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EVALUATION OF IN VITRO ANTI-HYPERLIPIDAEMIC ACTIVITY BY ETHANOLIC EXTRACT OF *Hydrocharis laevigaetum* HUMP & BONPL. SOUTH AMERICAN SPONGE PLANT

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ABSTRACT

The present study emphasizes the *in-vitro* studies on antihyperlipidemic activity and phytochemical analysis of an ethanolic extract of dried plant of *Hydrocharis laevigaetum* by pancreatic lipase activity. *Hydrocharis laevigaetum* is a floating aquatic plant commonly known as South American sponge plant. Plant was extracted by using ethanol as solvent by Soxhlet apparatus. From the preliminary phytochemical studies, the ethanolic extract shows maximum constituents like proteins and amino acids, alkaloids, tannins & phenols, flavonoids, terpenoids, saponins, glycosides, fixed oils, quinones, resins, carbohydrates respectively. The ethanolic extract of plant was analysed by pancreatic lipase activity using standard drug orlistat.

KEYWORDS: Hyperlipidemia, *Hydrocharis laevigaetum*, Orlistat, Phytochemical studies, Soxhlet extraction.

INTRODUCTION

Hyperlipidemia is defined as increase in the levels of fasting total cholesterol concentration which may be allied with triglyceride concentration. It is also known as hyperlipoproteinemia as lipids are insoluble in plasma and are transported in blood as particles known as lipoproteins. Hyperlipidemia is the prime cause of atherosclerosis and atherosclerosis induced conditions like coronary heart disease (CHD), ischemic cerebrovascular disease, and peripheral vascular disease. These conditions cause morbidity or mortality in a majority of midlife or older adults. Dyslipidaemias,

including Hyperlipidemia (hypercholesterolemia) and low levels of high density-lipoprotein cholesterol (HDL-C), are major causes of increased atherogenic risk; both genetic disorders and lifestyle (sedentary behaviour and diets high in calories, saturated fat, and cholesterol) contribute to the dyslipidaemias seen in countries around the world.

Hydrocharis laevigaetum

Hydrocharis laevigaetum is a perennial freshwater plant native to tropical regions of Central and South America, ranging from central Mexico to northern Argentina. This versatile species thrives in various aquatic habitats including rivers, lakes, swamps, canals, and ponds. Hydrocharis laevigaetum has been confirmed to naturalize and invade various regions, including North America, South America, Asia, Australia, and Africa, demonstrating its potential to establish self-sustaining populations and negatively impact ecosystems beyond its native range. H. laevigaetum has distinct root characteristics: rapidly growing main roots with long hairs and slower-growing, thinner secondary roots. Its short stems support rosette- shaped leaves with petioles, often featuring a thick, parenchymal pad on the underside. The plant reproduces both sexually, producing flowers and seeds, and asexually through clonal growth. H. laevigaetum stands out among native aquatic plants as a promising tool for wastewater phytoremediation. Its rapid growth and exceptional ability to reduce chemical oxygen demand (COD) by nearly 80% highlight its potential for environmentally friendly water purification.

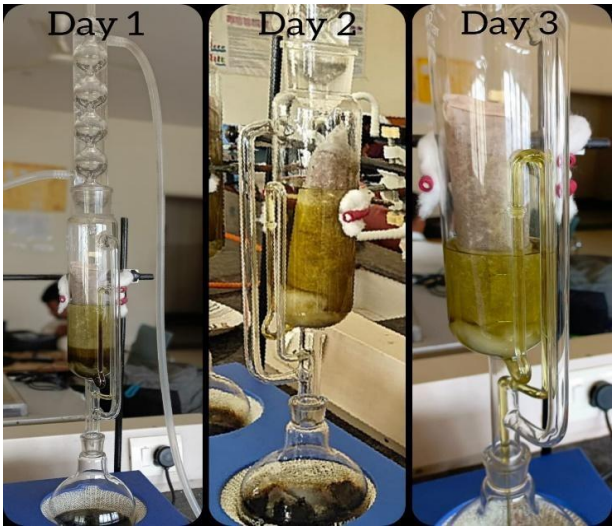
MATERIALS AND METHODS

Plant collection and authentication of plant material

Plant was sourced from nurseries of Sree Padma aqua flora located in Aluva, Kerala, India. This plant was identified and authentication was carried out by DR. Madhav Shetty, a botanist associated with S V university in Tirupathi, Andhra Pradesh, India. The herbarium was prepared and stored with specimen number 1047.

Preparation of plant extract and extraction process

The whole plant of Hydrocharis laevigaetum was washed with fresh water to remove the debris, epiphytes and adhered materials. It was shade dried in laboratory for 10 days and then dried plant was pulverized into coarse powder. Further it is sieved to remove debris and preserved in air tight glass container for further process. For extraction process 50g of coarsely powdered leaves of Hydrocharis laevigaetum plant was packed in a thimble which was placed in a central portion of Soxhlet apparatus. A condenser was installed above the central compartment and 300ml of ethanol was placed in the lower round bottom flask. At a steady temperature of 60°C, the solvent in the round bottom flask was heated to boiling. The vapour enters the reflux condenser, through the side arm and rises. In this location, the vapour condenses and drips into the thimble holding the substance to be extracted. The extract gradually collected in the thimble as the warm solvent percolates through the material along its walls. The Concentrated extract was collected in round bottom flask.



Evaporation

The ethanolic extract of Hydrocharis laevigaetum is taken into a China dish and subjected to heating in a water bath. Temperatures are maintained around 80 ℃ for 20 minutes for the evaporation of ethanolic solvent to get a pure extract of Hydrocharis laevigaetum. Extract is stored in the container for further studies.

In-vitro antihyperlipidemic of Hydrocharis laevigaetum by pancreatic lipase activity

Preparation of working solution: Phosphate buffer (40 mM) pH 6.9

(A) Sodium dihydrogen orthophosphate– 6.24g in 1000mL of de-ionized water.

(B) Disodium hydrogen phosphate dihydrate– 7.12g in 1000mL of de-ionized water.

Mix 45mL of solution A with 55 mL of solution B and make up to 200 mLwith deionised water. Dissolve 0.1 mg in 1mLof enzyme porcine pancreatic lipase pH 6.9.Dissolve 50 mg of Orlistat in 50 mL of phosphate buffer and dilute appropriately to get concentration of 50 µg/mL using phosphate buffer pH 6.9.

Procedure

Pancreatic lipase activity was determined by measuring the hydrolysis of p-nitrophenyl butyrate (p-NPB) to p-nitrophenol using a method. The 0.1 mg/ml of enzyme solution was prepared by reconstituting porcine pancreatic lipase using 0.1 M Tris-HCl buffer (pH 8). Then, 5 µl of test sample was mixed with 90 µl of enzyme buffer, and incubated for 15 min at 37°C. After incubation, 5 µl of 10 mM p-nitro phenylbutyrate (p-NPB) was added to enzyme mixture and the reaction was allowed to proceed for further 15 min at 37°C. After incubation, the absorbance of p-nitrophenol released was measured at 405 nm using a UV Visible spectrophotometer.

RESULT

Percentage Yield (% yield) of the extract

The dried powder of Hydrocharis laevigaetum was extracted using 95% v/v ethanol. The percentage yield of the extract of the plant was found to be 37% w/w

The percentage yield of Hydrocharis laevigaetum extract

Serial No.	Extract	Percentage yield
1	Ethanolic extract	37% w/w

Qualitative phytochemical screening of ethanolic extract of *Hydrocharis laevigaetum*

The extract has been tested to confirm the presence of Alkaloids, Glycosides, Proteins, Tannins, Flavonoids, Terpenoids, Saponins, Quinones, Fixed oils, Resins, Coumarins and Carbohydrates. The results are discussed in the table. The image for the phytochemical screening of Hydrocharis laevigaetum are given below.

Sl. No.	Test	Results
1	**Test for Proteins and amino acids**	
	Millon's test	+
2	**Test for Alkaloids**	
	Dragondroff's test	+
3	**Test for Tannins and phenols**	
	Ferric chloride test	+
4	**Test for Flavonoids**	
	Zn- HCl test	+
5	**Test for Steroids/ Terpenoids**	
	Salkowski test	+
6	**Test for Saponins**	
	Froth test	+
7	**Test for Glycosides**	
	Killer Kiliani test	+
8	**Test for Quinones**	
	NaoH test	+
9	**Test for Fixed oils**	
	Paper/Spot test	+
10	**Test for Resins**	
	Acetone test	+
11	**Test for Coumarins**	
	Fluorescence test	+
12	**Test for Carbohydrates**	
	Benedict's test	+

Note: (+) = Present, (-) = Absent

Qualitative phytochemical screening of ethanolic extract of Hydrocharis laevigaetum

Phytochemical screening ethanolic extract of Hydrocharis laevigaetum Hump and Bonpl

Percentage inhibition of Pancreatic lipase activity

This study evaluates the inhibitory effect of a sample on pancreatic lipase activity in comparison to the standard drug Orlistat. The percentage inhibition was calculated based on the reduction in absorbance at 405 nm.

Calculations

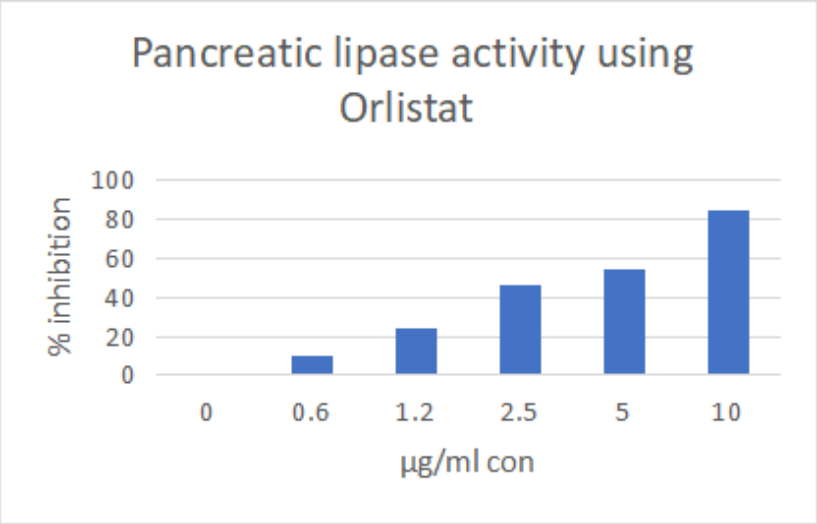
The percentage inhibition of pancreatic lipase activity is calculated as follows

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

Percentage inhibition of pancreatic lipase activity using Orlistat (standard)

SL no.	Concentration µg/ml	Optical density at 405nm	Percentage inhibition
1	0	0.8334	0
2	0.6	0.7514	9.84
3	1.2	0.6354	23.76
4	2.5	0.444	46.72
5	5	0.384	53.92
6	10	0.123	85.14

Percentage inhibiton of Orlistat by pancreatic lipase activity

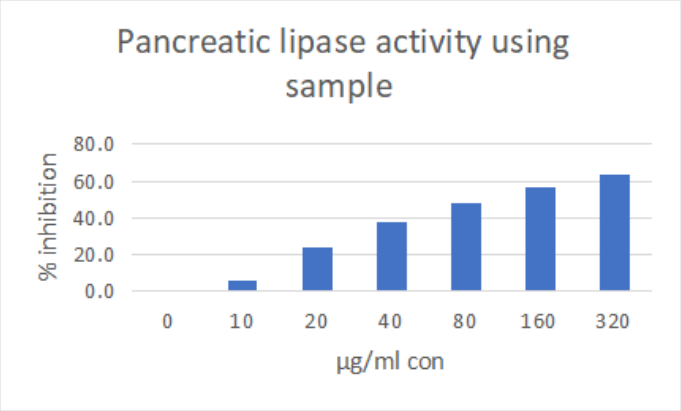


Graphical representation of pancreatic lipase activity using Orlistat

Pancreatic lipase activity using Sample

Sl no.	Concentration µg/ml	Optical density at 405nm	Percentage inhibition
1	0	0.852	0.0
2	10	0.799	6.2
3	20	0.647	24.1
4	40	0.529	37.9
5	80	0.441	48.2
6	160	0.367	56.9
7	320	0.31	63.6

Percentage inhibiton of Sample by pancreatic lipase activity



Graphical representation of Pancreatic lipase activity using Sample

Results showed a dose-dependent inhibition for both the sample and Orlistat, with the sample achieving 63.6% inhibition at 320 µg/mL and Orlistat achieving 85.14% inhibition at 10 µg/mL.

DISCUSSION

The primary goal of the present study was to evaluate the Antihyperlipidaemic activity of ethanolic extract of dried plant Hydrocharis laevigaetum. Hydrocharis laevigaetum is commonly known as South American sponge plant. It shows the potential as on antihyperlipidaemic, anticancer, antidiabetic, antimicrobial and antioxidant activity. Hyperlipidemia is a well-known risk factor for cardiovascular disease. Coronary artery disease is of the major cause of death in the world. In the present study ethanolic extract of dried plant of Hydrocharis laevigaetum was prepared by soxhlet extraction method and subjected to different qualitative chemical test for detection of phytochemical constituents and phytochemical analysis by using the method of Sofawara, Trease and Evans and Harborne. In- vitro antihyperlipidaemic of Hydrocharis laevigaetum is carried out by pancreatic lipase activity. Pancreatic lipase activity was determined by measuring the hydrolysis of P-nitro phenyl butyrate (p-NPB) to p-nitro phenol. This study evaluates the inhibitory effect of Hydrocharis laevigaetum sample on pancreatic lipase activity in comparison to standard drug orlistat. Orlistat is used to treat obesity with weight reduction by inhibition of lipase enzymes. The percentage inhibition was calculated based on the reduction in absorbance at 405nm. Orlistat exhibits good inhibitory action and pancreatic lipase activity compared to test sample. Test sample achieves 63.6% inhibition at 320 µg/mL and standard drug Orlistat achieving 85.14% inhibition at 10 µg/mL. The present investigation suggests that ethanolic extract of dried plant of Hydrocharis laevigaetum possess remarkable antihyperlipidemic activity.

CONCLUSION

At 10 µg/ml, the standard Orlistat exhibited good inhibitory action and pancreatic lipase activity shows 85% inhibition. With a 63.6% inhibition, the test sample demonstrated pancreatic lipase activity inhibition at 320 10 µg/ml. Table and the graph both include a list of the percentage inhibition values.

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