International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 20-11-2021, Accepted: 05-12-2021, Published: 21-12-2021
Volume 6, Issue 6, 2021, Page No. 1259-1264

Evaluation of antioxidant and pancreatic lipase inhibitory potential of *Polygala glaucoides* L. and *Polygala erioptera* DC

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Abstract
Obesity is the accumulation of abnormal or excessive fat that might make it difficult to maintain a healthy weight. *Polygala glaucoides* L and *Polygala erioptera* DC extracts were evaluated for their anti-pancreatic lipase and antioxidant activity. DPPH, ABTS, and ferric-reducing antioxidant power assays were used to determine the extracts' antioxidant activity. The leaf extract showed highest antioxidant potential for all three studied antioxidant assays in *P. glaucoides* whereas in *P. erioptera* stem has the highest antioxidant capacity. All extracts were found to inhibit pancreatic lipase activity, but among all extracts leaf showed maximum inhibitory potential for lipase i.e., 84.99 ±0.021 % in *P. glaucoides* and 74.59 ±0.02% in *P. erioptera.* The standard drug orlistat having 68.25 ±0.002% inhibitory activity for lipase. Plant extracts are showing more inhibitory activity for lipase as that of orlistat. In both the studied activities leaf extract showed more potential indicating it has compounds that can be used to treat obesity related to inflammation and oxidative stress.

Keywords: obesity, antioxidant, pancreatic lipase, orlistat, polygala

Introduction

Obesity is a worldwide epidemic that causes diseases and social biases. A body mass index (BMI) of 25 or higher is considered to be overweight, and a BMI of 30 or higher is considered as obese. According to the global burden of disease, the issue has reached epidemic proportions, with over 4 million people dying each year as a result of being overweight or obese in 2017.Obesity is defined by the World Health Organization as an abnormal accumulation of fat that can be harmful to one's health (Chedda *et al* 2016, Jee *et al* 2021) [1, 2]. Because appetising foods cause hyperphagia and excessive fat accumulation, as well as increased fatty acid oxidation in muscles and decreased anorexigenic hormones like cholecystokinin, food consumption is thought to modulate appetite modulating hormonal peptides in the brain and stomach. (Everard and Cani 2013). Obesity is linked to a variety of common diseases, including non-alcoholic fatty liver disease, hypertension, osteoarthritis, cardiovascular disease, stroke, type 2 diabetes, and cancer. There are several pathways in the human body that cause obesity, either directly or indirectly; these can be targeted to combat obesity (Wang *et al* 2017). Dietary triglycerides that cannot be absorbed are hydrolysed by pancreatic lipase, a pancreatic enzyme that separates triglycerides into monoacylglycerol and free fatty acids, which are then combined with bile acids, cholesterol, and lysophosphatidic acid (LPA) to form mixed micelles in the small intestine. Enterocytes ingest mixed micelles, and re-synthesize triglycerides stored in adipocytes (Mark E. Lowe 2002, Fu *et al* 2016).

One method for determining the potential efficacy of natural products as an anti-obesity treatment is to block pancreatic lipase. The mechanism involves inhibiting dietary triglyceride absorption, which is the primary source of extra calories. Pancreatic lipase hydrolysed 50-70 percent of the total dietary fat. Furthermore, pancreatic lipase inhibition has no effect on any central mechanism, making it an ideal approach for obesity treatment (Alias *et al* 2017). There are many anti-obesity medications, namely, orlistat, lorcaserin, sibutramine, and rimonabant. Sibutramine and Rimonabant have been withdrawn from the market due to cardiovascular effects and many psychiatric side effects (Ref). Orlistat is an actinobacterium-derived saturated form of lipstatin. It works as a reversible inhibitor of pancreatic triglyceride lipase, which prevents fat absorption (Derosa and Maffioli 2012). This function is beneficial not just in the prevention of obesity but also in the treatment of high blood pressure and type 2 diabetes. Orlistat's usefulness is restricted, because of its toxicity to various internal organs, including the kidney and liver (Chedda *et al* 2016) [1]. As a result, deadly adverse effects limit the use of synthetic anti-obesity medications, resulting in poor tolerability and patient compliance. This necessitates the urgent quest for agents that are less toxic and so provide higher safety and efficacy over time. Several bacterial, fungal, and marine species have been investigated for novel lipase inhibitory chemicals in the ongoing search for potent antiobesity medicines.

Polygala is a vast genus of flowering plants from the *Polygalaceae* family. Milkworts and snakeroots are two common names for the members of this genus. There are around 600 species in this genus, which are found all over the world (Ref?). Some species are employed as expectorants, anti-inflammatory agents, and to treat central nervous system disorders in local and traditional medicine. Plants in this genus are widely recognised for producing triterpene saponins and the chemotaxonomic significance is frequently emphasised. Xanthones, oligosaccharides, lignans, and other phenolic compounds are among the numerous specialised metabolites that have been discovered

(Klein Junior *et al*. 2012, Lacaille-Dubois *et al*., 2019). Recently *Polygala tenuifolia* and *Polygala japonica* Houtt had been utilized for their anti-adipogenesis activity by inhibiting lipid accumulation in adipogenic 3T3L1 cells (Wang *et al* 2017, Jee *et al* 2021) [2].

In present investigation, *Polygala glaucoides* (PG) L and *Polygala erioptera* (PE) DC are utilized for the antioxidant activity and antiobesity potential by using lipase inhibition assay. *P. glaucoides* and *Polygala erioptera* are erect herb with linear lanceolate leaves. Flowers in *P. glaucoides* are yellow-colored whereas in *P.* erioptera flowers are pink in colour. The available literature indicates that plant is rich source of phytocompounds and having various biological activities. This is first piece of report regarding the determination of antiobesity potential of *P. glaucoides* L and *Polygala erioptera* DC plants by using porcine pancreatic lipase inhibition.

Material and Methods

Collection of plant material

P. glaucoides L and *P. erioptera* DC plants were collected from various locations of Western Ghats, Maharashtra, India. A voucher specimen was submitted at the 'SUK' herbarium of the Department of Botany, Shivaji University, Kolhapur (Voucher Specimen No.- MPM 005 for *P. glaucoides* L and MPM 002 for *P. erioptera* DC).

Preparation of extract

The collected plants were extracted using two different solvents, i. e. methanol and water. Plant materials was cleaned and used for the preparation of fresh and dried extracts. Fresh leaves, stem, and root extracts were immediately prepared by blending in a blender and extracting with solvents. Leaves, stem, and root were separated for dry extraction, and shade dried for a day and kept in the oven for 2 days at 45^0C. Using a mechanical blender, the material was ground to powder after drying and then extracted with solvents. Accelerated solvent extraction method was adopted for extraction using (Accelerated Solvent Extractor-Dionex-350 system, Thermo-Scientific) with conditions viz. 1500 psi pressure, 70 mL/min flow rate, and 45^0C Temperature. The obtained extract was then evaporated using Rotatory Evaporator to concentrate the extracts. The concentrated extract was then kept at 45^0 C in the oven to obtain a dry residue which was then diluted (10mg/ml) to make working stock. The prepared working stock was used for further experimental purposes.

In vitro antioxidant activity

DPPH radical scavenging activity

In vitro DPPH radical scavenging activity was measured as the method described by Blois (1958) Rosidah *et al*., (2008) with slight modifications. 10 µl of plant extracts was added to the well and mixed 290 µl of DPPH. Then plate was incubated in dark for 20 mins. Absorbance was measured at 517nm. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution (DPPH without sample). Ascorbic acid was used as positive control. The ability to scavenge the DPPH radical was expressed as percentage inhibition and calculated using the following equation

DPPH scavenging activity (%) = [(OD1 – OD2) / OD1] X 100

Where, OD1 = Optical density of control, OD2 = Optical density of test sample

Ferric reducing antioxidant power (FRAP) assay

The ability to reduce ferric ions was analysed with minor modifications using the FRAP assay method described earlier (Benzie and Strain, 1996). The reaction mixture consists of 290µl (0.3 M acetate buffer, 5ml 10mM TPTZ, 5ml 20mM $FeCl_3$) and 10 µl (1mg / ml) plant extracts. For 15 minutes, the reaction mixture was incubated at 37^0C, and absorbance at 595 nm was measured. Ferric reduction was calculated from the linear calibration curve of Ascorbic acid, the antioxidant potential based on the ability to reduce ferric ions was expressed as mmol ascorbic acid equivalents per gm of fresh or dry extracts.

ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) scavenging activity

ABTS is a one of the common antioxidants that used to analyse scavenging potential of plant materials (Arnao *et al* 2001). In this, 20 µl (1mg / ml) of plant extracts were mixed with 180 µl of ABTS reagent. The reaction mixture was incubated at room temperature for 10 mins and scavenging activity was measured by checking absorbance at 734 nm. Ascorbic acid was used as standard and only ABTS as a blank control. The percentage of inhibition or scavenging was calculated by,

ABTS scavenging activity (%) = [(OD1 – OD2) / OD1] X 100

Where, OD1 = Optical density of control, OD2 = Optical density of test sample

Assessment of antiobesity activity by Pancreatic lipase inhibition

Preparation of Lipase and Substrate

Porcine pancreatic lipase (PPL) stock was prepared by dissolving 600 units of enzyme in to the 10 ml phosphate buffered saline (500mM Sodium phosphate buffer with 150mM NaCl) at pH 7.2. The Substrate p-nitrophenyl butyrate (PNB) was prepared by adding 8.5µl PNB in to the 10 ml of acetonitrile solution.

Pancreatic lipase inhibition assay

The plant extracts capacity to inhibit PPL was determined using a modified approach described by Lewis earlier. The lipase activity was evaluated using UV-transparent 96-well plates on an ELISA reader to measure the hydrolysis of pNPB to p-nitrophenol. Pancreatic lipase inhibition assay was carried out in 96 well plate, reaction mixture containing 100 µl of buffer, 50 µl of lipase enzyme, 25 µl of plant extracts (conc. 1mg to 5mg/ml) and 25 µl of p-nitrophenyl butyrate substrate. Orlistat was used as positive control at different concentrations ranging from 1mg to 5mg/ml. Distilled water and methanol were considered as negative control.

eaction mixtures were incubated at 37^0C for 30 min and optical density were recorded at 400 nm. Percentage inhibition is calculated by following formula,

% Inhibition = [(Control OD – Sample OD)/Control OD] x100

Results and Discussion

The antioxidative potential of both plant part extracts were determined using the DPPH, ABTS and FRAP assays. In *P. glaucoides*, dry leaf methanol extract showed more DPPH scavenging and Ferric reducing power i.e., 78.26±0.001 % and 205.29±0.01 mM equivalent to ascorbic acid per gm of material. Antioxidants that may oxidize ferric ion (Fe3+) to ferrous ion (Fe2+) generate the Fe2+TPTZ-1 blue complex when combined with TPTZ (Benzie and Strain, 1996). While ABTS scavenging activity was seen in dry leaf aqueous extract i.e., 89.65±0.06 % as shown in (Table-1). But in *P. erioptera*, fresh and dry stem methanolic extracts have more significant DPPH scavenging and ferric reducing power activity i.e. 77.71±0.003% and 446.63±0.010mM, whereas fresh leaf aqueous extract showed 83.71±0.017 % ABTS scavenging activity (Table-2). Results indicate the antioxidant molecules are soluble in both solvents but mechanism of action is varied with plant parts.

Table 1: The antioxidant activity of *P. glaucoides* (PG) DC plant parts. Standard deviation ± was calculated for all activities. S-Stem, L-Leaves, R- Root, F-Fresh, D- Dry, A- Aqueous, M-Methanol.

	DPPH scavenging activity equivalent to Ascorbic acid (%)	ABTS scavenging activity Equivalent to Ascorbic acid (%)	Ferric reducing power equivalent to Ascorbic acid (mM/gm)
PGSFA	70.66±0.007	82.20±0.05	112.71±0.06
PGLFA	66.27±0.024	13.29±0.02	121.31±0.02
PGRFA	38.79±0.06	69.25±0.02	53.57±0.02
PGSDA	70.37±0.003	62.35±0.03	156.47±0.01
PGLDA	77.59±0.020	89.65±0.06	176.69±0.01
PGRDA	12.20±0.070	36.24±0.02	81.53±0.05
PGSFM	77.84±0.007	28.75±0.01	140.56±0.05
PGLFM	67.24±0.005	31.43±0.03	139.91±0.04
PGRFM	27.69±0.026	51.06±0.01	105.83±0.01
PGSDM	75.81±0.002	57.54±0.01	96.58±0.02
PGLDM	78.26±0.001	67.08±0.02	205.29±0.01
PGRDM	47.36±0.016	25.00±0.04	118.19±0.03

Table 2: The antioxidant activity of *P. erioptera* (PE) DC plant parts. Standard deviation ± was calculated for all activities. S-Stem, L-Leaves, R- Root, F-Fresh, D- Dry, A- Aqueous, M-Methanol.

	DPPH Scavenging activity equivalent to Ascorbic acid (%)	ABTS Scavenging activity equivalent to Ascorbic acid (%)	Ferric reducing power equivalent to Ascorbic acid (mM/gm)
PESFA	71.18±0.02	74.84±0.015	153.17±0.007
PELFA	61.90±0.012	83.71±0.017	208.55±0.010
PERFA	30.91±0.006	74.80±0.056	94.71±0.006
PESDA	24.42±0.024	14.88±0.003	51.63±0.002
PELDA	67.34±0.009	62.52±0.020	377.01±0.021
PERDA	52.74±0.032	24.99±0.007	50.73±0.003
PESFM	77.71±0.003	25.54±0.015	140.35±0.016
PELFM	49.98±0.006	17.95±0.002	304.58±0.030
PERFM	51.44±0.011	20.26±0.001	183.04±0.018
PESDM	39.65±0.009	26.14±0.012	446.63±0.010
PELDM	30.87±0.013	10.92±0.004	40.47±0.001
PERDM	24.84±0.006	24.05±0.030	94.19±0.002

In present study leaf extracts from *P. glaucoides* and stem extracts from *P. erioptera* having highest antioxidant activity means they possess more redox potential showing free radicle compounds. The phytoconstituents are the generally responsible for such type of activity basically phenolic and their derivatives. Phenolic compounds possess redox properties, allowing them to serve as antioxidants. Considering that their free radical scavenging ability is promoted by their hydroxyl groups (Shoib A. Baba, 2014, Olamide E. Adebiyi, 2017). Such redox potential showing compounds are soluble in both the solvents.

There are a number of different approaches for measuring lipase activity, such as using natural or synthetic triglycerides as the substrate. Spectrophotometric, turbidimetric, titrimetric, chromogenic, and immunochemical detection are some of the methods used. Using the spectroscopic approach and pNPB as the substrate, a pancreatic lipase inhibition analysis of selected *Polygala glaucoides* L and *Polygala erioptera* DC plant parts was successfully employed in the present study (Figure- 1 and 2). Both plant parts extracts in two different solvents were prepared at variable concentration such as 1mg/ml to 5mg/ml and screened for pancreatic lipase inhibitory activity. Among the tested plant extracts fresh leaf aqueous extract at 5mg/ml concentration has 84.99 ±0.021 % lipase inhibition potential in *P. glaucoides* and fresh leaf methanol extract of *P. erioptera* shows 74.59 ±0.02 at 5mg/ml concentration. But as compared to stem and leaf extract in *P. glaucoides* root extract has less potent inhibitory activity. Similarly, in *P. erioptera* stem has less potent lipase inhibitory potential in aqueous solvent. Orlistat was used as standard lipase inhibitor which showing 68.25 ±0.002% at 5mg/ml concentration. As compared with other plant parts extracts of the *P. glaucoides* and *P. erioptera*, leaf has the highest pancreatic lipase inhibition potential means it has capability to avoid triglycerides accumulation in the adipogenic tissue. Even leaf extracts show more significant lipase inhibitory potential than standard drug orlistat. Orlistat is the one of the commonly used drugs available in the market which inhibit the lipase (Derosa and

Maffioli, 2012). There is no early report of lipase inhibition or anti-obesity activity of these studied plants except the anti-inflammatory activity. So, we use to screen all plant parts separately for their lipase inhibition activity. Previous report on anti-inflammatory activity of *P. erioptera* showed plant has significant antiinflammatory activity performed by using Carrageenan induced Paw Edema in Rats (Sammaiah *et al* 2008). The anti-inflammatory potential of this plant indicates the plant can be used to treat inflammation related diseases including obesity. Recently, *Polygala tenuifolia* (PTE) and *Polygala japonica* utilized for their antiobesity activity using 3T3L1 adipogenic cell lines. In PTE treatment to fully differentiated 3T3L1 adipocytes reduced lipid accumulation by lowering lipid production and triglyceride content while increasing lipase activity. Similarly, in high-fat diet (HFD) -induced obese mice, PTE had an inhibitory effect on lipid accumulation, resulting in a smaller body size, lower triglycerides level, lower body weight, and less epididymal fat after treatment with 250 mg/k for 5 weeks (Wang *et al* 2017). Similarly, antiobesity effect of Polygalin -C isolated from *P. japonica* was studied by Jee *et al* (2021) [2] in which he observed significantly decreased lipid accumulation compared to the control in 3T3L1 adipocytes with suppression of adipogenesis transcription factors including peroxisome proliferator activated receptor γ (PPAR γ) and CCAAT/enhancer-binding protein (C/EBP) α, and lipogenic factors respectively. The result obtained from previously studied two plants and primary screened two plants in this study indicates that Polygala species possesses antiobesity activity related to chronic inflammation.

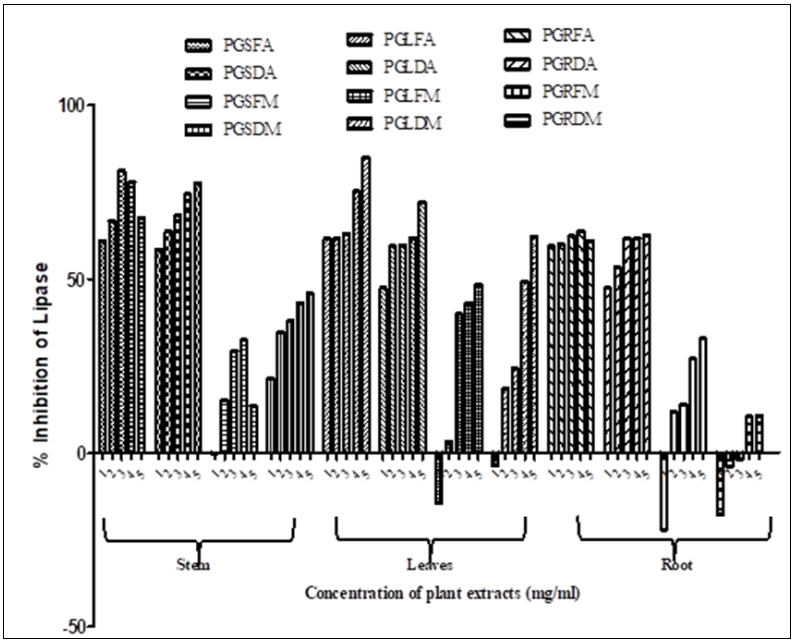

Fig 1: Porcine pancreatic lipase inhibitory potential of different parts of the *Polygala glaucoides* (PG) L plant extracts using two different solvents i.e., water and methanol. Two-way ANNOVA was used for statistical analysis. P values is < 0.0001 and it *** significant. S-Stem, L-Leaves, R- Root, F-Fresh, D- Dry, A- Aqueous, M-Methanol.

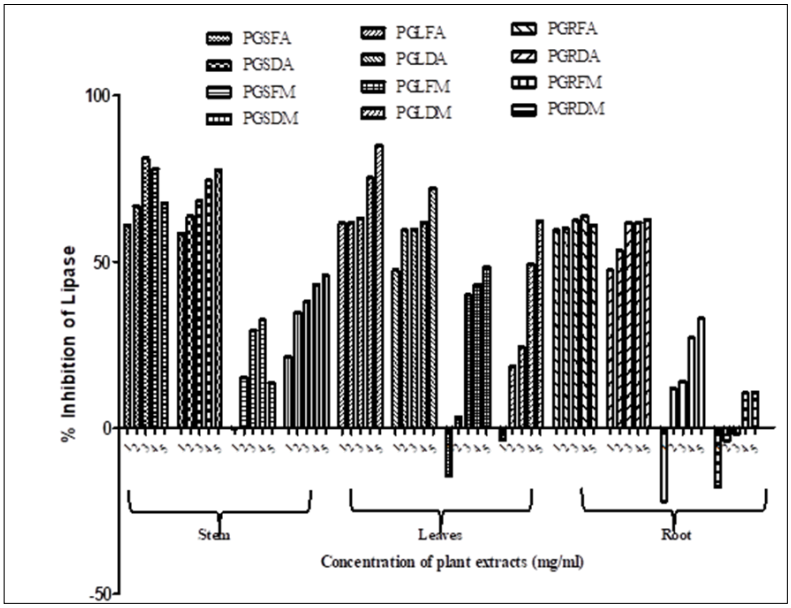

Fig 2: Porcine pancreatic lipase inhibitory potential of different parts of the *Polygala erioptera* (PE) L plant extracts using two different solvents i.e., water and methanol. Two-way ANNOVA was used for statistical analysis. P values is < 0.0001 and it *** significant. S-Stem, L-Leaves, R- Root, F-Fresh, D- Dry, A- Aqueous, M-Methanol.

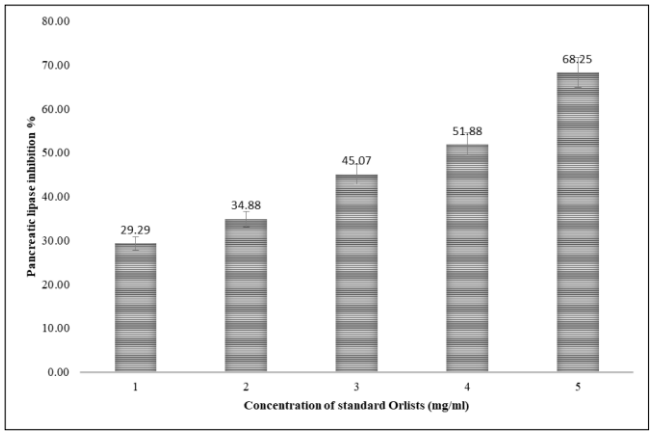

Fig 3: Porcine pancreatic lipase inhibitory activity of standard drug Orlistat. Standard deviation ± was calculated for all activities.

Conclusion

From the obtained results it can be conclude that, among the investigated different parts of the two species of *Polygala* i.e., *P. glaucoides and P. erioptera,* having significant antioxidant as well as pancreatic lipase inhibitory activity. Leaf extracts in both these plants are more potent in antioxidant and lipase inhibition potential as that of stem and root extracts. This indicate that leaf possess the high number of compounds that are responsible for such above studied activities. Further studies are necessary to purify such activity showing compound and to study actual mechanism of action at gene level expression by using adipogenic cells and rat model.

Statistical analysis

Each experiment was performed in triplicate, the average values are treated as single data points, and the data are expressed as mean ± standard deviation. Two-way ANNOVA was calculated by using Graph Pad prism.

Acknowledgement

Authors are thankful to Dr Vinod Shimple, Botanist, for authentication and Mr Mukund Kamble for help in the collection of Plant material. We are also thankful to Head, Department of Biotechnology, Shivaji University, Kolhapur for providing laboratory facilities.

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