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RESEARCH ARTICLE

Screening of Phytochemical and Quantitative Lipase Inhibition of Honey and Cinnamon Paste: A Synergistic Effect

Pavithra R C, Arun A*, Kanimozhi S
Assistant Professor, School of Hotel and Catering Management,
Vels Institute of Science Technology and Advanced Studies (VISTAS) Pallavaram, Chennai 117.
*Corresponding Author E-mail: **arunarticle2016@gmail.com**

ABSTRACT:

Objective: Obesity is one among the metabolic syndromes caused due to the disproportion in intake and disbursement of energy, it takes the reasons for many non-communicable diseases or chronic disorders like diabetics, heart diseases etc. An imbalanced diet with a composition of high fat foods are the common causative agents for obesity. There are many synthetic therapeutic methods to prevent and reduce obesity which always ends with side effects. The study involves in estimating the anti-obesity properties of honey and cinnamon individually and also their synergistic effect in inhibiting the pancreatic lipase. **Methods:** Ethanol extract was prepared for honey, cinnamon and their combinations, the qualitative phytochemical screening were done with their standard procedures. Porcine Pancreatic lipase activity was done for the samples at different concentrations from 10 to 60µg/ml. **Results:** The present study reveals the presence of all tested phytochemicals in the honey-cinnamon paste. With the increase in the concentration of extracts the higher inhibition of the lipase enzyme was observed. Percentage of inhibition ranged from honey 8.48±.041 to 41.49±0.14%, cinnamon 14.48±0.45 to 60.74±0.19% and honey-cinnamon pastes 23.48±0.20 to 87.37±0.20% respectively. **Conclusion:** The lipase inhibition effect reveals that the honey-cinnamon paste has a synergistic effect against lipase which could positively inhibits and reverts obesity which in turn eventually protects from obesity related non-contagious diseases.

KEYWORDS: Honey-Cinnamon paste, Lipase, Obesity, Phytochemicals.

INTRODUCTION:

Obesity is common issue faced globally, the World Health Organization announced that obesity is a universal problem now considered as a disease caused due to an increase in the size and number of adipose cells in the body. In India the prevalence of obesity is about 40.3 percentage and the rate is drastically increasing for the past four decades[1]. Proportion of obesity is higher among the women compared to men and with regard to children India takes the second position next to China[2].

Obesity is a destructive disorder that causes both social and economic issues for human beings, it is a result of multiple reasons like consumption of fatty and sugary foods with high calorific value in daily diet, genetics, low physical activities, lack of sleep etc[3]. Obesity is one of the most imperative risk factors for diabetes mellitus, kidney and lungs disorders, atherosclerosis and associated cardiovascular diseases. Bioactive compounds extracted from plants and microbial sources are alternatives to combat these conditions[4]. In India more than 2.8 million of death have been reported due to obesity and obesity related non-contagious diseases[5].

Many treatments are available in market to treat andprevent obesity, in traditional siddha or ayurvedic treatments natural medicines, and body massages are used to treat obesity[6] in western or allopathic treatment various drugs and surgeries example bariatric surgery is done to reduce obesity related issues[7]. A broad spectrum of daily activities like gymnasium, aerobics, yoga, clinical aromatic therapies, etc. are also followed to treat

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obesity[8]. Beside the existence of several natural or synthetic treatments are in existence the final outcome of treatment is undetermined. Practicing the consumption of food ingredients healthy and medically active into the daily diet always produces a healthy result for human beings. Cinnamon and honey are among those healthy and active ingredients to be considered into daily diet to control obesity and diseases related to it.

Honey has been used as natural sweetener from ancient period of time, it has been in the records of ancient civilization as traditional medicine used directly or indirectly to control and treat diseases[9]. Honey is a rich source of bioactive constituents like glycosides, terpenes, flavonoids, saponins and alkaloids;[10] it is considered a potential and common functional food with low glycemic index and hypo glycemic property that enhances its value in controlling obesity and diabetes issues[11]. Cinnamon takes a long history for its usage as spice and preservative in foods, various compounds have been identified in cinnamon like cinnamaldehyde, eugenol, coumarin and cinnamic acids that helps in controlling lipid and glycemic levels which controls high fat and sugar levels in blood[12].

Honey and cinnamon have an effective property to inhibit obesity and its related issues, the study encompasses in evaluating the combined effect of honey and cinnamon in synergistic way of inhibiting the obesity issues.

MATERIALS AND METHODS:

The study involves in finding out the synergistic effect of honey and cinnamon, it is initiates with procurement of ingredients honey and cinnamon. The ingredients are bought directly from organics stores at Kanyakumari, the cinnamon bought were dried and grinded into find powder using mixer.

a) Preparation of cinnamon and honey paste:

Add 5gram of cinnamon powder and 5gram of raw organic honey and mix well together to make a fine paste.

b) Preparation of ethanol extract:

The prepared honey-cinnamon paste was steeped into 100 ml of ethanol for 72 hours, then the supernatant liquid was filtered with Whatman filter paper and stored for further analysis at 4^0C.

The 5 gram of honey and 5gram of cinnamon powder are individually steeped into 50ml of ethanol for 72 hours and their extract is filtered and stored separately at the same 4°C and used for their analysis.

c) Qualitative Analysis of Phytochemical:

Phytochemical tests were carried out on the ethanolic extract of honey, cinnamon and honey-cinnamon paste using standard procedures to identify the phytochemical constituents.

Test for Phenols:

Ferric Chloride test was performed by using the method of (Sofowora, 1993)[13]. 2ml of ethanol extract of honey, cinnamon and honey-cinnamon paste was taken in a beaker. Then, 2ml of ferric chloride solution was added. A deep bluish green solution indicates presence of phenols.

Test for Carbohydrates:

Test was performed by using the method of (Sofowora, 1993)[13]. 3ml of the ethanol extract of honey, cinnamon and honey-cinnamon paste was added to 2ml of Molisch's reagent and the resulting mixture shaken. 2ml of concentrated sulfuric acid was poured carefully down the side of the test tube. Formation of a red or dull violet colour at the inter-phase of the two layers was indicative of positive test.

Test for Terpenoids:

Salkowski test was performed by using the method of (Edeoga*et al.,* 2005)[14]. 5ml of ethanol extract of honey, cinnamon and honey-cinnamon paste was mixed in 2ml of chloroform. Then 3ml of concentrated sulfuric acid was added to form a layer. A reddish browncoloration of interface indicated presence of terpenoids.

Test for Saponins:

Foam test was performed using the method of (Kokate, 1999)[15]. To 1ml of ethanol extract of honey, cinnamon and honey-cinnamon paste was diluted with 3mL of distilled water.The suspension was shaken in a graduated cylinder for 5min. A layer of foam indicated the presence of saponins.

Test for Glycosides:

Kellar – Kiliani test was performed by using the method of (Parekh and Chanda, 2007)[16]. 2ml of ethanol extract of honey, cinnamon and honey-cinnamon paste was added with 1ml of glacial acetic acid. Then 1ml of ferric chloride was added with 1ml concentrated sulfuric acid. Green-blue coloration of solution indicated the glycoside presence.

Test for Tannins:

Test was performed by using the method of (Kumar *et al.,* 2007)[17]. Alcoholic ferric chloride solution (10%) was added in 2-3ml of ethanol extract of honey, cinnamon and honey-cinnamon paste. The development of dark blue colour of solution indicated the presence of tannins

Test for Flavonoids:
Test was performed by using the method of (Harborne, 2005)[18]. To 1ml of ethanol extract of honey, cinnamon and honey-cinnamon paste was heated with 10ml ethyl acetate over a steam bath (40–50°C) for 5min. Filtrate was treated with 1ml dilute ammonia. A yellow coloration demonstrated positive test for flavonoids

Test for Steroids:
Identification of steroids was done by adopting the method described by (Edeoga*et al.,* 2005)[14]. To 1ml of ethanol extract of honey, cinnamon and honey-cinnamon paste, 2ml acetic anhydride and 2ml concentrated sulfuric acid was added, colour change from blue to dark green indicated the presence of steroids.

Test for Alkaloids:
Hager's test was performed using method described by (Wagner *et al.,* 1996)[19]. To 1 ml of ethanol extract of honey, cinnamon and honey-cinnamon paste, 1 or 2mL of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicates the presence of alkaloids.

d) Porcine Pancreatic Lipase (PPL) Inhibition Assay:
Pancreatic lipase plays avital role in digestion and absorption of dietary fat because the fat is not directly absorbed from intestine. So, it is possible to reduce the absorption of dietary fat by the inhibition of pancreatic lipase, thus it helps in prevention of obesity. Orlistat is one of the clinically approved drugs for obesity management in pancreatic lipase inhibition[20]. PPL inhibition assay Lipase activity was measured using DNPB as a substrate[21]. DNPB was synthesized using the method previously described by (Mosmuller*et al.,* 1992)[22]. PPL stock solutions (1mg/mL) were prepared in 0.1 mM potassium phosphate buffer (pH 6.0), and the solutions were stored at −20°C. To determine lipase inhibitory activity, the extracts (0.2mg/mL) or phytochemicals (at different concentrations) were pre-incubated with the enzyme for 1 hour in potassium phosphate buffer (0.1mM, pH 7.2, combined with 0.6mL/100mL Tween 80) at 30°C before assaying the enzyme activity. The reaction was then started by adding 0.1mL 25mM DNPB, all in a final volume of 5.0mL. After incubation at 30°C for 5 minutes, the amount of 2,4-dinitrophenol released in the reaction was measured at 360nm using the Evolution 300UV-Visible spectrophotometer. The activity of the negative controls was also checked with and without inhibitor. The inhibitory activity (I) was calculated according to the following formula (Sharma *et al.,* 2005)[23].

Inhibition % = [1 – (B-b)/(A-a) *100]

where A is the activity of the enzyme without inhibitor, a is the negative control without inhibitor, B is the activity of the enzyme with inhibitor, and b is the negative control with inhibitor.

The measurements were made in triplicate and the IC_{50} (Inhibitory Concentration at which 50% inhibition of enzyme activity occurs) values of the test samples were determined by performing the assay as above with varying concentrations of the test samples from 10 to 60 µg/mL. The IC_{50} values were determined from the plots of percentage inhibition Vs concentration.

Orlistat:
Orlistat (tetrahydrolipstatin) is an effective lipase inhibitor used to treat overweight and obese subjects[24] and is commonly used in lipase inhibition assays as a positive control[25,26]. In this study, orlistat is used as a control for assay validation. The solution is prepared by dissolving 5.2mg of orlistat into 1mL of DMSO.

e) Statistical analysis:
The results are expressed as mean±SD. Each sample of ethanol extract of honey, cinnamon and honey-cinnamon paste was tested in triplicate in three independent experiments. Statistical significance of the differences between means was established by testing an homogeneity of variance and a normality of distribution followed by ANOVA with Tukey's post hoc test. The P values below 0.01 were considered statistically significant. All analyses were performed using IBM SPSS Statistics version 25 software.

RESULTS AND DISCUSSIONS:

Phytochemical analysis:
Phytochemicals, are the chemicals components produced by the plants which is found in their fruits, grains, vegetables etc. these components are bio-active towards animal biochemistry and may provide necessary health benefits. The phytochemical helps in advert and recuperate from many chronic diseases. Evidence based reports suggests various beneficial roles for phytochemicals against many contagious diseases likes viral and parasitic infections and non-contagious diseases like ulcer, inflammation, cancer, diabetes, cardio-vascular diseases which is based on *in vitro* and *in vivo* assays[27]. Phytochemical components are rich in ant-oxidant values that helps in fighting against the oxidative stress and obesity related issues[28].

Table 1: Phytochemical analysis of ethanol extract of honey, cinnamon and honey-cinnamon paste*

S. No	Phytochemicals	Honey	Cinnamon	Honey-Cinnamon paste
1.	Phenol	-	+	+
2.	Carbohydrates	+	+	++
3.	Terpenoids	-	+	+
4.	Saponins	++	-	+
5.	Glycosides	+	+	++
6.	Tannin	-	+	+
7.	Flavonoids	+	-	++
8.	Steroids	-	+	+
9.	Alkaloids	-	+	+

*+ indicates presence, ++ indicates high concentration, - indicates absence

In the present study the phytochemical analysis for the three samples honey, cinnamon and honey-cinnamon paste has shown the result as on table 1. From the above table it is understood that the sample honey bought from the organic stores of Kanyakumari has shown positive phytochemical analysis results for carbohydrates, saponins, glycosides and flavonoids and negative results in phenol, terpenoid, tannins, steroids and alkaloids which is similar to the study of (Baba *et al.,* 2020)[10] where the ethanol extract of two varieties of honey has also shown the presence of phytochemicals namely; alkaloids, flavonoids, saponins, phenols, glycosides and absence of tannins.

The sample cinnamon extract showed the presence of phenol, carbohydrates, terpenoids, glycosides, tannins, steroids and alkaloids, but negative results in saponins and flavonoids. A reference study of (Adarsh *et al.,* 2020)[29] also showed a similar result in the presence of alkaloids, carbohydrates, terpenoids, steroids, reducing sugar, glycosides, phenols and tannins, also showed negative results in flavonoids, amino acids and saponins which is similar to the result of present study. The reference and current study report proves that there exists no copious difference in the presence of phytochemicals constituents of honey and cinnamon.

The phytochemical result for the paste honey and cinnamon has shown a very positive result with an improved synergistic effect of both honey and cinnamon with a proof in presence of all tested phytochemical constituents.

Effect of cinnamon and honey paste on weight loss (Lipid profile):

Pancreatic lipase, the main lipid digesting enzyme, removes fatty acids from the α and α′ positions of dietary triglycerides, which yield the lipolytic product β-monoglyceride and long chain saturated and polyunsaturated fatty acids. Inhibition of pancreatic lipase is an attractive targeted approach for the discovery of potent anti- hyper lipidemic agents[30].

The anti-obesity agents present in the three samples honey, cinnamon and honey-cinnamon paste is identified by the pancreatic lipase inhibition assay. In the present study the lipase was isolated from the porcine pancreas and the inhibitory activity was determined with different concentrations from 10 to 60 µg/ml of ethanol extracts of three samples.

The maximum inhibition observed in honey-cinnamon paste shown an inhibition of 87.4% with lipase activity at 60µg/ml, whereas cinnamon showed an inhibition of 60.86% with lipase activity at 60 µg/ml. The inhibition percentage observed in honey in ethanol extract showed an inhibition of 41.49% with lipase activity at 60µg/ml. From which it is authentically evident that there is a synergistic effect in honey and cinnamon paste. The IC_{50} values were determined to find the 50% inhibitory concentration against lipase. The values were for Honey 72.31µg/ml for the Cinnamon it is 56.51µg/ml and for the honey-cinnamon paste the value is 32.22µg/ml. As the concentration of the extract increases the lipase inhibition effect increases significantly at $p< 0.01$.

The IC_{50} value of the honey-cinnamon paste is about 32.22µg/ml, which is moreover nearest to the IC_{50} value of orlistat (26. 71µg/ml) the effective isolated form of synthetic drug used in treatment for obesity. Hereby the honey-cinnamon pastes a natural and home-made traditional and functional food has an effective impact as an alternative medicine in treating obesity.

Table 2: Percentage of Lipase inhibition activity of honey, cinnamon and honey-cinnamon paste

Concentration µg/mL	Honey (% of inhibition) Mean ± SD#	p value	Cinnamon (% of inhibition) Mean±SD#	p value	Honey -Cinnamon Paste (% of inhibition) Mean±SD#	p value
10	8.48±0.41[a]	0.00**	14.48±0.45[a]	0.00**	23.48±0.20[a]	0.00**
20	11.49±0.15[b]		21.54±0.05[b]		26.76±0.59[c]	
30	18.70±0.46[c]		26.76±0.20[c]		46.55±0.32[c]	
40	24.74±0.45[d]		39.79±0.36[d]		62.56±0.34[d]	
50	32.67±0.27[e]		44.24±0.12[e]		72.65±0.33[e]	
60	41.49±0.14[f]		60.74±0.19[f]		87.37±0.20[f]	

**Different letters (a-f) for each column symbolize Significant differences at $p <0.01$ by means of Tukey's HSD test.
#Values are the mean of triplicates

CONCLUSION:

Obesity has become a chronic disease among all humans with no regards to age, gender or any other demographic issues. Treating obesity with natural food as medicine is always a consistent and safe method beside many synthetic products and practices available in today's market. Hence the study, an attempt to check the synergistic effect of honey-cinnamon paste in inhibiting lipase the causative agent for obesity has proved positive with the presence of pharmacological active constituents.

CONFLICT OF INTEREST:

Nil.

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