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Anti-obesity and antioxidant activity of dietary flavonoids from *Dioscorea steriscus* tubers

Pamhidzai Dzomba*, Cexton Musekiwa

Chemistry Department, Faculty of Science, Bindura University of Science Education, P. Bag 1020, Bindura, Zimbabwe

PEER REVIEW

Peer reviewer

Dr. Luke Gwatidzo, Tswane University of Technology, P Bag X680, Pretoria 0001, South Africa.
Tel: 00263777663788
E-mail: lgwatidzo@buse.ac.zw

Comments

This is a valuable research in which the authors have shown the antioxidant, anti-lipase and α-amylase activity of *D. steriscus*. Antioxidant activity was assessed by DPPH antiradical activity while ant-obesity activity was assessed using enzyme inhibition activities by chromogenic methods. *D. steriscus* has been found to be a promising source of lead compounds anti-obesity drugs.
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ABSTRACT

Objective: To investigate antioxidant and anti-obesity activity of flavonoids extracted by solvent cold percolation and preparative thin liquid chromatography from *Dioscorea steriscus* tubers.
Methods: 1-diphenyl-2-picrylhydrazyl (DPPH) antiradical activity was employed to investigate antioxidant activity while chromogenic method was used to determine alpha amylase inhibition activity and spectrophotometric methods using triolein as a substrate was used to investigate lipase activity.
Results: Thin liquid chromatography profiling revealed eight different flavonoid types. Ethyl acetate extract yielded two types, R_f values 0.38 and 0.40; chloroform extract also yielded two types R_f values 0.06 and 0.51, while ethanol extract yielded four types with R_f values 0.16, 0.33, 0.65 and 0.96. All the extracted flavonoids exhibited antioxidant activity with ethanol extracts exhibiting the greatest antiradical activity. The order of enzyme inhibition capacity was ethyl acetate<chloroform<ethanol. Ethanol extract exhibited significantly greater anti-obesity activity as compared to herbex, a commercially anti-obesity medication sold in drug stores. Anti-α amylase activity and anti-lipase activity for herbex was (78.38±0.02)% and (76.07±0.09)% respectively, while that for ethanolic extract (R_f=0.96) was (93.66±0.00)% and (95.88±0.13)%.
Conclusions: Results of the present study show that *Dioscorea steriscus* consists of bioactive compounds that can act as lipase and α-amylase inhibitors and therefore can be useful for the development of functional foods against obesity. It can also be used as a source of lead compounds for designing new ant-obesity therapeuticals.

KEYWORDS
Anti-obesity activity, Antioxidant activity, Flavonoids, *Dioscorea steriscus*

1. Introduction

Flavonoids are bioactive compounds synthesized by plants through the shikimic acid and the mevalonate pathways. They have been extracted from many edible foods[1]. Several studies have reported the importance of consuming a diet consisting of flavonoids[2-4]. Flavonoids act as antioxidants to prevent diseases due to oxidative stress in the body. It has been shown that reactive oxygen and nitrogen species generated in the human body can cause diseases such as coronary heart diseases, obesity, diabetes type 2 and cancers[5]. Flavonoids act as antioxidants by neutralizing the effects of the reactive oxygen and nitrogen species thereby acting as protecting agents against diseases. Antioxidant activities of flavonoids from dietary plants have been studied by several researchers[6-8]. Examples of flavonoids that have been extracted from dietary plants include, quercetin aglycone, quercetin-3,4′-O-diglucoside and quercetin-4′-O-glucoside[9]. The flavonoid quercetin has demonstrated promising protective effect in reducing

*Corresponding author: Pamhidzai Dzomba, Bindura University of Science Education, Chemistry Department, Bag 1020 Bindura, Zimbabwe.
Tel: +263773474525.
Fax: +263 7536
E-mail: pdzomba@gmail.com, pdzomba@buse.ac.zw
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the risk of cardiovascular diseases. Quercetin forms the main antioxidant form of many green leafy vegetables. Other antioxidant forms that have been found in dietary plants include ascorbic acid, tocopherols, and phenolic acids.

Obesity is becoming a major public health problem in both developed and developing countries[10]. The number of children who are born obese is ever increasing in developing countries while the number of adults who are classified as obese in developed countries has reached alarming levels[10]. The World Health Organization reported that obesity is a disease that has been implicated with the genesis of many other diseases such as cancers, diabetes and hypertension[11]. Obesity is primarily caused by an energy imbalance due to a disorder in lipid metabolism[12]. A number of enzymes that are involved in lipid metabolism have been identified and these form a rich pool of potential drug targets for obesity remediation[11]. Targeting triacylglycerol acylhydrolase lipolytic and carbohydrates hydrolyzing enzymes is one of the approaches that can be used to assess the efficacy of drugs to treat obesity. Obesity drugs function by inhibiting pancreatic lipase and α-amylase[12]. Orlista is a drug that is currently used to treat obesity. It functions by inhibiting the action of gastrointestinal lipase and reduces absorption of dietary fats[12]. The drug has serious side effects including steatorrhea, stomach pain, irregular menstrual periods and headaches. This has provided an impetus to search for alternative drugs. Flavonoids have been shown to help in treating and reducing the risk of obesity[13-15]. In previous studies, plant catechins and anthocyanins significantly reduced the weight of abdominal adipose tissues[16]. In traditional medicine, *Dioscorea steriscus* (*D. steriscus*) is acclaimed to treat a variety of diseases which include hypertension, diabetes, heart problems, stomach pains and obesity (Table 1).

Table 1

Uses of *D. steriscus* in traditional medicine.

Plant part	Uses	Frequency of quote
Tubers	Relish, eaten with sadza	80%
Tubers	Dried powdered tubers mixed with maize or sorghum porridge to treat hypertension	68%
Tubers	Treat obesity	82%
Tubers	Treat cancers	75%
Tubers	Treat erectile dysfunction in man	90%
Tubers	Treat diabetes	62%
Leaves	Treat heart problems	61%

Frequency of quote was determined through face to face interviews of 100 people at Mazowe and Harare agricultural shows, Zimbabwe. Only uses which had a frequency of quote of ⩾60% are indicated. Uses with a frequency of quote that were below 60% were considered insignificant.

Scientific information to authenticate this claim is unavailable, therefore we provide the rationale by this study. The present study was designed to investigate anti-obesity and antioxidant activity of flavonoids extracted from *D. steriscus* tubers.

2. Materials and methods

2.1. Chemicals

Thin layer chromatography (TLC) silica gel plates, 1-diphenyl-2-picrylhydrazyl (DPPH) and triolein were purchased from Sigma Chemical Company (St. Louis, MO, USA). All solvents and other chemicals were analytical grade bought from SkyLabs, South Africa.

2.2. Plant material

D. steriscus tubers were bought from vendors around Bindura town and stored in a freezer until use. A branch and a tuber were taken to Harare National Herbarium for authentication. The plant in Figure 1 was grown in the Bindura Agricultural herbal garden for future reference.

Figure 1. Photo of *D. steriscus*.

2.3. Extraction

Fresh tubers 10 g were pounded into pastes using a hand mill and shaken with 100 mL of hexane to remove lipids. The residues were then sequentially extracted with different solvents, ethyl acetate, chloroform and ethanol overnight. The extracts were filtered using Whatman No. 1 filter and then concentrated to dryness on a Büchi rotary evaporator at 40 °C. After recording the mass, the extracts were redissolved in 1 mL 1% (v/v) original solvent and kept in a fridge at 0-5 °C before use.

2.4. TLC separation of flavonoids

TLC separation of phytocompounds was achieved by

employing a mobile phase consisting of ethyl acetate/ethanol/formic acid/distilled water in the 5:3:1:1. Plates of 20 cm×20 cm of TLC silica plate (TLC silica gel 60 F_{254}) were taken, and one end spotted in the form of bands of 5 mm width with extract. The plates were then air dried and kept for the development in chromatographic chamber containing 10 mL of the prepared solvent system. Sections consisting of flavonoids were determined by spraying with 1% ethanolic solution of $AlCl_3$ and heating at 100 °C for 5 min. R_f values were marked and used to separate the sections by scratching into different test tubes. Sections with the same R_f values were combined and redissolved in their extracting solvents. The contents were filtered and the filtrate concentrated in a Büchi rotary evaporator at 40 °C. Resultant masses were measured and recorded. The contents were redissolved in 5 mL of their respective solvents and kept in a fridge.

2.5. DPPH antiradical activity

DPPH antiradical activity was evaluated following a slightly modified method described previously[2]. DPPH solution (100 mmol/L) dissolved in ethanol was mixed with 1 mL of extract/ascorbic acid. The reaction mixture was incubated in the dark for 30 min and there after the optical density was recorded at 517 nm. For the control, 1 mL of DPPH solution in ethanol was mixed with 1 ml of each solvent without extract and optical density of solution was recorded after 30 min. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate percentage antiradical activity.

$$\text{Percentage antiradical activity (\%)}=\frac{A_c-A_s}{A_c}\times 100$$

Where A_s is the absorbance of sample after 30 min and A_c is the absorbance of control after 30 min.

2.6. Alpha amylase inhibition activity

Alpha amylase inhibition activity was assayed by a chromogenic methods reported in previous studies. Herbex, a commercially found anti-obesity drug, was bought from a local pharmacy and was used as standard drug. Samples and herbex 1 mL were added to buffer solutions (pH 6.9) consisting of α-amylase. The solutions were incubated at 25 °C. This was followed by addition of 1% starch solution. The mixtures were incubated at 25 °C for 35 min. The reaction was then stopped by addition of 1 mL 3, 5 dinitrosalicylic acid. The contents were boiled in boiling water bath for 5 min and cooled to room temperature. The contents were diluted by addition of 10 mL of distilled water and absorbance determined. The results were compared to a control (100% enzyme activity) which was conducted in the same way by replacing extract with 1 mL of respective solvents. Inhibition of starch hydrolysis by α-amylase inhibitor resulted in a diminished absorbance at 546 nm in comparison. Percentage inhibition was determined by the equation;

$$\text{Percentage inhibition (\%)}=\frac{A_c-A_s}{A_c}\times 100$$

2.7. Lipase inhibition activity

Lipase activity was measured using triolein as a substrate. The reaction mixture was prepared by mixing 0.1 mL of lipase (200 units/mL in 0.1 mL phosphate buffer pH 6.8) with 0.1 mL of the sample/standard solutions. The reaction mixture was kept at 37 °C. After incubation for 1 min, the amount of oleic acid released by the lipase was measured at 340 nm[17] using the UV-vis instrument, GENESYS 10S UV-Vis v4.003 2L9Q129001. The inhibitory activity was calculated as

$$\text{Inhibitory activity (\%)}=\left(1-\frac{B}{A}\right)\times 100$$

Where A and B are the activities of the enzyme without and with sample respectively.

2.8. Correlation between antioxidant activity and antiradical activity

The relationship between antioxidant activity and anti-obesity activity was investigated by comparing the activities of the different concentrations 0-100 mg/mL of extracts (R_f= 0.96).

2.9. Statistical analysis

The data on antioxidant anti-obesity activity was reported as mean±SD of three determinations. Student's t-test (P=0.05) was applied to compare antioxidant activity of extracts and ascorbic acid and ant-obesity activity of extracts with that of herbex. Ascorbic acid was taken as a standard antioxidant since it is widely added to foods. ANOVA analysis and least significant difference test were applied to compare antioxidant and anti-obesity of extracts.

3. Results

3.1. TLC profiling

In this study, presence of flavonoids were determined by spraying with a revealing agent, 1% ethanolic solution of $AlCl_3$ and heating at 100 °C for 5 min. A total of eight different flavonoid types were observed (Table 2). The ethyl acetate

extract yielded two types with R_f values 0.38 and 0.40. Chloroform extracts also yielded two types of flavonoids at R_f values at 0.05 and 0.51. Ethanol extracts yielded the greatest number of flavonoids at R_f values of 0.16 (2); 0.33 (3), 0.65 (4) and 0.96 (5) (Figure 2). Spots that gave negative results with the revealing agent were designated as unknowns.

Table 2

TLC profiling of flavonoids in *D. steriscus*.

Solvents	R_f values	Assigned substance
Ethyl acetate	0.24	Unknown
	0.38	Flavonoid 1
	0.40	Flavonoid 2
	0.62	Unknown
	0.88	Unknown
Chloroform	0.05	Flavonoid 1
	0.16	Unknown
	0.44	Unknown
	0.51	Flavonoid 2
Ethanol	0.11	Unknown
	0.16	Flavonoid 1
	0.29	Unknown
	0.33	Flavonoid 2
	0.65	Flavonoid 3
	0.70	Unknown
	0.84	Unknown
	0.96	Flavonoid 4

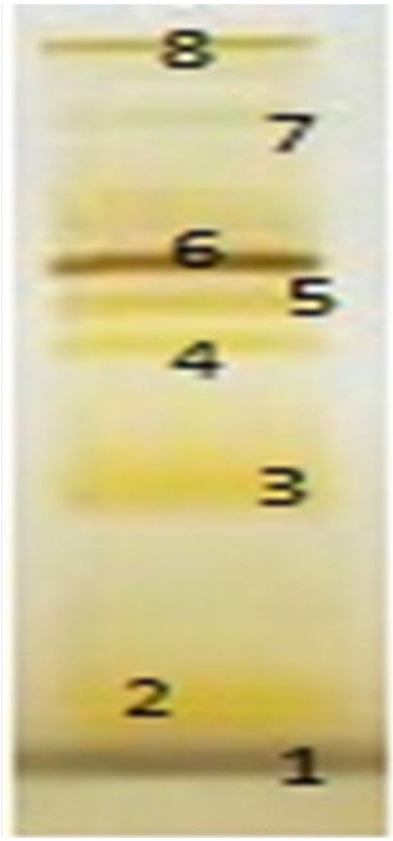

Figure 2. TLC profiling of flavonoids (ethanol extract) in *D. steriscus*.

3.2. DPPH assay

Antioxidant activity of the extract was tested by analyzing the DPPH free radical scavenging activity. All the identified flavonoids depicted antioxidant activity (Table 3). Ethanol extract revealed the greatest antiradical activity [(99.22 ±0.10)%, R_f 0.96] which was significantly greater than antiradical activity of standard antioxidant ascorbic acid [(82.62±0.07)%]. Ethyl acetate and chloroform extracts showed inferior antiradical activity, (33.63±0.09)% and (46.12±0.25)% respectively.

Table 3

Antioxidant activities of flavonoids in *D. steriscus*.

Solvent	R_f values	Assigned substance	Antioxidant activity (%)
Ethyl acetate	0.38	Flavonoid 1	21.55±0.15
	0.40	Flavonoid 2	33.63±0.09*
Chloroform	0.05	Flavonoid 1	24.09±0.17
	0.51	Flavonoid 2	46.12±0.25*
Ethanol	0.16	Flavonoid 1	89.41±0.05
	0.33	Flavonoid 2	91.00±0.33
	0.65	Flavonoid 3	97.13±0.03*
	0.96	Flavonoid 4	99.22±0.10*
Water	–	Ascorbic acid	82.62±0.07*

Data are expressed as mean±SD, n=3. *: Results showed significantly difference in t-test and ANOVA (P=0.05).

3.3. Anti-obesity assay

Anti-obesity activity of isolated flavonoids was determined by investigating enzymes lipase and α-amylase inhibitory activity. The order of inhibitory activity was ethyl acetate<chloroform<ethanol. Ethanol extract exhibited significantly greater anti-obesity activity as compared to herbex, a commercially anti-obesity medication sold in drug stores. Anti-α amylase activity and anti-lipase activity for herbex was (78.38±0.02)% and (76.07±0.09)% respectively while that of ethanolic extract (R_f=0.96) was (93.66±0.00)% and (95.88 ±0.13)% (Table 4).

Table 4

Alpha amylase inhibition activity of *D. steriscus*.

Solvent	R_f values	Assigned substance	Anti-α amylase activity (%)
Ethyl acetate	0.38	Flavonoid 1	11.66±0.12
	0.40	Flavonoid 2	19.44±0.10*
Chloroform	0.05	Flavonoid 1	21.09±0.04
	0.51	Flavonoid 2	38.38±0.21*
Ethanol	0.16	Flavonoid 1	75.09±0.03
	0.33	Flavonoid 2	86.00±0.07*
	0.65	Flavonoid 3	87.03±0.13*
	0.96	Flavonoid 4	93.66±0.00*
Water	–	Herbex	78.38±0.02*

Data are expressed as mean±SD, n=3. *: Results showed significantly difference in t-test and ANOVA (P=0.05).

3.4. Correlation between antioxidant activity and anti obesity activity

Figure 3 shows the relationship between ant-obesity

activity and antioxidant activity of flavonoids (R_f=0.96). Computed results showed a significant correlation between antiradical activity versus anti-lipase activity and anti-α amylase activity R^2 =0.995 and 0.983 respectively. Results in Tables 2-5 also show that there was a significant relationship between antioxidant activity and ant-obesity activity.

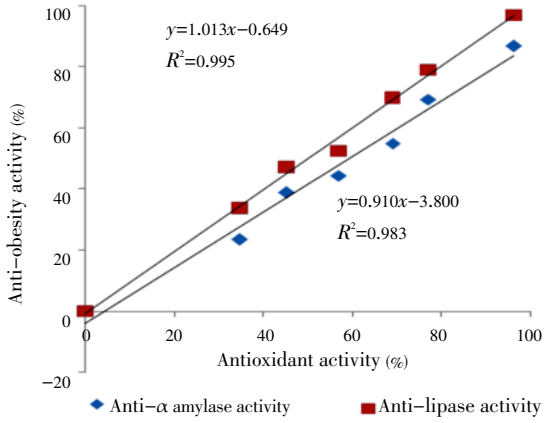

Figure 3. Relationship between ant-obesity activity and antioxidant activity.

Table 5

Lipase inhibition activity of Dioscorea steriscus

Solvent	R_f values	Assigned substance	Anti-lipase activity (%)
Ethyl acetate	0.38	Flavonoid 1	26.42±0.13
	0.40	Flavonoid 2	45.41±0.05*
Chloroform	0.05	Flavonoid 1	24.53±0.03
	0.51	Flavonoid 2	38.11±0.12*
Ethanol	0.16	Flavonoid 1	84.66±0.25*
	0.33	Flavonoid 2	93.34±0.14*
	0.65	Flavonoid 3	95.12±0.07*
	0.96	Flavonoid 4	95.88±0.13*
Water	–	Herbex	76.07±0.09*

Data are expressed as mean±SD, n=3. *: Results showed significantly difference in t-test and ANOVA (P=0.05).

4. Discussion

Antioxidants have been found to be the bioactive compounds in most medicinal plants[18]. Flavonoids exert antioxidant activity by acting as free radical scavengers through the donation of a hydrogen atom[19]. This property is attributed to the phenolic hydroxyl groups attached to the ring moiety. Oxygen radicals are constantly generated in the body to participate in metabolic processes. If an imbalance occurs in the body, the free radicals may attack lipids in cell membranes, tissue proteins, enzymes and DNA to cause oxidative damage. Oxidative damage has been implicated in the pathology of a series of human ailments such as cancers, heart diseases, obesity and aging[17]. The magnitude of antioxidant activity depends on chemical structure and type of the flavonoid. Previous studies have found out that some plant based food stuffs such as the soybeans proteins and compounds from green tea are lipase inhibitors[20]. Some plant species such as *Camelia sinensis* and *Cassia mimosoides* have also been found to have lipase inhibitors[20]. Inhibition of lipase prevents metabolism of lipids. Studies on flavonoids have increased over the years since the discovery of the French paradox. French people have one of the lowest incidents of cardiovascular diseases despite high intake of saturated fats[21]. This has been explained in terms of their regular intake of red wine which consists of large quantities of flavonoids. Consumption of food rich in flavonoids has been associated with many health promoting effects including cancer preventing and anti-obesity activity[20,21]. Flavonoids can act as enzymes inhibitors if they complex metals such as copper, magnesium and zinc required for the maintenance of the three dimensional conformation of enzymes. The enzyme α-amylase is one of the important enzymes in the human body that breaks down starch to simple sugars. Flavonoids enzyme inhibitors in *D. steriscus* can delay carbohydrate metabolism and reduce the rate of glucose absorption.

The present results reveal that *D. steriscus* consist of lipase and α-amylase inhibitors and therefore can be useful for the development of functional foods against obesity. It can also act as a source of lead compounds for the design of new ant-obesity therapeuticals.

Conflict of interest statement

Authors declare that there is no conflict of interest.

Acknowledgements

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Comments

Background

Obesity is implicated in the genesis of many health problems such as cancers and heart diseases in both developing and developed countries. Currently drugs in use have side effects and sometimes lack efficacy. There is therefore a need for potent anti-obesity therapeutics.

Research frontiers

The present work shows anti-lipase and α-amylase activity of *D. steriscus* extracts of ethanol, chloroform and ethyl acetate. *D. steriscus* is used to treat many diseases

including obesity in folk medicine. It has been eaten as relish for some time. Currently there is no scientific information to authenticate its traditional medical use.

Related reports

Flavonoids have been reported to have *in vitro* and *in vivo* anti-obesity activity in literature. *D. steriscus* flavonoids have been demonstrated in this work to exhibit high anti-lipase and α-amylase activity.

Innovations and breakthroughs

D. steriscus is a plant that grows in the wild in Southern Africa. It has been used to treat several diseases in traditional medicines by the local people. There is no scientific evidence on its nutritional or flavonoid content. In the present work, authors demonstrated antioxidant, anti-lipase and α-amylase activity of this plant for the first time.

Applications

Since *D. steriscus* has been used as relish and medicine by the local people, it could be applied for the development of neutraceuticals to treat obesity.

Peer review

This is a valuable research in which the authors have shown the antioxidant, anti-lipase and α-amylase activity of *D. steriscus*. Antioxidant activity was assessed by DPPH antiradical activity while ant-obesity activity was assessed using enzyme inhibition activities by chromogenic methods. *D. steriscus* has been found to be a promising source of lead compounds anti-obesity drugs.

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