Pancreatic Lipase inhibition assay of various extracts of leaves of Murraya Koenigii in southern areas of Goa

Abstract.
The objective of the study was to assess the lipase inhibitory activities of chloroformic, methanolic and aqueous extracts from the commonly available Murraya koenigii (L.) Spreng leaves (Rutaceae) in southern villages of Goa, for potential use in the treatment of obesity. Extracs of the leaves of this plant were evaluated for lipase inhibitory activity using porcine pancreatic lipase (PPL: triacylglycerol lipase) and per-nitrophenyl butyrate in an in vitro assay. Among the three extracts screened, chloroformic extract exhibited the highest pancreatic lipase inhibitory activity of 53.42%, followed by methanolic extract (51.88%) and aqueous extract (36.42%), respectively. Chloroformic extract has not been screened for its pancreatic lipase inhibition assay. All the Crude extracts of leaves of Murraya koenigii (L.) Spreng leaves (Rutaceae) have potential as pancreatic lipase inhibitory agents. Chloroformic extract was found to be most effective and hence can be used as a potent anti-obesity agent to combat hyperlipidemia.

Keywords: Murraya koenigii, pancreatic lipase inhibitory activity, Soxhlet extraction, per-nitrophenyl butyrate, anti-obesity agent

1 Introduction

Obesity is one of the leading causes for metabolic disorders that is an outcome of imbalance between food intake, physical activity, metabolic rate or could be drug induced. Several approaches have been implied for the treatment of obesity targeting at specific mechanisms, which include lipase inhibition, suppressive effect on food intake, stimulatory effects on energy expenditure, inhibition of adipocyte differentiation and the regulatory effect on lipid metabolism. The irregularities seen with respect to lipid levels such as increases in total and low-density lipoprotein (LDL) cholesterols, low concentrations of high-density lipoprotein (HDL) cholesterols, and high triglyceride levels is termed as Dyslipidemia. Drug induced dyslipidemia in particular increases the risk of cardiovascular disease and metabolic dysfunction. Research shows that improving antioxidant status and arresting the accumulation of lipids in the hepatocytes improves blood lipid profile. This is possibly the most effective way for combating Cardiovascular disorders and liver disorders. Currently there are plenty of therapeutic drugs but with limited efficacy and undesirable side effects. One of the most widely studied approach is the inhibition of pancreatic lipase. Natural plant sources can interrupt the lipase as well as adipocyte activity, thus, bringing about inhibition of fat absorption and/or fat accumulation in the body. The pancreatic lipase enzyme is a crucial enzyme in the human digestive system for breaking down dietary fat. Interfering with fat absorption along the gastrointestinal tract is one of the potential ways for treating obesity. Pancreatic lipase has a major role in digestion of triglycerides. Pancreatic lipase inhibitors are substances that reduce the activity of the enzyme in the small intestine, primarily by decreasing fat absorption. Inhibition of pancreatic lipase activity is the most widely studied approach to find potential anti-obesity agents.
In recent years, greater attention has been paid to the use of *M. koenigii* in traditional medicines and home remedies [10]. This plant tolerates any soil, preferably bit loose and sandy type. Villages in south Goa have red sandy soil with good drainage which is best for a good yield. The optimum temperature of Goa is between 26 to 37 degrees which is again favorable for the growth of this plant. The leaves have characteristic flavor and aroma. Phytochemicals like tannins, flavonoids and saponins have been progressively considered due to their anti-obesity and antihyperlipidemic properties [11], eventually found to be inhibitors of pancreatic lipase [12].

It has been postulated that pancreatic lipase in the process of assimilation of Triacylglycerol in the small intestine in tur leads to a glucose surge post a meal. Subsequently, it has been observed this hyperglycemia mediates in insulin and glucose resistance [13]. In a study of comparison of *Murraya koenigii* with other medicinal plants for their inhibitory effect of enzymes linked in glucose and lipid metabolism, hydroalcoholic extract of *Murraya koenigii* showed potent α-amylase inhibition [14]. Interestingly, pancreatic lipase inhibition assay with hydroalcoholic extract of *Murraya koenigii* gave the best results too [14].

A study in 2007 by Vinuthan et al, who used aqueous and methanol leaf extract of *Murraya koenigii* to evaluate the hypolipidemic effects on male Sprague Dawley rats. There was a significant decrease in plasma cholesterol, triglycerides, and phospholipids in the treatment group. The decrease in the lipid profile was attributed to the constituents present in the leaf which were found to stimulate insulin secretion [17].

Xie et al, 2006 observed the tendency of body weight reduction after curry leaf treatment [xie et al]. Other than leaves, stem bark extract of *Murraya koenigii* [kant upadhay] has shown to exert hypolipidemic effect which is similar to that effect of leaves. The results are pertaining to lipid profiles only [20].

Similar lipid lowering effect has been effectively shown in spite of different solvents used for extraction like chloroform [12], dichloromethane, ethyl acetate [21] and ethanol [22] apart from aqueous and methanol extracts.

Given the abundance of evidence on *Murraya koenigii*, it is imperative to explore its phytochemicals in combating obesity. According to the literature survey, chloroformic extract has not been screened for its pancreatic lipase inhibition assay.

In this study, the organic and aqueous extracts of *Murraya koenigii* plant, collected from southern parts of Goa were tested for its efficacious lipase inhibition.

## 2 Materials and methods

### 2.1 Plant material

Leaves of *Murraya koenigii* were collected from different regions of South Goa. A herbarium was prepared and specimen was identified by Botany department of Goa University, Dona Paula, Goa, India. Leaves were washed and shade dried for 2 weeks. After drying, the leaf material was ground into a fine powder using a blender and stored in an airtight container for further use.

### 2.2 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 Goa.

### 2.3 Instrumentation

EVOLUTION 300 UV-Vis spectrophotometer, Semi auto biochemistry analyser, Shaker device, filter paper, digital weighing scale, Soxhlet extractor, Rotary evaporator

### 2.4 Preparation of Plant extract

About 25gms. of dried and grounded *Murraya koenigii* leaves were taken for the preparation of crude extract using Soxhlet apparatus and was allowed to run continuously for 10 reflex cycles each. Three different organic solvents (chloroform, methanol, and distilled water) were used for the solvents diffuse into the plant material and solubilize the material. The extraction was allowed to run for 10 reflex cycles each. Three different organic solvents (chloroform, methanol, and distilled water) were used for the solvents diffuse into the plant material and solubilize the material.
2.5 Phytochemical screening

The bioactive components of *Murraya koenigii* were determined for all the crude extracts derived from methanol, chloroform, and aqueous solvents after condensation in the rotary evaporator. The bioactive components like alkaloids, saponins, tannins, terpenoids, flavanoids, phenols, steroids, glycosides, and anthraquinones were tested using standard methods.

2.5.1 Test for Alkaloids

Mayer’s test:

- 3 ml sample was stirred with 3 ml of 1% HCl on steam bath. Mayer reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloid.

2.5.2 Test for Anthraquinones (Born Trager’s reaction for free Anthraquinones).

- One gram of the powdered plant was placed in a dry test tube and 20 mL of chloroform was added. This was heated in steam bath for 5 min. The extract was filtered while hot and allowed to cool. To the filtrate was added with an equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink coloration as indicative of the presence of Anthraquinones. Control test were done by adding 10 mL of 10% ammonia solution in 5 ml chloroform in a test tube.

2.5.3 Test for Flavonoids

Ferric chloride test for flavonoids: About 0.5 of each portion was boiled with distilled water and then filtered. To 2 ml of the filtrate, few drops of 10% ferric chloride solution were then added. A green-blue or violet coloration indicated the presence of a phenolic hydroxyl group.

2.5.4 Test for Phenol:

- Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.5.5 Test for Glycosides:

- A small amount of alcoholic extract was taken in 1 mL of water in a test tube and a few drops of aqueous NaOH were added. A yellow coloration indicates the presence of glycosides.

2.5.6 Test for Saponins

- 5 ml of sample was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

2.5.7 Test for Tannins
About 2ml of the sample was stirred with 2ml of distilled water and few drops of FeCl3 solution were added. Formation of green precipitate was indication of presence of tannins.

2.5.8 Test for Terpenoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H2SO4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

2.6 Pancreatic Lipase Inhibition assay

Pancreatic lipase activity was determined by measuring the hydrolysis of p-nitrophenyl butyrate (p-NPB) to p-nitrophenol using a method reported previously [26]. 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-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 Vis spectrophotometer [26]. Furthermore, a positive control, Orlistat, was used to ensure the reliability of results.

Relative pancreatic lipase activity (%) was calculated as [(the activity of the compound with the substrate — the activity of the compound without the substrate) / (activity without the compound and with the substrate — negative control without the compound and substrate)] x 100.

Statistical analysis

All results were expressed as Mean ± Standard deviation (n=3). Significance of difference from the control was determined by TUKEY test and a p value 3

3 Results And Discussion

3.1 Percentage yield of extracts

Three extracts were prepared from leaves of Murraya koenigii taken from Cortalim village of south Goa, and tested for their anti-lipase potential at various concentrations using porcine lipase inhibition assay.

The total yield of the Murraya leaves was estimated using a weighing scale. The dry yield was measured by weighing sample leaves from three different areas of weight ranging from 500g, 400g and 450g. Around 87gms, 70gms and 75gms of dry yield was obtained respectively (Table: 2). A mean ± SD of 77 ± 8.7 gm was obtained from those specimens. 17.1% of dry yield was obtained from the Murraya leaf sample.

Table 2

<table>
<thead>
<tr>
<th>Wet weight (gm)</th>
<th>Period of drying</th>
<th>Dry weight (gm)</th>
<th>Yield percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>Shade dried at RT for 3 weeks</td>
<td>87</td>
<td>17.4</td>
</tr>
<tr>
<td>400</td>
<td></td>
<td>70</td>
<td>17.5</td>
</tr>
<tr>
<td>450</td>
<td></td>
<td>75</td>
<td>16.6</td>
</tr>
<tr>
<td>Mean ± SD:</td>
<td>77 ± 8.7</td>
<td></td>
<td></td>
</tr>
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</table>

Statistical analysis

All results were expressed as Mean ± Standard deviation (n=3). Significance of difference from the control was determined by TUKEY test and a p value 3
About 2.75, 1.5, and 7.1 gm of crude extract were obtained from dried Murraya leaves using methanol, chloroform, and water respectively. A mean ± SD of 2.67± 0.09 (10.6% yield), 1.5 ± 0.05 (6.0% yield), and 3.2 ± 0.02 (12.8% yield) was obtained from 25gms. (Table 3) of dried leaves of *Murraya koenigii*. Crude extracted through the aqueous solvent obtained a greater yield of 3.2 gm which was followed by the methanolic extract. Comparatively a lesser yield from the chloroform extract.

Table 3  
<table>
<thead>
<tr>
<th>sample quantity in gm</th>
<th>solvents</th>
<th>yield in gm</th>
<th>Mean ± SD</th>
<th>yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>methanol</td>
<td>2.75</td>
<td>2.67± 0.09</td>
<td>10.6%</td>
</tr>
<tr>
<td>25</td>
<td>chloroform</td>
<td>1.56</td>
<td>1.5 ± 0.05</td>
<td>6.00%</td>
</tr>
<tr>
<td>25</td>
<td>water</td>
<td>3.2</td>
<td>3.2 ± 0.02</td>
<td>12.8%</td>
</tr>
</tbody>
</table>

3.2 Preliminary phytochemical screening

Preliminary phytochemical screening reveals the presence of Phenols, flavonoids, glycosides, saponins, tannins and terpenoids. whereas, alkaloids were tested negative in all three different extracts (Table 4).

Table 4  
<table>
<thead>
<tr>
<th>Constituents</th>
<th>Methanol (Solvent)</th>
<th>Chloroform (solvent)</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Coumarins</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Phenol</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Saponins</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Steroids</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Tannins</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>

3.3 Porcine Pancreatic Lipase Inhibition Assay (PPL)
Three extracts of Murraya koenigii leaves were prepared and tested for pancreatic lipase inhibition at various concentrations of 200, 400, 600, 800 and 1000 micrograms/ml.

The inhibitory activities of chloroformic, methanolic and aqueous extracts towards pancreatic lipase are recorded in graph 1.

As shown in Fig. 1, the chloroformic extract of Murraya koenigii showed an activity of 53.42% at a concentration of 1 mg/ml, proving to be most effective in inhibiting pancreatic lipase followed by methanolic extract that reported 51.88%. The aqueous extract does show inhibitory activity of 36.42% but to a lesser extent.

Values obtained with reference to Fig. 1 are represented in Table 1.

### Table 1. Values obtained with reference to Fig. 1.

<table>
<thead>
<tr>
<th>SOLVENT</th>
<th>Mean Difference</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloroform</td>
<td>209.9400</td>
<td>11.0706</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>278.2067</td>
<td>11.0706</td>
<td>0.000</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Aqueous</td>
<td>209.9400</td>
<td>11.0706</td>
<td>0.000</td>
</tr>
<tr>
<td>Methanol</td>
<td>Aqueous</td>
<td>278.2067</td>
<td>11.0706</td>
<td>0.000</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Methanol</td>
<td>68.2667</td>
<td>11.0706</td>
<td>0.001</td>
</tr>
<tr>
<td>Methanol</td>
<td>Aqueous</td>
<td>278.2067</td>
<td>11.0706</td>
<td>0.000</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Methanol</td>
<td>68.2667</td>
<td>11.0706</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Based on observed means. The error term is Mean Square(Error) = 183.837.

*. The mean difference is significant at the 0.05 level.
4 Conclusion

Murraya koenigii

5 References


H. Rouhi et al., “Pharmacological Interventions to Treat Antipsychotic Obesity Potential of Natural Products,” *Front. Psychiatry*


Tjyybjb.Ac.Cn et al., “Obtaining of New Antioxidant and Antimicrobial Peptides Derived from Human Hemoglobin by Peptide Hydrolysis and Comparison with These Obtained by Bovine Hemoglobin,” 2023, doi:

Kopaei, “Herbs and their potentials in metabolic syndrome, obesity, inflammation, and oxidative stress: A review,” *ARYA Atheroscler.*

et al., “Medicinal profile, phytochemistry, and pharmacological activities of murraya koenigii and its primary bioactive compounds,” *Tffyjh.Ac.Cn*


P. Gaur, K. Shanker, and A. Plants, “In vitro Antidiabetic and Hypolipidemic Activity of Selected Medicinal Plants for the management of hyperlipidemia,” *NPC Natural Product Communications,* vol. 1, no. 4, pp. 94–98.


