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Inhibition of *Candida rugosa* Lipase by Secondary Metabolites Extracts of Three Algerian Plants and their Antioxydant Activities

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Abstract: Lipase inhibitors have generated a great interest because they could help in the prevention or the therapy of lipase-related diseases. Therefore, the aim of this work was to evaluate the inhibitory effect of secondary metabolites extracts such as phenolic compounds and saponins of three Algerian medicinal plants: *Achillea santolina, Inonotus hispidus* and *Zizyphus lotus*, indeed their antiradicalaire activity using DPPH• (1, 1-diphenyl-2-picryl-hydrazyl). The phenolic extracts have shown a strong antiradicalaire activity than the saponin extracts with EC50 values ranged from 6 to 11 µg/ml and from 51 to 82 µg/ml, respectively. The enzymatic inhibition produced by these plant extracts is described here for the first time. The results have shown that the phenolic extracts are more potent than the saponin extracts with Ki values ranged from 0.011 mg/ml to 0.027 mg/ml for phenolic extracts, and ranged from 0.071 mg/ml to 0.69 mg/ml for saponin extracts. The nature, mechanism and possible physiological relevance of lipase inhibition by extract components are discussed.

Keywords: *Achillea santolina*, *Inonotus hispidus*, *Zizyphus lotus*, *Candida rugosa* lipase, inhibition, saponins extracts, phenolic extracts, DPPH•.

1. INTRODUCTION

Lipase is an enzyme that hydrolyzes triacyl glycerols (TGs). The digestion and absorption of natural lipids begin with hydrolysis by pancreatic lipase. The activity of this enzyme greatly affects the metabolism of fat and the concentration of TAG in blood [1]. Obesity is caused as a result of an imbalance between energy intake and expenditure. Excess energy is stored in fat cells, which enlarge or increase in number.

Human obesity is one of the most serious health problems. Obesity is associated with an increased risk of several serious diseases including hypertension, coronary heart disease, type II diabetes, stroke, osteoarthritis and cancer. Consumption of the dietary fat is an important contributor to human obesity. Gastric and pancreatic lipases (PL) are the principal lipolytic enzymes in the gastrointestinal (GI) tract that are responsible for the hydrolysis of triacyl glycerides (TAG). Thus, inhibition of lipase in the GI tract reduces the amount of fat that can be absorbed [2]. It is generally thought that a potent and specific inhibitor of PL could be useful in the treatment of obesity [3]. Orlistat, a potent inhibitor of PL and one of the best-selling drugs worldwide, has proved useful in the treatment of obesity, but side effects due to its potency, such as fecal incontinence, have also arisen [3]. Indeed, *Propioni bacterium acnes* lipase and its inhibition by antiacne compounds have been studied because the fatty acids produced by *P. Acnes* lipase activity on sebaceous triglycerides induce severe inflammation. Tetracyclines have been the most common systemic therapy for acne due to their beneficial clinical effects, their inhibition of lipase activity of *P. acnes* as well as their inhibition of *P. acnes* chemotaxis [4]. Therefore, research on new lipase inhibitors for the therapy of these diseases and also for other pathologies like ulcer and cutaneous diseases [5] has generated a great interest. Recently, inhibitors of lipase and lipid absorption have been isolated from natural sources with the aim of preventing and treat ingmetabolic syndrome. For our knowledge, No report has been found for evaluating the inhibitory activity of these three Algerian medicinal plants. In the present study, we report the inhibitory effect of phenolic and saponins extracts of three Algerian medicinal plants on *Candida rugosa* lipase for the first time.

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2. MATERIALS AND METHODS

2.1. Materials

Achillea santolina, Inonotus hispidus and *Zizyphus lotus* were collected in April 2009 in Laghouat City, South of Algeria. The plant material (*Achillea santolina* and *Zizyphus lotus*) was identified by two of the authors (Benarous and Yousfi). *Inonotus hispidus* was identified by Bernard Duhem, Museum National d'Histoire Naturelle, Laboratoire de Cryptogamie, 12 Buffon street, 75231 Paris, FRANCE. Voucher specimens were deposited in the laboratory of fundamental sciences of Laghouat University. *Candida rugosa* lipase, p-nitrophenyllaurate, 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) and all other reagents were purchased from Sigma-Aldrich. All other chemicals and solvents used were of analytical grade.

2.2. Phytochemical Tests

Before the extraction procedure, phytochemical tests have been realized using standard phytochemical procedures [6-11] to prove that the plants mentioned above contain

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phenolic compounds and saponins. These tests have confirmed that the three Algerian plants *Achillea santolina, Inonotus hispidus* and *Zizyphus lotus* contain phenolic compounds which are mentioned in previous works [12, 13]. In this work, Saponins have been found in the fungus *Inonotus hispidus* and *Achillea santolina* for the first time. Indeed, we have found that Algerian *Zizyphus lotus* is rich on saponins which is mentioned for the Tunisian plant by Renault *et al*. (1997) [14].

2.3. Preparation of Plant Extracts

The plants were air dried and ground into fine powder. The powdered material (5 g) was extracted for 30 h with 100 ml of alcohol mixture: 5/4/1 (v/v/v) ethanol/ methanol/water at room temperature. After removal of methanol/ethanol under reduced pressure in a rotary evaporator at 40°C, the remaining aqueous solution of the extraction is defatted twice with petroleum ether to remove lipids and pigments. Then, the aqueous solution obtained is extracted with ethyl acetate. The ethyl acetate (Et-O-Ac) fraction is dried by addition of a sufficient amount of anhydrous sodium sulphate, and then evaporated to dryness using a rotary evaporator. The residue is dried, dissolved in 10 ml of methanol and kept at +4°C, this fraction contain phenolic compounds. The aqueous solution obtained is extracted twice with butanol. The butanol (BuOH) fraction is evaporated to dryness using a rotary evaporator. The residue is dried, dissolved in 10 ml of aqueous ethanol 50 % and kept at +4°C, this fraction contain saponins. All of these extracts have been tested phytochemically using the same procedure mentioned in section 3.1 to prove the existence of phenolic compounds in Et-O-Ac fractions and saponins in BuOH fractions.

2.4. Quantification of Total Phenols, Flavonoids and Saponins

The amount of total phenolics in plant extracts was estimated by the Folin–Ciocalteu reagent [15, 16], which is considered as the best method for total phenolics (included tannins) determination [17]. The total phenolic content of the ethyl acetate and butanol extracts was expressed as gallic acid equivalents (GAE). Whereas, the total flavonoid content in the ethyl acetate and butanol extracts was determined by a slightly modified version of the method of Zhishen *et al.* (1999) and Dewanto *et al.* (2002) [18, 19]. Flavonoid contents were calculated using a standard calibration curve, prepared from quercetin. The total saponins content of the butanol extracts was estimated by the method of Hiai *et al.* (1976) [20] and was expressed as digitonin equivalents.

2.5. Free Radical Scavenging DPPH•

The method is based on the reduction of alcoholic DPPH• solutions in the presence of a hydrogen donating antioxidant. DPPH• solutions show a strong absorption band at 515 nm with a deep violet color. The absorption vanishes and the resulting decoloration is stochiometric with respect to degree of reduction. The remaining DPPH•, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant [21-23]. Radical scavenging activity of plant extracts against stable DPPH• (2,2-diphenyl-2- picrylhydrazylhydrate) was determined spectrophotometrically by the slightly modified method of Brand Williams *et al*. (1995) [24], 1 ml of various concentrations of sample solution diluted in methanol were added to 1 mL of methanolic DPPH• radical solution (250 μM). The mixture was then shaken vigorously and allowed to stand at room temperature in obscurity for 30 min. The decrease in absorption was measured at 515 nm against a blank. Inhibition of DPPH• free radical in percent (I %) was calculated as follows:

$$I\% = \left(1 - \left({A_{sample}}/{A_{blank}}\right)\right) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The antioxidant activity of the extract was expressed as an EC50 value defined as the concentration (in μg/ml) of the extract that inhibited the formation of DPPH• radicals by 50%. Triplicate measurements were carried out and their activity was calculated by the percentage of DPPH• scavenged. The DPPH• radical scavenging activity obtained for each plant extract was compared with that of Vitamin C, Trolox, gallic acid and quercetin.

2.6. Assay of *Candida rugosa* Lipase Inhibition

The method of Prim *et al*. (2000); Prim *et al*. (2003) and Ruiz *et al*. (2006) [5, 25, 26] was adopted for assays inhibition with a lot of modifications. The non-colored substrate p-NPL was dissolved at 10 mM in 2-propanol by vortex for 3 min. A 1:10 (v/v) dilution in phosphate saline buffer with pH 7 was prepared with gentle agitation until an optically clear and stable emulsion was achieved. Briefly, 100 μl of enzyme solution (2.5 mg/ml) were added into 100 μl of diluted plant extracts (as inhibitor) which it was previously preincubated for 15min at 37 °C. Then, 900 μl of the substrate mixture were added, this reaction mixture were incubated for 15 additional minutes at 37 °C and the reaction was terminated by addition of 100 μl of 0.1 M Na_2CO_3. Released p-nitrophenol (the yellow-colored product of the reaction) was immediately determined by measuring the absorbance at λ = 405 nm in a Spectophotometer (Jenway 6405). All assays were performed within the linear range of absorbance of the p-nitrophenol (p-NP) calibration curve, which was obtained under the same assay conditions. One unit of activity was defined as the amount of enzyme that released 1 μmol of p- NP per minute under the assay conditions described.

Inhibition kinetics were performed using varying concentration of phenolic and saponin extracts in the assay mixture. Reciprocal initial velocity plots versus inhibitor concentration were used to determine the mode of inhibition. The inhibition constants (Ki) were calculated according to the Dixon model from curve fits using Microsoft Excel 2010. The concentrations yielding a lipase inhibition of 50% (IC50) were calculated from the inhibition vs. plant extract concentration curves by regression analysis. All experiments were done at least in triplicate.

3. RESULTS AND DISCUSSION

3.1. Quantification of Total Phenols, Flavonoids and Saponins

Solvent extraction is a commonly used method to obtain secondary metabolites from plant materials. However, it has been suggested that no single solvent can extract all the secondary metabolites from plants because of its variation in solubility and polarity [27-30]. For this reason and after many experiments we have used the solvent mixture ethanol/methanol/water with 5/4/1 (v/v/v). The extraction yields in both fractions (Et-O-Ac and BuOH) ranged from 1.46 to 9.21 % where BuOH extracts are showing a higher ratio than the Et-O-Ac extracts (Table **1**). This result could interpret by the polarity, high molecular weight and solubility of phenolic compounds, flavonoids and saponins to be extracted with BuOH solvent [31-34].

The amount of total phenolic varied in the three plants with the both solvent extraction and ranged from 2.66±0.18 to 6.35±0.3 mg GAE/g of dry material for Et-O-Ac fractions, and ranged from 0.26±0.04 to 0.82±0.03 mg GAE/g of dry material for BuOH fractions (Table **1**). The Et-O-Ac extracts have shown higher total phenolic and total flavonoids content than the BuOH extracts. We have marked that even the BuOH fraction showed the higher ratios of dry residue, it showed the lower quantities of total phenolic and flavonoid content than the Et-O-Ac fractions which prove that the BuOH has extracted the compounds with high molecular weight but without phenolic structures, we admit that these molecules are saponins. It has been noted that the amount of total phenolic compounds in Asteraceae varieties which is represented here by *Achillea santolina* is higher than the other families Rhamnaceae and Basidiomycetes. However, this phenolic content of *Achillea santolina* is lower than other plants in the same family such as: *Artemisia campestris* with 20.38±0.30 mg GAE/g dw and *Globularia alypum* with 21.54±0.81 mgGAE/g dw. But this amount is higher than *Artemisia arboresens* with 3.42±0.50 mg GAE/g dw and *Ruta Montana* with 3.13±0.30 mg GAE/g dw [35, 36]. The total phenolic content in the plants tested in this study was lower than those reported for the most of other Asian medicinal and common dietary plants [37-41].

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolic. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties [36]. In this study, the content of flavonoids (mg/g), in quercetin equivalent which varied from 0.1 to 0.97 has shown higher values in the Et-O-Ac extracts than the BuOH extracts. Therefore, these amounts presented lower values than the most of African and Asian medicinal plants [35-37].

As we have already mentioned that these plants contain saponins, we have estimated the quantity of saponins in the BuOH fractions. Saponins are widely distributed among plants and have a wide range of biological properties. The more recent investigations and findings on their biological activities were summarized [42]. The amount of saponins of the three plants was ranged from 3.84±0.38 to 123.7±5.69 mg DE/g dw. Total saponins content has presented the highest value for the *Zizyphus lotus* with 123.7±5.69 mg DE/g dw which is higher than *Tribulus terrestris* [43], and the lowest value for *Inonotus hispidus* with 3.84±0.38 mg DE/g dw but it is higher than *Ipomoea batatas* [44].

These saponins quantities in the three Algerian plants are higher than the total phenolic and flavonoids quantities in both fractions (Et-O-Ac and BuOH).

3.2. Free Radical Scavenging DPPH•

The aim of finding potent antioxidants is to reveal a relationship between antiradicalaire activity and lipase inhibitory activity. The 1,1-diphenyl-2-picrylhydrazine (DPPH•) radical scavenging is one of the most extensively used antioxidant assays for plant samples [45]. The phenolic and saponins extracts were evaluated for their antiradicalaire activity using a chemical method (DPPH• assay). The DPPH• was used in this assay to assess the free radical scavenging (antioxidant) properties of plant extracts and Trolox, vitamin C, gallic acid and quercetin, well known as a potent antioxidant controls [46-48]. A parameter introduced recently for the interpretation of the results from the DPPH• method is the ‘‘efficient concentration” or EC50 value. This is defined as the concentration of the substrate that causes 50% loss of the DPPH• activity. The EC50 values were calculated by the linear regression method of plots of the percent of antiradical activity against the concentration of the tested compounds [36]. The concentration of inhibitors has been calculated in µg/ml. The results obtained are summarized in (Table **2**). All the Et-O-Ac extracts showed a beneficial effect against free radical damage compared with the antioxidant controls but total saponin extracts in BuOH

Table 1. Extraction Yield, Total Phenols, Total Flavonoids and total Saponins Contents for the Studied Plants mg/g Dry Weight

Plant	Part	Fraction	*Extraction Yield %*	Total Phenols (mg GAE /g dw)	Total Flavonoids (mg QE /g dw)	Total Saponins (mg DE /g dw)
Achillea santolina	Leaves and flowers	Et-O-Ac	5.20	6.35±0.30	0.97±0.00	-
		BuOH	8.90	0.82±0.03	0.28±0.02	21.90±2.09
Zizyphus lotus	Leaves	Et-O-Ac	4.94	6.09±0.31	0.83±0.01	-
		BuOH	9.21	0.63±0.38	0.69±0.04	123.70±5.69
Inonotus hispidus	fungus	Et-O-Ac	1.46	2.66±0.18	0.53±0.01	-
		BuOH	4.20	0.26±0.04	0.10±0.00	3.84±0.38

Et-O-Ac: ethyl acetate fraction, **BuOH:** n-butanol fraction, **GAE:** Gallic acid equivalent, **QE:** Quercetin equivalent, **DE:** Digitonin equivalent, **dw**: dry weight.

Table2. Free Radical (DPPH•) Scavenging Activity of the Plant Extracts Compared to the Antioxidant Standards

Plant	Fraction	EC 50 (µg/ml)
Achillea santolina	Et-O-Ac	11 ± 1,58
	BuOH	51 ± 2,65
Zizyphus lotus	Et-O-Ac	6 ± 0,80
	BuOH	82 ± 11,68
Inonotus hispidus	Et-O-Ac	8 ± 0,90
	BuOH	75 ± 1,59
Gallic acid		5 ± 0,14
Quercetin		4 ± 0,20
Trolox		7 ± 0,40
Vitamin C		6 ± 0,04

fraction exhibited a moderate antiradicalaire activity than the phenolic compounds (gallic acid and quercetin) and the synthetic antioxidants (trolox and vitamin C). It has been reported in many researches that saponins showed a moderate antioxidant activity [44, 49, 50]. The phenolic compounds and the flavonoids have registered a higher activity than the most medicinal plants mentioned in Djeridane *et al*. (2010) [36] such as: *Artemisia campestris* with EC50 of 09.61 ± 0.02 µg/ml, *Cleome arabica* 13.15 ± 0.01 µg/ml, *Deverra scoparia* 14.70 ± 0.03 µg/ml and *Ononis angustissima* 20.83 ± 0.08 µg/ml and *Teucrium-polium* flavonoids in Sharififar *et al*. (2009) [51]. Furthermore, the phenolic compounds of *Inonotus hispidus* have shown a powerful activity and we suggest that it is due to the potent phenols such as Hispolon and Hispidin as registered by Yousfi *et al*. (2009) [13]. It can be observed that the phenolic content of the extracts had no significant correlation with their antiradical activity as measured by the DPPH• assay (R^2<0.1).

3.3. Assay of *Candida rugosa* Lipase Activity

The effect of several polyphenol, flavonoids and saponins extracts on *C. rugosa* lipase was analyzed to evaluate their potential as antilipase drugs. Recently, inhibitory effects of these natural secondary metabolites on pancreatic lipase were reported to be involved in anti-obese effects such as flavonoids (e.g. Mangiferin, Hesperidin) [52-54] and saponins (e.g. the asaponins, chikusetsusaponins) [55, 56]. In this study, *Candida rugosa* lipase has shown normal Michaelis kinetics on p-nitrophenyllaurate (p-NPL) and for each extract the IC50 values were calculated from the plot of the enzyme activity as a function of phenolics and saponin concentration. Among the extracts tested, Et-O-Ac extracts were more active than BuOH extracts and the only inactive one was the BuOH extract of *Inonotus hispidus* (Table **3**, Fig. **1**); the different inhibition produced by these extracts is probably related to their substances with different chemical structures and physicochemical properties.

Table 3. IC50, Ki Values and Type Inhibition for Each Extract

Plant	Organic Fraction	Ext	IC_{50} (mg/ml)	Ki (mg/ml)	Inhibition Type
Achillea santolina	**Ac-O-Et**	**dry res**	2.32±0.25	0.14±0.01	***Uncompetitive***
		phe	0.37±0.00	0,02±0.00	
	BuOH	**dry res**	4.83±0.15	0.30±0.00	***Uncompetitive***
		phe	0.05±0.00	0.00±0.00	
		sap	1.19±0.03	0.07±0.00	
Zizyphus lotus	**Ac-O-Et**	**dry res**	3.70±0.00	0.22±0.00	***Uncompetitive***
		phe	0.45±0.00	0.03±0.00	
	BuOH	**dry res**	8.27±0.23	0.50±0.01	***Uncompetitive***
		phe	0.06±0.00	0.01±0.00	
		sap	11.53±0.46	0.69±0.03	
Inonotus hispidus	**Ac-O-Et**	**dry res**	2.22±0.03	0.04±0.00	***Uncompetitive***
		phe	0.56±0.02	0.01±0.00	

Dry res: dry residue, **phe**: phenols, **sap**: saponins

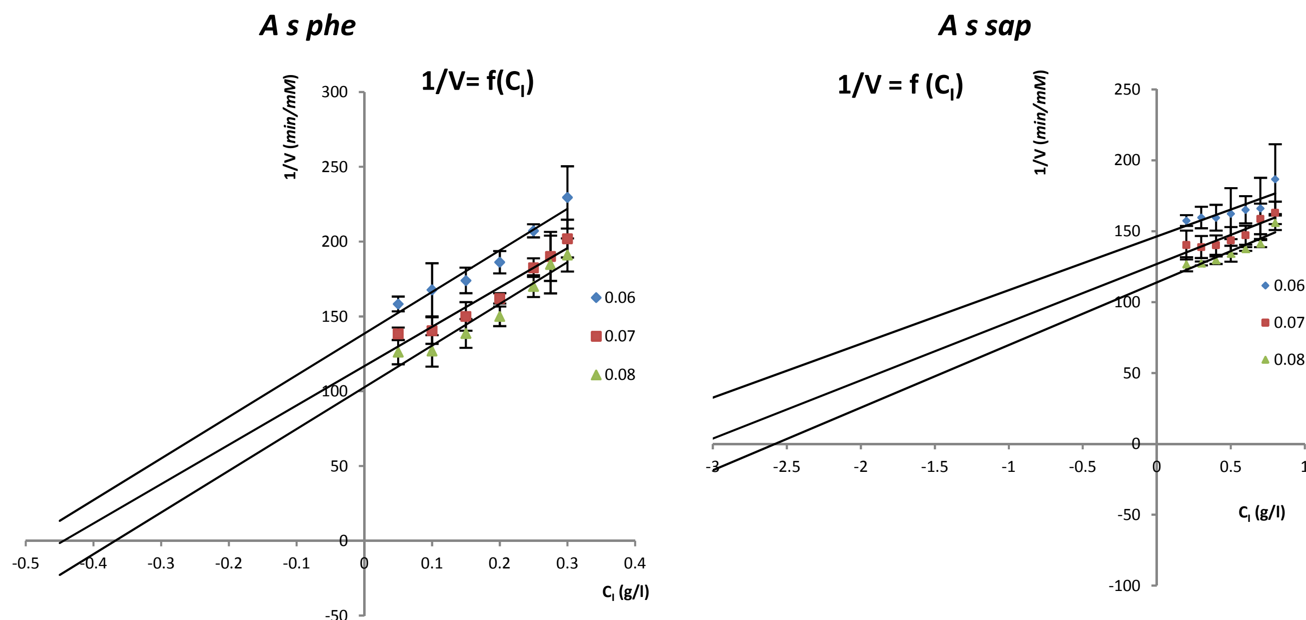

Fig. (1). Dixon plots of inhibition of *Candida rugosa* lipase by the plant extracts of *Achillea santolina (As)* expressed in g/l.phe: Et-O-Ac extract, sap: BuOH extract. Substrate concentrations: 0.06 mM (S1), 0.07mM (S2) and 0.08 mM (S3). The graph represents the means of three experiments.

Many scientific researches and studies have been done for evaluating anti-obesity and anti-acne effects of the medicinal plants extracts on lipase activity aiming to find a new natural drug for both diseases without secondary effects. In this study, we have done the same and we want to compare our plant extracts with those works (Table **4**). In the aim to show which plant family contains the most potent species as lipase inhibitors, we have grouped in Table **4** from different literature sources the most powerful plants with their family and their IC50, we reported that in the family of *Compositae* the Et-O-Ac extract of *Achillea santolina* have presented a potent lipase inhibition than the 50 % EtOH extract of *Gynura pseudochina* which it is already used for the acne treatment (Table **4**). For *Inonotus hispidus* and *Zizyphus lotus*, we have not found any researches for species in the same families but compared with the other plants mentioned in Table **4**, our plants extracts are less potent lipase inhibitors than the other species of families: *Fabaceae*, *Zingiberaceae*, *Sapindaceae*, *Moraceae*, *Saxifragaceae*, *Lamiaceae* and *Araliaceae*. On the other hand, the polyphenols of the Et-O-Ac extracts of *A. santolina*, *Z. Lotus* and *I. hispidus* are very powerful lipase inhibitors than polyphenols of *Salacia reticulate* which it is used for obesity treatment, catechin and kaempferol. Furthermore, the saponins of the BuOH extracts of *A. santolina* and *Z. Lotus* are more effective inhibitors than cyclocariosides (Dammarane saponins) of *Cyclocarya paliurus* but less effective than gypsogenin 3-O-b -D-glucuronide (triterpenoid saponin) of *Acanthopanax senticosus*. Among all extracts, there is no extract more active than the anti-obese drug Orlistat.

We have tried to propose the active molecules in the previous extracts basing on the literature; the Et-O-Ac extract of *A. santolina* is rich with flavonoids such as quercetin and luteolin [57]. Quercetin is known as a potent lipase inhibitor (Table **4**) so we suggest that this molecule is maybe responsible for the inhibition. The BuOH extract of *Z.lotus* is probably containing the dammarane saponins [58] which are known for their inhibitory effect and we suggest that they may be responsible for this inhibition. The Dixon plots of inhibition of *Candida rugosa* lipase for each extract have shown one inhibition type which it is uncompetitive. The uncompetitive inhibition occurs when the inhibitor binds to a site distinct which only becomes available after the substrate (S1) has bound to the active site of the enzyme. This inhibition is most commonly encountered in multi-substrate reactions [59]. In fact, we suggest that *C. rugosa* lipase contains another site than the active one which it is constituted of characteristics amino acids; this site may fixe the inhibitor changing the protein conformation and inhibiting the reaction catalysis.

CONCLUSION

All the plants presented in this work are already used in Arab traditional medicine for treatment of diseases but it is the first time that they have been studied for their anti obesity, anti-acne and antioxidant effects. The results demonstrated that extracts showed certain activities against lipase, and were efficient free radical scavengers. No relation has been found between the antioxidant activity and the antilipase effect. The results from this study give a scientific support to the use of these plants in folklore medicine for the treatment of obesity and acne, and show for the first time, the potential role of lipase inhibition in its activity. For this, further studies should be achieved to identify the active molecules responsible for the *C. rugosa* lipase inhibition and to explain their reaction mechanism. Indeed, identifying the

Table 4. IC50 Values of Extract Plants and Molecules

Solvent	Plant		IC50 (mg/ml)	Reference
	Family	Specie		
50% EtOH	*Compositae*	*Gynura pseudochina**	0.4400 ± 0.0075	Batubara *et al*. 2009 [60]
MeOH	*Fabaceae*	*Caesalpinia sappan*	0.1500 ± 0.0011	Batubara *et al*. 2009 [60]
50% EtOH	*Fabaceae*	*Intsia palembanica*	0.0830 ± 0.0070	Batubara *et al*. 2009 [60]
50% EtOH	*Zingiberaceae*	*Curcuma longa**	0.0193 ± 0.0012	Batubara *et al*. 2009 [60]
50% EtOH	*Sapindaceae*	*Lepisanthes amoena**	0.1679 ± 0.0058	Batubara *et al*. 2009 [60]
EtOH	*Moraceae*	*Morus bombycis*	0.0280 ± 0.0090	Kim *et al*. 2010 [61]
Et-o-Ac	*Saxifragaceae*	*Bergenia crassifolia*	0.0033 ± 0.0002	Ivanov *et al*. 2011 [62]
Molecules from plants				
(+)-catechin 3,5-di-O-gallate (hydrolysable tannins)	*Saxifragaceae*	*Bergenia crassifolia*	0.0004±0.0000	Ivanov *et al*. 2011 [62]
carvacrol (monoterpene)	*Lamiaceae*	*Monarda punctata L* °	0,0270±0.0000	Yamada *et al*. 2010 [1]
gypsogenin 3-O-b -D-glucuronide (triterpenoid saponin)	*Araliaceae*	*Acanthopanax senticosus*	0.0004±0.0000	Li *et al*. 2007 [63]
cyclocariosides (Dammarane saponins)	*Juglandaceae*	*Cyclocarya paliurus*	9.1000±0.0000	Kurihara, 2003 [64]
polyphenols (mangiferin, catechins)	*Hippocrateacaeae*	*Salacia reticulata* °	264±0.0000	Yoshikawa *et al*. 2002 [65]
Catechin			1.8300±0.0000	Ruiz *et al*. 2006 [5]
kaempferol			2.1800±0.0000	Ruiz *et al*. 2006 [5]
Quercetin			0.2700±0.0000	Gatto *et al*. (2002) [66]
Other inhibitors				
Orlistat	Synthetic antiobesity drug		0.0002 ± 0.0000	Jang *et al*. 2008 [3]
Valilactone	*Streptomyces albolongus (bacteria)*		$0.14 * 10^{-6}$	Birari and Bhutani, 2007 [67]
Vibralactone (b-lactone-type)	*Boreostereum virans (fongus)*		0.4000± 0.0000	Dong-Ze *et al*. 2006 [68]
Chloramphenicol	antiacne drug (antibiotic)		0.2188 ± 0.0028	Batubara *et al*. 2009 [60]
Tetracycline	antiacne drug (antibiotic)		0.4713 ± 0.0055	Batubara *et al*. 2009 [60]

* used for acne treatment, ° used for obesity treatment, EtOH: ethanol, MeOH: methanol.

active molecules for the antioxidant activity aiming to introduce these natural compounds in the drugs for the treatment of diseases mentioned above.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Yamada, K.; Murata, T.; Kobayashi, K.; Miyase, T.; Yoshizaki, F. A lipase inhibitor monoterpene and monoterpene glycosides from Monarda punctate. *Phytochemistry*, **2010**, *71*,1884-1891.

[2] Kumar, A.; Chauhan, G.S. Extraction and characterization of pectin from apple pomace and its evaluation as lipase (steapsin) inhibitor. *Carbohydr. Polym*., **2010**, *82*, 454–459.

[3] Jang, D.S.; Lee, G.Y.; Kim, J.H.; Lee, Y.M.; Kim, J.M.; Kim, Y.S.; Kim, J.S. A new pancreatic lipase inhibitor isolated from the roots of *Actinidia arguta*. *Arch. Pharm. Res.,* **2008**, *31*, 666-670.

[4] Higaki, S. Lipase inhibitors for the treatment of acne, *J. Mol. Catal. B: Enzym.,* **2003**, *22*, 377–384.

[5] Ruiz, C.; Falcocchio, S.; Xoxi, E.; Villo, L.; Nicolosi, G.; Pastor, F.I. J.; Diaz, P.; Saso, L. Inhibition of *Candida rugosa* lipase by saponins, flavonoids and alkaloids. *J. Mol. Catal. B: Enzym*., **2006**, *40*, 138-143.

[6] Bruneton, J. Pharmacognosy, Phytochemistry and Medicinal Plants, second Ed., Intercept, London, Paris and New York, **1999**.

[7] Sofowora, A. Medicinal Plants and Traditional Medicine in Africa, first ed., John Wiley and Sons Ltd. Chichester, **1982**.

[8] Harborne, J.B. Phytochemical Method: A guide to Modern Techniques of Plants Analysis, second ed., Chapman and Hall, New York, **1983**.

[9] Evans, W.C. Trease and Evans Pharmacognosy, 13th ed., E. LBS with Bailliere Tindall, **1989**.

[10] Harbone, J.B. Phytochemical Methods, first ed., Chapman and Hall Ltd, London, UK, **1973**.

[11] Tadhani, M.; Subhash, R. Preliminary studies on Stevia rebaudiana leaves: proximal composition, mineral analysis and phytochemical screening. *Int. J. Med. Sci.*, **2006**, *6*, 321-326.

[12] Ardestani, A.; Yazdanparast, R. Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. *Food Chem.*, **2007**, *104*, 21-29.

[13] Yousfi, M.; Djeridane, A.; Bombarda, I.; Hamia, C.; Duhem, B.; Gaydou, E.M. Isolation and Characterization of a New Hispolone

derivative from antioxidant extracts of Pistacia atlantica. *Phytother. Res.*, **2009**, *23*, 1237-1242.

[14] Renault, J.H.; Ghedira, K.; Thepenier, P.; Lavaud, C.; Zeches-Hanrot, M.; Le Men-Olivier, L. Dammarane saponins from *Zizyphus lotus*. *Phytochem.*, **1997**, *44*, 1321-1327.

[15] Singleton, V.L.; Ross, J.A. Colorimetry of total phenolics with phosphomolybdic– phosphotungstic acid reagent. *Am. J. Enol. Vitic.*, **1956**, *16*, 144-158.

[16] Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. *Methods Enzymol.*, **1999**, *299*, 153-178.

[17] Engelheardt, U. Flavonoids-analysis. *CRC Crit. Rev. Food Sci. Nutr.*, **2000**, *41*, 398–399.

[18] Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.,* **1999**, *64*, 555-559.

[19] Dewanto, V.; Wu, X.; Adom, K.K.; Liu, R.H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.,* **2002**, *50*, 3010-3014.

[20] Hiai, S.; Oura, H.; Nakajima, T. Color reaction of some sapogenins with vanillin and sulfuric acid. *Planta Med.*, **1976**, *29*, 116-122.

[21] Cuendet, M.; Hostettmann, K.; Potterat, O. Iridoid glucosides with free radical scavenging properties from Fagraea blumei. *Helv. Chim. Acta*, **1997**, 80, 1144-1152.

[22] Mensor, L.L.; Menezes, F.S.; Leitão, G.G.; Reis, A.S.; Dos Santos, T.C.; Coube, C.S.; Leitao, S.G. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH• free radical method. *Phytother. Res.*, **2001**, 15, 127-130.

[23] Gourine, N.; Yousfi, M.; Bombarda, I.; Nadjemi, B.; Stocker, P.; Gaydou, E.M. Antioxidant activities and chemical composition of essential oil of Pistacia atlantica from Algeria. *Ind. Crop. Prod.*, **2010**, *31*, 203–208.

[24] Williams, W.B.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel - Wissenschaft und - technologie, Lebensmittel - Wissenschaft und technologie*, **1995**, 28, 25–30.

[25] Prim, N.; Blanco, A.; Martínez, J.; Pastor, F.I.J.; Díaz, P. estA, a gene coding for a cell- bound esterase from Paenibacillus sp. BP-23, is a new member of the bacterial subclass of type B carboxylesterases. *Res. Microbiol.,* **2000**, *151*, 303-312.

[26] Prim, N.; Sánchez, M.M.; Ruiz, C.; Pastor, F.I.J.; Díaz, P. Use of methylumbeliferyl-derivative substrates for lipase activity characterization. *J. Mol. Catal. B: Enzym.,* **2003**, *22*, 339-346.

[27] Garcia-Alonso, M.; Pascual-Teresa, S.D.; Santos-Buelga; Rivas-Gonzalo, C.J.C. Evaluation of the antioxidant properties of fruits. *Food Chem.,* **2004**, *84*, 13-18.

[28] Sun, T.; Ho, C.T. Antioxidant activities of buckwheat extracts, *Food Chem.,* **2005**, *90*, 743-749.

[29] Iqbal, S.; Bhanger, M.I. Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chem.*, **2007**, *100*, 246-254.

[30] Juan, M.Y.; Chou, C.C. Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with Bacillus subtilis BCRC14715. *Food Microbiol.*, **2010**, *27*, 586-591.

[31] Kwon, J.H.; Bélanger, J.M.R.; Paré, J.R.J.; Yaylayan, A.V. Application of the microwave-assisted process (MAPTM*) to the fast extraction of ginseng saponins. *Food Res. Int.*, **2003**, *36*, 491-498.

[32] Spingo, G.; Tramelli, L.; De Faveri, D.M. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng.*, **2006**, *81*, 200-208.

[33] Engelberth, A.S.; Clausen, E.C.; Carrier, D.J. Comparing extraction methods to recover ginseng saponins from American ginseng (Panax quinquefolium), followed by purification using fast centrifugal partition chromatography with HPLC verification. *Sep. Purif. Technol.*, **2010**, *72*, 1-6.

[34] Mujwah, A.A.; Mohammed, M.A.; Ahmed, M.H. First isolation of a flavonoid from Juniperus procera using ethyl acetate extract. *Arab. J. Chem.*, **2010**, *3*, 85-88.

[35] Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chem.*, **2006**, *97*, 654-660.

[36] Djeridane, A.; Yousfi, M.; Brunel, J.M.; Stocker, P. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chem. Toxicol.*, **2010**, *48*, 2599-2606.

[37] Kaur, C.; Kapoor, H.C. Antioxidant activity and total phenolic content of some Asian vegetables *Int. J. Food Sci. Technol.*, **2002**, *37*, 153-161.

[38] Katalinic, V.; Milos, M.; Kulisi, C.T.; Jukic. M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem.*, **2006**, *94*, 550–557.

[39] Tawaha, K.; Alali, F.Q.; Gharaibeh, M.; Mohammad, M.; El-Elimat, T. Antioxidant activity and total phenolic content of selected Jordanian plant species. *Food Chem.*, **2007**, *104*, 1372-1378.

[40] Hua-Bin, L.; Ka-Wing, C.; Chi-Chun, W.; King-Wai, F.; Feng, C.; Yue, J. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chem.*, **2007**, *102*, 771-776.

[41] Siddharthan, S.; Yi-Zhong, C.; Harold, C.; Sun, M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chem.*, **2007**, *102*, 938-953.

[42] Sparg, S.G.; Light, M.E.; Staden, J.V. Biological activities and distribution of plant saponins. *J. Ethnopharmacol.*, **2004**, *94*, 219-243.

[43] Dinchev, D.; Janda, B.; Evstatieva, L.; Oleszek, W.; Aslani, M.R.; Kostova, I. Distribution of steroidal saponins in Tribulus terrestris from different geographical regions. Phytochemistry., **2008**, *69*, 176–186.

[44] Dini, I.; Tenore, G.C.; Dini, A. Saponins in Ipomoea batatas tubers: Isolation, characterization, quantification and antioxidant properties. *Food Chem.,* **2009**, *113*, 411–419.

[45] Krishnaiah, D.; Sarbatly, R.; Nithyanandam, R. A review of the antioxidant potential of medicinal plant species, food and bioproducts processing. *Food Bioprod. Process*, **2010**. doi:10.1016/j.fbp.2010.04.008.

[46] Maret, G.T.; Jeffrey, A. Vitamin E, antioxidant and nothing more. *Free Radic. Biol. Med.*, **2007**, *43*, 4-15.

[47] Jun, T.; Hiroaki, K.; Kenji, I.; Kazutaka, M.; Eiichi, G.; Itaru, Y.; Akihiro, T. Inhibition of free radical-induced erythrocyte hemolysis by 2-O-substituted ascorbic acid derivatives. *Free Radic. Biol. Med.*, **2007**, *43*, 1156-1164.

[48] Giovanni, B. Development of a quantitative method for the analysis of total L-ascorbic acid in foods by high-performance liquid chromatography. *J. Chromatogr. A.*, **2007**, *1154*, 97-102.

[49] Gülcin, I.; Vakhtang, M.; Akcahan, G.; Riad, E. Antioxidant activity of saponins isolated from ivy: α-Hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F. *Planta Med.*, **2004**, *70*, 561-563.

[50] Nzowa, L.K.; Barboni, L.; Teponno, B.R.; Ricciutelli, M.; Lupidi, G.; Quassinti, L.; Bramucci, M.; Tapondjou, A.L. Rheediinosides A and B, two antiproliferative and antioxidant triterpene saponins from Entada rheedii. *Phytochemistry,* **2010**, *71*, 254–261.

[51] Sharififar, F.; Nudeh, D.G.; Mirtajaldini, M. Major flavonoids with antioxidant activity from Teucrium polium L. *Food Chem.*, **2009**, *112*, 885-888.

[52] Kawaguchi, K.; Mizuno, T.; Aida, K.; Uchino, K. Hesperidin as an inhibitor of lipases from porcine pancreas and Pseudomonas. *Biosci. Biotechnol. Biochem.,***1997**, *61*, 102-104.

[53] Shin, J.E.; Han, M.J.; Kim, D.H. 3-Methylethergalangin isolated from Alpinia officinarum inhibits pancreatic lipase. *Biol. Pharm. Bull.*, **2002**, *25*, 1442-1445.

[54] Moreno, D.; Ripoll, C.; Ilic, N.; Poulev, A.; Aubin, C.; Raskin, I. Inhibition of lipid metabolic enzymes using Mangifera indica extracts. *J. Food Agric. Environ.*, **2006**, *4*, 21-26.

[55] Han, L.K.; Kimura, Y.; Kawashima, M.; Takaku, T.; Taniyama, T.; Hayashi, T.; Zheng, Y.N.; Okuda, H. Anti-obesity effects in rodents of dietary teasaponin, a lipase inhibitor. *Int. J. Obesity.*, **2001**, *25*, 1459-1464.

[56] Han, L.K.; Zheng, Y.N.; Yoshikawa, M.; Okuda, H.; Kimura, Y. Anti-obesity effects of chikusetsusaponins isolated from Panax japonicus rhizomes. *BMC Complem. Altern. M.,* **2005**, *5*, 9.

[57] Urmanova, F.F.; Komilov, K.M. Flavonoids of *Achillea santolina*. *Chem. Nat. Compd.*, **1999**, *35*, 241.

[58] Renault, J.H.; Ghedira, K.; Thepenier, P.; Lavaud, C.; Zeches-Hanrot, M.; Le Men-Olivier, L. Dammarane saponins from *Zizyphus lotus*. *Phytochem.*, **1997**, *44*, 1321-1327.

[59] Nelson, D.L.; Cox, M.M. Lehninger principles of biochemistry, fourth Edition, W. H. Freeman, University of Wisconsin–Madison, **2005**.

[60] Batubara, I.; Mitsunaga, T.; Ohashi, H. Screening antiacne potency of Indonesian medicinal plants: antibacterial, lipase inhibition, and antioxidant activities. *J. Wood Sci.*, **2009**, *55*, 230-235.

[61] Kim, Y.S.; Lee, Y.M.; Kim, H.; Kim, J.; Jang, D.S.; Kim, J.H. Anti-obesity effect of Morus bombycis root extract: Anti-lipase activity and lipolytic effect. *J. Ethnopharmacol.*, **1999**, *130*, 621-624.

[62] Ivanov, A.S.; Nomura, K.; Malfanov, I.L.; Sklyar, I.V.; Ptitsyn, L.R. Isolation of a novel catechin from Bergenia rhizomes that has pronounced lipase-inhibiting and antioxidative properties. *Fitoterapia*, **2011**, *82*, 212-218.

[63] Li, F.; Li, W.; Fu, H.; Koike, K. Pancreatic Lipase-Inhibiting Triterpenoid Saponins from Fruits of Acanthopanax senticosus. *Chem. Pharm. Bull.*, **2007**, *55*, 1087-1089.

[64] Kurihara, H. Hypolipemic effect of Cyclocarya paliurus (Batal) Iljinskaja in lipid-loaded mice.*Biol. Pharm. Bull.*, **2003**, *26*: 383–385.

[65] Yoshikawa, M.; Shimoda, H.; Nishida, N.; Takada, M.; Matsuda, H. Salacia reticulata and its polyphenolic constituents with lipase inhibitory and lipolytic activities have mild antiobesity effects in rats. *J. Nutr.*, **2002**, *132*, 1819-1824.

[66] Gatto, M.T.; Falcocchio, S.; Grippa, E.; Mazzanti, G.; Battinelli, L.; Nicolosi, G.; Lambusta, D.; Saso, L. Antimicrobial and anti-lipase activity of quercetin and its C2-C16 3-O-acyl-esters, *Bioorg. Med. Chem.*, **2002**, *10*, 269-272.

[67] Birari, R.B.; Bhutani, K.K. Pancreatic lipase inhibitors from natural sources: unexplored potential. *Drug Discov. Today*, **2007**, *12*, 879-889.

[68] Dong-Ze, L.; Fei, W.; Tou-Gen, L.; Jian-Guo, T.; Wolfgang, S. Vibralactone: a lipase inhibitor with an unusual fused lactone produced by cultures of the *Basidiomycete Boreostereum* vibrans. *Org. Lett.*, **2006**, *8*, 5749-5752.

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