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Lipase inhibitory activity of *Lagenaria siceraria* fruit as a strategy to treat obesity

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ABSTRACT

Objective: To explore pancreatic lipase inhibitory activity under different extraction conditions in order to track the most potent extract.

Methods: The methanolic extract and its fractions in solvents of increasing polarity, ether, chloroform, ethyl acetate, *n*-butanol and water, were made through cold maceration. Extracts in ethanol, ethyl acetate, acetone and chloroform were similarly prepared. Aqueous extract was prepared through hot decoction method. A reported method was used to determine lipase inhibitory activity of extracts and fractions over wide ranges of concentrations.

Results: The extracts and fractions exhibited concentration dependent activity. The IC_{50} (μg/mL) values of methanolic, ethanolic, chloroform, ethyl acetate, acetone, ethyl acetate (after washing with water) and aqueous decoction were 293.40, 266.47, 157.59, 182.12, 352.34, 257.00, and 190.00, respectively. The activity of chloroform, ethyl acetate and aqueous extracts were close to that of the drug orlistat (IC_{50} 146 μg/mL). Out of the fractions of the methanolic extract, the chloroform fraction was most active (IC_{50} 189.6 μg/mL). The order of inhibitory activity of the fractions was as follows: chloroform > ether > *n*-butanolic > aqueous > ethyl acetate. The GC/MS analysis of the most active chloroform faction showed the presence of hexadecanoic acid, methyl hexadecanoate, isopropyl palmitate, methyl 9,12-octadecadienate, and methyl 9,12,15-octadecatrienoate.

Conclusions: The study suggests that *Lagenaria siceraria* has potential to inhibit pancreatic lipase activity, suppressing lipid digestion and thereby diminishing entry of lipids into the body. Regular intake of aqueous decoction of the fruit may therefore be recommended for control of obesity. Fatty acids and their esters may play role as inhibitors of lipase.

1. Introduction

Obesity has become one of the major health concerns throughout the world as it is associated with a number of fatal metabolic disorders including diabetes, hypertension, stroke, osteoarthritis, cancer, cardiovascular diseases, sleep breathing disorders [1,2]. It consumes about 2%–6% of the total health care expenditure in many developed nations [3]. According to the recent reports of World Health Organization (WHO, 2015), since 1980, the number of obese people has more than doubled globally, and in 2014, over 1.9 billion adults were overweight and more than 600 million were suffering from obesity. Obesity, which is characterized by a disproportionate accumulation of fat in the body, is a result of an imbalance in the intake of calories and their utilization by the body. Clinically, a person is considered obese if his/her body weight is at least 20 percent higher than normal. Overweight and obesity are related but different conditions. If the Body Mass Index (BMI) of a person is between 25 and 29.9, he/she is considered overweight. Obesity, on the other hand, is characterized by BMI 30 or higher [4].

The accompanying life threatening risks of obesity necessitates on the part of scientists to explore remedies for its treatment. One of the strategies used to treat obesity is to suppress digestion and, therefore, absorption of dietary lipids in the gastrointestinal tract. This can be accomplished by inhibiting pancreatic lipase enzyme (triacylglycerol lipase) that is responsible for digestion of 50%–70% fats consumed as part of diets [5]. The anti-obesity drug

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Orlistat follows the same strategy. This powerful lipase inhibitor, however, suffers from safety issues. It has some serious side effects like steatorrhea, fecal incontinence, and flatulence [6–9]. It also has risks of vitamin deficiencies and liver diseases. Its use is also restricted by contraindication in pregnancy, patients with malabsorption disorders and reduced gallbladder function [7].

In view of the above, there is a need to explore safer alternative and complementary therapy to combat obesity. Plant based remedies offer a natural choice due to a number of factors including safety, efficacy and affordability. Consequently, the recent years have witnessed an influx of studies exploring plants for their lipid lowering or controlling effect. Plants have a wide variety of natural chemical compounds having diverse structural features making many of them potentially compatible with pancreatic lipase modulating mechanisms.

Lagenaria siceraria (*L. siceraria*) (LS, Family Cucurbitaceae) is a climber or trailer of Asian and African origin with subglobose ellipsoid or lageniform fruit [10]. The plant is cultivated for its fruit, which is used as vegetable [11]. It has highly rich ethnomedicine and is recognized to have cardiotonic, hepatotonic, anti-hyperglycemic, and anti-hyperlipidemic properties [12–14]. The fruit has also been exhibited to possess fibrinolytic [15], antithrombotic [16], and anti-atherosclerotic activity [17]. Antioxidant properties of the fruit have been studied in detail demonstrating it having remarkable antioxidative and free radical scavenging potential [18]. It possesses considerable anti-microbial properties against a number of microorganisms [19,20]. It has also been shown to possess antihyperlipidemic properties in animal models [21–23].

The fruit has been found to contain ascorbic acid, caffeoylquinic acid, cucurbitacins, pectin, β-carotene, iso-fucosterol, campesterol, spinasterol, kaempferol, palmitic acid, oleanolic acid, linoleic acid, quercetin and iso-quercetin [11,19,24–27].

Although the plant has been extensively studied for various therapeutic properties, there is no literature report on its lipase inhibitory activity. The ethno-medicinal repute of the plant for its fat lowering effect [14,28] stimulated our attention for this study. In view of the fact that composition and hence efficacy of a plant extract depends on the solvent used for its extraction, we employed a variety of solvents for the purpose.

2. Materials and methods

2.1. Chemicals

All chemicals used in these experiments were of analytical grade. Porcine pancreatic lipase typeII, *p*-Nitrophenyl palmitate (*p*-NPP) and Orlistat was purchased from Sigma-Aldrich (USA). Methanol, ethanol, acetone, *n*-butanol, chloroform, ethoxyethane, ethyl acetate, dimethyl sulfoxide (DMSO) and Tris buffer were purchased from Merck (Germany).

2.2. Preparation of methanolic extract

LS fruit (2 kg) was collected from a local market of Lahore, Pakistan, in December 2015. The fruit was peeled off, crushed and ground into a fine paste like material. Cold maceration method was used to obtain methanolic extract. The paste (1500 g) was extracted in 2 L methanol for 15 d at ambient temperature (25–30) °C. The extract obtained by filtration (Whatman filter paper 41) was concentrated by evaporating the solvent on a rotary evaporator under reduced pressure at 30 °C yielding 200.34 g of methanolic extract as a gummy material. The methanolic extract (200 g) was placed in distilled water (200 mL) and fractionated successively into the solvents of increasing polarity, *i.e.*, ether, chloroform, ethyl acetate and *n*-butanol. As a result, fractions were obtained in these solvents along with the left over aqueous.

2.3. Preparation of ethyl acetate extract after washing with water

The fruit (2 kg) was peeled off, crushed and ground. To remove water-soluble components, the ground fruit material was soaked in 2 L distilled water and placed on a shaker for 8 h. It was filtered and the residue was dried in an incubator at 25 °C for 30 min. The dried residue (180 g) was extracted with ethyl acetate (3 L) by shaking on a shaker for 24 h. Then, the extract was filtered. The ethyl acetate extract (1.2 g) was obtained after evaporating the solvent *in vacuo*.

2.4. Preparation of aqueous decoction

To obtain its aqueous decoction, 500 g ground fruit material was boiled in 500 mL distilled water for 2 h. The decoction was filtered, and water was removed *in vacuo* to obtain aqueous extract (3.2 g).

2.5. Preparation of chloroform, ethanol, acetone and ethyl acetate extracts

The ground material (1 kg) was soaked in chloroform (1 L) and kept for 7 d at ambient temperature (25–30) °C with occasional shaking. The extract then was filtered and the filtrate so obtained was concentrated *in vacuo*.

The ethyl acetate, ethanol and acetone extracts were obtained in the same manner.

2.6. Determination of enzyme inhibitory activity

The porcine pancreatic lipase inhibitory activities of LS fruit samples were determined according to a reported method [3] with minor modification, using *p*-nitrophenyl palmitate (*p*-NPP) as a substrate. The enzyme under the reaction conditions hydrolyses *p*-NPP to release *p*-nitrophenol, which is a colored substance and can be monitored at 410 nm. Serial dilutions of each extract and fractions were prepared in DMSO (25–600) μg/mL. Lipase (0.1 mg) was dissolved in Tris-buffer (50 mM, pH 8). The mixture was stirred for 15 min and centrifuged at 2000 rpm for 10 min. The clear supernatant was recovered. In a test tube, 1 mL fruit sample (or, Orlistat) was mixed with 0.5 mL lipase solution. It was incubated for 30 min at 37 °C. Then, 1 mL substrate *p*-NPP (3 mM in 2-propanol) was added into it. After incubating the mixture for 2 h at 37 °C, its absorbance was recorded at 410 nm against a blank. The percent inhibition was calculated using the following formula:

$$\%\ \text{Activity} = [\text{Ac} - \text{As}/\text{Ac}] \times 100$$

where Ac and As are the absorbance of control and sample, respectively. The control contained all constituents except a test sample. Orlistat was used as a positive control.

2.7. GC–MS analysis

The equipment used for GC–MS (gas chromatography-mass spectrometry) investigation was Agilent GC7890A/MS5975. The GC column was HP-5 MS (30 m, 0.25 mm, 0.25 μm); helium was used as a carrier gas at the flow rate of 1 mL/min. The injector temperature was 230 °C; oven temperature was automated from 50 °C then 10 °C/min to 300 °C for 5 min at hold. In the MS, the solvent delay time was 4.00 min, relative voltage 71 eV, scan parameters (40–800) amu, MS source temperature 240 °C and MS quad temperature was 150 °C. NIST 05 library was used to identify the phytochemicals.

2.8. Statistical analysis

The enzyme inhibitory activity of each sample was determined at least thrice for better reliability and statistical mean was calculated using Microsoft Excel 2007 with SD. The IC_{50} values were calculated using the same program.

3. Results

3.1. Extraction and fractionation

The yield of the methanolic extract (4.7%) was calculated based in dried fruit powder used for extraction, while the yield of fractions were calculated based on the methanolic extract used for fractionation.

Methanol is considered a solvent of choice when a wide array of natural products is to be extracted. Cold maceration was selected because it is less destructive to most chemical bonds as compared to hot extraction. In case of water, however, hot extraction was employed in order to simulate the cooking conditions used for the vegetable. Nonpolar solvents, diethyl ether and chloroform extracted about 18% and 10.7% of the constituents of the methanolic extract, respectively. Moderately polar ethyl acetate extracted 21.5% while more polar *n*-butanol 15.5%. The most polar solvent water was able to retain more than 22% of the methanolic extract. LS fruit thus contains a broad range of nonpolar to highly polar phytochemicals.

Seeing that ethyl acetate is taking up a large chunk of the methanolic extract, we used another strategy for its extraction. The finely divided paste of the fruit was first cold extracted with water followed by extraction in ethyl acetate (0.7%). For comparison, direct aqueous decoction was also obtained (0.64%). Inspired by the observation that the chloroform fraction was the most powerful inhibitor of lipase, direct chloroform extract (yield about 1.0%) was also done. In a similar manner, direct extraction in ethyl acetate, ethanol and acetone was also carried out and the yields were 1.4%, 1.1%, 4.0%, respectively.

3.2. Lipase inhibitory activity of methanolic extract and its fractions

The lipase inhibitory activities of the methanolic extract and its fraction were investigated, and the findings are displayed in Figure 1. Their IC_{50} values are displayed in Figure 3. The methanolic extract and its ether, ethyl acetate, *n*-butanolic, and aqueous fractions showed dose dependent lipase inhibitory activities with IC_{50} (μg/mL) 293.4, 231.7, 189.6, 370.0, 252.2 and 261.9, respectively. For comparison, the IC_{50} of the standard drug Orlistat was 145.7 μg/mL. As the results indicate, the chloroform fraction was most potent and ethyl acetate fraction least potent.

3.3. Lipase inhibitory activity of extracts in different solvents

The lipase inhibitory activities of the extracts of LS fruit in a number of solvents were also evaluated in order to figure out the best solvent (Figure 2). As Figure 2 shows, all the extract exhibited dose dependent efficacy. Again, chloroform proved to be the most suitable solvent to extract lipase inhibitors from the fruit. Acetone was least suitable. The IC_{50} (μg/mL) values of methanolic, ethanolic, chloroform, ethyl acetate, acetone, ethyl acetate (after washing with water) and aqueous decoction were 293.4, 266.47, 157.59, 182.12, 352.34, 257.0, and 190.0, respectively (Figure 4).

The methanolic extract and its fractions displayed lipase inhibitory activities in a concentration dependent manner like the

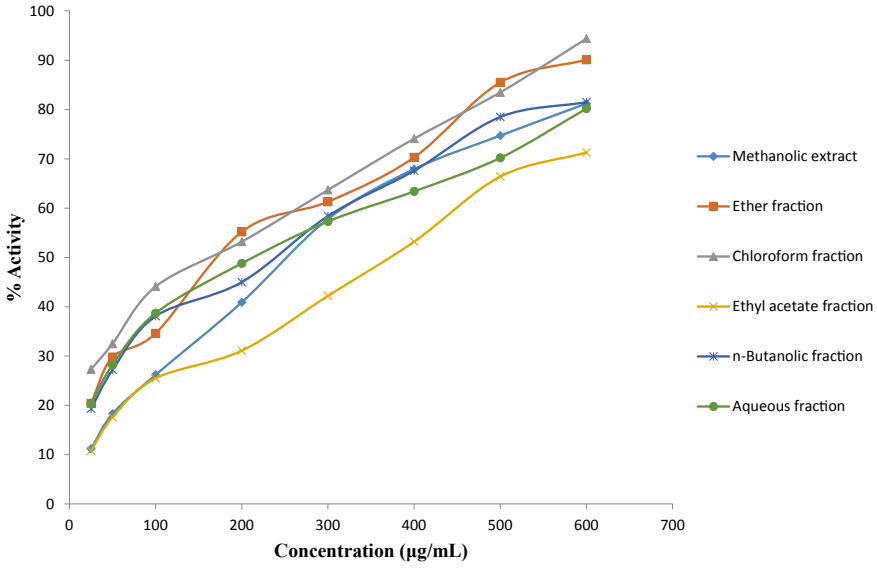

Figure 1. Lipase inhibitory activities (%) of methanolic extract of the fruit of *Lagenaria siceraria* and its fractions (n = 3).

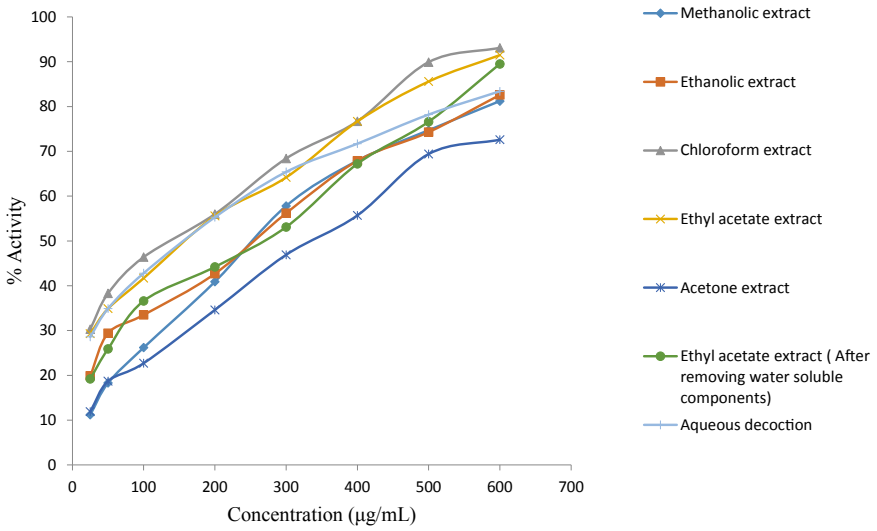

Figure 2. Lipase inhibitory activities (%) of extracts of the fruit of *Lagenaria siceraria* in different solvents (n = 3).

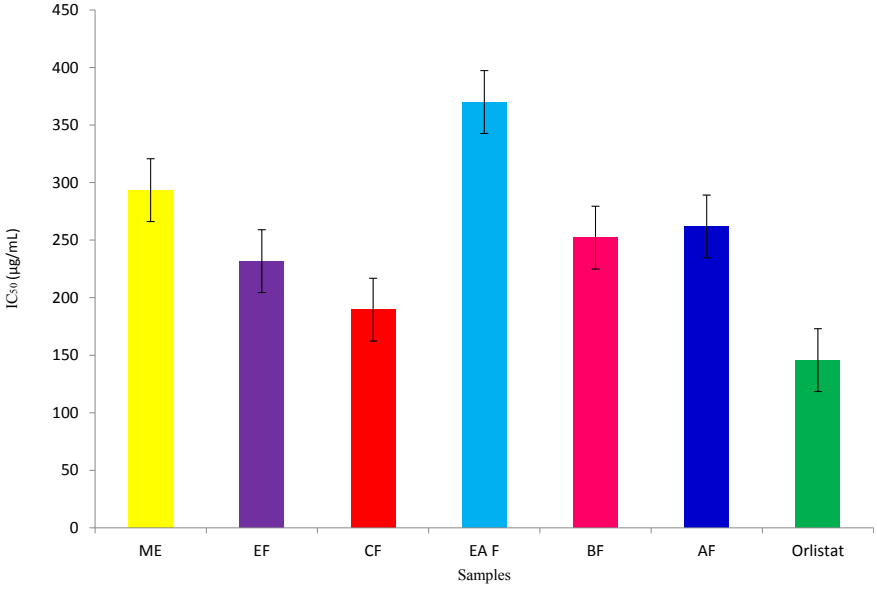

Figure 3. IC_{50} values of methanolic extract (ME) of the fruit of *Lagenaria siceraria* and its ether (EF), chloroform (CE), ethyl acetate (EAF), *n*-butanolic (BF) and aqueous (AF) fractions against lipase in comparison with Orlistat (n = 3).

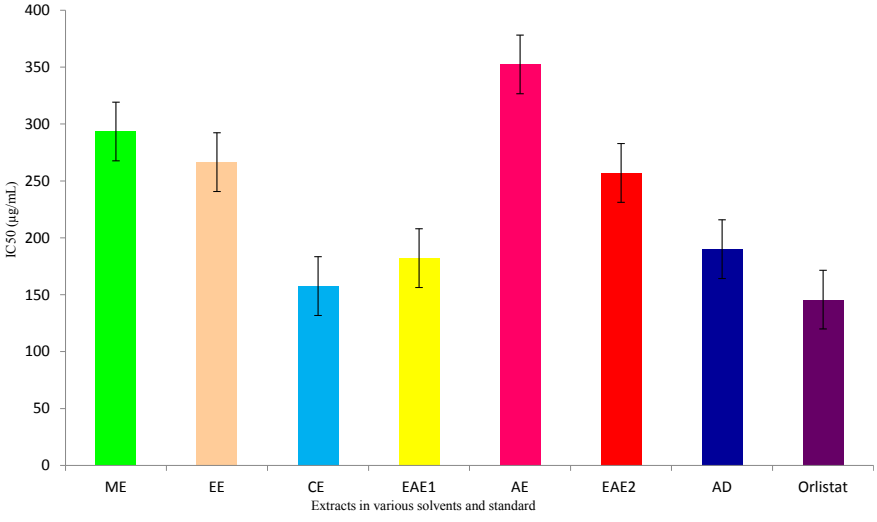

Figure 4. IC_{50} values of methanolic (ME), ethanolic (EE), chloroform (CE), acetone (WE), aqueous (AD), ethyl acetate (EAE1) and ethyl acetate after washing with water (EAE2) extracts of *Lagenaria siceraria* in different solvents against lipase in comparison with Orlistat (n = 3).

Table 1
List of the compounds identified in chloroform extract and fraction of *Lagenaria siceraria* fruit with the help of GC–MS.

Extracts	Retention time (min)	Compounds identified	Mol. mass	Total (%)
Chloroform extract	16.869	Hexadecanoic acid	256	32.70
	18.512	9,12-Octadecadienoic acid, (Z,Z)-	280	14.40
	18.555	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278	14.30
	21.704	Hexadecanoic acid, 2-hydroxy-1-(hydroxylmethyl)ethyl ester	330	7.60
	23.063	9,12-Octadecadienic acid, (Z,Z)-, 2-hydroxy-1-(hydroxylmethyl ethyl ester	354	4.40
Chloroform fraction	15.525	Hexadecanoic acid, methyl ester	270	7.38
	16.293	Hexadecanoic acid	256	11.60
	16.448	Isopropyl palmitate	298	1.20
	17.205	9,12-Octadecadienoic acid, (Z,Z)- methyl ester	294	21.80
	17.299	Alpha-lenolenic acid methyl ester	292	25.10
	17.884	9,12-Octadecadienoic acid, (Z,Z)-	280	6.70
	21.059	Hexadecanoic acid, methyl ester	278	21.60

standard drug Orlistat, indicating a possible similarity in their mechanism of action. The order of efficacy of the extract and its fractions was as follows: Chloroform > ether > *n*-butanolic > aqueous > methanolic > ethyl acetate.

Chloroform fraction was thus the most potent inhibitor of the enzyme, and its efficacy was close to that of Orlistat. The ethyl acetate fraction, on the other hand, was least active.

3.4. Identification of phytochemicals by GC/MS analysis

List of the chemical compounds identified by GC/MS analysis is given in Table 1.

4. Discussion

Inhibition of pancreatic lipase is a viable strategy to combat obesity [1,29]. Side effects, cost and availability of synthetic drugs demand for safer, affordable and readily available alternative. Consequently, more and more attention is being paid to natural plant based inhibitors [30,31]. Fruits and vegetables having desirable medicinal properties are preferred for long term consumption as they pose no side effects being already compatible with our body. *L. siceraria* (bottle gourd, calabash) is a fruit vegetable cultivated on large scale in many parts of the world including Pakistan and India [10,12,32,33].

Inspired by the immense repute of the ethno-medicinal applications of the plant as antihyperlipidemic, antihyperglycemic, cardiotonic and hepatotonic agent, the present study was designed. As the choice of solvent plays key role in the extraction of phytochemicals from a plant sample, multiple solvents were used for extraction. Methanol is a solvent of choice for indiscriminate extraction of phytochemicals of all kinds. To segregate the methanol soluble constituents of the fruit based on their polarity, the methanolic extract was successively fractionated into various solvents with increasing polarity.

As the results indicated, the chloroform fraction showed maximal inhibitory activity against lipase enzyme. The ethyl acetate fraction was the least active. Interestingly, the activity of the aqueous extract (IC_{50} 190.0 μg/mL) was much higher than the aqueous fraction (IC_{50} 261.9 μg/mL) of the methanolic extract. This means, to obtain lipase inhibitory constituents of the fruit, water, under hot extraction conditions, is a better solvent than methanol. Similarly, ethyl acetate extract after washing with water (IC_{50} 257.0 μg/mL) and ethyl acetate extract obtained directly (IC_{50} 182.12 μg/mL) was more active than its fraction (IC_{50} 370.0 μg/mL) alluding to a similar conclusion.

The chemical constituents of the chloroform fraction, as identified by GC–MS, included esters of some fatty acids, *i.e.*, hexadecanoic acid methyl ester, isopropyl palmitate, 9,12-octadecadienoic acid methyl ester, and alpha-linolenic acid methyl ester. These compounds may contribute towards lipase inhibitory activity in an additive or synergistic manner.

Since LS is a vegetable, its consumption along with fat containing foods is expected to inhibit pancreatic lipase inhibitory activity suppressing fat digestion and thereby diminishing entry of lipids into the body. The combination of LS or its decoction with meals may therefore be recommended for control of obesity. The fact that the fruit is also famed for its antihyperglycemic, cardiotonic and hepatotonic activity, its consumption should impart added benefit for the patients of diabetes, cardiovascular disorders and liver diseases. Unlike the medicine, the fruit can be used by anybody at any time for any duration. Moreover, the fruit is readily available to people of all economic sectors as it can easily be grown under most conditions and is cultivated in many parts of world.

L. siceraria fruit possess good ability to inhibit action of pancreatic lipase. Chloroform was a better solvent to extract chemical components of the fruit having better potential to inhibit the enzyme. Aqueous decoction also showed good activity. It might be recommended to make this fruit–vegetable a regular part of our meals in order to reduce the calorie intake.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

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