**Comparative study between aqueous extract of two species of *Amorphophallus* for Antilipase potential**

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**ABSTRACT**

Obesity is one of the main public health problems in developed countries. It is considered to be a risk factor associated with the genesis or development of major chronic diseases, including cardiovascular disease, diabetes, and cancer. A marked inhibition of pancreatic lipase activity by aqueous extracts of Amorphophallus peaoniifolius and Amorphophallus konjac was observed to be similar in this study. The inhibitory role could be attributed to the presence of various secondary metabolites in the extract. The extract may be used as an anti-obesity drug. The substances that are used to reduce the activity of lipases found in the intestine are called lipase inhibitors. They bind to lipase enzymes (secreted from the pancreas, which are related to dietary triglyceride absorption and catalyze the digestion of dietary triglycerides) in the intestine. Therefore, lipase inhibitors prevent the hydrolysis of dietary triglycerides to monoglycerides and fatty acids, so no absorption takes place in the intestine and fat is excreted in the feces rather than being absorbed for use as a source of caloric energy. This mechanism could be used for the treatment of obesity. An example of a lipase inhibitor is Orlistat, which was used in our current study as a reference substance and tends to block absorption of 30% of the total fat intake from a meal. Lipase inhibitors have many side effects, like oily spotting, in addition to abdominal cramps and hypertension. These side effects could be controlled by reducing the consumption of dietary fats in a suitable form. Further studies on the isolation of active principles from the extract and their inhibitory efficacy against lipases are under investigation.

Keywords- Obesity, Antilipase potential, *Amorphophallus peaoniifolius, Amorphophallus konjac*

**I INTRODUCTION**

There are countless active therapeutic compounds found in plants that can be used to treat a variety of ailments. In contrast to currently prescribed chemical drugs, which may be more expensive and potentially more hazardous, herbal medicines typically have few adverse effects and are inexpensive [1]. As early as 3000 BC, Chinese and Egyptian texts discussed the therapeutic use of plants [2]. Then, as scientists created their own versions of plant substances, the usage of herbal treatments gradually decreased in favor of conventional medications. Herbal remedies are actually pharmaceuticals and as such should only be recommended by qualified, licensed healthcare professionals. They are not always safe. Today, nevertheless, more medical professionals are starting to consider using herbal treatments for several common maladies. Herbs have gained popularity in the medical community since some doctors utilize them to counteract the negative effects of medications [3]. Due to worries about negative side effects and potential addiction, there seems to be a shift away from several prescription medications. Since there are few medicinal plants that may be harvested from the wild, there has been an upsurge in the cultivation of medicinal herbs [3].

Body mass index (BMI) is a standard metric used to measure adult overweight and obesity [4 and 5]. Obesity and overweight are associated with an increase in BMI (>30 kg/m2). BMI over 35 is associated with non-communicable diseases such endometrial, breast, ovarian, prostate, liver, gall bladder, kidney, and colon cancer as well as musculoskeletal disorders (osteoarthritis) and cardiovascular diseases. Systemic oxidative stress can be raised by hyperlipidemia associated with obesity. [6] Because obesity is the root cause of many ailments, treating it is a herculean undertaking for clinicians. [7]

The guidelines for treating obesity emphasize that a comprehensive approach to weight management is necessary. This approach should involve multiple disciplines and include lifestyle changes, behavioral therapy, medication, and possibly bariatric surgery. Anti-obesity medications (AOM) are prescribed for individuals who have a body mass index (BMI) of 30 kg/m2 or higher. They may also be prescribed if the BMI is 27 kg/m2 or higher and the person has one or more co-morbidities (8). A reliable strategy for the prevention or treatment of metabolic diseases involves drug intervention of lipid metabolism. Triacylglycerides in the duodenum are hydrolyzed by the important enzyme pancreatic lipase, which has been identified as the primary target that controls lipid absorption. The pancreas secretes pancreatic lipase, and it has been shown that inhibiting pancreatic lipase and controlling lipid absorption are successful approaches for finding new medications to treat metabolic diseases. Currently, the only pancreatic lipase inhibitor that has been approved is orlistat, a hydrogenated derivative of lipstatin. Orlistat has a strong anti-lipase effect, but it also has a number of adverse effects, including stomach discomfort, oily spotting, and fecal incontinence. Because herbal medications have demonstrated pleasing safety profiles in long-term medical treatments, there has recently been a significant deal of interest in the screening of possible pancreatic lipase inhibitors derived from herbal medicines. (9)

In this study, we evaluated the potential anti-obesity properties of the aqueous extracts from *Amorphophallus konjac* and *Amorphophallus paeoniifolius*. We specifically focused on their ability to inhibit lipase activity. Based on review of literature (11-26), it appears that these plant extracts have not undergone prior screening to determine their lipid inhibitory activity.

**II MATERIALS AND METHODS**

1. **Materials and Reagents**

The corm of *Amorphophallus peaoniifolius* was bought from a nearby market, and Dr. M. Niranjan Babu, professor of pharmacognosy at Seven Hills College of Pharmacy in Tirupati, taxonomically certified the plant material. The corms were dried, cut into small pieces, and ground into a coarse powder with an electric mill. This powder was then placed in an airtight container for later usage. .*Amorphophallus konjac* root aqueous extract was bought from Vital Herbs Pvt. Ltd. in Delhi. Chicken Pancrease, orlistat, olive oil was purchased at a local market, all other reagents were available in the college.

1. **Extraction**

Tubers of Amorphophallus peaoniifolius were carefully cleansed with clean water before being sliced into little pieces and sun-dried. Using an electric blender, the dried plant material was crushed into a fine powder. 10 g of powder material were soaked in 100 ml of water and incubated at room temperature for 3 days with intermediate stirring with a mechanical stirrer. The extract was evaporated in a rotating vacuum evaporator after being filtered through Whattmann filter paper No. 1. The dried crude extracts were weighed and saved for further research. The extracted extract's percentage yield was calculated. (27)

1. **Phytochemical Screening**

The presence of phytoconstituents (alkaloids, phenol, tannins, carbohydrate, glycosides, saponins, steroids, flavonoids, terpenoids, resins, and proteins) in aqueous extracts of both species was determined by preliminary phytochemical analysis (qualitative). (28)

1. **Total phenol content**

Secondary aromatic plant metabolites with one or more hydroxyl substituents are known as phenolic compounds. Polyphenols, which occur naturally, offer favorable health-related features due to their high antioxidant activity. According to current research, many polyphenol-rich extracts are excellent pancreatic lipase inhibitors (29). The total phenol content was evaluated by making a 1 mg/ml extract and preparing a reaction mixture with 0.5 ml of the extract. 2.5 mL of water-dissolved 10% Folin-Ciocalteu's reagent and 2.5 mL of 7.5% NaHCO3 aqueous solution The samples were incubated for 45 minutes on a thermostat set to 45 degrees Celsius. A spectrophotometer with a wavelength of 765 nm was used to measure the absorbance. For each analysis, the samples were produced in triplicate, and the mean absorbance value was determined. The same method was followed with the standard gallic acid solution, and a calibration curve was created. The concentration of phenol content was quantified in terms of gallic acid equivalent (mg of GA/g of extract) based on the measured absorbance.(30)

1. **Pancreatic Lipase Inhibition Assay**

Olive oil was used as a substrate to test the inhibitory action of CPL (Chicken pancreatic lipase). The method for measuring pancreatic lipase activity was modified from that described before by Sandhya et al.(31)

Extraction lipase from Chicken- Chicken pancreas tissue samples were taken. Pancreatic tissues were cleaned, deposited in an ice-cold sucrose solution for 30 minutes, then homogenized in phosphate buffer with a mortar and pestle. After homogenization, it was centrifuged for 10 minutes at 4°C at 6000 rpm. After collecting the supernatant in a separate beaker, the sample was left to perform the experiment.

Determination of Chicken Lipase Activity- Pipette in millilitres the following reagents as shown in table 1 in to the conical flask. Swirl the mixture and equilibrate to 37° C for 10 minutes before adding the enzyme solution to the test flask only. Incubate for 30 minutes at 37°C after thoroughly mixing. Then, to both the test and blank solutions, add 95% ethanol. Shake thoroughly before adding 4 drops of phenolphthalein indicator to both the test and the blank. Titration of the solution against 0.05M NaOH (standardized by potassium hydrogen phthalate as per IP) was used to determine the released fatty acids.

The following formula was used to calculate enzyme activity: Equation 1

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Units/ml enzyme = (NaOH consumed)(Molarity of NaOH) (1000) (2) ( dilution factor) / volume of enzyme used.

**Table 1. Reagents for chicken lipase activity**

|  |  |  |
| --- | --- | --- |
| Reagents | Test (ml) | Blank (ml) |
| Di ionized water | 2.5 | 2.5 |
| Phosphate buffer | 1.0 | 1.0 |
| Olive oil | 3.0 | 3.0 |

Lipase inhibitory action of *Amorphophallus peaoniifolius* and *Amorphophallus konjac* aqueous extracts- A conical flask was used to combine orlistat (the reference medication), aqueous extracts of *Amorphophallus peaoniifolius* and *Amorphophallus konjac* at various doses (1 mg, 2 mg, 3 mg, 4 mg, and 5 mg), 3 ml of olive oil, 1 ml of phosphate buffer, and 2.5 ml of distilled water. For ten minutes, incubate. Add 1 ml of lipase enzyme, then wait 30 minutes before analyzing. Phenolphthalein was used as an indicator while the conical flake's contents were titrated against the standardized 0.05M NaOH solution until a pink color was achieved. The formula was used to determine the proportion of lipase activity that was inhibited. (31) Equation 2

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where A is lipase activity, B is activity of lipase when incubated with the extract.

Extracts Half maximal inhibitory concentration, IC50 values were calculated at concentrations of 25, 50, and 100 g/ml. As a positive control, orlistat was utilized. In µg/mL, the concentration at 50% inhibition was calculated.

**III RESULTS AND DISCUSSION**

1. **Percentage yield of *Amorphophallus peaoniifolius***

The extract was prepared by macerating Amorphophallus peaoniifolius. The percentage yield ofaqueous extract of Amorphophallus peaoniifolius was found to be 11.3%

1. **Phytochemical Analysis**

A preliminary phytochemical analysis was conducted to identify various phytochemicals in *Amorphophallus peaoniifolius* and *Amorphophallus konjac*. The analysis revealed the presence of saponins, flavonoids, and polyphenols in both plants as presented by Table 2

**Table 2 Phytochemical screening of aqueous extracts of *Amorphophallus peaoniifolius* and *Amorphophallus konjac***

|  |  |  |  |
| --- | --- | --- | --- |
| **S.No** | **Constituents** | 1. ***peaoniifolius*** | ***A. konjac*** |
| **1** | Alkaloids | + | ++ |
| **2** | Saponins | + | \_ |
| **3** | Tannins | ++ | ++ |
| **4** | Quinones | \_ | \_ |
| **5** | Glycosides | ++ | ++ |
| **6** | Flavonoids | ++ | ++ |
| **7** | Polyphenols | ++ | ++ |
| **8** | Terpenoids | ++ | ++ |
| **9** | Proteins | ++ | ++ |
| **10** | Sterols | ++ | ++ |

1. **Total phenol content**

In this study, the total phenol content was calculated in 1mg of plant extract from Amorphophallus peaoniifolius and Amorphophallus konjac from calibration curve of gallica acid (Figure 1), as shown in Table 3.

**Figure 1. Calibration curve of gallic acid**

**Table 3 Total Phenolic content of *Amorphophallus peaoniifolius* and *Amorphophallus konjac***

|  |  |
| --- | --- |
| ***Plant extract*** | ***Total phenolic content***  ***(mg gallic acid/ grams of plant extract*** |
| *Amorphophallus peaoniifolius* | 26.4 |
| *Amorphophallus konjac* | 27.3 |

1. **Antilipase activity of *Amorphophallus peaoniifolius* and *Amorphophallus konjac***

Lipase activity of Chicken Pancreatic Lipase- The activity of chicken Pancreatic lipase was determined, and the results are shown in Table 4

**Table 4 Chicken pancreatic Lipase Activity**

|  |  |  |  |
| --- | --- | --- | --- |
| **S.no** | **Solutions** | **NaOH Consumed** | **Enzyme activity of test solution** |
| 1 | Blank | 0.5ml | 250units/ml |
| 2 | Test | 3ml |

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Antilipase activity *of Amorphophallus peaoniifolius* and *Amorphophallus konjac*-   The activity of these plants in treating obesity was determined. Antilipase activity was detected using the reference drug Orlistat. The results are shown in Tables 5,6 and 7.

**Table 5 .Enzyme inhibitory activity of *Amorphophallus peaoniifolius***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Powdered drug concentration** | **NaOH consumed (burette readings)** | **Enzyme activity (units/ml)** | **Enzyme inhibitory activity percentage** | **IC 50 micrograms/ml** |
| 1 mg | 1 ml | 100 | 60 | 211.1 |
| 2 mg | 0.6ml | 60 | 76 |
| 3 mg | 0.4 ml | 40 | 84 |
| 4 mg | 0.2ml | 20 | 92 |
| 5 mg | 0.1 ml | 10 | 96 |

**Table 5 .Enzyme inhibitory activity of *Amorphophallus peaoniifolius***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Powdered drug concentration** | **NaOH consumed(burette readings)** | **Enzyme activity (units/ml)** | **Enzyme inhibitory activity percentage** | **IC 50 micrograms/ml** |
| 1 mg | 1.5 ml | 150 | 40 | 176.9 |
| 2 mg | 1ml | 100 | 60 |
| 3 mg | 0.6 ml | 60 | 76 |
| 4 mg | 0.4 ml | 40 | 84 |
| 5 mg | 0.2 ml | 20 | 92 |

**Table 7 Enzyme inhibitory activity of Orlistat**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Powdered drug concentration** | **NaOH consumed(burette readings)** | **Enzyme activity (units/ml)** | **Enzyme inhibitory activity percentage** | **IC 50 micrograms/ml** |
| 1 mg | 0.9ml | 90 | 64 | 255.5 |
| 2 mg | 0.7ml | 70 | 72 |
| 3 mg | 0.4 ml | 40 | 84 |
| 4 mg | 0.2 ml | 20 | 92 |
| 5 mg | 0 ml | 0 | 100 |

**IV CONCLUSION**

Obesity is a significant public health issue in developed countries. It is widely recognized as a risk factor linked to the onset or progression of major chronic diseases such as cardiovascular disease, diabetes, and cancer. In this study, it was observed that the aqueous extracts of *Amorphophallus peaoniifolius* and *Amorphophallus konjac* had a similar effect in inhibiting pancreatic lipase activity. The presence of various secondary metabolites in the extract could be attributed to the inhibitory role. The extract has the potential to be utilized as a drug for combating obesity. Lipase inhibitors are substances used to decrease the activity of lipases found in the intestine. In the intestine, they attach themselves to lipase enzymes. These enzymes are secreted by the pancreas and play a role in the absorption of dietary triglycerides, as well as catalyzing their digestion. Lipase inhibitors work by preventing the breakdown of dietary triglycerides into monoglycerides and fatty acids. As a result, the absorption of these fats in the intestine is blocked, leading to their excretion in the feces instead of being utilized as a source of caloric energy. This mechanism has the potential to be utilized in the treatment of obesity. In our current study, we used Orlistat as a reference substance. Orlistat is an example of a lipase inhibitor, which blocks the absorption of approximately 30% of the total fat intake from a meal. Lipase inhibitors can cause various side effects, including oily spotting, as well as abdominal cramps and hypertension. One way to manage these side effects is by moderating the intake of dietary fats in an appropriate manner. Additional research is required to investigate the isolation of active compounds from the extract and determine their effectiveness in inhibiting lipases.

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