obesities

MDPI

Review

Methods to Evaluate the Antiobesity Effects of Medicinal Plants Using Enzyme Assays

Subhani Bandara *, Shelby Devereaux and Aruna Weerasooriya

Cooperative Agricultural Research Center, Prairie View A&M University, Prairie View, TX 77446, USA
* Correspondence: suba17s@yahoo.com; Tel.: +1-936-261-5045

Abstract: Obesity is a chronic disease affecting both adults and children worldwide. One major cause of obesity is high-calorie intake due to overconsumption of foods rich in fat and carbohydrates. Hence, obesity can be controlled by controlling the diet and by other lifestyle changes that increase energy expenditure. However, this is not always possible for individuals who are already overweight and suffering from other diseases. Therefore, certain drugs have been developed to assist with weight reduction. One major avenue for drug development involves the inhibition of enzymes that break down fat and carbohydrates from the diet. This can reduce the bioavailability and absorption of dietary lipids and carbohydrates, allowing for the management of obesity. Although there are synthetic drugs available on the market to inhibit these enzymes, plant-based natural drugs may provide a better alternative to treat obesity due to fewer side effects and a lower cost. In this review, different methods that can be used to screen medicinal plant extracts for inhibitors of those digestive enzymes and certain limitations of those methods are discussed. Currently, there is limited research on the effects of varying conditions on enzyme assays, and this is an area that can be addressed in future research.

Keywords: obesity; enzyme assay method; medicinal plants; enzyme inhibitors; pancreatic lipase; cholesterol esterase; α-amylase; α-glucosidase

Citation: Bandara, S.; Devereaux, S.; Weerasooriya, A. Methods to Evaluate the Antiobesity Effects of Medicinal Plants Using Enzyme Assays. *Obesities* **2023**, *3*, 13–35. https://doi.org/10.3390/obesities3010003

Academic Editor: Sara Baldassano

Received: 3 November 2022
Revised: 14 January 2023
Accepted: 16 January 2023
Published: 19 January 2023

1. Introduction

The level of obesity worldwide has increased from about a sixth of the world population to around one-third of the world population from 1980 to 2016 [1], showing the immense need to seek solutions to this global issue. Additionally, in the United States, obesity-related health issues cost about $147 billion, or 9.1% of annual healthcare costs [2]. The current pandemic situation has further proven the importance of addressing obesity, as it has been declared a risk factor for COVID-19 [3]. The prevalence of obesity in the USA is 40%, which is remarkably high compared with other countries [3]. A survey conducted at hospitals in the USA has shown that most of the younger patients admitted to the ICU for COVID-19-related complications were obese. Obesity is known to block ventilation, impair immunity to viral infections, enhance inflammation, and adversely affect cardiovascular functions, making people more susceptible to infectious diseases [3]. Individuals with a Body Mass Index (BMI) higher than 30 kg/m^2 are considered obese, while those with a BMI higher than 40 kg/m^2 are categorized as extremely obese [4]. Obesity also leads to other metabolic disorders such as type 2 diabetes, liver, kidney, cardiac, and gallbladder diseases, high blood pressure, and cancer [4]. Hence, obesity is a significant issue that can lead to a myriad of other health-related problems and should be addressed promptly.

Other than lifestyle changes and exercise, additional treatment methods are necessary to provide effective solutions to control obesity. Bariatric surgery is a successful procedure used to enhance the life expectancy of obese patients [5]. However, this surgical intervention can be limited to certain individuals or countries, making it less suitable as a global approach. Therefore, other types of treatments or drugs are required to address obesity

and related health issues. Anti-obesity drugs that act via peripheral or central pathways have been used historically to manage obesity, but most of these drugs were removed from the market due to serious side effects. The history of different types of weight loss drugs and their associated side effects is discussed in the review article by Muller et al., 2022 [5]. Despite the challenges in developing pharmacotherapy to control obesity, four types of drug targets, including leptin, ghrelin, mitochondrial uncouplers, and growth differentiation factor 15, were advanced to clinical trials due to the efficacy observed at the initial screenings [5].

Obesity is strongly linked to elevated levels of lipids, cholesterol, and glucose in the blood arising from the diet. Hence, delaying the digestion and absorption of fat and carbohydrates from the diet provides a path to control obesity [1]. Inhibiting the fat-metabolizing enzymes, such as pancreatic lipase, pancreatic cholesterol esterase, as well as carbohydrate metabolizing enzymes such as α-amylase and α-glucosidase, can accomplish this [1,6]. The drugs that are currently approved in the United States as antiobesity pharmacotherapies include orlistat, naltrexone/bupropion, liraglutide 3 mg, semaglutide 2.4 mg, and phentermine/topiramate [5]. From this list, orlistat is the only drug that acts by inhibiting the metabolic enzyme lipase. But it is also known to cause liver injury and gastrointestinal issues [5]. The modes of action of the other approved drugs mentioned above are discussed in detail elsewhere [7]. Acarbose and miglitol are alpha-glucosidase inhibitors that are approved by the FDA in the United States to manage type II diabetes mellitus as adjuncts to diet and exercise [8] and acarbose has also shown promising weight management effects in humans [9]. Due to the safety and efficacy-related challenges associated with most of the pharmacological therapeutics, researchers have turned towards medicinal plant-based antiobesity drugs to discover potential natural compounds with reduced side effects in a cost-effective manner. Furthermore, enzyme inhibitory drugs do not cause serious side effects similar to the drugs that act on the central or sympathetic nervous system to elicit their effects by suppressing appetite or improving energy expenditure [9,10]. Therefore, enzyme inhibitory drugs can be promising in long-term usage for controlling obesity. Plants can be ideal candidates to find lead compounds for drug development due to the presence of a wide array of phytochemicals and their proven safety from long-term usage. Polyphenols and glycoproteins present in plant extracts are some of the main compounds known for their enzyme-inhibitory effects [11,12]. A variety of secondary metabolites found within plants with potential enzyme inhibitory properties are suggested to have a defensive role in plants against predators [13]. The phytochemicals important in antiobesity effects and their action mechanisms related to metabolism had been reviewed previously [14]. Due to the above-mentioned properties of the plant extract-based drugs, it is important to know the methods to screen for plant extracts as potential anti-obesity drug targets.

The focus of this review is to provide an overview of different methods used to study the antiobesity effects of medicinal plants via the inhibition of fat and carbohydrate metabolizing enzymes. Furthermore, certain limitations that can be encountered during these assays and ways to address some of the limitations are discussed here.

To construct this review, the Web of Science database was searched using the keywords "antiobesity medicinal plants" and after the exclusion of the review articles, a total of 125 articles were obtained. The studies demonstrating the enzyme inhibitory effects of medicinal plant extracts were selected for this review (Table 1). Additional articles were incorporated to discuss other considerations and possible limitations of these assays. This review provides insights into method development for the researchers searching for natural, medicinal plant-based drugs to combat obesity via the inhibition of important metabolic enzymes.

Table 1. Medicinal plants, plant parts, and the extraction solvents used for the screening of inhibitors of different metabolic enzymes.

Plant Name	Parts Used	Extraction Solvent	Enzyme Inhibited				Ref
			Pancreatic Lipase	Pancreatic α-Amylase	α-Glucosidase	Cholesterol Esterase	
Sweet gale (*Myrica gale* L.)	Stems & leaves	Hot water extract and ethanol extract	–	√	√	–	[15]
Roseroot (*Rhodiola rosea* L.)							
Sheep sorrel (*Rumex acetosa* L.)							
Stinging nettles (*Utrica dioica* L.)							
Dandelion (*Taraxacum officinale* L.)							
39 plant species from several different families	Different parts	70% ethanol	√	–	–	–	[16]
Bugloss (*Echium angustifolium* Mill.)	Leaves	Methanol, water, acetone and hexane as separate extracts	√	√	√	–	[17]
23 plant species from few different families	Different parts	Water extract and methanol extract	√	√	–	–	[18]
Cang zhu (*Atractylodes lancea* (Thunb.) DC.)	rhizome	Methylene chloride/methanol mix (1:1)	√	–	–	–	[19]
Guinea pepper (*Aframomum melegueta* K.Schum.) and Toothache plant (*Spilanthes acmella* (L.) L.)	Seeds or flower buds	70% ethanol	√	–	–	–	[20]
Lotus lily (*Nelumbo nucifera* Gaertn.)	Flower petals	Methanol extraction followed by water extraction	√	–	–	–	[21]
Siamese cassia (*Cassia siamea* Lam.)	roots	Ethyl acetate	√	–	–	–	[22]
Large-fruited juniper (*Juniperus macrocarpa* Sm.) and Greek Juniper (*Juniperus excelsa* M.Bieb.)	Branches, fruits and leaves	Water, ethyl acetate and methanol (2.5% *w*/*v*) as separate extracts	√	√	√	–	[23]
Spreading pellitory (*Parietaria judaica* L.)	Leaves	Methanol, water, acetone and hexane as separate extracts	√	√	–	–	[24]
Golden shower tree (*Cassia fistula* L.)	Leaves	20% to 80% hydroethanol	√	–	–	–	[25]
Bitter melon (*Momordica charantia* L.) and Babchi (*Psoralea corylifolia* L.)	Leaves	Successive extraction with chloroform, acetone and 70% ethanol	√	–	–	–	[4]
Mixture of Chinese mulberry (*Cudrania tricuspidata* (Carrière) Bureau ex Lavallée), Blue Honeysuckle (*Lonicera caerulea* L.), and Soybean (*Glycine hispida* (Moench) Maxim.)	Fruits/seeds	80% methanol	√	–	–	–	[26]
Red everlasting (*Helichrysum sanguineum* (L.) Kostel.)	Aerial parts	Hexane, acetone, methanol and water as separate extracts	√	√	–	–	[27]
Egyptian broomrape (*Orobanche aegyptiaca* Pers.)	Aerial parts	Successive extraction with petroleum ether, methylene chloride, chloroform, methanol and collected separately	√	√	–	–	[28]
Yellow camellia (*Camellia nitidissima* C.W.Chi)	Flower	90% ethanol	√	–	–	√	[1]
Saltcedar (*Tamarix dioica* Roxb. ex Roth)	Aerial parts	Methanol (containing 10% water)	–	–	√	–	[25]

Table 1. *Cont.*

Plant Name	Parts Used	Extraction Solvent	Enzyme Inhibited				Ref
			Pancreatic Lipase	Pancreatic α-Amylase	α-Glucosidase	Cholesterol Esterase	
Baboon's cucumber (*Kedrostis africana* (L.) Cogn.)	Tuber	Ethanol extract and water extract	√	√	√	–	[29]
Common guava (*Psidium guajava* L.)	Leaves	Water	√	√	√	–	[30]
Mangaba fruit (*Hancornia speciosa* Gomes)	Leaves	96% ethanol	√	√	√	–	[31]
East Indian lotus (*Nelumbo nucifera* Gaertn.)	Leaves	75% ethanol	√	–	√	–	[32]
Atlas mastic tree (*Pistacia atlantica* Desf.)	Leaves	100% methanol	√	√	√	√	[33]
Southern blue gum (*Eucalyptus globulus* Labill.)	Leaves	Sequential extraction with hexane, ethyl acetate and methanol	√	√	–	–	[34]
White shrubby horsetail (*Ephedra alata* Decne.)	Fruits	Sequential extraction with hexane, acetone, methanol and water	√	√	√	–	[35]
Wild coffee (*Psychotria densinervia* (K.Krause) Verdc.)	Leaves and Bark	70% ethanol and 30% water mix	√	√	√	√	[36]

2. Inhibition Assays for Fat Metabolizing Enzymes

Lipids contribute to a large part of the calories in the diet. Hence, inhibition of the fat-digesting enzymes with phytochemicals is used as an approach to control obesity. Pancreatic lipase and cholesterol esterase are key enzymes required for digestion and the absorption of fat from the diet. Triglycerides are a major component of dietary fat, with a small contribution from phospholipids [37]. Pancreatic lipase is involved in the digestion of triglycerides, releasing β-monoglycerides and free fatty acids. Human lipase consists of pre-duodenal and extra-duodenal lipases and contributes to the digestion of 50 to 70% of the fat in the diet [38]. Hence, plant extracts that inhibit pancreatic lipase have been the main target of natural product researchers searching for antiobesity drugs. Pancreatic cholesterol esterase is primarily involved in digesting cholesterol esters from the diet in the small intestine and produces free cholesterol and free fatty acids [39]. Additionally, this enzyme is known to facilitate the formation of mixed micelles and aid in the uptake of cholesterol from these micelles into enterocytes [40,41]. Hence, the inhibition of this enzyme will result in reduced bioavailability and absorption of dietary cholesterol [40].

2.1. Assay Methods to Screen for Pancreatic Lipase Inhibitors

A variety of different assay methods were utilized for the screening of pancreatic lipase inhibitors (Table 2). The most common method utilized by researchers for the determination of pancreatic lipase inhibition uses *p*-nitrophenyl esters linked to organic acids as the substrate, providing a direct and continuous measurement of the enzyme activity via spectrophotometry [42]. When these *p*-nitrophenyl esters, such as *p*-nitrophenyl butyrate, *p*-nitrophenyl laurate, *p*-nitrophenyl palmitate, *p*-nitrophenyl phosphate, etc., are used as the substrate during the pancreatic lipase assay, they give *p*-nitrophenol and the corresponding organic acid as the products (Figure 1). *p*-nitrophenol is converted to yellow color *p*-nitrophenolate in the alkaline buffer media, and the color intensity can be measured using UV-Vis spectrophotometry in the wavelength range of 405–410 nm [43]. Furthermore, researchers used the oleate ester of 4-methylumbelliferone as the substrate to evaluate the lipase activity using fluorometry [18,21,34]. In this method, the enzyme activity generates 4-methylumbelliferone, which is quantified using the excitation and emission wavelengths set at 360 nm and 455 to 465 nm, respectively. Jiao et al., 2014 used 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin)-ester (DGGMR) as the substrate to study the

enzyme activity of recombinant human pancreatic lipase [19]. DGGMR is converted to an unstable dicarbonic acid ester with the action of lipase. This unstable compound is spontaneously broken down to form glutaric acid and methylresorufin under alkaline conditions. The color intensity of methylresorufin, which is a bluish-purple compound, is measured at 580 nm to calculate enzyme activity [44]. The lipase PS™ assay kit is another choice to evaluate pancreatic lipase inhibition by plant extracts. This measures the formation of quinone diamine dye that absorbs at 550 nm [20]. Aabideen et al., 2021 used a titration-based method to quantify the lipase activity of *Cassia fistula* extract [25]. In this method, a homogenized substrate emulsion containing Arabic gum and olive oil was reacted with the enzyme; the reaction was stopped, and the free fatty acids released were quantified by titrating with 0.02 M NaOH until the pH reached 9.4.

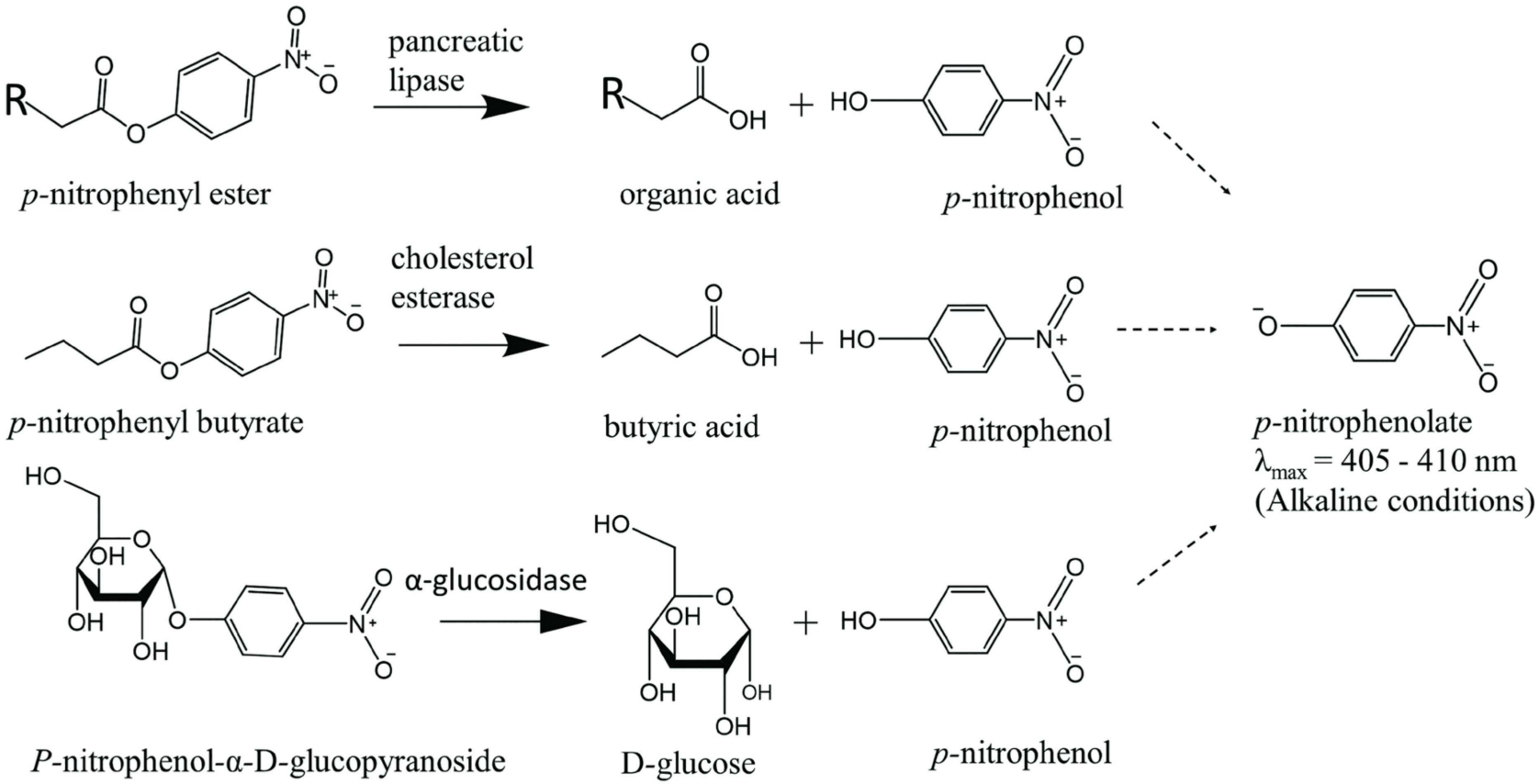

Figure 1. Common enzymatic reactions used to screen medicinal plants for inhibitors of pancreatic lipase, cholesterol esterase, and α-glucosidase. All these reactions lead to the formation of *p*-nitrophenol as one of the products. *p*-nitrophenol will be converted to yellow color *p*-nitrophenolate under alkaline conditions. The figure was generated using ChemDraw Professional software.

During the pancreatic lipase inhibitory assays, enzyme concentrations of 1 mg/mL were mostly used, while 0.5 mg/mL, 4 mg/mL, and 10 mg/mL concentrations were also used. The method of preparation of the enzyme for the assay using crude porcine pancreatic lipase type II (Sigma, St. Louis, MO, USA) was described in a few research articles. Jamous et al., 2018 added the crude enzyme to a 20 mM tris-HCl buffer at pH 8 to obtain a 10 mg/mL solution, stirred it for 15 min, centrifuged it at 1500× *g* for 10 min, and retrieved the supernatant to use in assays [16]. Similarly, Zhang et al., 2020 dissolved the enzyme in the assay buffer containing 13 mM Tris-HCl, pH 8.0, 1.3 mM $CaCl_2$, and 150 mM NaCl at 1 mg/mL concentration and centrifuged for 15 min at 3200× *g* at 25 °C and usedthe supernatant for the reaction [1]. Al-Rimawi, et al., (2020) prepared the enzyme stock solution by dissolving 25 mg of enzyme powder in 10% DMSO [17]. The same group also used 10% DMSO to initially dissolve the plant extract and dilute it with Tris-HCl buffer to obtain the required concentrations for the lipase assay. It is important to obtain clear and particle-free solutions for colorimetric assays, as the particles can alter the spectrophotometric readings.

Table 2. Conditions used during the screening for pancreatic lipase inhibitors using plant extracts.

Enzyme Concentration	Substrate Used	Product Formed	Buffer Used	Extract Concentration	Preincubation (Before Substrate Addition)	Incubation	Solution/Method Used for Termination	Wavelength (nm)	Ref
10 mg/mL (crude) in buffer	100 mM *p*-nitrophenyl butyrate in acetonitrile	*p*-nitrophenol	20 mM Tris-HCl buffer, pH 8	5 mg/mL in ethanol	37 °C for 5 min	Not given	N/A	410	[16]
1 mg/mL in 10% DMSO	20.9 mg *p*-nitrophenyl butyrate in 2 mL acetonitrile	*p*-nitrophenol	Tris-HCl buffer	20 to 400 µg/mL in 10% DMSO and buffer	37 °C for 15 min	37 °C for 30 min	N/A	405	[17]
0.5 mg/mL in buffer	0.5 mM 4-methylumbelliferyl oleate	4-methylumbelliferone	13 mM Tris-HCl buffer, pH 8.0	2.5 mg/mL final concentration in DMSO	10 min (temperature not specified)	37 °C for 30 min	N/A	Em/Ex = 465/360	[18]
Recombinant human pancreatic lipase (concentration Not given)	1,2-O-dilaury-rac-glycero-3-glutaric acid-(6′-methylresorufin)-ester (concentration not given)	methylresorufin	Assay buffer, pH 8.4	120 µg/mL	10 min (temperature not specified)	37 °C for 30 min	N/A	580 nm	[19]
Human pancreatic lipase 248 units/L	Reconstituted substrate solution	Quinone diamine dye	Not given	Not given	N/A	37 °C for 5 min followed by 37 °C for 3 min after adding activator reagent	N/A	550 nm	[20]
Conc. Not given	4-methyl umbelliferyl oleate	4-methyl umbelliferone	Tris buffer	12.5 to 200 µg/mL	Not given	25 °C for 30 min	N/A	Em/Ex = 460/360	[21]
1 mg/mL in buffer (0.1 mg/mL final conc.)	4-nitrophenyl palmitate stock solution (10 mM) in acetonitrile and diluted in ethanol (1:2 *v*/*v*) to 3.33 mM (0.167 mM final conc.)	*p*-nitrophenol	0.1 mM Tris-HCl buffer, pH 8.5	250 µg/mL final conc. in buffer and DMSO	Not given	37 °C for 30 min	N/A	405 nm	[22,45]
Dissolved In 4-morpholinepropanesulfonic acid (10 mM) and ethylenediaminetetraacetic acid (EDTA, 1 mM) buffer solution pH 6.8 (concentration Not given)	4-nitrophenyl butyrate	*p*-nitrophenol	Tris–HCl, 100 mM, and $CaCl_2$, 5 mM, pH 7.0	80% *w*/*v* in ethanol at logarithmic concentrations	37 °C for 15 min	37 °C for 30 min	N/A	405	[23]
1 mg/mL stock solution in buffer	20.9 mg 4-nitrophenyl butyrate in 2 mL acetonitrile	*p*-nitrophenol	Tris–HCl buffer	50 to 400 µg/mL in 10% DMSO	37 °C for 15 min	37 °C for 30 min	N/A	410	[24]
1 mg/mL (25 units/mL)	Substrate solution containing Arabic gum (10 g) and olive oil (10% *w*/*v* in buffer)	Free fatty acids	0.01 Tris-HC1 buffer	In buffer (Conc. Not given)	4 °C for 30 min	37 °C for 30 min	Added acetone: ethanol (1:1) mixture	N/A	[25]
1 mg/mL in 0.1 mM in potassium phosphate buffer, pH 6.0	20.9 mg para-nitrophenyl butyrate in 2 mL acetonitrile	*p*-nitrophenol	Tris-HC1 buffer, pH 7.4	50 to 400 µg/mL in 10% DMSO	25 °C for 15 min	37 °C for 30 min	N/A	405	[4]

Table 2. *Cont.*

Enzyme Concentration	Substrate Used	Product Formed	Buffer Used	Extract Concentration	Preincubation (Before Substrate Addition)	Incubation	Solution/Method Used for Termination	Wavelength (nm)	Ref
Prepared in buffer (conc. Not given)	10 mM 4-nitrophenyl butyrate	*p*-nitrophenol	0.1 M Tris-HC1 buffer, pH 8	Not given	37 °C for 15 min	37 °C for 15 min	N/A	405	[26]
1 mg/mL stock in buffer	20.9 mg para-nitrophenyl butyrate in 2 mL acetonitrile	*p*-nitrophenol	Tris-HCl	1 mg/mL stock in 10% DMSO diluted to 50 to 400 µg/mL	37 °C for 15 min	37 °C for 30 min	N/A	405	[27]
1 mg/mL stock	20.9 mg para-nitrophenyl butyrate in 2 mL acetonitrile	*p*-nitrophenol	Tris-HC1 buffer, pH 7.4	1 mg/mL stock in 10% DMSO diluted to 50 to 400 µg/mL	37 °C for 15 min	37 °C for 30 min	N/A	405	[28]
1 mg/mL in buffer	2 mg/mL para-nitrophenyl butyrate in buffer	*p*-nitrophenol	13 mM Tris-HC1 buffer, pH 8, 1.3 mM $CaCl_2$ and 150 mM NaCl	Not given	37 °C for 10 min	37 °C for 20 min	N/A	405	[1]
Added at four times the volume of test samples (conc. Not given)	para-nitrophenyl butyrate in buffer	*p*-nitrophenol	Not given	50 to 200 µg/mL in DMSO	37 °C for 15 min	37 °C for 25 min	N/A	405	[29]
Not given	4 mM *p*-nitrophenyl laurate in buffer	*p*-nitrophenol	0.05 mM Tris-HCl, pH 8.0 buffer containing 0.5% Triton-X100	Not given	37 °C for four periods of time	Not specified	Transferred to an ice bath and added 0.05 mM Tris-HCl, pH 8.0	410	[30]
1 mg/mL	para-nitrophenyl butyrate	*p*-nitrophenol	0.1 mM potassium phosphate buffer, pH 7.2 with 0.1% Tween 80	2.5 to 35 µg/mL	30 °C for 1 h	30 °C for 5 min	N/A	405	[31]
Not given	10 mM para-nitrophenyl phosphate	*p*-nitrophenol	0.1 M phosphate buffer, pH 7.4	Various concentrations of sample in buffer	37 °C for 5 min	37 °C for 20 min	N/A	405	[32]
1 mg/mL in 10 mM MOPS and 1 mM EDTA buffer	10 mM para-nitrophenyl butyrate in acetonitrile	*p*-nitrophenol	100 mM Tris-HCl and 5 mM $CaCl_2$, pH 7.4	Not given	37 °C for 15 min	37 °C for 30 min	N/A	405	[33]
2 units/mL in buffer	0.5 mM 4-methylumbelliferyl oleate in buffer	4-methylumbelliferone	50 mM Tris-HCl, pH 8.0	Not given	Not given	37 °C for 30 min	N/A	Em/Ex = 455/360	[34]
1 mg/mL in buffer	20.9 mg para-nitrophenyl butyrate in 2 mL acetonitrile	*p*-nitrophenol	Tris-HCl buffer	50 to 400 µg/mL	37 °C for 15 min	37 °C for 30 min	N/A	410	[35]
4 mg/mL	10 mM para-nitrophenyl butyrate in dimethylformamide	*p*-nitrophenol	Phosphate buffer	3.125 to 200 µg/mL	37 °C for 37 min	37 °C for 30 min	N/A	405	[36]

2.2. Assay Methods to Screen for Pancreatic Cholesterol Esterase Inhibitors

Provided the search criteria used for this review, only three articles were discovered using pancreatic cholesterol esterase inhibition assays to study the antiobesity effects of plant extracts (Table 3). In all of these studies, the enzyme assay utilized *p*-nitrophenyl butyrate as the substrate, which was also the major substrate used during pancreatic lipase assay. Similarly, the formation of *p*-nitrophenolate was spectrophotometrically analyzed at 405 nm to assess the enzyme activity. In contrast to the lipase assay, pancreatic cholesterol esterase needs to be activated by primary bile salts to hydrolyze various substrates containing long-chain fatty acid moieties, including cholesterol esters, triacylglycerols, phospholipids, and ceramides [41]. To accommodate this, researchers used sodium taurocholate bile salt in the reaction buffer during the cholesterol esterase assay [1,33,36]. It was shown that bile salts such as sodium taurocholate elicit a protective effect on the enzyme by forming a bile acid-enzyme complex and avoiding enzyme degradation by proteases [46].

Table 3. Conditions used during the screening for cholesterol esterase inhibitors using plant extracts.

Enzyme Concentration	Substrate Used	Buffer Used	Extract Concentration	Preincubation (Before Enzyme)	Incubation	Wavelength (nm)	Ref
0.163 units/mL in 0.1 M sodium phosphate, pH 7.0	1 mg/mL para-Nitrophenyl Butyrate in buffer	100 mM NaCl, 5.16 mM sodium taurocholate, 100 mM sodium phosphate, pH 7.0	Not given	37 °C for 10 min	37 °C for 20 min	405	[1]
Enzyme in buffer (concentration not given)	5 mM para-Nitrophenyl Butyrate in dimethylformamide	100 mM phosphate buffer, pH 7.0	Samples dissolved in 80% methanol (concentration not given)	Room temperature for 5 min	15 min (temperature not specified)	405	[33]
Not given	0.2 M para-Nitrophenyl Butyrate	Not given	3.12 to 200 µg/mL	25 °C for 10 min	25 °C for 5 min	405	[36]

3. Inhibition Assays for Carbohydrate Metabolizing Enzymes

Other than lipids, carbohydrates are another main source of calories in the diet that contribute to weight gain. Carbohydrates are broken down and maltose and oligosaccharides are formed because of the action of pancreatic and salivary α-amylases by hydrolyzing α-1,4 glycosidic bonds [25]. The final step of starch hydrolysis is catalyzed by the enzyme α-glucosidase, which is found in the brush border of the small intestinal lining [13]. α-glucosidase hydrolyses the terminal α-1,4 linkages of maltose and oligosaccharides, producing glucose. Hence, when these enzymes are inhibited, it delays the digestion of carbohydrates, leading to lower levels of blood glucose and providing a path to developing therapies for conditions such as obesity, diabetes, and cancer [47].

3.1. Assay Methods to Screen for α-Glucosidase Inhibitors

The substrate *p*-nitrophenol-α-D-glucopyranoside is used to assess the effect of plant extracts on α-glucosidase (Table 4). The enzyme's action will convert the substrate into *p*-nitrophenol (Figure 1). In this method, sodium carbonate is added to end the reaction while providing alkaline conditions to convert the product into *p*-nitrophenolate, which has an intense yellow color. This is the same product that was observed during the pancreatic lipase reactions where a 4-nitrophenyl ester was used as the substrate. α-glucosidase was mostly used at a concentration from 0.5 to 1 units/mL and was usually prepared in the corresponding buffer solution used in the assay. However, in certain journal articles, the solvent used to prepare the enzyme and the substrate are not clearly stated. Additionally, Al-Rimawi, et al., 2020 used 10% DMSO and buffer solution to prepare plant extracts for both lipase and α-amylase assays, while only buffer was used during the α-glucosidase assay [17]. This could be due to the enzyme α-glucosidase's sensitivity to DMSO.

3.2. Assay Methods to Screen for α-Amylase Inhibitors

Researchers used different methods to screen for α-amylase inhibitors in medicinal plant extracts (Table 5). From these assays, 3,5-dinitrosalicylic acid (DNS)-based method was used by the majority of the researchers (Figure 2). DNS reacts with the reducing sugars produced by the action of the enzyme to form 3-ammino-5-nitrosalicylic acid, which absorbs light at 540 nm [48]. After the addition of DNS, the reaction is usually stopped by boiling. A fluorescence-based EnzChek® Ultra Amylase Assay Kit is another choice for screening for α-amylase inhibitors in a high throughput format [18]. The kit contains a starch derivative tagged with BODIPY™ FL dye, which can be digested by α-amylase to release highly fluorescent products. The increase in the fluorescence due to the degradation of starch is proportional to α-amylase activity, providing a highly sensitive method for measuring enzyme activity/inhibition using fluorescence spectrometry. In another method, starch azure, which is also known as insoluble blue starch, was used as the substrate to quantify α-amylase activity [31]. This substrate is made by attaching the Remazol Brilliant Blue dye to insoluble corn starch via a covalent bond [49]. The enzymatic reaction drives the formation of soluble colored fragments, and the color intensity will be measured spectrophotometrically after the exclusion of the remaining substrate by centrifugation or filtration. Starch azure can be a good choice when dealing with crude enzyme extracts, as *exo*enzymes that may be present in crude extracts do not act on this substrate [49]. Jerbi et al., 2017 used 2-chloro-4-nitrophenol-α-D-maltotrioside as the substrate to test the α-amylase activity [34]. During this reaction, 2-chloro-4-nitrophenol and maltotriose are released as products, and the absorbance of the 2-chloro-4-nitrophenol can be measured at 405 nm to quantify the enzyme activity. Additionally, the amount of remaining starch after the reaction can be used to measure the enzyme activity by reacting the starch with Lugol's solution [36]. Lugol's reagent contains iodine and potassium iodide. Iodine in the solution makes a deep blue color complex with starch, which can be quantified spectrophotometrically around 620 nm. Buchholz and Melzig et al., 2015 mentioned they used the same buffer for both α-amylase and lipase assays, which is inconsistent with the optimum pH requirement for α-amylase versus pancreatic lipase enzyme activity [18]. The pH required for α-amylase is

usually around 6.9 and for lipase, it is around pH 8 and the buffer they used for the lipase assay is consistent with the required pH. They may have used the buffer provided with the kit for the α-amylase assay, although they reported it incorrectly.

α-amylase was mostly used at 2 units/mL concentration and was usually prepared in the assay buffer solution. But some researchers have utilized different methods to prepare the enzyme. Al-Rimawi, et al., 2020 prepared α-amylase for the assay by initially dissolving it in a small volume of 10% DMSO and diluting it with the buffer solution to obtain 2 units/mL solution [17]. The same group also used 10% DMSO and the buffer to dissolve their plant extract samples for the α-amylase assay.

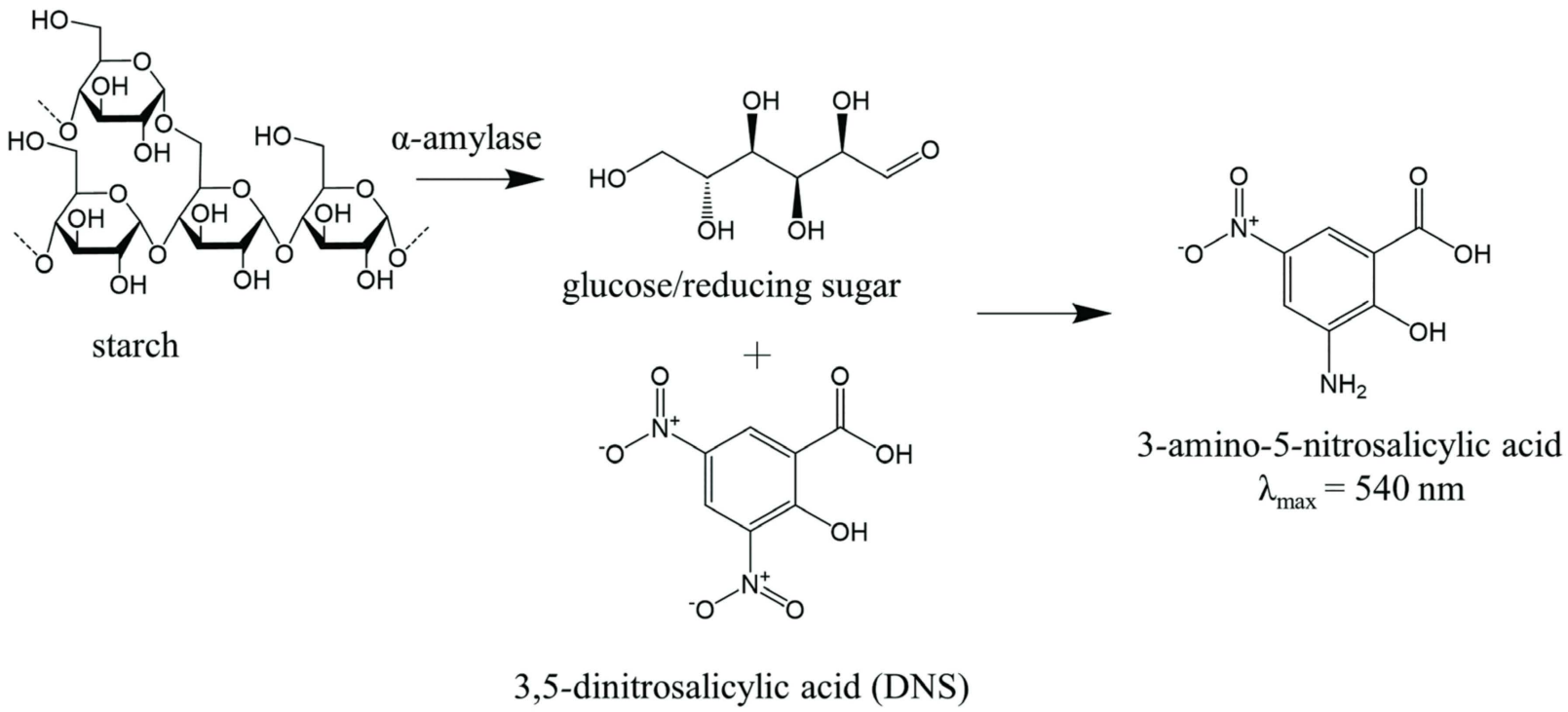

Figure 2. The DNS-based method used to evaluate α-amylase inhibitors in medicinal plant extracts. The figure was generated using ChemDraw Professional software.

Table 4. Conditions used during the screening for α-glucosidase inhibitors using plant extracts.

Enzyme Concentration	Substrate Used	Buffer Used	Extract Concentration	Preincubation (Before Substrate)	Incubation	Solution/Method Used for Termination	Wavelength (nm)	Ref
0.5 units/mL	5 mM *p*-nitrophenyl-α-D-glucopyranoside	10 mM potassium phosphate buffer (pH 6.8)	Various concentrations in buffer	37 °C for 15 min	37 °C for 15 min	200 mM sodium carbonate	405	[15]
1 unit/mL	*p*-nitrophenyl-α-D-glucopyranoside	Phosphate buffer	100 to 500 μg/mL in buffer	37 °C for 15 min	37 °C for 20 min	0.1 M sodium carbonate	405	[17]
Not given	20 mM *p*-nitrophenyl-α-D-glucopyranoside	0.5 M phosphate buffer, pH 6.5	Not given	37 °C for 15 min	37 °C for 35 min	Not given	405	[23]
1 unit/mL in buffer	5 mM *p*-nitrophenyl-α-D-glucopyranoside	50 mM phosphate buffer, pH 6.8	100 μg in 70 μL buffer	37 °C for 10 min	37 °C for 30 min	Not given	405	[50]
50 μg/mL	*p*-nitro-phenyl-a-D-glucopyranoside	Not given	Not given	37 °C for 5 min	37 °C for 30 min	sodium carbonate solution	405	[29]
Not given	5 mM *p*-nitrophenyl-α-D-glucopyranoside	0.1 M Citrate-phosphate buffer, pH 7.0	Not given	37 °C for four periods of time	Not given	0.05 M sodium hydroxide	410	[30]
0.2 units/mL	0.5 mM *p*-nitrophenyl-α-D-glucopyranoside	0.1 M phosphate buffer, pH 7.0	25 to 85 μg/mL	N/A	37 °C for 30 min	0.2 M sodium carbonate	410	[31]
0.26 units/mL in buffer	0.3125 mM *p*-nitrophenyl-α-D-glucopyranoside	0.1 M phosphate buffer, pH 6.8	Various concentrations of diluted sample in buffer	37 °C for 15 min	37 °C for 15 min	0.2 M sodium carbonate	405	[32]
Enzyme dissolved in buffer (concentration not given)	20 mM *p*-nitrophenyl-α-D-glucopyranoside	0.5 M phosphate buffer, pH 6.5	Samples dissolved in 80% methanol	37 °C for 15 min	37 °C for 35 min	Not given	405	[33]
1 unit/mL	5 mM *p*-nitrophenyl-α-D-glucopyranoside	100 mM phosphate buffer, pH 6.8	100 to 500 mg/mL	37 °C for 15 min	37 °C for 20 min	0.1 M sodium carbonate	405	[35]
1 unit/mL	*p*-nitrophenyl-α-D-glucopyranoside	Not given	3.12 to 200 μg/mL	37 °C for 10 min	37 °C for 30 min	0.2 M sodium carbonate	405	[36]

Table 5. Conditions used during the screening for α-amylase inhibitors using plant extracts.

Enzyme Concentration	Substrate Used	Buffer Used	Extract Concentration	Preincubation (Before Substrate)	Incubation	Reagent Added to Generate Color	Solution/Method Used for Termination	Wavelength (nm)	Ref
1 unit/mL in buffer	0.5% starch in buffer	20 mM sodium phosphate buffer with 6 mM NaCl (pH 6.9)	15.6 to 250 mg/L in buffer	37 °C for 15 min	37 °C for 15 min	3,5-dinitrosalicylic acid	boiling for 5 min	540	[15]
2 units/mL in 10% DMSO and buffer	1 g/100 mL corn starch in water	Not given	5 to 500 µg/mL in 10% DMSO and buffer	30 °C for 10 min	30 °C for 3 min	3,5-dinitrosalicylic acid	Boiling 85–90 °C for 10 min	540	[17]
1.25 µg/mL in buffer	200 µg/mL DQ™ starch from corn, BODIPY® FL conjugate	Same buffer used in Lipase activity assay *	2.5 mg/mL in DMSO (final concentration)	none	37 °C for 30 min	none	N/A	Em/Ex = 535/485	[18]
Not given	0.5% potato starch in buffer	Phosphate buffer, pH 6.9	Not given	37 °C for 5 min	37 °C for 3 min	96 mM 3,5-dinitrosalicylic acid in 5.31 M sodium potassium tartrate, 2 M NaOH	85 °C heater for 15 min	540	[23]
2 units/mL in 10% DMSO	1% starch solution	0.02 M sodium phosphate, 0.006 M NaCl, at pH 6.9	10 to 500 µg/mL in 10% DMSO and buffer	30 °C for 10 min	30 °C for 3 min	3,5-dinitrosalicylic acid	Boiling at 90 °C for 10 min	540	[24]
2 units/mL in buffer	1% starch mixed with buffer	0.02 M sodium phosphate buffer, pH 6.9	10 to 500 µg/mL	25 °C for 10 min	25 °C for 10 min	3,5-dinitrosalicylic acid	Boiling for 5 min	540	[27]
2 units/mL stock prepared by dissolving 25 mg in 10% DMSO and diluting in buffer up to 100 mL	1% corn starch in water	Not given	1 mg/mL stock in 10% DMSO diluted to 10 to 500 µg/mL	30 °C for 10 min	30 °C for 3 min	3,5-dinitrosalicylic acid	Boiling 85–90 °C for 10 min	540	[28]
0.1 mg/mL	Starch solution	Phosphate buffer	Not given	37 °C for 10 min	37 °C for 30 min	Iodine reagent	Added 1 M HCl	580	[29]
Not given	1% starch in buffer	0.05 M Tris, pH 7.0 buffer with 38 mM NaCl and 0.1 mM $CaCl_2$	Not given	37 °C for 20 min	37 °C for four periods of time	3,5-dinitrosalicylic acid	Not given	540	[30]
2.0 units/mL in buffer	Starch azure solution in buffer	50 mM Tris-HCl buffer, pH 6.9, containing 10 mM $CaCl_2$	20 to 100 µg/mL in 25% DMSO	37 °C for 10 min	37 °C for 10 min	N/A	Added 50% acetic acid	595	[31]
0.25 units/mL in buffer	2.5% *w*/*v* potato starch in buffer	20 mM phosphate buffer, pH 6.9	Samples in 80% methanol (concentration not given)	Room temperature for 5 min	15 min (temperature not specified)	3,5-dinitrosalicylic acid	80 °C for 40 min	540	[33]
50 mg enzyme in 100 mL buffer	2-chloro-4-nitrophenol-α-D-maltotrioside	40 mM phosphate buffer, pH 6.9	25 to 100 µg/mL in DMSO	N/A	37 °C for 5 min	N/A	N/A	405	[34]

Table 5. *Cont.*

Enzyme Concentration	Substrate Used	Buffer Used	Extract Concentration	Preincubation (Before Substrate)	Incubation	Reagent Added to Generate Color	Solution/Method Used for Termination	Wavelength (nm)	Ref
2 units/mL in 10% DMSO	1% starch solution	0.02 M Na_2HPO_4/NaH_2PO_4, 0.006 M NaCl, pH 6.9	10 to 500 µg/mL	30 °C for 10 min	30 °C for 3 min	3,5-dinitrosalicylic acid	90 °C for 10 min	540	[35]
500 µg/mL	0.25% starch solution	Not given	3.12 to 200 µg/mL	37 °C for 10 min	37 °C for 30 min	Lugol's solution	1 M HCl	620	[36]

* The type of buffer used here may not be accurately stated. Refer to the discussion under α-amylase assay.

4. Additional Considerations and Limitations for the Enzyme Inhibitory Assays

During the development of the assay methods to screen for enzyme inhibitors in medicinal plant extracts, several factors such as temperature, buffer, PH, the solubility of compounds, and the use of organic solvents and additives must be considered. According to the parameters listed in the tables for different enzyme assays, these conditions sometimes have a wide variation, but there has been little research on how these variations can affect the results of the inhibitory assay experiments. Based on the literature, some factors to be considered for the most commonly used assay method for pancreatic lipase, cholesterol esterase, α-amylase, and α-glucosidase inhibitory assays are described below.

4.1. Pancreatic Lipase Inhibitory Assays

The most commonly used assay for the detection of pancreatic lipase inhibition involves the use of *p*-nitrophenyl esters as the substrate. During this reaction, if a long-chain organic acid/fatty acid is linked to *p*-nitrophenol, turbidity can result from the release of the insoluble organic acid with the enzyme action [51]. To overcome this issue, researchers have precipitated the fatty acid using $CaCl_2$ or using emulsifiers such as 2% Triton X-100 (TX-100), 5 mM sodium deoxycholate (SDC), or 0.01% gum Arabic to avoid turbidity. However, the use of gum arabic and TX-100 has been shown to reduce enzyme activity, and the addition of $CaCl_2$ necessitates another step to remove the precipitate. SDC seems to be a good choice for this purpose as it has been shown to improve enzyme activity in addition to removing turbidity [42].

When reviewing the literature, it was discovered that reaction temperatures ranging from 25 °C to 37 °C and incubation times ranging from 5 min to 30 min have been used for the lipase assay (Table 2). Vo et al., 2022, had shown that the lipase activity decreased to half of the initial rate after 5 min of reaction at 37 °C due to enzyme instability [42]. Furthermore, they have reported that at 25 °C lipase activity is reduced by 1.7 times compared with 37 °C, but 25 °C can be a good alternative for prolonged incubations considering the stability of the enzyme. Additionally, the chemical hydrolysis of *p*-nitrophenyl esters is known to be high at increased temperatures [52]. Hence, the selection of the temperature and the incubation times should be carried out with proper controls for the correct interpretation of the inhibitory activity of the plant extracts.

According to the literature, pH ranging from 7.0 to 8.5 was used for the pancreatic lipase assay with Tris-HCl or phosphate buffers. This pH range is usually selected as the highest enzyme activity is observed in this range when the formation of *p*-nitrophenolate is measured around 410 nm [25]. Lipase assay conducted at pH 7.0 had shown to become turbid after 10–15 min, even in the presence of the emulsifier SDC while remaining transparent at higher pH values [42]. When *p*-nitrophenyl esters are used as the substrate, the highest lipase activity was observed with a pH 8.0 sodium phosphate buffer and pH 9.0 Tris-HCl buffer [42]. Based on the same study, the use of a pH 8.0 buffer was recommended for the lipase reaction, as that is closer to physiological pH and a higher pH can increase the chance of spontaneous hydrolysis of the ester substrate. However, the change in pH can cause significant errors when the measurements are made around 405–410 nm to detect *p*-nitrophenol [25]. One way to overcome this issue is to take the readings at either 348 nm or 268 nm, which are isosbestic points for *p*-nitrophenolate that are not affected by the pH. The isosbestic point of 348 nm is more suitable for the measurements as it is furthest away from the 280 nm absorption maximum for proteins and has a direct correlation with the concentration of the product [52]. Additionally, the molar extinction coefficient of *p*-nitrophenol, determined at varying pH values, can be used to obtain the concentration using the Beer-Lambert equation [53,54].

The solubility of the plant-based inhibitor or the commercial enzyme can be another issue during the enzyme assays. Most of the time, organic solvents that are mixable with aqueous media are used to address this problem. During a study of suitable co-solvents to be used in the lipase assay, researchers found that Ethanol (EtOH), Methanol (MeOH), Isopropyl alcohol (IPA), Dimethyl sulfoxide (DMSO), and Acetonitrile (AcCN) can be used

at 10% (*v*/*v*) without inducing turbidity or affecting the enzyme reaction. Additionally, EtOH could be used at 20% (*v*/*v*) and DMSO and MeOH were tolerated at 30% (*v*/*v*) concentrations for the lipase assay [42].

Lipase activity was also shown to be enhanced by calcium and sodium ions based on the substrate used and the enzyme source. When *p*-nitrophenyl palmitate was used as the substrate, the highest lipase activity was observed at 5 mM NaCl and 1 mM $CaCl_2$ and the activity decreased with higher concentrations of these salts [42].

It is usually recommended to prepare the lipase enzyme freshly before usage every day to avoid degradation or denaturation. However, for routine laboratory usage, the enzyme may need to be stored for a longer period. 10% glycerol was found to be effective for the storage of lipase at −20 °C for three weeks without precipitation or losing activity at 100 mg/mL stock concentration in sodium phosphate buffer, pH 8.0 [42].

According to the literature review, in most cases where *p*-nitrophenyl esters are used as the substrate, acetonitrile is used to prepare the substrate stock solution. In a few instances, the buffer was used as the solvent for the substrate. Vo et al., 2022, recommended the use of acetonitrile at a 10% or lower final concentration [42]. Hence, the stock solution can be diluted in the buffer to obtain the final concentration in the preferred range, and the use of appropriate controls will be helpful to eliminate calculation errors caused by the negative effects of solvents.

4.2. Cholesterol Esterase and α-Glucosidase Inhibitory Assays

The formation of *p*-nitrophenolate is commonly used to assess the enzyme activity of pancreatic cholesterol esterase and α-glucosidase, similar to pancreatic lipase. These assays involve varying pH conditions while measuring the product formation in the wavelength range of 405–410 nm. For example, according to the literature, pH 6.5 to 7.0 is used in α-glucosidase assays to provide the optimum pH for the enzyme activity. Furthermore, 0.1 M to 0.2 M sodium carbonate is added to the reaction mixture to provide the alkaline conditions necessary to measure *p*-nitrophenolate levels. During cholesterol esterase assays usually, pH 7.0 buffers are used without further adjusting the final pH with an alkaline solution. As the extinction coefficient of the *p*-nitrophenolate is pH dependent, the results indicating the concentration of this product can be misleading [52]. To avoid this issue, approaches such as taking the readings at isosbestic points or calculating the extinction coefficient at the conditions used can be involved, similar to pancreatic lipase assays.

4.3. α-Amylase Inhibitory Assays

According to our literature review, the major method used for α-amylase assay involves DNS. The free carbonyl groups on the reducing sugars released from the enzyme action undergo an oxidation-reduction reaction with DNS producing orange-red color 3-amino-5-nitro salicylic acid. The polyphenols in plant extracts, which have a reducing potential, may interfere with this assay method. Nyambe-Silavwe et al., 2015 tested the interference of polyphenols, (-)-epigallocatechin gallate (EGCG), gallic acid, and phlorizin on DNS-based α-amylase assays [55]. It was found that EGCG, which has a higher number of OH groups compared with other compounds, shows the most interference, followed by gallic acid and phlorizin. Therefore, the use of proper control experiments without the enzyme is essential in this type of assay to correct for such interferences. Additionally, the α-amylase concentration of 0.5 units/mL has resulted in 7 to 8-fold lower IC_{50} values when acarbose was used as the inhibitor, compared with 3.0 units/mL concentration, which is closer to the values used by many researchers [55]. Also, when 0.5 units/mL of enzyme solution were used, enzyme activity showed a linear relationship with respect to time up to 15 and 12 min for amylose and amylopectin substrates, respectively. Hence, choosing the proper enzyme concentration is important to obtain accurate results, and a series of enzyme concentrations can be tested to select the right value under the given conditions.

5. Conclusions

Researchers have studied the ability of different medicinal plant extracts to inhibit the vital metabolic enzymes pancreatic lipase, cholesterol esterase, α-amylase, and α-glucosidase using various methods. For pancreatic lipase inhibitor screening, both spectrophotometric and fluorometric assays were used, and the most common assay type was the spectrophotometric measurement of the formation of *p*-nitrophenolate in the wavelength range from 405 to 410 nm. Cholesterol esterase and α-glucosidase inhibitor screening also involve the spectrophotometric measurement of *p*-nitrophenolate. The most commonly used substrate for pancreatic lipase and cholesterol esterase was *p*-nitrophenyl butyrate, possibly due to the solubility of the short-chain butyric acid that is produced by the enzyme action. This can avoid turbidity issues that are normally observed when long-chain fatty acid esters are used in the assays. However, the cholesterol esterase assay usually requires a bile salt such as sodium taucholate as an enzyme activator. The α-glucosidase inhibitory assays used *p*-nitrophenyl-α-D-glucopyranoside as the substrate specific for the enzyme. The α-amylase inhibitory assays were carried out using spectrophotometric methods involving different substrates and fluorescence-based assay kits. The DNS-based spectrophotometric assay was the most common one used with the α-amylase enzyme. The temperature of 37 °C was mostly used for the incubation for all the assays, although some variations were also observed. Complications can arise during the enzyme assay methods due to the formation of insoluble products and the insolubility of the plant extract or the enzyme at the required concentrations. Hence, solvents such as DMSO were commonly used during the preparation of these samples. The enzyme concentration, incubation temperature and time, buffer, pH, use of organic solvents and additives, and interferences from the metabolites in the plant extracts are other main factors to consider when developing an assay method. There is limited research on how the variation of these factors can affect different enzyme assays, and this can be a direction for future research. When suitable drug targets are discovered using these screening methods, in vivo experiments are required to evaluate the efficacy and safety of those compounds. Usually, the plant extracts/compounds of interest are orally administered to mouse models, and the change in body weight, adipose tissue weight, lipid profile, and the expression of related genes are measured during in vivo studies [56].

Author Contributions: S.B.: Conceptualization, writing—review and editing. S.D.: preparing figures, review and editing, A.W.: review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the USDA-NIFA Evans Allen Fund.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zhang, H.L.; Wu, Q.X.; Wei, X.; Qin, X.M. Pancreatic lipase and cholesterol esterase inhibitory effect of Camellia nitidissima Chi flower extracts in vitro and in vivo. *Food Biosci.* **2020**, *37*, 100682. [CrossRef]
2. Malik, V.S.; Popkin, B.M.; Bray, G.A.; Després, J.P.; Hu, F.B. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation* **2010**, *121*, 1356–1364. [CrossRef]
3. Kass, D.A. COVID-19 and Severe Obesity: A Big Problem? *Ann. Intern. Med.* **2020**, *173*, 840–841. [CrossRef] [PubMed]
4. Karale, P.; Dhawale, S.C.; Karale, M.A. Quantitative Phytochemical Profile, Antioxidant and Lipase Inhibitory Potential of Leaves of Momordica charantia L. and Psoralea corylifolia L. *Indian J. Pharm. Sci.* **2022**, *84*, 189–196. [CrossRef]
5. Müller, T.D.; Blüher, M.; Tschöp, M.H.; DiMarchi, R.D. Anti-obesity drug discovery: Advances and challenges. *Nat. Rev. Drug Discov.* **2022**, *21*, 201–223. [CrossRef] [PubMed]

6. Spínola, V.; Castilho, P.C. Assessing the In Vitro Inhibitory Effects on Key Enzymes Linked to Type-2 Diabetes and Obesity and Protein Glycation by Phenolic Compounds of Lauraceae Plant Species Endemic to the Laurisilva Forest. *Molecules* **2021**, *26*, 2023. [CrossRef]
7. Williams, D.M.; Nawaz, A.; Evans, M. Drug Therapy in Obesity: A Review of Current and Emerging Treatments. *Diabetes Ther.* **2020**, *11*, 1199–1216. [CrossRef]
8. Dahlén, A.D.; Dashi, G.; Maslov, I.; Attwood, M.M.; Jonsson, J.; Trukhan, V.; Schiöth, H.B. Trends in Antidiabetic Drug Discovery: FDA Approved Drugs, New Drugs in Clinical Trials and Global Sales. *Front. Pharmacol.* **2021**, *12*, 807548. [CrossRef]
9. Tucci, S.A.; Boyland, E.J.; Halford, J.C. The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: A review of current and emerging therapeutic agents. *Diabetes Metab. Syndr. Obes.* **2010**, *3*, 125–143. [CrossRef]
10. Rufino, A.T.; Costa, V.M.; Carvalho, F.; Fernandes, E. Flavonoids as antiobesity agents: A review. *Med. Res. Rev.* **2021**, *41*, 556–585. [CrossRef]
11. Tundis, R.; Loizzo, M.R.; Menichini, F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: An update. *Mini Rev. Med. Chem.* **2010**, *10*, 315–331. [CrossRef] [PubMed]
12. Herranz-López, M.; Olivares-Vicente, M.; Encinar, J.A.; Barrajón-Catalán, E.; Segura-Carretero, A.; Joven, J.; Micol, V. Multi-Targeted Molecular Effects of Hibiscus sabdariffa Polyphenols: An Opportunity for a Global Approach to Obesity. *Nutrients* **2017**, *9*, 907. [CrossRef] [PubMed]
13. Barrett, M.L.; Udani, J.K. A proprietary alpha-amylase inhibitor from white bean (Phaseolus vulgaris): A review of clinical studies on weight loss and glycemic control. *Nutr. J.* **2011**, *10*, 24. [CrossRef]
14. Saad, B.; Ghareeb, B.; Kmail, A. Metabolic and Epigenetics Action Mechanisms of Antiobesity Medicinal Plants and Phytochemicals. *Evid.-Based Complement. Altern. Med.* **2021**, *2021*, 9995903. [CrossRef]
15. Sekhon-Loodu, S.; Rupasinghe, H.P.V. Evaluation of Antioxidant, Antidiabetic and Antiobesity Potential of Selected Traditional Medicinal Plants. *Front. Nutr.* **2019**, *6*, 53. [CrossRef] [PubMed]
16. Jamous, R.M.; Abu-Zaitoun, S.Y.; Akkawi, R.J.; Ali-Shtayeh, M.S. Antiobesity and Antioxidant Potentials of Selected Palestinian Medicinal Plants. *Evid.-Based Complement. Altern. Med.* **2018**, *2018*, 8426752. [CrossRef]
17. Al-Rimawi, F.; Jaradat, N.; Qneibi, M.; Hawash, M.; Emwas, N. Free radicals and enzymes inhibitory potentials of the traditional medicinal plant Echium angustifolium. *Eur. J. Integr. Med.* **2020**, *38*, 101196. [CrossRef]
18. Buchholz, T.; Melzig, M.F. Medicinal Plants Traditionally Used for Treatment of Obesity and Diabetes Mellitus—Screening for Pancreatic Lipase and alpha-Amylase Inhibition. *Phytother. Res.* **2016**, *30*, 260–266. [CrossRef]
19. Jiao, P.; Tseng-Crank, J.; Corneliusen, B.; Yimam, M.; Hodges, M.; Hong, M.; Maurseth, C.; Oh, M.; Kim, H.; Chu, M.; et al. Lipase Inhibition and Antiobesity Effect of Atractylodes lancea. *Planta Med.* **2014**, *80*, 577–582. [CrossRef]
20. Ekanem, A.P.; Wang, M.; Simon, J.E.; Moreno, D.A. Antiobesity properties of two African plants (Afromomum meleguetta and Spilanthes acmella) by pancreatic lipase inhibition. *Phytother. Res.* **2007**, *21*, 1253–1255. [CrossRef]
21. Velusami, C.C.; Agarwal, A.; Mookambeswaran, V. Effect of Nelumbo nucifera Petal Extracts on Lipase, Adipogenesis, Adipolysis, and Central Receptors of Obesity. *Evid.-Based Complement. Altern. Med.* **2013**, *2013*, 145925. [CrossRef] [PubMed]
22. Kumar, D.; Karmase, A.; Jagtap, S.; Shekhar, R.; Bhutani, K.K. Pancreatic Lipase Inhibitory Activity of Cassiamin A, a Bianthraquinone from Cassia siamea. *Nat. Prod. Commun.* **2013**, *8*, 195–198. [CrossRef] [PubMed]
23. Gok, H.N.; Orhan, N.; Ozupek, B.; Pekacar, S.; Selvi, S.N.; Orhan, D.D. Standardization of Juniperus macrocarpa Sibt. & Sm. and Juniperus excelsa M. Bieb. Extracts with Carbohydrate Digestive Enzyme Inhibitory and Antioxidant Activities. *Iran. J. Pharm. Res.* **2021**, *20*, 441–455. [CrossRef] [PubMed]
24. Qadi, M.; Jaradat, N.; Al-lahham, S.; Ali, I.; Abualhasan, M.N.; Shraim, N.; Hussein, F.; Issa, L.; Mousa, A.; Zarour, A.; et al. Antibacterial, Anticandidal, Phytochemical, and Biological Evaluations of Pellitory Plant. *BioMed Res. Int.* **2020**, *2020*, 6965306. [CrossRef]
25. Aabideen, Z.U.; Mumtaz, M.W.; Akhtar, M.T.; Raza, M.A.; Mukhtar, H.; Irfan, A.; Raza, S.A.; Touqeer, T.; Nadeem, M.; Saari, N. Cassia fistula Leaves; UHPLC-QTOF-MS/MS Based Metabolite Profiling and Molecular Docking Insights to Explore Bioactives Role towards Inhibition of Pancreatic Lipase. *Plants* **2021**, *10*, 1334. [CrossRef]
26. Suh, D.H.; Jung, E.S.; Park, H.M.; Kim, S.H.; Lee, S.; Jo, Y.H.; Lee, M.K.; Jung, G.; Do, S.G.; Lee, C.H. Comparison of Metabolites Variation and Antiobesity Effects of Fermented versus Nonfermented Mixtures of Cudrania tricuspidata, Lonicera caerulea, and Soybean According to Fermentation In Vitro and In Vivo. *PLoS ONE* **2016**, *11*, e0149022. [CrossRef]
27. Jaradat, N.; Qneibi, M.; Hawash, M.; Sawalha, A.; Qtaishat, S.; Hussein, F.; Issa, L. Chemical Composition, Antioxidant, Antiobesity, and Antidiabetic Effects ofHelichrysum sanguineum (L.) Kostel. from Palestine. *Arab. J. Sci. Eng.* **2021**, *46*, 41–51. [CrossRef]
28. Jaradat, N.; Qadi, M.; Ali, I.; Hussein, F.; Issa, L.; Rashdan, D.; Jamoos, M.; Najem, R.; Zarour, A.; Arar, M. Phytochemical screening, antiobesity, antidiabetic and antimicrobial assessments of Orobanche aegyptiaca from Palestine. *BMC Complement. Med. Ther.* **2021**, *21*, 256. [CrossRef]
29. Unuofin, J.O.; Otunola, G.A.; Afolayan, A.J. In vitro alpha-amylase, alpha-glucosidase, lipase inhibitory and cytotoxic activities of tuber extracts of Kedrostis africana (L.) Cogn. *Heliyon* **2018**, *4*, e00810. [CrossRef]

30. Simao, A.A.; Marques, T.R.; Marcussi, S.; Correa, A.D. Aqueous extract of Psidium guajava leaves: Phenolic compounds and inhibitory potential on digestive enzymes. *Anais Acad. Bras. Cienc.* **2017**, *89*, 2155–2165. [CrossRef]
31. Dos Santos, U.P.; Tolentino, G.S.; Morais, J.S.; Souza, K.D.; Estevinho, L.M.; dos Santos, E.L. Physicochemical Characterization, Microbiological Quality and Safety, and Pharmacological Potential of Hancornia speciosa Gomes. *Oxidative Med. Cell. Longev.* **2018**, *2018*, 2976985. [CrossRef] [PubMed]
32. Zhang, H.; Chen, G.L.; Zhang, Y.L.; Yang, M.; Chen, J.M.; Guo, M.Q. Potential hypoglycemic, hypolipidemic, and anti-inflammatory bioactive components in Nelumbo nucifera leaves explored by bioaffinity ultrafiltration with multiple targets. *Food Chem.* **2022**, *375*, 131856. [CrossRef] [PubMed]
33. Pekacar, S.; Orhan, D.D. Investigation of Antidiabetic Effect of Pistacia atlantica Leaves by Activity-Guided Fractionation and Phytochemical Content Analysis by LC-QTOF-MS. *Front. Pharmacol.* **2022**, *13*, 826261. [CrossRef]
34. Jerbi, A.; Derbali, A.; Elfeki, A.; Kammoun, M. Essential Oil Composition and Biological Activities of Eucalyptus globulus Leaves Extracts from Tunisia. *J. Essent. Oil Bear. Plants* **2017**, *20*, 438–448. [CrossRef]
35. Jaradat, N.; Dacca, H.; Hawash, M.; Abualhasan, M.N. Ephedra alata fruit extracts: Phytochemical screening, anti-proliferative activity and inhibition of DPPH, alpha-amylase, alpha-glucosidase, and lipase enzymes. *BMC Chem.* **2021**, *15*, 41. [CrossRef]
36. Mba, J.R.; Zouheira, D.; Dairou, H.; Yadang, F.S.A.; Gael, N.N.; Ayong, L.; Kuiate, J.R.; Agbor, G.A. In Vitro Antioxidant, Anti-Inflammatory, and Digestive Enzymes Inhibition Activities of Hydro-Ethanolic Leaf and Bark Extracts of Psychotria densinervia (K. Krause) Verdc. *Adv. Pharmacol. Pharm. Sci.* **2022**, *2022*, 8459943. [CrossRef] [PubMed]
37. Ramírez, M.; Amate, L.; Gil, A. Absorption and distribution of dietary fatty acids from different sources. *Early Hum. Dev.* **2001**, *65*, S95–S101. [CrossRef] [PubMed]
38. Birari, R.B.; Bhutani, K.K. Pancreatic lipase inhibitors from natural sources: Unexplored potential. *Drug Discov. Today* **2007**, *12*, 879–889. [CrossRef]
39. Carlier, H.; Bernard, A.; Caselli, C. Digestion and absorption of polyunsaturated fatty acids. *Reprod. Nutr. Dev.* **1991**, *31*, 475–500. [CrossRef]
40. Ngamukote, S.; Mäkynen, K.; Thilawech, T.; Adisakwattana, S. Cholesterol-Lowering Activity of the Major Polyphenols in Grape Seed. *Molecules* **2011**, *16*, 5054. [CrossRef]
41. Pietsch, M.; Gütschow, M. Synthesis of Tricyclic 1,3-Oxazin-4-ones and Kinetic Analysis of Cholesterol Esterase and Acetylcholinesterase Inhibition. *J. Med. Chem.* **2005**, *48*, 8270–8288. [CrossRef]
42. Vo, C.-V.T.; Luu, N.V.H.; Nguyen, T.T.H.; Nguyen, T.T.; Ho, B.Q.; Nguyen, T.H.; Tran, T.-D.; Nguyen, Q.-T. Screening for pancreatic lipase inhibitors: Evaluating assay conditions using p-nitrophenyl palmitate as substrate. *All Life* **2022**, *15*, 13–22. [CrossRef]
43. Pohanka, M. Biosensors and Bioassays Based on Lipases, Principles and Applications, a Review. *Molecules* **2019**, *24*, 616. [CrossRef] [PubMed]
44. Lim, S.; Xenoulis, P.; Stavroulaki, E.; Lidbury, J.A.; Suchodolski, J.S.; Carrière, F.; Steiner, J.M. The 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester (DGGR) lipase assay in cats and dogs is not specific for pancreatic lipase. *Vet. Clin. Pathol.* **2020**, *49*, 607–613. [CrossRef] [PubMed]
45. Birari, R.; Roy, S.K.; Singh, A.; Bhutani, K.K. Pancreatic lipase inhibitory alkaloids of Murraya koenigii leaves. *Nat. Prod. Commun.* **2009**, *4*, 1089–1092. [CrossRef]
46. Vahouny, G.V.; Weersing, S.; Treadwell, C.R. Function of specific bile acids in cholesterol esterase activity in vitro. *Biochim. Biophys. Acta (BBA) Lipids Lipid Metab.* **1965**, *98*, 607–616. [CrossRef]
47. Assefa, S.T.; Yang, E.-Y.; Chae, S.-Y.; Song, M.; Lee, J.; Cho, M.-C.; Jang, S. Alpha Glucosidase Inhibitory Activities of Plants with Focus on Common Vegetables. *Plants* **2020**, *9*, 2. [CrossRef]
48. Keharom, S.; Mahachai, R.; Chanthai, S. The optimization study of α-amylase activity based on central composite design-response surface methodology by dinitrosalicylic acid method. *Int. Food Res. J.* **2016**, *23*, 10–17.
49. Lehoczki, G.; Kandra, L.; Gyémánt, G. The use of starch azure for measurement of alpha-amylase activity. *Carbohydr. Polym.* **2018**, *183*, 263–266. [CrossRef]
50. Niaz, A.; Adnan, A.; Bashir, R.; Mumtaz, M.W.; Raza, S.A.; Rashid, U.; Tan, C.P.; Tan, T.B. The In Vitro alpha-Glucosidase Inhibition Activity of Various Solvent Fractions of Tamarix dioica and H-1-NMR Based Metabolite Identification and Molecular Docking Analysis. *Plants* **2021**, *10*, 1128. [CrossRef]
51. Gupta, R.; Rathi, P.; Gupta, N.; Bradoo, S. Lipase assays for conventional and molecular screening: An overview. *Biotechnol. Appl. Biochem.* **2003**, *37*, 63–71. [CrossRef] [PubMed]
52. Hriscu, M.; Chris, L.; Tosa, M.; Irimie, F.D. pH-Profiling of thermoactive lipases and esterases: Caveats and further notes. *Eur. J. Lipid Sci. Technol.* **2013**, *115*, 571–575. [CrossRef]
53. Hotta, Y.; Ezaki, S.; Atomi, H.; Imanaka, T. Extremely stable and versatile carboxylesterase from a hyperthermophilic archaeon. *Appl. Environ. Microbiol.* **2002**, *68*, 3925–3931. [CrossRef] [PubMed]
54. Kademi, A.; Aït-Abdelkader, N.; Fakhreddine, L.; Baratti, J. Purification and characterization of a thermostable esterase from the moderate thermophile Bacillus circulans. *Appl. Microbiol. Biotechnol.* **2000**, *54*, 173–179. [CrossRef] [PubMed]

55. Nyambe-Silavwe, H.; Villa-Rodriguez, J.A.; Ifie, I.; Holmes, M.; Aydin, E.; Jensen, J.M.; Williamson, G. Inhibition of human α-amylase by dietary polyphenols. *J. Funct. Foods* **2015**, *19*, 723–732. [CrossRef]
56. Mopuri, R.; Islam, M.S. Medicinal plants and phytochemicals with anti-obesogenic potentials: A review. *Biomed. Pharmacother.* **2017**, *89*, 1442–1452. [CrossRef]