and orlistat.*

Scientific name	Family name	IC_{50} (µg/mL)
Pistacia lentiscus	*Anacardiaceae*	2.8 ± 0.2 *
Olea europaea	*Oleaceae*	9.7 ± 1.3 ***
Marrubium vulgare	*Lamiaceae*	50.2 ± 1.3 ***
Orlistat		0.11 ± 0.0

Results are mean ± S.D (n = 3 independent replicates). The IC_{50} of each extract was compared with that of orlistat by one-way ANOVA followed by Dunnett's multiple comparisons test. (*) $P<0.05$, (***) $P<0.001$.

As illustrated in Fig. 2 and Table 1, orlistat showed a significant inhibition of PL activity with an IC_{50} value of 0.11 ± 0.0 µg/mL. Comparable to orlistat performance, *P. lentiscus* extract showed a very potent anti-lipase activity with an IC_{50} value of 2.8 ± 0.2 µg/mL, which is in accordance with our earlier *in vivo* results that revealed a decrease in plasma triglycerides levels in alloxan-induced diabetic rats, due to administration of *P. lentiscus* methanol extract at a dose of 300 mg/kg [16].

Similar results were observed by Foddai et al. [11] for *P. lentiscus* and *P. terebinthus* grown in Sardinia, where crude aqueous extracts of leaves and fruits were evaluated for their PL inhibitory activity. In this study, a marked anti-lipase activity was reported for leaves extracts of these two *Pistacia* species with IC_{50} values of 6.1 ± 0.2 µg/mL for *P. lentiscus* and 9.0 ± 0.4 µg/mL for *P. terebinthus.* The fruit extracts however were not as efficient as the leaves extracts in inhibiting PL activity since IC_{50} values were much higher (230.7 ± 38.4 µg/mL for *P. lentiscus* and 125.2 ± 12.1 µg/mL *P. terebinthus*).

Similarly, *O. europaea* demonstrated a potent activity against PL, corresponding to an IC_{50} value of 9.7 ± 1.3 µg/mL. Only a few studies have evaluated the PL inhibitory activity of *O. europaea* before the present study. Among these, a survey was conducted by Danış et al. [17] to examine the inhibitory effect of PL by the crude methanol extracts of 23 different organs of some culinary, herbal, and aquatic plants, including *O. europaea* leaves, with claimed weight reducing effects in Turkish folk medicine. The authors classified *O. europaea* extract as a poor PL inhibitor since it only displayed 37% inhibition of PL activity. The accurate concentration used was nevertheless not specified. These discrepancies in activity between Algerian *O. europaea* and Turkish one may be related to qualitative and quantitative variation in active components, influenced by environmental and many other factors such as genetic factors, the chemotype of the species, soil characteristics, and the season during which the plants were collected [18]. In addition, methods of drying, extraction, and analytical conditions contribute to the differences in the chemical composition of plant extracts [19].

The present study revealed that *M. vulgare* extract exhibits a moderate anti-lipase activity (IC_{50} = 50.2 ± 1.3 µg/mL) in comparison to orlistat and the two other extracts. However, this result seems to be much better than that reported by Villa-Ruano and his colleagues [20] who investigated the anti-lipase activity of the crude ethanol extract of *M. vulgare,* grown in Mexico. To the best of our knowledge, this was the only work that evaluated the anti-lipase activity of *M. vulgare* before the present investigation. The authors reported that PL was inhibited by 13.02 ± 0.6% when 100 µg/mL of the extract

was used. They also confirmed the presence of flavonoids and tannins in *M. vulgare* extract

Qualitative analysis of the tested extracts by TLC showed the occurence of luteolin hyperoside, rutin and vitexin in both, *P. lentiscus* and *O. europaea* (Fig. 3, Table 2). The interesting anti-lipase activity shown by these extracts may probably be linked to the presence of these active compounds which may act synergistically.

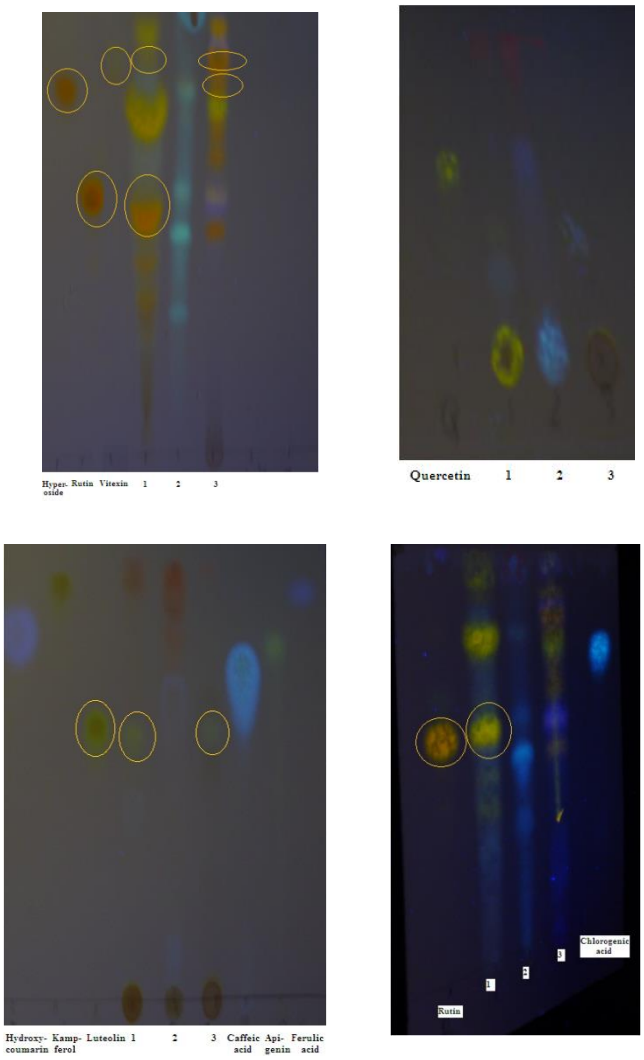

Fig. 3. *Thin layer chromatographic profiles of methanol extracts of Olea europaea (1), Marrubium vulgare (2) and Pistacia lentiscus (3). Circles indicate the presence of phenolic compounds.*

Table 2. *Coexistent phytocomponents in Pistacia lentiscus, Olea europaea and Marrubium vulgare extracts according to TLC chromatogram.*

	P. lentiscus	***O. europaea***	***M. vulgare***
Rutin	-	+	-
Vitexin	+	+	-
Hyperoside	+	-	-
Luteolin	+	+	-
Chlorogenic acid	-	-	-
Kampferol	-	-	-
Caffeic acid	-	-	-
Apigenin	-	-	-
Quercetin	-	-	-
Ferulic acid	-	-	-
Hydroxycoumarin	-	-	-

Previous reports indicated that phenolic compounds such as luteolin and rutin were found as the main active components in *Prunella vulgaris* L., one of the most promising Chinese herbs for lipase inhibition (74.7% inhibition at 200 μg/mL), studied by Zheng et al. [14]. Rutin isolated from *P. vulgaris* displayed an increased inhibitory effect on PL at concentrations of 25 μg/mL, 50 μg/mL, and 100 μg/ml (11.0 %, 24.4 % and 30.8 % respectively). The IC_{50} values were however not calculated.

The findings of the current study are in agreement with those of Yu et al. [21], where 75 % ethanol extracts of Chinese peanut shells showed 45.3 % inhibition of PL activity at 0.29 mg/mL. High performance liquid chromatography (HPLC) method, used for the qualitative determination of polyphenols in peanut shells extracts, revealed the presence of a complex mixture of polyphenols where luteolin was the major component. This latter compound was also found at high levels in three promising Chinese herbs with inhibitory activity of 17.3% on PL at a concentration of 25 μg/mL when tested by Zheng et al. [14].

The present results support those of Aruna et al. [22] who studied the effect of rutin administration in rats receiving a high-fat diet (HFD) and ethanol (EtOH) on serum lipase as a marker enzyme of pancreatic function. Their survey indicated that the co-administration of rutin caused a decrease in serum lipase level in a dose-dependent manner, which indicates a decrease in pancreatic damage, when compared to the control animals that received HFD-EtOH only. The optimum activity of rutin was observed at 100 mg/Kg of body weight.

Al-Hallaq et al. [15] reported that rutin isolated from *Crataegus aronia* L. exhibited higher efficacy in PL inhibition (IC_{50} = 77.3 ± 1.7 μg/mL), compared to different aqueous extracts of *C. aronia* (IC_{50} = 225.2 ± 33.4 μg/mL for aerial part and 286.1 ± 37.4 μg/mL for fruits-extracts).

In vitro modulation of proliferative activity in colorectal cancer cell lines by tested plant extracts

The current study evaluated the cytotoxic effect of the selected plant extracts on five different obesity-related colorectal cancer cell lines (HT29, HCT116, SW620, CACO2 and SW480). Cell viability was assessed using the SRB assay and the cytotoxic effect of the test extracts and cisplatin (antineoplasic drug) was determined (Fig. 4). The effect of

each compound on human periodontal ligament fibroblasts (PDL) was also assessed in order to evaluate their safety. The IC_{50} values were calculated and the results are shown in Table 3.

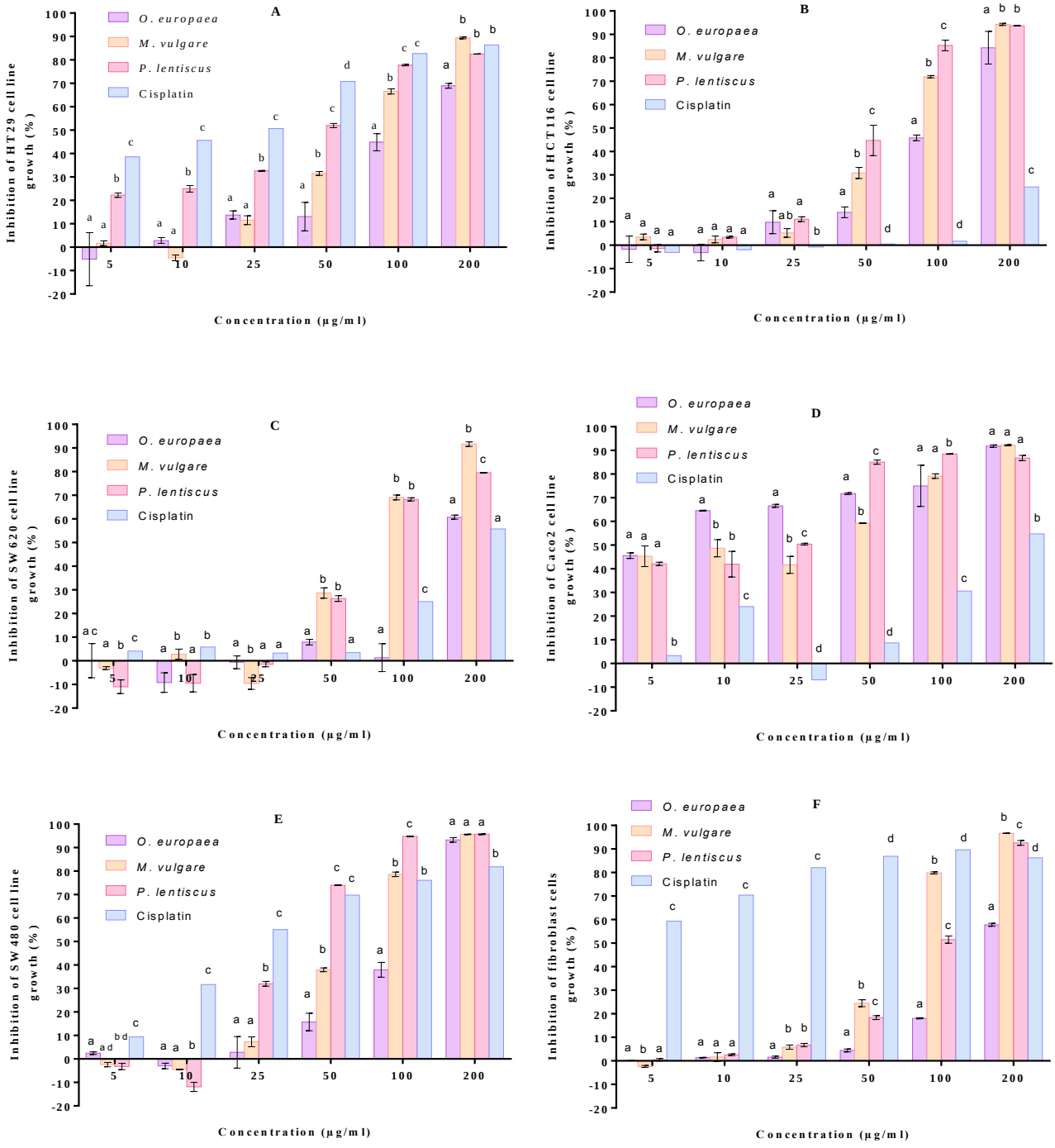

Fig. 4. *In vitro cytotoxic activity of O. europaea, M. vulgare and P. lentiscus methanolic extracts against colorectal cancer cell lines. A: HT29, B: HCT116, C: SW620, D: Caco2, E: SW480, F: periodontal ligament fibroblasts. Results are expressed as Mean ± SD, (n=3). Comparison was carried out between all groups at each concentration using Tukey's multiple comparisons test. Results were considered significant when p<0.05. Bars labeled with the same letters present no significant difference.*

Table 3. *IC_{50} values (µg/mL) of in vitro antiproliferative activity of Olea europaea, Marrubium vulgare and Pistacia lentiscus methanolic extracts and cisplatin on colorectal cancer cell lines.*

	IC_{50} value µg/Ml					
Treatment	**HT29**	**HCT116**	**SW620**	**CACO2**	**SW480**	**Fibroblasts**
Olea europaea	128.3±19.7	101.6±10.9 Iii	174.7±23.5 a, v	**11.8±1.2** Vii	101.6±15.4 Viii	176.1±5.3 a
Marrubium vulgare	65.5±8.1 b, i	**69.9±2.4** b, iii, iv	**75.2±5.8** b, vi	**36.6±4.3** b, vii	**70.3±2.5** b, viii, ix	71.8±1.6 b, x
Pistacia lentiscus	**33.5±1.4** i, ii	**47.8±7.2** c, iv	**75.3±6.1** c, vi	**18.0±1.2** Vii	**43±4.7** c, ix	81.4±5.5 c, x
Cisplatin	19.72±1.62 d, ii	878.12±105.4	173.8±21 v	175.41±21.05	26.9±0.061 d, ix	1.52±0.18 d

Results are mean ± SD (n = 4 independent replicates). On the same row, each cell mean is compared with the control cell mean (fibroblasts) using two-way ANOVA followed by Dunnett's multiple comparisons test. Values labeled with the same letter (a-d) are not significantly different from fibroblast values. On the same column, each cell mean is compared with every other cell mean using two-way ANOVA followed by Tukey's multiple comparisons test. Values labeled with the same roman numeral (i-x) present no significant difference.

As shown in Fig. 4 and Table 3, crude methanol extracts of *P. lentiscus* and *M. vulgare* exhibited an interesting antiproliferative activity against all the colorectal cancer cell lines studied with IC_{50} values ranging from 18.0±1.2 µg/mL to 75.3±6.1 µg/mL for *P. lentiscus* and from 36.6±4.3 µg/mL to 75.2±5.8 µg/mL for *M. vulgare*. They were particularly active on Caco2 cell line. This later was also sensitive to *O. europaea* treatment with an IC_{50} equal to 11.8±1.2 µg/ml. However, *O. europaea* was less effective on the other cell lines (IC_{50} values ranging from 101.6±10.9 µg/mL to 174.7±23.5 µg/mL).

P. lentiscus was the most effective extract since it showed the smallest IC_{50} values among the three tested extracts (18.0±1.2 µg/mL on Caco2, 33.5±1.4 µg/mL on HT29, 43±4.7 µg/mL on SW480 and 47.8±7.2 µg/mL on HCT116). In addition, there were no statistically significant differences between the IC_{50} obtained for *P. lentiscus* on HT29 and SW480 and those obtained for cisplatin on the same cell lines (19.72±1.62 µg/mL and 26.9±0.061, respectively). On the other hand, cisplatin was only active on HT29 (IC_{50} = 19.72±1.62 µg/mL) and on SW480 (IC_{50} = 26.9±0.061 µg/mL). In fact, the other cell lines were cisplatin-resistant with IC_{50} values of 173.8±21 µg/mL on SW620, 175.41±21.05 µg/mL on Caco2 and 878.12±105.4 µg/mL on HT116. Besides, it exerted an unselective cytotoxicity in fibroblast incubations, translated by a very minor IC_{50} value (1.52±0.18 µg/mL).

Unlike cisplatin, the test extracts exhibited higher IC_{50} values on PDL (176.1 ± 5.3 µg/mL for *O. europaea*, 81.4 ± 5.5 µg/mL for *P. lentiscus* and 71.8 ± 1.6 µg/mL for *M. vulgare*) than those determined against the selected cancer cell lines. This suggests their relative preferential selectivity for cancer tissues over normal ones.

Currently, there is an increasing interest in the phenolic compounds in olive leaves, due to their countless biological properties. Zeriouh et al. [23] have investigated the effects of the phenolic fraction of the olive leaves on colon tumor development in HCT116 xenograft athymic nude mice, and on the growth of the HCT116 and HCT8 colon cancer cell lines. They observed that olive leaf polyphenol-rich extract limited growth of the HCT116 tumor *in vivo* and induced apoptosis in both colorectal cancer cell lines *via* activation of caspase-3, -7, and -9, in addition to mitochondrial production of reactive oxygen species.

Five groups of phenolic compounds were principally found in the olive leaves: oleuropeosides (oleuropein and verbascoside); flavones (luteolin-7-glucoside, apigenin-7-glucoside, diosmetin-7- glucoside, luteolin, and diosmetin); flavonols (rutin); flavan-3-ols (catechin), and substituted phenols (tyrosol, hydroxytyrosol, vanillin, vanillic acid, and caffeic acid) [24].

The most abundant compound in olive leaves, oleuropein, has been widely investigated for its antiproliferative properties and shown to exhibit good activity. Morana et al. [25] have studied the activity of oleuropein on two human osteosarcoma cell lines (MG-63 and Saos2) and found it exerts toxic effects on both cell lines (IC_{50} range from 247.4 - 475.0 μM for MG63 cells and from 798.7 - 359.9 μM for Saos2 cells) in a concentration- and time-dependent manner.

Oleuropein has also been reported to reduce the cell viability of LNCaP and DU145 prostate cancer cell lines by inducing necrotic cell death and apoptosis *via* a dose-dependent increase in the levels of reactive oxygen species (ROS). In addition to its pro-oxidant effect on cancer cells, it has exhibited an antioxidant effect on non-malignant control cells, suggesting that oleuropein behaves as an antioxidant in normal cells and as a pro-oxidant in neoplastic cells [26].

Due to the presence of oleuropein and hydroxytyrosol in olive oil, the oil has been reported to possess anti-proliferative effects *in vitro* on HT-29 and SW620 colorectal cancer cell lines. The inhibition of proliferation of SW620 cells by hydroxytyrosol was found to be mediated by inhibiting the expression and enzymatic activity of fatty acid synthase (FAS), a key anabolic enzyme of biosynthesis of fatty acids, which was reported to be highly expressed in various types of common human cancers, including colon cancer. The anti-proliferative effect in HT-29 cells was FAS-independent. Oleuropein has been found to exert a non-dependent anti-proliferative effect on SW620 cells only. In addition, both olive oil polyphenols were found to induce apoptosis in both cell lines through the block of the cell cycle in the S phase [27].

Balan et al. [28] demonstrated that the hexane extract of crude Chios mastic gum (He-CMG), a resin produced by *P. lentiscus* var. *Chia* (grown in the southern region of Chios island, Greece) can induce G1-phase arrest, loss of adhesion to the substrate, and subsequent apoptosis in human colon HCT116 cancer cells *in vitro*. In fact, they observed that the presence of different concentrations of He-CMG (25, 50, and 100 μg He-CMG/ml) resulted in a markedly visible and concentration-dependent disturbance in the cell cycle including a decrease in G2, the presence of more cells progressing through the S-phase at the expense of the G2-fraction, and appearance of apoptotic cells. They concluded that the hexane extract of crude Chios mastic gum can induce the anoikis (inappropriate or loss of epithelial cell adhesion to the extracellular matrix, provoking apoptosis) form of apoptosis in HCT116 human colon cancer cells *in vitro*, associated with the activation of caspases-8 and -9. However, the chemical composition of He-CMG has not been analyzed and the active constituents have not been identified.

Further studies conducted by Dimas et al. [29] extended this research to investigate the *in vivo* anticancer activity of He-MG in human HCT116 tumors xenografted in immunodeficient SCID mice model. He-MG was injected intraperitoneally to the HCT116 tumor bearing SCID mice at different administration schedules and doses ranging from 100 to 220 mg/kg of body weight, then tumor size was checked. The results indicated that He-MG administered at a dose of 200 mg/kg inhibited tumor growth by approximately 35% in the absence of toxicity (side-effects) after 35 days.

Also, *M. vulgare* has been studied by many researchers for its cytotoxic effect against a variety of cancer cell lines such as MCF7 (breast cancer cell line), HELA (cervix cancer cell line), HEPG2 (Liver cancer cell line), SK-N-BE(2)-C (human bone marrow neuroblastoma cells) and WEHI-164 (mouse fibro sarcoma) [30,31]. However, only few reports about its cytotoxic effect on human colorectal cancer cell lines are available in the literature. Belayachi and his colleagues [32] have screened different extracts of *M. vulgare* collected in Morocco across a panel of human cancer cell lines, among them two colorectal adenocarcinomas (SW620 and SW480). Their results indicated that the crude methanol extract of *M. vulgare* was totally inactive against both cell lines, whereas dichloromethane fraction showed promising activity against the SW620 cell line with an IC_{50} value of 10.58 ± 4.37 µg/mL. They suggested that treatment with the extract increased cellular necrosis, without the presence of a detectable apoptotic cell fraction.

Many researchers focused on the potential efficacy of pure flavonoids as antiproliferative agents. They realized that luteolin was among the most effective flavonoids studied, and that the block of the development of cancer cells *in vitro* (IC_{50} value between 3 and 50 µg/ml) as well as *in vivo* (IC_{50} value between 5 and 10 mg/ kg) was conducted through a variety of mechanisms such as the protection from carcinogenic stimuli, the inhibition of tumor cell proliferation, the induction of cell cycle arrest and the induction of apoptosis *via* intrinsic and extrinsic signaling pathways [33].

Lim et al. [34] observed cell cycle arrest in the G2/M phase by regulation of cyclin B1 expression, cell division cycle activity, and apoptosis in HT-29 human colon cancer cells at concentrations varying from 20 to 60 µM, contributing significantly to the antiproliferative effect of luteolin. Chen et al. [35] have investigated the influence of luteolin on diethylstilbestrol (DES)-induced cell proliferation, nuclear tyrosine kinase activity, and expression of insulin-like growth factor-I (IGF-I) receptor in Syrian hamster renal epithelial cells. Their results revealed that co-treatment with luteolin inhibited cell proliferation through the inhibition of both nuclear tyrosine kinase activity and of IGF-I receptor expression.

Manju et al. [36] studied the chemopreventive effect of luteolin on bacterial enzymes β-glucuronidase and mucinase in a colon carcinogenesis model induced by dimethylhydrazine (DMH) in rats. In fact, an increase in β-glucuronidase activity enhances the hydrolysis of glucuronide conjugates, liberating toxins, while an increase in the mucinase activity induces hydrolysis of protective mucins in colon. Oral administration of luteolin at different doses caused a significant decrease of β-glucuronidase and mucinase activities in colon bacteria, leading to a reduction of both tumors' number and size by 70 to 90 %, thus exerting chemopreventive and anticarcinogenic effects against DMH-induced colon cancer.

The results of a study conducted by Volate et al. [37] showed that diet containing rutin was able to suppress aberrant crypt foci (ACF) in an azoxymethane-induced rat

colon cancer model. Histological analysis of the colon mucosa revealed that rutin supplements induced apoptosis, leading the authors to suggest that rutin supplements may exert significant and potentially beneficial effects on decreasing the amount of precancerous lesions in the colon.

Yang et al. [38] investigated the anticancer effects of rutin on LoVo human colon cancer cell lines *via* assessment of cell viability, cytotoxicity, apoptosis, and intracellular H_2O_2 and $O^{2\bullet-}$ production, as well as cell cycle analysis. They observed that rutin inhibited the proliferation of LoVo cancer cells in a dose-dependent manner, with an IC_{50} value of 29.1 μM. Furthermore, they revealed that this antiproliferative activity of rutin could be mediated through cell-cycle arrest in the S phase and cell apoptosis induction via the generation of reactive oxygen species (ROS).

Guon and Chung [39] have demonstrated that hyperoside and rutin isolated and purified from the roots of *Nelumbo nucifera* significantly decreased cell viability in HT-29 colon cancer cell lines by inducing mitochondrial apoptosis through a caspase-dependent mechanism. The authors suggested that the activation of the mitochondria-dependent apoptotic pathway by hyperoside and rutin occurs via modulation of expression of apoptosis regulatory proteins Bcl-2-associated X protein (Bax) and B-cell lymphoma 2 (Bcl2). In fact, hyperoside and rutin increased the expression of Bax (proapoptotic) but decreased the expression of Bcl-2 (antiapoptotic), each in a dose-dependent manner, leading to the activation of cleaved caspases-3, -8, and -9 and cleaved poly-(ADP-ribose) polymerase (PARP). Hyperoside and rutin however did not affect the proliferation of colon normal cells.

CONCLUSION

The crude methanol extracts of the three selected plants from the Algerian traditional medicine were tested *in vitro* for their effects on pancreatic lipase activity, and against five obesity-related human colorectal cancer cell lines, namely HT29, HCT116, SW620, CACO2, and SW480. To the best of our knowledge, these traditional medicinal plants, grown in Algeria have not been previously screened for their anti-lipase and anti-proliferative activities against the selected colorectal cancer cell lines, except for *O. europaea* which has already been tested against HT29.

The findings of the present investigation indicated that the studied extracts showed potent inhibitory activity against pancreatic lipase, in particular, *P. lentiscus* and *O. europaea* which can be therefore considered as promising sources of anti-obesity agents for body weight management.

The screening of methanolic extracts of the three medicinal plants against a panel of obesity-related human colorectal cancer cell lines revealed that the CACO2 cell line was sensitive to the three extracts while HT29, SW480 and HCT116 cell lines were especially sensitive to *P. lentiscus.* The other cell lines, however, showed moderate sensitivity to the extracts. Remarkably, the extracts exhibited a pertinent privileged selectivity for cancer cell lines over normal ones, in contrast to the commercial drug used as a control (cisplatin).

Although phytochemicals possessing anti-lipase and anti-proliferative activities were found to coexist in the selected plants, we could not conclude whether or not these substances were the only contributors to their excellent properties. Therefore, the main effective components of each extract as well as the molecular mechanisms by which these extracts exert their activities should be further investigated. Furthermore, more research is necessary to investigate *in vivo* bioactivity and cytotoxicity of the extracts to

explore in more depth their potential beneficial use in the treatment of obesity and cancer.

Acknowledgements. The authors would like to thank the University Mohammed Seddik Benyahia-Jijel and the General Direction of Scientific Research and Technological Development (Algeria) for financial support of this research. The authors are also grateful to Professor Nizar Mhaidat (The Jordan University of Science and Technology, Jordan) and Professor Yasser Bustanji (The University of Jordan, Jordan) for providing the cell lines, and to Dr. Hanane Khennouf (The University of Jijel, Algeria) for botanical identification of the plant material.

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