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In vitro pancreatic lipase inhibition potential of commonly used *Dioscorea* species

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Abstract
To minimize energy intake through gastrointestinal pathways, the production of nutrient digestion and absorption inhibitors is considered as an essential strategy among the current treatments for obesity. Pancreatic lipase is one of the key enzymes, which act on the dietary fat and help in absorption of dietary fat. Inhibition of the lipase enzyme helps in the reduction of fat absorption and obesity management. The extracts of four different *Dioscorea* species were screened for Primary phytochemicals like phenolic, flavonoids highest phenolic content was found in *D. alata* dry root methanolic extract and flavonoids was found in *D pentaphylla* dry aerial part extract. Different plant part extracts in different solvent were assessed for the lipase inhibition activity. The highest activity was observed in *D pentaphylla* aqueous extract of dry aerial part 76.71±0.024% with IC50 value 0.850mg/gm. The activity differs with different solvent and plant parts. *Dioscorea* species exhibit strong lipase inhibition potential hence can be used for the obesity management.

Keywords: *Dioscorea*, phytochemicals, lipase inhibition, antiobesity

Introduction

Obesity is on the rise at an alarming rate, yet presently there are just a few treatments available to treat it. Antiobesity medicines might be developed by specifically targeting the enzymes involved in this process. Natural materials have a large number of Pancreatic Lipase (PL) inhibitors that might be transformed into therapeutic treatments. It is a serious and chronic disease with associated increased risk of insulin resistance, type 2 diabetes, cardiovascular disease, cancer, gallstones, fatty liver disease, osteoarthritis, and oxidative stress and inflammation based pathologies in human population.

In order to minimize energy consumption of fat, nutrient digestion and absorption should be minimized. Since fat contributes to unwanted calorie deposition more than protein or carbohydrate, inhibition of fat absorption is considered as the most common target to decrease energy intake. To minimize energy intake through gastrointestinal pathways, the production of nutrient digestion and absorption inhibitors are considered essential strategies among the current treatments for obesity. For the production of antiobesity agents, inhibition of digestion and absorption of dietary lipids can be targeted by inhibitory action on pancreatic lipase. (seyedan et at 2015, Yang et al. 2014).

Lipases are enzymes that catalyze the hydrolysis of ester bonds of triacylglycerol's i.e. fats and oils. After action of lipase on the fat free fatty acid have been absorbed by the body are responsible for the development of obesity. The passage of triacylglycerol from the intestinal lumen into the body can be reduced if lipases are blocked. An anti-obesity drug that inhibits digestive lipases might be effective (Ado *et al* 2013).

In the market drugs like Orlistat are available, which reduces fat absorption through inhibition of pancreatic lipase.

Some plant extract have also been reported to inhibit the pancreatic lipase, (F Lei *et al* 2007), (Roh and Jung 2012).

Dioscorea is a genus that belongs to the *Dioscoreaceae* family. Antiobesity properties have been reported from several *Dioscorea* species. Several *Dioscorea* species have been shown to have antibacterial, antifungal, anti-diabetic, anti-inflammatory, and anti-lipase properties. (Panda and Padhan 2020). The anti-obesity properties of *Dioscorea* species are rarely investigated (Jeong et al 2016, Kwon et al 2003). The current study examines the pancreatic lipase inhibition effect of different extracts from four *Dioscorea species: D. alata, D. bulbifera, D. pentaphylla, and D. oppositifolia*

Methods and Material

A. Collection of plant material

Four *Dioscorea* species viz. *Dioscorea oppositifolia, Dioscorea bulbifera, Dioscorea alata, Dioscorea pentaphylla* were collected from various locations from Kolhapur district Maharashtra India. Voucher specimens were deposited in the herbarium of Department of Botany. Shivaji University Kolhapur. *D. oppositifolia, D. bulbifera, D. alata, D. pentaphylla* (Voucher specimen no RSP001, RSP002, RSP003, RSP004 respectively). Tubers were planted in pots and maintained at Botanical Garden of Shivaji University, Kolhapur.

B. Preparation of plant extract

Extraction was carried out by modified method of (Satyajit and Lutfun, 2012) for extraction dry/fresh plant powder (10gm) and diatomaceous earth was mixed in equal volume and filled in to 66 ml stainless steel extraction cell. The cell was loaded in accelerated solvent extractor (Dionex™ ASE™ 350 Thermo Scientific). The extraction was carried out at 45^0c with 5 min heating and 5 min static time under pressure 1300- 1500 PSI. These extracts were evaporated by rotary evaporator under vacuum, methanolic and aqueous extract evaporated at 64^0c and 100^0c respectively. After evaporation dry residue was removed from flask. Collected

residue was dissolved in respective solvent to obtain concentrations of 1mg/ ml for each assay

C. Total phenolic

Total phenolic content were estimated as modified method of Wolfe et al 2003 total reaction volume had 300 µl 12.5 µl of plant extract and 12.5 µl of folin – cocatteau reagent (1:10 diluted with water) was mixed and incubated at room temperature for 10 min. after incubation 7% sodium carbonate was added and reaction volume was make up to with D/W and incubated in dark at 37 for 90 min. then optical density was measured at 760 nm on 96well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific). Phenolic content was determined by calibration curve of standard Gallic acid (20 to 100 µg/ml). (Wolfe et al 2003)

D. Total flavonoids

Total flavonoids content was estimated by modified method of luximon-Ramma et al (2002) 100 µl of plant extract and 100 µl of 2% aluminum chloride mixed together and incubated at room temperature for 10 min. then optical density was measured at 368 nm on 96 well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific). Flavonoid content was determined by calibration curve of standard quercetin (20 to 100 µg/ml).

E. Pancreatic lipase activity

The activity of Porcine Pancreatic Lipase (PPL, type II) was evaluated using the substrate p-nitrophenyl butyrate (p-NPB). Kim, et al. and Zheng, et al. earlier published a technique for assessing pancreatic lipase activity, which was adapted for this study. PPL stock solutions (0.6 mg/mL) were produced in a 0.5 mM sodium phosphate buffer (pH 7.2) containing 150 mM NaCl and kept at 20 °C. The extracts (concentrations 1mg/ml to 5 mg/ml) or Orlistat (at same concentrations) were used to assess the lipase inhibitory activity For the lipase inhibition reaction, 100 l of buffer, 50 l of lipase enzyme, 25 l of plant extract and Orlistat, and 25 l of p-nitrophenyl butyrate substrate were added, and the optical density at 400 nm was measured using a spectrophotometer (Multiskan sky 96well spectrophotometer, thermo scientific) for control reaction 125 µl buffer 50 µl of lipase enzyme 25 µl of p-nitrophenyl butyrate substrate was added The inhibitory activity was calculated according to the following formula:

$$\% \text{ Inhibition} = [(\text{Control OD} - \text{Sample OD})/\text{Control OD}] \times 100$$

F. Statistics

The data were shown as mean ± standard deviation and analyzed using Two way ANOVA was calculated p value is statically significant ($P =< 0.0001$) was considered as level of significance. The data were analyzed using GraphPad Prism 5 Software, San Diego, California, USA.

Results and Discussion

A. Total phenolic content

The total phenolic content of various parts of four yam species is shown in the table below (fig 1). The phenolic content of various plant parts of four yam species differed significantly. The *D. alata* dry root methanolic extract has the highest phenolic content, at 283.6±0.005 mg/gm GAE of dry weight, while the root methanolic extract has the lowest, at 16.1±0.003 mg/gm GAE of fresh weight. When comparing the extraction solvents, the methanolic extract had a higher phenolic content than the aqueous extract of the plants. When comparing the phenolic content of the dry and fresh plant parts, the dry part has a greater phenolic content than the fresh plant part. Secondary aromatic plant metabolites, Phenolics are widely distributed throughout plants. Antioxidant and other biological activity features have been linked to Phenolics. Bhandari et al. (2004) investigated the four *Dioscorea* species found in Nepal: *D. bulbifera*, *D. versicolor*, *D. deltoidea*, and *D. triphylla*, and found that *D. bulbifera* has the greatest phenolic content, while *D. triphylla* has the lowest.

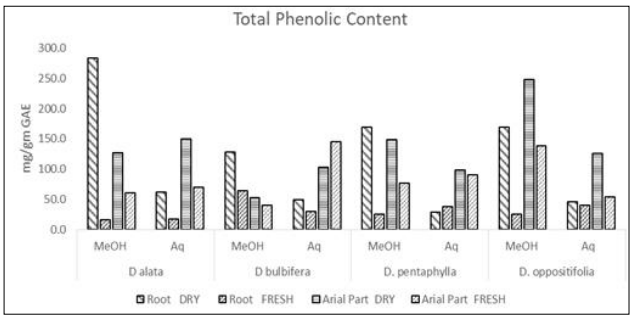

Fig 1: Comparison of the total phenolic content estimated in the different *Dioscorea* extracts

B. Total flavonoids content

Flavonoids are a class of naturally occurring polyphenol chemicals that are distinguished by their flavone nucleus. It is currently regarded an essential component in a wide range of nutraceutical, pharmaceutical, medical, cosmetic, and other products. Flavonoids promote good health and prevent illness. (Karak 2019) While comparing the flavonoid content in different *Dioscorea* extract (fig 2). The maximum flavonoid content was found in *D. pentaphylla* dry aerial part extract i.e. 234.83±0.030 mg/gm rutin equivalent of dry weight and the lowest content found in *D. alata* fresh root aqueous extract i.e. 21.63±0.002 mg/gm rutin equivalent of fresh weight.

C. Lipase inhibition activity

Pancreatic lipase is primarily responsible for the hydrolysis of triglycerides into free fatty acids and monoglycerides. It's a lipolytic enzyme that catalysis the hydrolysis of the triacylglycerol's' ester bonds. The enzyme operates by removing the fatty acids from sites 1 and 3 of the triglyceride, leaving a 2-monoglyceride and two free fatty acids in their place. (Mark Lowe 1997)

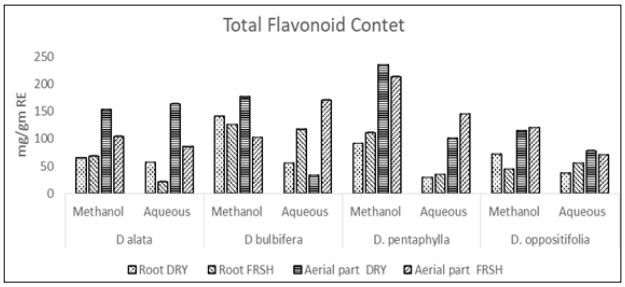

Fig 2: Comparison of the total flavonoid content estimated in the different *Dioscorea* extracts. Values expressed in mg/gm rutin equivalent

In present study 32 different extracts were prepared from four *Dioscorea spices* and their anti-lipase activity was investigated at concentration of 5mg/ml for PPL inhibition. The inhibitory activities towards pancreatic lipase are represented in table-1. Among the 32 extracts 4 crude extracts showed above 70% lipase inhibition and 18 extracts shows inhibition activity between 50 to 70% and 10 extracts has below 50% activity at concentration 5 mg/ml (table no 1). The significant inhibition of Pancreatic lipase was observed in *D. pentaphylla* dry aerial part aqueous extract 76.71±0.024% with IC50 0.8508 mg/ml, although in *D. pentaphylla* fresh aerial part methanolic extract 70.71±0.018% with IC50 0.8986 mg/ml. In case of *D. alata* dry aerial part methanolic extracts shows 74.81±0.023 % inhibition with IC50 3.9650 mg/ml and *D. bulbifera* dry aerial part aqueous extract shows 70.26±0.020% with IC50 1.1165mg/ml. The orlistat was used as positive control it shows the 86.89% inhibition at 5mg/ml concentration with IC50 value 0.9525 mg/ml Orlistat, a Hydrogenated derivative of lipstatin, is the pancreatic lipase inhibitor currently approved for a long-term treatment of obesity. (Fig 6). Kwon et al. (2003) isolated Dioscin, diosgenin, and saponins from *D. nipponica* and tested for lipase inhibitory efficacy in rats, finding a substantial inhibitory effect. Bioactive flavonoids were extracted from *D. steriscus* tubers that can inhibit lipase and -amylase, making them useful for the development of anti-obesity therapeutics. (Dzomba and Musekiwa 2014). Juong et al (2016) has reported n butanolic extracts of *D. oppositifolia* has bioactive compound which inhibit lipase and reduces fat absorption in high fat induced mice. Although correlating with the phenolic and flavonoid content of the extracts, no such trend was seen in the study that high phenolic or flavonoid containing extracts exhibited the highest lipase inhibitory ability. The observed inhibitory action might be attributed to the presence of all phytochemicals in the crude extracts. It is still necessary to separate the bioactive components from the crude extracts.

Table 1: Lipase inhibition activity of all *Dioscorea* extract values expressed in % with ±SE

Sr.No.	Plant Name	Solvent	Root		Aerial Part	
			Dry	Fresh	Dry	Fresh
1	*D. alata*	MeOH	43.71±0.050	64.86±0.042	74.81±0.023	57.66±0.078
		Aq	58.76±0.011	55.49±0.056	65.05±0.040	58.14±0.011
2	*D. bulbifera*	MeOH	60.94±0.037	46.92±0.084	64.13±0.021	66.68±0.046
		Aq	37.25±0.023	53.41±0.074	70.26±0.020	63.54±0.007
3	*D. pentaphylla*	MeOH	65.15±0.024	53.09±0.048	64.97±0.023	70.71±0.018
		Aq	56.61±0.082	64.18±0.030	76.71±0.024	67.17±0.041
4	*D. oppositifolia*	MeOH	18.93±0.018	9.71±0.032	19.49±0.019	6.54±0.043
		Aq	36.27±0.046	29.90±0.046	45.11±0.170	66.36±0.073

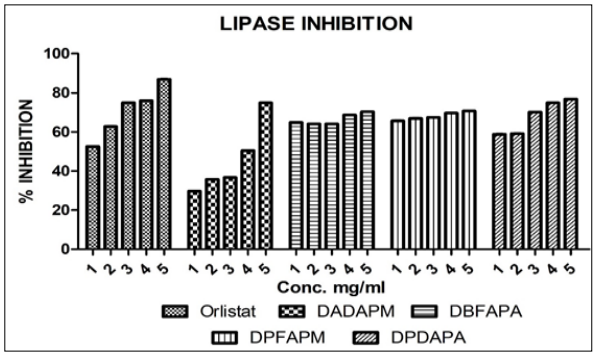

Fig 3: Inhibitory effect of orlistat and plant extract pancreatic lipase activity. DADAPM: - *D. alata* dry aerial part methanolic extract, DBFAPA: - *D. bulbifera* fresh aerial part aqueous extract, DPFAPM: - *D. pentaphylla* fresh aerial part methanolic extract, DPDAPA: - *D. pentaphylla* Dry aerial part aqueous extract. Two way ANOVA was calculated p value is statically significant (*P=< 0.0001)*

Conclusion

All the extract's exhibit varied degree of lipase inhibition**.** Over all Comparison between screened the extracts and solvents the all *D. pentaphylla* extracts shows the above 50% activity. While considering this need to isolate bioactive compounds from *D. pentaphylla* and evaluate for the lipase inhibition. Cured extract might be useful to manage obesity and associated problems.

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