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

Charumathi Salva a\*, Bhanusree.P , Chakrapani. P Divya Sree. S, Jhansi.S, Sravani.T, Sonali. R Department of Pharmaceutical Analysis Seven Hills College of Pharmacy, Venkataramampuram, Tirupati, Andhra Pradesh, India salvacharu87@gmail.com

Rajitha Galla b

Department of Pharmaceutical Chemistry

Institute of Pharmaceutical Technology

Sri Padmavathi Mahila Viswa VIdyalayam, Tirupati, Andhra Pradesh, India

**ABSTRACT**

Obesity is a significant public health concern inside developed nations. The presence of this factor is widely recognized as a significant contributor to the onset and progression of various chronic illnesses, such as cardiovascular disease, diabetes, and cancer. In this study, it was discovered that aqueous extracts of Amorphophallus peaoniifolius and Amorphophallus konjac both significantly inhibited pancreatic lipase activity. The presence of numerous secondary metabolites in the extract may be responsible for the inhibitory effect. As an anti-obesity medication, the extract may be utilized. Lipase inhibitors are drugs that are used to lower the activity of lipases found in the intestine They bind to lipase enzymes, which are released by the pancreas and involved in the breakdown of dietary lipids. These enzymes are found in the intestine. Because lipase inhibitors stop the breakdown of dietary triglycerides to monoglycerides and fatty acids, there is no absorption of fat in the intestine and it is not used as a source of caloric energy. Instead, fat is expelled in the stool. This mechanism could potentially be used to treat obesity. Orlistat, which was used as a reference substance in our present study, is an example of a lipase inhibitor and tends to prevent absorption of 30% of the total fat intake from a meal. Lipase inhibitors have numerous adverse effects, including oily spotting, abdominal cramps, and hypertension. In this study*, Amorphophallus peaoniifolius* and *Amorphophallus konjac* were evaluated for their anti-obesity properties. It was hypothesized that it would possess a comparable anti-obesity effect. The antilipase activity of the aqueous extracts of Amorphophallus peaoniifolius and Amorphophallus konjac was evaluated. Compared to orlistat (100%), it inhibits chicken Pancreatic lipase enzyme by 96% and 92% at 5 mg/ml. The present study demonstrates that aqueous extracts of *Amorphophallus peaoniifolius* and *Amorphophallus konjac* have comparable antilipase activity in obesity.

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. Unlike now prescribed 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 adverse side effects and the possibility of 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].

The body mass index (BMI) is a standard metric for measuring overweight and obesity in adults. [4 and 5]. Obesity and overweight are related to a higher BMI (>30 kg/m2). BMI greater than 35 kg/m2 is associated with noncommunicable diseases, including endometrial, breast, ovarian, prostate, liver, gall bladder, renal, 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]

A holistic strategy for weight management is recommended, as stated in the guidelines for managing obesity. Modifications to one's way of living, behavioral therapy, medication, and even bariatric surgery may all be part of this comprehensive strategy. When a person's body mass index (BMI) is 30 kg/m2 or greater, medical professionals may decide to administer anti-obesity drugs. The presence of one or more co-morbidities and a body mass index of 27 kg/m2 or more may also warrant their prescription. (8). A reliable strategy for the prevention or treatment of metabolic diseases involves drug intervention of lipid metabolism. Pancreatic lipase is the key enzyme responsible for hydrolyzing duodenal triacyl glycerides, the principal target that regulates lipid absorption. Pancreatic lipase is an enzyme secreted by the pancreas; blocking this enzyme or regulating fat absorption has been proven to be effective in treating metabolic disorders. 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. Recently, there has been a great deal of interest in the screening of potential pancreatic lipase inhibitors generated from herbal medicines. This is because herbal pharmaceuticals have showed appealing safety profiles in long-term medical therapy. (9)

*Amorphophallus konjac* and *Amorphophallus paeoniifolius* aqueous extracts were compared for their potential anti-obesity effects in this study.. 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**

Alkaloids, phenol, tannins, carbohydrates, glycosides, saponins, steroids, flavonoids, terpenoids, resins, and proteins were identified in early phytochemical investigation of aqueous extracts from both species. (qualitative). (28)

1. **Total phenol content**

Phenolic chemicals are secondary aromatic plant metabolites that include one or more hydroxyl substituents. The significant antioxidant activity of naturally occurring polyphenols has positive implications for human health. The polyphenols in many extracts are excellent pancreatic lipase inhibitors, according to recent studies. (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 450 C. The absorbance was measured using a spectrophotometer at a wavelength of 765 nm. The samples were made in triplicate for each analysis, and the average absorbance was calculated. The same procedure was used using the reference gallic acid solution to generate a calibration curve. Gallic acid equivalent (mg of GA/g of extract) was used to express the phenol content concentration after absorbance was determined..(30)

1. **Pancreatic Lipase Inhibition Assay**

Olive oil was used as a substrate to test the inhibitory action of CPL (Chicken pancreatic lipase). Pancreatic lipase activity was measured in a way different from what was 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

$Enzyme Activity=\left(NaOH consumed\right)\left(Molarity of NaOH\right)100×2×\frac{dilutionfactor}{volime of enzyme used}$-----------1

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

$Lipase Inhibition=\frac{A-B}{A}$ ---------------------------2

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 This research looked at the effectiveness of *Amorphophallus peaoniifolius* and *Amorphophallus konjac* in combating obesity. It was speculated that it would have a similar effect on weight maintenance. 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. *Amorphophallus peaoniifolius* and *Amorphophallus konjac* were subjected to preliminary qualitative phytochemical investigation using established procedures. The phytoconstituents (glycosides, flavonoids, and polyphenols) were abundant in both aqueous extracts. Both *Amorphophallus peaoniifolius* and *Amorphophallus konjac* have high levels of phenolic compounds, with a total phenolic content of 26.4 and 27.3 mg gallic acid/gm of plant extract, respectively. *Amorphophallus peaoniifolius and Amorphophallus konjac* aqueous extracts were tested for their antilipase activities. In comparison to orlistat (100%), it inhibited chicken pancreatic lipase by 96% and 92% at 5mg/ml. The aqueous extracts of *Amorphophallus peaoniifolius* and *Amorphophallus konja*c both exhibit comparable antilipase action in obesity, according to the current study. It is necessary to conduct more research to find out how to separate the extract's active ingredients and assess how well they inhibit lipases..

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