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Pancreatic Lipase Inhibitory Activity of Saraca Asoca bark Extract

Sweety Tiwari[*1], Rajesh Gour[1], Akhlesh Kumar Singhai[1]

[1]School of Pharmacy, LNCT University, Bhopal, M.P

***Corresponding Author**

Email ID: sweetytiwari2989@gmail.com

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ABSTRACT

Inhibition of pancreatic lipase is the mechanism that has received the greatest attention from researchers in the search for promising viable anti-obesity ingredients. For the purpose of locating possible anti-obesity drugs, the mechanism that has received the greatest attention from researchers is the inhibition of pancreatic lipase. Orlistat is the only medicine that has been licensed by the Food and Drug Administration (FDA) and is accessible for the treatment of obesity. Other than treatments that reduce obesity by acting on the central nervous system, orlistat is the only drug that inhibits pancreatic lipase. This naturally occurring chemical, known as lipstatin, was the starting point for the discovery of orlistat. The research that is being done to find novel pancreatic lipase inhibitors that do not have any negative side effects has been inspired by the effectiveness of naturally occurring substances that are used for the treatment of obesity. From this point forward, a great number of plant extracts and isolated substances have been found for their ability to inhibit pancreatic lipase. The stem bark of the Asoka tree is astringent, and it also functions as a uterine tonic and has a stimulating impact on the tissue of the ovary and the endometrium. Burning sensations, fever, and dyspepsia are among conditions that may be helped by the bark. This research was conducted to evaluate the methanol and aqueous extracts of *Saraca asoca* bark for pancreatic lipase inhibition activity.

Keywords: *Pancreatic lipase inhibition, Saraca asoca bark*

1. INTRODUCTION

It is well acknowledged that obesity is a significant lifestyle-related problem, particularly in societies that have already progressed. Obesity is a condition of lipid metabolism that continues to be a global concern. Overall, obesity ranks fifth in terms of fatalities that occur across the globe. The development of diabetes, cardiovascular problems, musculoskeletal illnesses, and some forms of cancer are also associated with it [1]. Obesity is thought to be the result of a chronic imbalance between the amount of energy that is consumed and the amount of energy that is expended. It is possible to produce anti-obesity drugs that target the digestion and absorption of dietary lipids via pancreatic lipase, which is a significant source of excess calorie intake but may also be targeted for development. The alarming rise in the prevalence of obesity and metabolic illnesses that are associated with it has given rise to significant worries over the health of the general population. Industrialization, increased consumption of fast food, and a decline in physical activity are the primary contributors to the alarming rate at which obesity is spreading in emerging nations [2]. Obesity is mostly believed to be a lifestyle condition that is prevalent in industrialized countries.

At the same time as a problem with weight and a breakdown of lipid homeostasis coexist, a wide variety of health concerns also occur. There are serious consequences associated with this interconnected network of metabolic disorders and their co-morbidities, including cardiovascular abnormalities (such as heart failure, hypertension, and pulmonary embolism), endocrine imbalances (such as insulin resistance, glucose intolerance, and hypothyroidism), arthritis, urinary incontinence, and gastrointestinal complications (such as gastroesophageal reflux disease, colon cancer, and hepatic steatosis, among others). Additionally, obesity and metabolic problems that are associated to it disrupt lifestyles in a variety of ways, including physically, financially, and mentally. Differentiating a person from society may be accomplished by psychological effects such as social prejudice, sadness, physical incapacity, and so on [3].

Inhibition of pancreatic lipase is the mechanism that has received the greatest attention from researchers in the search for promising viable anti-obesity ingredients. It is well knowledge that pancreatic lipase is an essential enzyme for the process of lipid absorption via the breakdown of total dietary lipids. Orlistat is the only medicine that has been licensed by the FDA

and is now accessible for the treatment of obesity. Other than treatments that fight obesity by acting on the central nervous system, orlistat is the only drug that inhibits pancreatic lipase. The naturally occurring chemical known as lipstatin was the starting point for the discovery of orlistat [4, 5].

The study that is being done to find novel pancreatic lipase inhibitors that do not have any negative side effects has been inspired by the effectiveness of naturally occurring substances that have been used to treat obesity. From this point forward, a great number of plant extracts and isolated substances have been found for their ability to inhibit pancreatic lipase. In addition to that, a wide variety of microbial products and isolated compounds, basic protein protamines, polysaccharides such as chitosan, dietary fibers derived from wheat bran and cholestyramine, soya proteins, and synthetic compounds, among other things, have been investigated for their ability to inhibit pancreatic lipase [6]. In spite of this, isolated compounds derived from plants and microorganisms have been extensively researched and reported for their ability to inhibit pancreatic lipase. Considering that dietary materials and edible plants are the primary factors of concern, the most extensive research has been conducted on the anti-obesity activity of these substances, with the goal of incorporating them into the typical diet. The screening of plant extracts has been regarded to be one of the effective methodologies [7,8] in the quest for new pancreatic lipase inhibitors that may be derived from natural resources. The stem bark of the Asoka tree is astringent, and it also functions as a uterine, it has a stimulating impact on the tissue of the ovary and the endometrium. Dyspepsia, fever, and a burning feeling are among conditions that may be helped by the bark. Menorrhagia, leucorrhoea, internal bleeding, hemorrhoids, and hemorrhagic dysentery are some of the other conditions that may be treated with this medication [9, 10]. In this experiment, the extract of *Saraca asoca* bark was tested for its ability to inhibit the activity of pancreatic lipase.

2. EXPERIMENTAL

Chemicals: Para nitrophenyl butyrate, Orlistat, Porcine pancreatic lipase (PPL, Type II) was purchased from Sigma- Aldrich (USA). All other chemicals and solvents were of analytical grade and purchased from a local dealer in Bhopal.

Instrumentation: UV -Vis spectrophotometer (Shimadzu) Semi auto biochemistry analyser, Shaker device, filter paper, digital weighing scale, Soxhlet extractor, Rotary evaporator

Plant Materials

The *Saraca asoca* bark, were purchased from the local market of Bhopal, M.P. Studies were conducted on the organoleptic characteristics of the *Saraca asoca* bark, which included color, odor, appearance, taste, and texture, among other characteristics. An assortment of solvents with varying degrees of polarity, including petroleum ether, chloroform, ethyl acetate, and methanol, were used in order to ascertain the extractive values. It was determined that a continuous soxhlet extraction was performed on ten grams of dried and coarsely powdered *Saraca asoca* bark using one hundred milliliters of petroleum ether, chloroform, ethyl acetate, and methanol as solvents. It was possible to get an aqueous extract by using the maceration procedure with water. A rotary evaporator was used to concentrate the extracts, and a vacuum desiccator was used to dry that concentrate. The extractive values were determined by looking at the air-dried medication and calculating the proportion of solvent-soluble extractive in terms of weight-to-weight ratio.

The screening of phytochemicals:

Following the condensation process in the rotary evaporator, the bioactive components of *Saraca asoca* bark were analyzed for all of the crude extracts that were obtained from petroleum ether, chloroform, ethyl acetate, and methanol solvents. A variety of bioactive components, including anthraquinones, alkaloids, saponins, tannins, terpenoids, flavanoids, phenols, steroids, and glycosides, were ascertained by the use of conventional techniques. [11]

Total phenolic compound estimation:

The Folin-Ciocalteu technique was used, with certain modifications, in order to ascertain the phenolic content of certain extracts. The preparation of the calibration curve for gallic acid included dissolving 10 mg of gallic acid (the standard) in 10 ml of methanol in order to get a stock solution with a concentration of 1 mg/ml. Through the use of stock solution, a range of aliquots ranging from 5 to 25µg/ml were created in methanol. By utilizing an ultraviolet-visible spectrophotometer, the calibration curve was created by plotting concentrations against absorbance at a wavelength of 765 nm. Ten milligrams of dried extract were extracted and filtered with ten milliliters of methanol. After diluting the Folin-Ciocalteu reagent with distilled water at a ratio of 1:10 volume/volume, 2 milliliters of the extract or standard were combined with 1 milliliter of sodium carbonate at a concentration of 7.5 grams per liter. During the 15-second vortexing process, the mixture was left to stand at a temperature of 40 degrees Celsius for 15 minutes. To determine the absorbance, a spectrophotometer was used at a wavelength of 765 nm. Utilizing the equation that was derived from the calibration curve, the total phenolic content was determined to be grams per hundred milligrams of gallic acid equivalent.

Pancreatic Lipase inhibitory activity

Pancreatic Lipase inhibitory activity of the extracts was determined according to protocols described by Tang et al., 2016 [12]. Pancreatic Lipase activity was measured using the release of 4-methylumbelliferone (4-MU) from the substrate, which

is 4-methylumbelliferyl oleate (4-MU oleate). A 25 µL aliquot of samples (assay activity of 0.5–2.5 mg/mL) dissolved in Tris-buffer (13 mM Tris-HCl, 150 mM NaCl and 1.3 mM $CaCl_2$, pH = 8) and 225 µL of a 0.5 mM 4-MU oleate solution were mixed in a 96-well microplate and incubated for 15 min at 37°C. An enzyme blank was prepared with the substrate omitted. After incubation, 25 µL of PL solution (assay concentration = 3.125 U/mL) was added to start the enzyme reaction and then incubated at 37°C for 1 h. After incubation, the amount of 4-methylumbelliferone released by the lipase was measured with a fluorimeter at an excitation wavelength of 340 nm and emission wavelength of 450 nm. Orlistat (PL inhibitory drug at 0.25 mg/mL) served as a positive control and was analyzed using same protocol. The PL inhibitory activity (%) was calculated using the equation:

$$\text{Inhibition}(\%)=[(Ac-Aeb)-As]/(Ac-Aeb)\times 100$$

Ac = Absorbance of the negative control (uninhibited reaction),

As = Absorbance of the sample (inhibited reaction) and

Aeb = Absorbance of the enzyme blank (substrate omitted).

The concentration of extract that reduced enzyme activity by 50% (IC50) was obtained by non-linear regression analysis of a plot of PL inhibition (%) vs. the sample concentrations.

3. RESULTS AND DISCUSSION

Percentage yield of extracts

Various polarity extracts were prepared from *Saraca asoca* bark and extractive value was calculated

Table 1: Solvent extractive values (%w/w) of *Saraca asoca* bark

S No.	Name of extract	Extractive values (%w/w)
1	Petroleum ether Extract	4.91
2	Chloroform Extract	4.32
3	Ethyl acetate Extract	5.78
4	Methanol Extract	9.78
5	Aqueous extract	11.23

Preliminary phytochemical screening

Preliminary phytochemical screening reveals the Phytochemical screening of *Saraca asoca* bark showed the presence of triterpenes in chloroform extract; triterpenes, tannins and flavonoids in ethyl acetate extract; carbohydrate, alkaloids, tannin flavonoid, protein and saponin in methanol extract.

Table 2: Phytochemical analysis of *Saraca asoca* bark

S. No.	Chemical class	Chemical test	Pet ether	Chloroform	Ethyl acetate	Methanol	Aqueous
1	Alkaloids	Dragendorff's test	-	-	-	+	-
2	Napthoquinone	Juglone test	-	-	-	-	-
3	Steroids	Salkowaski test	+	-	-	-	-
4	Carbohydrate	Molish test	-	-	-	+	+
5	Triterpenes	Vanillin-sulphuric	+	+	+	-	-

		acid test					
6	Tannin	Ferric chloride test	-	-	+	+	+
7	Glycoside	Keller-killani test	-	-	-	+	+
8	Proteins	Biuret test	-	-	-	+	+
9	Flavonoids	Shinoda Test	-	-	+	+	+
10	Saponins	Lead acetate test	-	-	-	+	+

Where + is Present and – is Absent

Total phenolic compound estimation: Phenolic content of *Saraca asoca* bark was determined by Folin-Ciocalteu method. Calibration curve of gallic acid were prepared using UV spectrophotometer

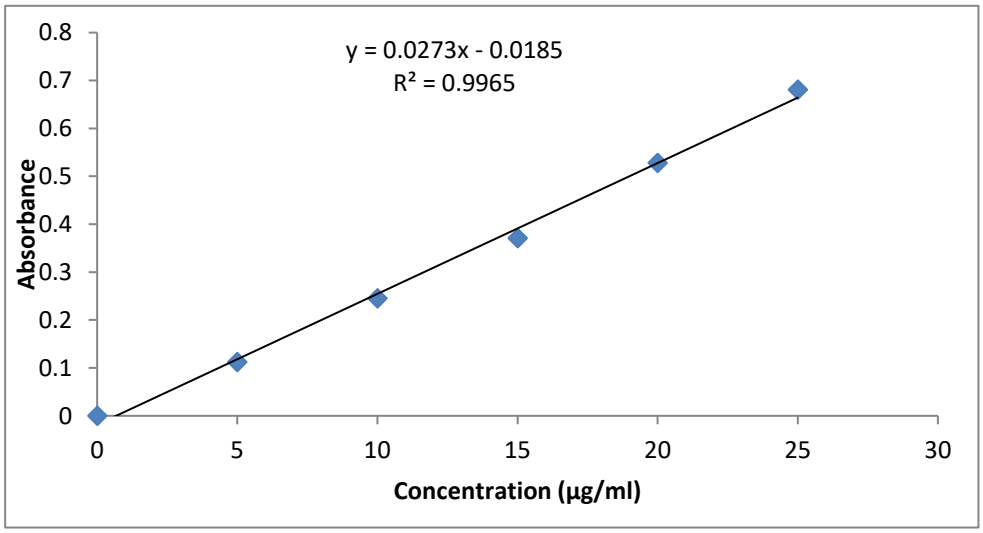

Figure 1: Calibration curve of Gallic acid (at 256 nm)

Table 3: Total phenolic contents of the *Saraca asoca* bark extracts

S. No.	Name of extract	TPC (mg GAE /g)	
1	Petroleum ether Extract	1.18 ± 0.14	0.75 ± 0.09
2	Chloroform Extract	9.45 ± 1.23	6.38 ± 0.86
3	Ethyl acetate Extract	46.48 ± 1.38	42.48 ± 1.13
4	Methanol Extract	102.02 ± 3.17	97.14 ± 4.28
5	Aqueous extract	69.31 ± 2.19	62.29 ± 1.92

Utilizing the equation that was derived from the calibration curve, the total phenolic content was determined to be grams per hundred milligrams of gallic *acid* equivalent. The total phenolic content of the methanol extract of *Saraca asoca* bark was found to be 102.02 ± 3.17 mg GAE /g, which was higher than the amounts found in other extracts. It was discovered that the total phenolic content of the methanol extract was greater than that of the chloroform and ethyl acetate extracts together. The percentage of total phenolic content that is found in petroleum ether extract is the lowest.

Lipase inhibitory action in the pancreas of porcine origin: Due to the fact that methanol and aqueous extract of *Saraca asoca* bark exhibit the highest total phenolic content, these two extracts were chosen for further investigation into their ability to block the activity of porcine pancreatic lipase. The pancreatic lipase inhibitor activity of *Saraca asoca* bark methanol extracts was equivalent to that of orlistat, and it increased rapidly with an increase in concentration. At a concentration of 100 µg/ml, the porcine pancreatic lipase activity of MESA is discovered to be 83.39%, while the IC50 value is found to be 62.25 µg/ml.

Table 4: Porcine pancreas lipase inhibitory activity of *Saraca asoca* bark extracts

S. No.	Concentration	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
1	Orlistat	40.41	58.42	64.31	83.21	91.24
2	MESA (methanol extract of *Saraca asoca* bark)	20.13	36.12	48.05	71.09	83.39
3	AQSA (aqueous extract of *Saraca asoca* bark)	16.95	27.98	38.87	56.15	61.26

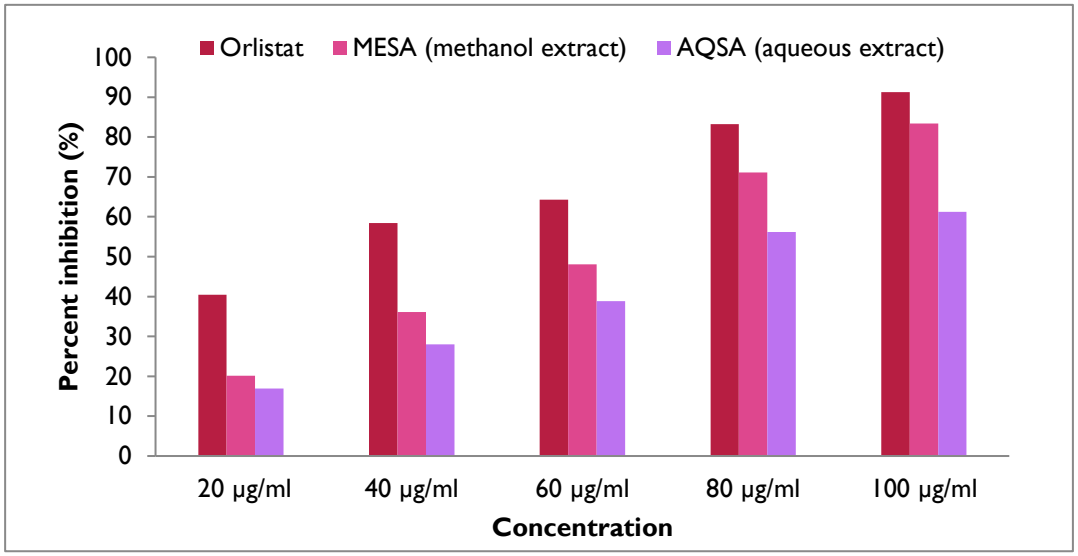

Figure 2: Porcine pancreas lipase inhibitory activity of *Saraca asoca* bark extracts

It is possible that the pancreatic lipase activity of the plant extracts is explained by the phytochemicals that are contained in the plant extracts [13]. Several of the earlier investigations have shown that flavonoids and phenols have the ability to limit activity by binding to the enzyme substrate complex. This, in turn, results in a reduction in the rate at which lipids are absorbed. There is no evidence that phytochemicals have the power to lower cholesterol levels just because they are there; rather, it is the concentration of these phytochemicals that plays a role in contributing to their anti-lipidemic effect. These polyphenols have been shown to be responsible for the conformational changes that occur in the structure of the lipase enzyme [14]. The amino acids tyrosine and tryptophan are the most important ones that are related in the binding process. The process of lipolysis in fat cells of adipose tissue is triggered by abnormalities in cAMP levels, which in turn activates protein kinase A and substrates such as hormone-sensitive lipase and perilipin [15]. Hormone sensitive lipase (HSL) is an important enzyme in the mobilization of lipids, and it is one of the substrates that is activated by lipolysis. In the sense that the heterodimer remains connected, it is known that calcium and colipase contribute to the stability of pancreatic lipase. On the other hand, the use of herbal medications such as *Saraca asoca* bark causes the heterodimer of the enzyme to become dissociated.

4. CONCLUSION

A comparison was done between the pancreatic lipase inhibition experiments performed with methanol and those performed with an aqueous extract of *Saraca asoca* bark in the current investigation. Because it was shown to be the most effective, methanol extract has the potential to be used as a powerful anti-obesity agent in the fight against hyperlipidemia. One hypothesis is that the anti-hyperlipidemic ability of this plant is due, in part, to the presence of tannins, saponins, and

flavonoids, which are the primary components of this plant. There are bioactive phytochemicals in the bark of the *Saraca asoca* plant that are responsible for its anti-obesity properties.

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