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Anti-lipase and antioxidant properties of 30 medicinal plants used in Oaxaca, México

Nemesio Villa-Ruano[1*], Guilibaldo G. Zurita-Vásquez[1], Yesenia Pacheco-Hernández[2], Martha G. Betancourt-Jiménez[3], Ramiro Cruz-Durán[4] and Horacio Duque-Bautista[1]

[1] Universidad de la Sierra Sur, Guillermo Rojas Mijangos S/N, Ciudad Universitaria, CP70800, Miahuatlán de Porfirio Díaz, Oaxaca, México.
[2] Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, Col. Centro, CP 8500, Ciudad Obregón, Sonora, México.
[3] Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato. Km 9.6 Libramiento Norte, Carretera Irapuato-León, CP 36000, Irapuato México.
[4] Facultad de Ciencias UNAM, Ciudad Universitaria, México DF, Ciudad Universitaria, CP 04510, Del. Coyoacán, México, D.F.

ABSTRACT

We report the results of *in vitro* anti-lipase and antioxidant assays using crude ethanolic extracts from 30 plants grown in Oaxaca, México. Anti-lipase tests were performed by using porcine pancreatic lipase (PPL) [EC 3.1.1.3] from Affymetrix/USB. The extracts of *Solanum erianthum, Salvia microphylla, Brungmansia suaveolens* and *Cuphea aequipetala* showed up to 60% PPL inhibition. The effect of these extracts on the kinetic parameters of PPL (K_m= 0.36 mM, and V_{max}=0.085 mM min^{-1}) revealed that the alcoholic preparations of *S. erianthum* and *C. aequipetala* engendered a non-competitive inhibition (V_{max}=0.055 mM min^{-1}; V_{max}= 0.053 mM min^{-1}), whereas those of *S. microphylla* and *B. suaveolens* produced a mixed inhibition (K_m= 0.567 mM, V_{max}=0.051 mM min^{-1}; K_m=0.643 mM, V_{max}= 0.042 mM min^{-1}). In addition to these findings, seven extracts from different plants were able to inhibit PPL in the range of 30-50%. Antioxidant tests against 2,2-Diphenyl-1-picryl hydrazyl (DPPH) confirmed that *Arctostaphylos pungens, Gnaphalium roseum, Crotalaria pumila, Cuphea aequipetala, Rhus chondroloma,* and *Satureja laevigata* possess relevant antioxidant activity (IC_{50}=50-80 µg mL^{-1}). The general composition of the most effective ethanolic extracts was obtained in order to confirm their known chemistry reported by previous works. Comprehensive chemical analysis of the ethanolic extracts and their poisoning effects suggests that *S. microphylla*, *C. aequipetala* and *A. pungens* could be considered as the best sources with both desired properties.

Key terms: Phytotherapy, anti-obesity, antioxidant, Oaxacan ethnobotany.

INTRODUCTION

High prevalence illnesses such as diabetes mellitus, cancer and neurodegenerative diseases are induced by oxidative stress and obesity. These physiological disorders are commonly associated with improper diets and sedentary lifestyle. Current statistical studies demonstrate an alarming increase in obesity and neurodegenerative diseases in the past five years in Mexico (Rojas-Martínez *et al.*, 2012). This fact has turned into a serious health problem that requires special attention. Scientific evidence suggests that neurological disorders, respiratory diseases and premature ageing are the result of continuous oxidative stress which is aggravated by alcoholism, depression, intensive work, smoking and being overweight (Reuter *et al.*, 2010). Being overweight or obese should be prevented with constant exercise and dietary routines. Nevertheless when the latter techniques fail in obtaining 10% weight loss, pharmacological treatment is required. Despite the fact that this alternative is highly recommended, there are controversial results on the use of some pharmacological agents such as sibutramine (Villa-Ruano *et al.*, 2011). Synthetic derivatives of lipstatin that inhibit triglyceride hydrolysis are strongly recommended for controlling weight disorders. Unfortunately, Orlistat ® and related competitive inhibitors have side effects; however, they are relatively less aggressive compared with those of sibutramine. To date, the pharmaceutical market is limited to a short list of anti-obesity drugs. As a consequence of the need for new and more efficient anti-lipase agents,

exhaustive plant screenings from Asiatic, African and European ethnobotanical sources have been performed (Birari and Bhutani 2007; Ekanem *et al.*, 2007; Slanc *et al.*, 2009; Gholamhoseinian *et al.*, 2010; Zheng *et al.*, 2010). Nonetheless, there is no information about the efficacy of Latin American medicinal plants on this topic. Mexico is a privileged country in possessing extensive information about the use and therapeutic properties of many plants. Within México, Oaxaca is considered one of the most biodiverse and multicultural states, but also highly marginalized (Caballero *et al.*, 2004). Currently, people from rural communities of this state still depend on traditional folk medicine to cure their illnesses. In these places, the latter alternative is in many cases more accepted than allopathic medicine by native people. Here we report the results of *in vitro* anti-lipase and antioxidant assays using ethanolic extracts from plants of Oaxacan ethnomedicine in order to reveal some species with potential use in preventing obesity and cellular oxidative stress.

METHODS

Source of plants

The plant material was collected in San Andres Paxtlán, Oaxaca, México (16°13.081' N 096°30.331' W; 1996 m elevation) in April 2012 (Spring 2012). The identity of 30 species was corroborated at the Herbarium of the FCME-UNAM México, or by dichotomous keys (Martinez, 1979; Calderón y Rzedowski,

2005). 50 g of distinct plant organs (Table I) were excised and immediately extracted with 100 mL of absolute ethanol for 15 days at room temperature in darkness. The crude extracts were filtered with Ahlstrom grade 642 filter paper and concentrated using a Buchi R-200 rotovaporator to approximately 10 mL. These preparations were completely dried in a centrifuge

concentrator (RVC 2-25 CD) for 3 h. The resultant powder was stored in amber glass flasks at 4 °C until used. 3% DMSO was employed for resuspending the powder and to prepare different stocks (this solution did not show anti-lipase activity by itself).

TABLE I

Anti-lipase and antioxidant activities of 30 plants from Oaxacan ethnnomedicine.

	Scientific name	Family	Organs*	LI (%) [¶]	AA (IC50 μg mL^{-1})
1	*Chyrsanthemum parthenium* (L.) Sch. Bip.	*Asteraceae*	Shoots and leaves	5.7±2.3[§ a]	125.1±8.5[§g]
2	*Gnaphalium roseum*Kunth.	*Asteraceae*	Shoots and leaves	6.3±0.5[a]	72.9±3.2[b]
3	*Baccharis salicifolia* (Ruiz et Pav.) Pers.Lis	*Asteraceae*	Shoots and leaves	6.6±0.9[a]	154.4±6.0[i]
4	*Heimia salicifolia* Link.	*Lythraceae*	Shoots and leaves	7.6±0.2[a]	245.3±11.3[m]
5	*Borago officinalis* L.	*Boraginaceae*	Shoots, leaves and flowers	9.3±0.5[b]	115.23±3.4[f]
6	*Passiflora ligularis* Juss.	*Passifloraceae*	Leaves and tendrils	9.3±0.4[b]	386.8±12.7[r]
7	*Montanoa tomentosa* (Cerv.)	*Asteraceae*	Shoots and leaves	10.2±0.4[b]	356.2±13.2[q]
8	*Crotalaria pumila* Ort.	*Fabaceae*	Shoots and leaves	12.0±0.3[b]	75.3±2.8[b]
9	*Lippia alba* (Mill.) N.E. Br.	*Verbenaceae*	Shoots and leaves	12.4±1.1[b]	89.6±2.4[d]
10	*Marrubium vulgare* L.	*Laminaceae*	Shoots and leaves	13.2±0.6[b]	102.6±3.5[e]
11	*Diphysa floribunda* Peyr.	*Fabaceae*	Shoots, leaves and flowers	15.3±0.7[c]	289.3±13.1[o]
12	*Anrendera cordifolia* (Ten.) Steenis	*Basellaceae*	Leaves and aerial tubers	18.6±2.0[d]	223.7±19.2[l]
13	*Montanoa hibiscifolia* Benth.	*Asteraceae*	Shoots and leaves	19.7±1.5[d]	87.2±3.4[d]
14	*Physalis philadelphica* Lam.	*Solanaceae*	Shoots, leaves and flowers	22.6±1.8[e]	209.5±3.5[j]
15	*Artemisia ludoviciana* Nutt.	*Asteraceae*	Shoots and leaves	23.4±2.2[e]	215.6±11.5[k]
16	*Ipomoea wolcottiana* Rose	*Convolvulaceae*	Shoots and leaves	26.5±2.4[f]	445.8±5.9[s]
17	*Satureja laevigata* Standl.	*Laminaceae*	Shoots and leaves	28.5±1.3[f]	85.4±2.5[d]
18	*Microsechium helleri* (Peyr.) Cogn.	*Cucurbitaceae*	Tuberous roots and axillary buds	32.8±1.5[g]	256.3±4.5[n]
19	*Loeselia mexicana* (Lam.) Brand.	*Polemoniaceae*	Shoots and leaves	35.2±2.5[g]	468.4±23.8[t]
20	*Sambucus mexicana* Presl.	*Caprifoliaceae*	Shoots and leaves	37.1±2.1[g]	135.1±4.1[h]
21	*Chiranthodendron pentadactylon* Larreat	*Malvaceae*	Shoots and leaves	39.9±2.5[g]	102.6±6.6[e]
22	*Phaseolus coccineus* L.	*Fabaceae*	Shoots, leaves and flowers	41.3±0.4[g]	256.4±12.5[n]
23	*Cestrum anagyris* Dunal	*Solanaceae*	Shoots and leaves	45.6±0.6[h]	132.8±4.3[h]
24	*Rhus chondroloma* Standl.	*Anacardiaceae*	Shoots, leaves, flowers and fruits	49.4±1.4[i]	78.6±1.1[c]
25	*Arctostaphylos pungens* Kunt	*Ericaceae*	Shoots, leaves and stem bark	53.4±2.1[i]	56.4±3.3[a]
26	*Salmea scandens* (L.) D.C.	*Asteraceae*	Stem bark and leaves	55.9±0.9[i]	344.5±17.8[p]
27	*Cuphea aequipetala* Cav.	*Lythraceae*	Shoots and leaves	60.5±0.2[j]	76.5±2.3[b]
28	*Brungmansia suaveolens* (Humb. et Bonpl.) Berch et Presl.	*Solanaceae*	Flowers	65.3±2.7[k]	356.1±5.6[q]
29	*Salvia microphylla* Kunth	*Laminaceae*	Shoots and leaves	67.1±0.3[k]	99.2±1.1[e]
30	*Solanum* erianthum *D. Don.*	*Solanaceae*	Shoots, leaves and flowers	67.8±3.2[k]	104.8±3.4[e]
31*	Orlistat			97.6±0.4[l]	
32*	BHT				8.3±0.01[u]
33*	Ascorbic Acid				4.2±0.03[v]

* Mix of plant organs; [§] Means with standard deviation from experiments performed in quintuplicate. LI, % of lipase inhibition; AA, antioxidant activity expressed in IC$_{50}$. [¶] The percentage of PPL inhibition corresponds to the results of using 100 µg mL^{-1} from each extract. Means with different letters are statistically different according to an ANOVA-Tukey Test (p <0.05).

Anti-lipase activity

The turbidimetric method proposed by Shihabi and Bishop (1971) was used to determine the triolein hydrolysis under controlled conditions. The preparation of triolein emulsion was achieved as described by the same authors. Porcine pancreatic lipase (PPL) [EC 3.1.1.3] was purchased from Affymetrix/ USB and adjusted to a final concentration of 250 µg mL^{-1} (~ 6 units mL^{-1}) in 100 mM Tris buffer pH 9.0, containing triolein (0.3 mM) sodium deoxycholate (20 mM), colipase (3 mg L^{-1}) and calcium chloride (0.02 mM). Enzymatic reactions had a final volume of 1 mL and were initiated by its incubation at 37 °C for 30 min with two different concentrations of ethanolic extracts (50 and 100 µg mL^{-1}). Changes in turbidity were measured at 340 nm by spectrophotometry (Beckman DU 7400). The percentage of inhibition was calculated according to Gholamhoseinian et al., (2010) as a semi-quantitative parameter to estimate the effectiveness of each extract. Orlistat ® (Medimart Labs) was employed as the reference standard of inhibition. Kinetic parameters of PPL were determined by saturation curves using triolein as a substrate (0.2, 0.4, 0.8, 1.6, 2.0 and 2.2 mM) and adjusted by non-linear regression using the Solver® software as described by Villa-Ruano *et al.* (2009). The specific inhibitory effect of ethanolic extracts on PPL kinetics was determined from different substrate concentrations (0.2-2.2 mM) and two extract concentrations (50 and 100 µg mL^{-1}). Double-reciprocal Lineweaver-Burk plot regressions were performed with SigmaPlot 10.0 (Systat Software, Inc). All the experiments were assayed in quintuplicate.

Antioxidant activity

Antioxidant activity was determined according to Shafaghat *et al.* (2011), using butylated hydroxytoluene (BHT, Sigma-Aldrich Co.) and vitamin C (Sigma-Aldrich Co.) as reference standards. 2,2-Diphenyl-1-picryl hydrazyl (DPPH) was also obtained from Sigma-Aldrich Co. Dose-response curves (20-500 µg mL^{-1}) were performed for each extract in order to obtain the respective half maximum inhibitory concentration (IC$_{50}$). All the experiments were performed in quintuplicate.

Comparative phytochemical screening

To obtain the general chemical composition of the most effective plant extracts in both biological activities (22), a comparative phytochemical screening was performed. These extracts were directly processed to determine flavonoids, tannin/phenols, terpenes, steroids, saponins, alkaloids and cardiac glycosides according to general methods developed by Harborne (1998) and Háuad-Marroquín (2010). Semi-quantitative content was estimated for some metabolites in order to get an approach to their abundance in ethanolic extracts. Flavonoid content was determined at 450 nm by calibration curves using quercetin as a flavonoid standard. Total tannin/phenolic content was determined using gallic acid as internal standard and using the reagent of Folin-Ciocalteau (750 nm) according to Nugroho *et al.* (2013). Alkaloid content was calculated with Dragendorff's reagent at 435 nm according to Villa-Ruano *et al.* (2012). Total saponin content was determined at 408 nm considering digitonin as reference standard. Cardiac glycosides were determined at

486 nm based on their reaction with picric acid in alkaline media using digitoxine as reference standard (Solich et al, 1992). Results were expressed as abundant for the case of ≥500 µg gFW^{-1} (fresh weight), low for 50-400 µg gFW^{-1} or absent (undetectable). Terpene and steroid determination was only qualitative. All the reference standards were obtained from Sigma-Aldrich Co. and the experiments were performed in triplicate.

Statistical analysis

The anti-lipase and antioxidant data were processed by an ANOVA coupled to a Tukey Test ($p<0.05$) using SigmaPlot 10.0 software. All results were expressed as mean ± standard deviation (n=5). In order to compare the two biological parameters, the results were plotted in a stacked bar graph.

RESULTS

Table I summarizes the results of antioxidant and anti-lipase assays using ethanolic extracts from 30 medicinal plants commonly grown in family plots in rural communities of Oaxaca's Southern Lowlands. These medicinal plants are orally or topically administered to treat diverse diseases. Crude ethanolic extracts from *Solanum erianthum, Salvia microphylla, Brungmansia suaveolens* and *Cuphea aequipetala*, were the most effective, showing up to 60% but less than 70% inhibition on PPL. On the other hand, *Salmea scandens*, *Arctostaphylos pungens*, *Rhus chondroloma, Cestrum anagyris*, *Phaseolus coccineus*, *Chiranthodendron pentadactylon* and *Sambucus mexicana* showed a range inhibition of 40-56%. The latter extracts never achieved the characteristic *in vitro* inhibition of Orlistat ® (>90%), but showed a strong inhibitory effect on the activity of PPL. Results of the antioxidant screening reveal that the ethanolic extract of *A. pungens* was the best for the stability of DPPH radical (IC$_{50}$=56.4 µg mL^{-1}). Other alcoholic extracts from *Gnaphalium roseum, Crotalaria pumila, Cuphea aequipetala, Rhus chondroloma* and *Satureja laevigata* had a high antioxidant activity against the DPPH radical (IC$_{50}$=73-85 µg mL^{-1}) but only some of these species showed significant anti-lipase activity (>40%). Figure 1 shows that anti-lipase activity is not necessarily associated with antioxidant activity, but there were interesting coincidences for the case of *S. erianthum*, *C. aequipetala* and *A. pungens*. According to our results, *Lippia alba, Crotalaria pumila, Marrubium vulgare* and *Chrysanthemum parthenium* could be considered as alternatives to antioxidants, but not as anti-lipase sources (Figure 1). The ANOVA coupled to a Tukey test revealed significant differences among the effectiveness of ethanolic extracts ($p<0.05$) in the two biological activities (Table I). The comparative phytochemical screening of the most relevant extracts (22) showed diverse components dissolved in each ethanolic preparation (Table II). Nevertheless, the presence and high abundance of flavonoids and/or phenolic compounds was evident in all the samples. Alkaloid content was abundant mainly for *S. eriathum*, *B. suaveolens, Ipomoea wolcottiana* and *S. scandens*. Terpenes, saponins and steroids were present in almost all the ethanolic extracts but only the samples of *S. erianthum, R. chondroloma S. mexicana* and *C. pumila* gave positive results for cardiac glycosides.

The effect of the four most effective plant extracts on kinetic parameters of PPL is shown in Table III. Under our laboratory

conditions, the K_m value of triolein for this enzyme was 0.36 mM and V_{max} was 0.085 mM min^{-1} (Table III). Lineweaver-Burk plot analysis performed for the *S. erianthum* extract showed a non-competitive inhibition where only V_{max} was significantly decreased by both assayed concentrations (Fig. 2, Table III). The *S. microphylla* extract revealed a mixed inhibition on PPL (Fig. 3, Table III). Similar results were obtained for the alcoholic extract of flowers from *B. suaveolens* (Fig. 4, Table III). The effect of these extracts was clearly reflected in the modification of the K_m and V_{max} values (Table III). The corresponding extract of *C. aequipetala* had a non-competitive effect on kinetics of PPL, like *S. erianthum* (Figure 5, Table III).

DISCUSSION

According to our results, the kinetic parameters of PPL (Affymetrix/USB) were as follows: K_m = 0.36 mM and V_{max} = 0.085 mM min^{-1}. The absolute values of these parameters are

Figure 3. Lineweaver-Burk plot analysis for PPL at two concentrations of the ethanolic extract from *Salvia microphylla* (50 and 100 µg mL^{-1}), using different triolein concentrations (0.2-2.2 mM).
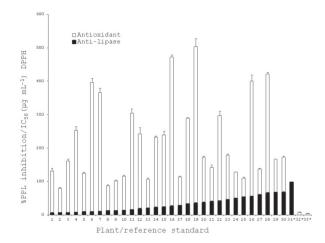

Figure 1. Effect of 30 ethanolic extracts on PPL activity and DPPH stability. Numbers correspond to the order of Table I. Number with asterisks indicate the reference standards used to normalize both biological activities. Bars represent the standard deviation from five repetitions.
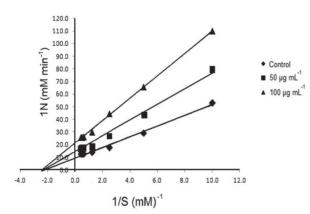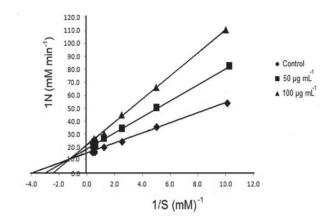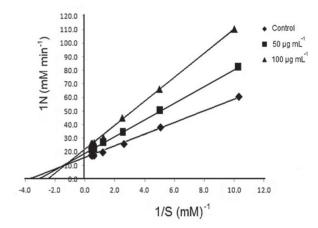

Figure 4. Lineweaver-Burk plot analysis for PPL at two concentrations of the ethanolic extract from *Brungmansia suaveolens* (50 and 100 µg mL^{-1}), using different triolein concentrations (0.2-2.2 mM).

Figure 2. Lineweaver-Burk plot analysis for PPL at two concentrations of the ethanolic extract from *Solanum eriathum* (50 and 100 µg mL^{-1}), using different triolein concentrations (0.2-2.2 mM).
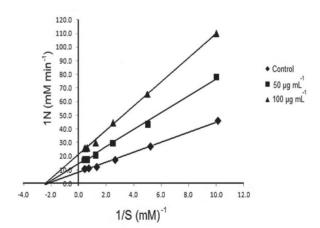

Figure 5. Lineweaver-Burk plot analysis for PPL at two concentrations of the ethanolic extract from *Cuphea aequipetala* (50 and 100 µg mL^{-1}), using different triolein concentrations (0.2-2.2 mM).

similar to those reported by Gholamhoseinian *et al.* (2010) but slight variations in K_m and V_{max} could be caused by the assay buffer (pH 9.0) and its composition. Considering the biochemical evidence, four plants exhibited very strong *in vitro* anti-lipase activity (>60%), *S. erianthum* being the most effective. *S. erianthum* is currently used in Oaxaca State as an oral analgesic to treat stomach ache and as a cutaneous antimicrobial agent. The chemistry of *S. erianthum* consists of sesquiterpenes as well as high endogenous levels of steroidal-alkaloid glycosides, tropane-alkaloids and tannins in the aerial parts (Essien *et al.*, 2012; Maurya *et al.*, 2013). Some strong anti-lipase activities are apparently associated with pentacyclic-triterpene glycosides as is the case with teasaponins, which share an association in chemical structure with *S. erianthum* glycosides (Birari and Bhutani 2007; Tucci *et al.*, 2010). The results of phytochemical screening support the presence of tannins, terpenes, steroids and saponins which could be involved in those effects (Table II). However, the non-competitive inhibition by the crude ethanolic extract suggests that many compounds may be involved in the observed biochemical behavior. Despite the aerial parts of this plant having evident anti-lipase properties, the toxic endogenous levels of tropane and *Solanum* alkaloids (Table II) must be considered before performing *in vivo* trials (Huang *et al.*, 2009). Further chromatographic isolation of the bioactive molecules and their spectroscopic confirmations are necessary to determine the anti-lipase molecules of *S. erianthum*. *S. microphylla* was the second-best anti-lipase source. Phenolic compounds, sesquiterpenes, and 12-methoxycarnosic acid, as well as triterpene aglycones such as oleanolic acid, lupeol and β-sitosterol are the main constituents of this species (Aydogmuş *et al.*, 2006). Our phytochemical tests confirm the incidence of flavonoids tannins, terpenes, steroids and saponins that could include the latter compounds (Table II). Carnosic acid isolated from *Salvia offcinalis* exhibits a strong *in vitro* and *in vivo* PPL inhibition (Tucci *et al.*, 2010). According to our results a mixed inhibition was observed by the ethanolic extract of *S. microphylla*. This fact suggests the putative involvement of 12-carnosic acid (as a possible competitive inhibitor) and other compounds such as pentacyclic diterpene glycosides and phenolic compounds as non-competitive inhibitors that bind to the enzyme-substrate complex. Carnosic acid and its derivatives seem to be chemo-taxonomic markers of the *Salvia* genus. Nevertheless, previous reports on anti-

TABLE II

Comparative phytochemical screening of the most effective anti-lipase and antioxidant ethanolic extracts

Plant species	Flavonoids	Tannins/phenols	Terpenes	Steroids	Saponins*	Alkaloids	Cardiac Glycosides
Solanum erianthum	+	++	√	√	++	++	++
Salvia microphylla	++	++	√	√	+	-	-
Brungmansia suaveolens	+	+	√	√	++	++	-
Cuphea aequipetala	++	++	√	√	+	+	-
Salmea scandens	+	++	√	-	-	++	-
Arctostaphylos pungens	++	++	√	√	-	-	-
Rhus chondroloma	++	++	√	√	-	+	+
Cestrum anagyris	++	+	-	√	+	-	-
Phaseolus coccineus	++	+	-	√	+	+	-
Chiranthodendron pentadactylon	++	++	√	√	-	-	-
Sambucus mexicana	++	++	-	√	-	+	+
Gnaphalium roseum	++	+	-	√	+	-	-
Satureja laevigata	++	++	√	√	+	-	-
Lippia alba	++	+	√	√	+	-	-
Crotalaria pumila	++	+	√	√	+	+	+
Marrubium vulgare	++	++	√	√	+	-	-
Chyrsanthemum parthenium	++	++	√	√	-	+	-
Loeselia mexicana	++	++	√	√	-	+	-
Microsechium helleri	++	+	√	√	++	-	-
Ipomoea wolcottiana	+	++	√	√	-	++	-
Artemisa ludoviciana	++	+	√	√	-	-	-
Physalis philadelphica	++	+	√	√	-	+	-

[+] 50-400 µg gFW [-1] [++] ≥500 µg gFW[-1]; [-] Undetectable under those conditions. * Triterpene saponins. √ Both steroid aglycones and terpene occurrences were qualitatively determined.

TABLE III

Effect of the four best alcoholic extracts on the kinetic parameters of PPL

Extract concentration	K_m(mM)		V_{max}(mM min^{-1})	
	50 μg mL^{-1}	100 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}
Without inhibitor*	0.364±0.012 §	0.364±0.012 §	0.085±0.0017 §	0.085±0.0017 §
Solanum erianthum	0.362±0.022	0.364±0.013	0.069±0.0024	0.055±0.0019
Salvia microphylla	0.465±0.017	0.567±0.035	0.066±0.0035	0.051±0.0011
Brugmansia suaveolens	0.452±0.034	0.643±0.014	0.050±0.0017	0.042±0.0021
Cuphea aequipetala	0.365±0.014	0.362±0.019	0.061±0.0045	0.053±0.0016

§ Means with standard deviation from experiments performed in quintuplicate. *Results from saturation curves performed under laboratory conditions without any inhibitor

lipase properties of *Salvia rhytidea* and *Salvia miltiorrhiza* suggest that only some *Salvia* species possess worthwhile levels of efficacy (Gholamhoseinian *et al.*, 2010, Zheng *et al.*, 2010). Geographical location, altitude, soil composition, weather and specific biotic elicitors may be involved in the biosynthesis of terpene and phenolic compounds in these plants. The high abundance of phenolic metabolites (Table II) is probably related to the significant antioxidant activity (IC$_{50}$= 99.2 μg mL^{-1}) of *S. microphylla*. The ethanolic extract of flowers from *B. suaveolens* had a mixed inhibition effect on PPL. This species produces classical tropane alkaloids with medicinal uses whose hyoscine is hydrolyzed by this enzyme under buffering conditions (Henry, 1949). Our phytochemical studies also confirm high endogenous levels of alkaloids and saponins as well as a low level of phenolic compounds in *B. suaveolens* flowers (Table II). This evidence could support the participation of alkaloids and saponins as the main anti-lipase agents. The mixed inhibitory effect on the kinetics of PPL suggests that some tropane alkaloids could interact with both the active site of the enzyme and the enzyme-substrate complex. According to our results, the fourth most efficient species with anti-lipase activity was *C. aequipetala*, a native Mexican medicinal plant. The plant is commonly known as "hierba del cancer" and has been traditionally administered for centuries without formal reports of poisoning (Waizel-Bucay *et al.*, 2003). According to previous phytochemical studies the aerial parts contain high endogenous levels of fatty acids, mainly miristic acid (in oil fraction), as well as flavonoids and triterpene glycosides in the alcoholic extracts (Hirsinger, 1980; Waizel-Bucay *et al.*, 2003). Detection of phenolics, terpenes, steroids and saponins in the phytochemical screening confirm the possible incidence and involvement of the latter compounds in the effects here reported (Table II). Considering the results of this study and the chemistry of *C. aequipetala*, the significant antioxidant properties (IC$_{50}$= 76.5 μg mL^{-1}) should be related to phenolic compounds and the strong non-competitive inhibition on PPL (Figure 5, Table III) could be associated with triterpene glycosides and steroids. Further experiments are required to verify this hypothesis. Due to *C. aequipetala* demonstrating interesting nutritional properties and containing low levels of alkaloids (Table II), this plant could be considered a good candidate for *in vivo* trials. Another plant with strong anti-lipase activity is *S. scandens*. Currently this species is being studied by our research group and some of the chemical compounds from the stem bark are being isolated,

characterized and quantified by chromatographic and spectroscopic methods (data not shown). Interestingly, the phytochemical screening reveals an alkaloid content that could be linked to the presence of specific alkylamides that contain nitrogen and probably interact with Dragendorff's reagent. *A. pungens* represents another desirable source with anti-lipase and antioxidant properties. This study confirms the previous findings of Slanc *et al.* (2009) where this *Ericaceae* showed a similar inhibition degree on PPL. The intrinsic chemistry of *A. pungens* reveals the presence of phenolic compounds such as quercetin, catechins and tannins (Winkelman, 1989) which are strongly involved in both biological activities (Zheng *et al.*, 2010). The general composition here found corresponds to high amounts of flavonoids and related phenolics but also with some steroids (Table II). Further chromatographic separations are required to determine the anti-lipase agents of this plant. *R. chrondoloma* and *C. anagyris* showed good anti-lipase and acceptable antioxidant activities; nevertheless, their chemistry have been poorly studied (Young, 1979). According to our results *R. chondroloma* showed high levels of flavonoids and tannins as well as terpenes and steroids (Table II). The moderate antioxidant activity of its ethanolic extract is probably linked to the high flavonoid and phenolic content (Table II) whereas the anti-lipase effects could be also associated with the latter compounds but possibly with other metabolites such as steroids. Additionally, *R. chondroloma* sowed an interesting presence of cardiac glycosides. A similar condition is observed for the case of *C. anagyris* (except for cardiac glycosides and alkaloids). *Phaseolus coccineus* contains a great variety of flavonoids that could participate in the observed anti-lipase activity (Williams *et al.*, 1995); the general composition of its ethanolic extract sustains this finding (Table II). *Chiranthodendron pentadactylon* ("Flor de Manita") is one of the most relevant plants in Mexican ethnobotany because of its anti-secretory properties (Velázquez *et al.*, 2012). The anti-lipase effect reported in the present work contributes to the discovery of novel uses for this native plant that are strongly related to its antioxidant catechins and flavonoids (Table II). The ethanolic extract of leaves from *Sambucus mexicana* showed similar anti-lipase effects to those of *C. pentadactylon*. High endogenous levels of anthocyanins and other phenolic compounds have been reported in this species (Lee *et al.*, 2007). The general phytochemical composition of those extracts endorses the occurrence of phenolic metabolites, steroids and low levels of alkaloids (Table II) but detailed experimentation is required to

reveal the anti-lipase agents of *S. mexicana*. Additionally, the presence of cardiac glycosides was detected in the leaf extracts of this plant; this finding could be considered as novel phytochemical information for the plant (Table II). Gholamhoseinian *et al.* (2010) reported a moderate anti-lipase activity of *Artemisia santolina* as we do for *A. ludoviciana*. This latter plant contains high flavonoid content (Table II) that should be participating in the moderate antioxidant activity reported in this study. The terpene content of *A. ludoviciana* (Table II) could include small quantities of sesquiterpene lactones that perhaps interact with the active site of PPL. It is known that the lactone moiety of Orlistat plays an important function in the irreversible inhibition of PPL. There is no information about the possible involvement of sesquiterpene lactones on this matter so far. Also, the same authors report the same low anti-lipase activity for *Marrubium anisodon* as we do for *M. vulgare*. Although this species showed good phenolic content (Table II) and antioxiadant activity (Table I), its anti-lipase activity was non-significant compared to other plant extracts. The ethanolic preparations of *M. vulgare* and *C. parthenium* clearly show that high phenolic content is not necessarily connected with relevant anti-lipase activity. *Loeselia mexicana*, *Microsechium helleri*, *S. laevigata*, *I. wolcottiana* and *Physalis philadelphica* showed a moderate anti-lipase activity and almost all of them except *I. wolcottiana* and *L. mexicana* demonstrated an acceptable antioxidant activity (Table I). Current phytochemical studies in *M. helleri* revealed the presence of many triterpene saponins (Hernández-Carlos *et al.*, 2012), some of them perhaps with anti-lipase properties. Presence and high abundance of saponins in this plant is also demonstrated in our phytochemical screening (Table II). *C. pumila* is an edible plant used to prepare traditional foods; the high phenolic content of this species (Table II) is strongly linked to its characteristic antioxidant activity (Table I). The presence of cardiac glycosides in the ethanolic extract of *C. pumila* could be a new chemical finding for this plant (Table II).

As a final conclusion, we report on some medicinal plants used in the state of Oaxaca-Mexico that possess noteworthy anti-lipase and antioxidant properties. Some of those plant species might be considered as a cheap herbal therapy for the prevention of high prevalence diseases originated by oxidative stress and obesity. The known chemistry and general composition of some species was considered to explain their respective biological activities. Chemical composition and the correlation of the two biological activities demonstrate that plants with anti-lipase activity are not necessarily good antioxidant agents and vice versa. In addition, this study remarks on the need for performing accurate phytochemical experimentation in less-studied plants in order to identify and isolate their bioactive compounds. Comprehensive analyses of the chemistry and toxic risks of these plants leads us to consider *S. microphylla*, *C. aequipetala* and *A. pungens* as the best medicinal plants with both desired properties.

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