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Additive inhibitory effect of the peels of *Citrus limon* and *Citrus sinensis* against amylase and glucosidase activity

Rangarajan N[1], Sangeetha R[2], Mohanasundaram S[*1], Sampath V[1], Porkodi K[1], Dass Prakash MV[1]

[1]Department of Biochemistry, Sri Sankara Arts and Science College (Autonomous), Enathur, Kanchipuram – 631561, Tamil Nadu, India
[2]Department of Biochemistry, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai – 600 117, Tamil Nadu, India

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ABSTRACT

Diabetes mellitus is a major health problem and it is a metabolic disorder characterized by hyperglycemia. Decreased utilization of glucose by the body cells and continuous raise in the plasma glucose levels are the major characteristic symptoms of diabetes. Uncontrolled hyperglycemia is associated with pathological conditions, mostly micro and macrovascular. Treatment for diabetes is aimed at the management of hyperglycemia using oral hypoglycemic agents which inhibit carbohydrate degrading enzymes, namely amylase and glucosidase. The purpose of this study was to investigate the naturally available inhibitors of amylase and glucosidase present in the peels of citrus fruits such as lemon and orange. The hydroalcoholic extract of the fruit peels were studied for their amylase and glucosidase inhibitory potential and the extract of lemon peel was found to exhibit better inhibition than the orange peel. The extracts were further evaluated for their additive effect. The combined effect of the extracts (50% inhibition achieved at 40 and 80mcg) proved to exhibit better inhibitory potential than the individual effect (50% inhibition achieved at 80 and 160mcg). Thus, from this study, it was very clear that our choice drug sources is containing potential antidiabetic principles that need to be studied further to understand the mechanism of action in detail.

[*]Corresponding Author

Name: Mohanasundaram S
Phone:
Email: sbmohan2007@gmail.com

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INTRODUCTION

Diabetes Mellitus is a disorder characterized by hyperglycemia and metabolic abnormalities. Elevated blood glucose levels are symptomatic of diabetes mellitus as a consequence of defective pancreatic insulin secretion or defective response of the target cells to the insulin that is produced (Piero, 2015). Itis a lifelong disease and a silent killer affected by millions of people. It is the second leading cause of blindness and renal disease worldwide. Diabetes is a disorder which is associated with long term damage such as malfunction of eyes, heart, kidneys, nervous and vascular system (ADA, 2007). It is also integrated with health complications like renal failure, foot ulcers, sexual dysfunction, cardiac diseases, stroke and blindness (Riaz, 2009).

Presently available treatment options in modern medicine have several undesired harmful side effects, so there is a need for safe and effective treatment modality for diabetes. Medicinal plants play a prime role in the treatment of diabetes in developing countries where resources are scanty (Wachtel-Galor and Benzie, 2011; Kumar

et al., 2014).

Herbal medicine have been used for the treatment of diabetes since a long time and are presently accepted as an alternative therapy for diabetic treatment. In the indigenous Indian system of medicine, a good number of medicinal plants has been mentioned in literature for the treatment of diabetes, and some of them have been experimentally assessed and active principles isolated (Aswathy and C, 2017). World Health Organization in 1980, has also recommended the evaluation of the efficacy of plants in conditions where there are no modern safety drugs. The ethnobotanical information reports states that about 800 plants have an antidiabetic effect. (Satoskar *et al.*, 1999)

Fruits are the vital dietary components which contain biologically active ingredients like flavonoids, phenols, and carotenoids. Evidence have been proposed to reveal that a diet which is rich in fruits and vegetables has an impacts on reducing the risk of a chronic and severe disease condition such as diabetes, cardiovascular disease and cancer (Chatterjee, 2014).

Fruit peels help to relieve chronic diseases as they are natural sources of important constituents with medicinal value. As many diseases like cancer, arthritis, liver diseases, have no complete cure in western medicine such as allopathy, fruit peels play a vital role in the development of modern herbal medicine (Nair *et al.*, 2013).

Citrus (*Citrus limon* and *Citrus sinensis*) is a plant belonging to the family Rutaceae and is distributed world-wide (Wadhwa and Bakshi, 2013). Traditionally, citrus has been used to treat ulcer, coronary heart diseases, cardiovascular diseases and diabetes mellitus. The medicinal value of the peels of citrus fruits (Lemon and Orange) can be attributed to the presence of secondary metabolites like flavonoids and other bioactive compounds in them (Clement *et al.*, 2007). This study was conducted to evaluate the anti-diabetic potential of the peels of lemon and orange in-vitro by assessing the inhibition of the enzymes alpha-amylase and alpha-glucosidase.

MATERIALS AND METHODS

Collection of plant material

The fruit peels (lemon and orange) were collected from suburban areas of Kancheepuram district, Tamil Nadu, India, during the month of January 2019. The plant materials were taxonomically identified by Dr. S. Jayaraman, Director, Plant Anatomy Research Institute, Tambaram, Chennai (Voucher specimen – 2019/PARC/4124, 25).

Preparation of plant extracts

The fruit peels of lemon and orange were shade dried and coarsely powdered. One kilogram of the coarse powder was subjected to exhaustive cold maceration in 70% ethanol for a duration of 72 h. The solvents were filtered and concentrated in a rotary evaporator and used for assays.

Alpha amylase inhibition assay

The amylase inhibition was done in-vitro following the method of Bernfeld, 1955. Hundred microliter of the peel extract was allowed to react with 200 μL of α- amylase enzyme (Hi media Rm 638) and100 μL of phosphate buffer (2mM, pH 6.9). After incubating for 20 mins, 100μL of 1% starch solution was added. 200 μL of the buffer served as the control. To both control and test solutions, 500μL of dinitrosalicylic acid reagent was added and incubated for 5 min at 60 °C. The standard inhibitor used here was Acarbose (Sigma). The absorbance was recorded at 540 nm.

Alpha-glucosidase inhibition assay

The assay of α-glucosidase inhibition was performed in-vitro by pre-incubating equal volumes of peel extract, sodium phosphate buffer (1 mM, pH 6.9) and α-glucosidase enzyme (Sigma) for 5 min. To the pre-incubated tubes, 0.1 ml of -nitrophenyl-α-D-glucopyranoside (Sigma) was added, followed by incubation for 10 min at 25 °C. Acarbose was used as the standard inhibitor. The absorbance was recorded at 405 nm and the percentage of inhibition was calculated.

Calculation of the percentage of inhibition

The percentage inhibition of α-amylase enzyme was calculated using the formula

$$Inhibition(\%) = \frac{100 \times (Absorbance\ of\ Control - Absorbance\ of\ test)}{Absorbance\ of\ Control}$$

Statistical analysis

The observations are expressed as a mean of six experiments $\pm$ standard deviation. The IC_{50} values were calculated by regression analysis.

RESULTS AND DISCUSSION

Diabetes mellitus is a chronic metabolic disorder in which defective regulation of the homeostasis of the carbohydrate, protein and lipid metabolism by the pancreatic hormone insulin leads to increased blood glucose levels. High blood glucose level, termed hyperglycemia, is a clinical condition which interferes with metabolic pathways and thus contributing to several micro and macrovascular complications (Deshmukh and Chinmay, 2015). Alpha amylase and alpha-glucosidase inhibition assay was per-

formed for both lemon peel and orange peel extract individually and in combination.

Inhibitory activity of extracts against alpha-amylase and glucosidase

Lemon peel extract was assayed for potential inhibitory activity at the concentration range of 10 – 1280 μg/ml. The enzyme inhibition was observed to be between 18% to 85% for amylase and 24% to 80% for glucosidase. Similarly, the range of inhibition by orange peel extract at the same concentrations was between 12% to 78% for amylase and 14% to 70% for glucosidase (Figure 1 and Figure 2).

The hydroalcoholic extracts of lemon and orange peels exhibited 50% inhibition of amylase and glucosidase at a concentration of 160 μg and 80 μg for lemon peel and 160 μg for orange peel. The IC 50 value of both the extract against the enzymes were determined. For lemon peel, the IC50 concentration was 98.6 μg and 32.8 μg for amylase and glucosidase, whereas it was 80.16 μg and 43.73 μg, respectively, for orange peel.

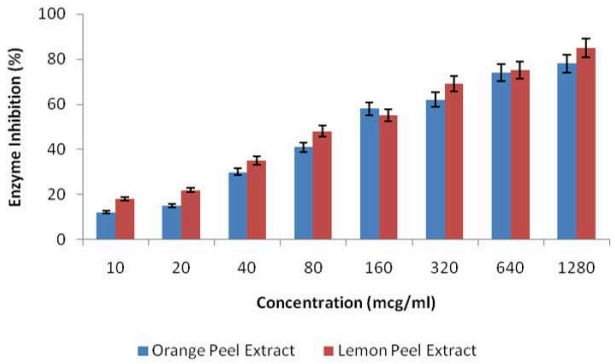

Figure 1: Amylase Inhibition by extracts All values areexpressed as Mean ± S.D (n = 3)

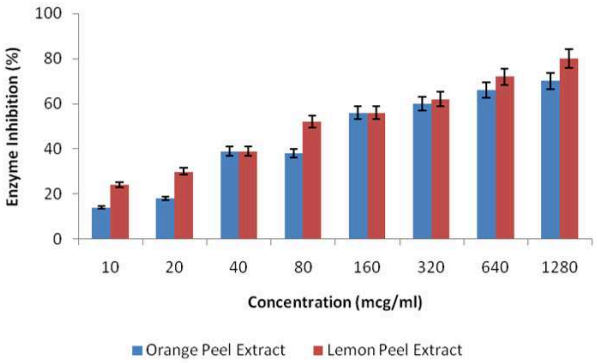

Figure 2: Glucosidase Inhibition by extracts All values areexpressed as Mean ± S.D (n = 3)

An additive effect of each extract against α-amylase and glucosidase

Investigation of the effect of both extracts, in combination, against the two enzymes, was carried out.

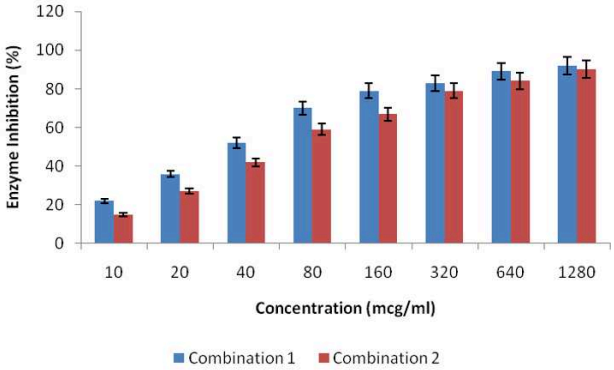

Figure 3: Combined Amylase Inhibitory effect of extracts All values are expressed as Mean ± S.D (n = 3)
Combination 1 – Fixed concentration of orange extract (80 mcg) and varying concentrations of lemon
Combination 2 – Fixed concentration of lemon extract (99 mcg) and varying concentrations of orange

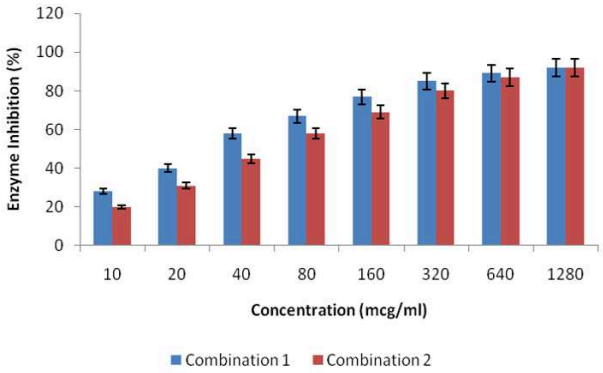

Figure 4: Combined Glucosidase Inhibitory effect of extracts All values are expressed as Mean ± S.D (n = 3)
Combination 1 – Fixed concentration of orange extract (43 mcg) and varying concentrations of lemon
Combination 2 – Fixed concentration of lemon extract (33 mcg) and varying concentrations of orange

The study was done using two combinations. In Combination 1, the extract under study contained varying concentrations (10 – 1280 μg) of orange peel extract and lemon extract in quantity equal to its IC 50 values, i.e., 98.6 μg for amylase inhibitory study and 32.8 μg for glucosidase inhibitory study. In Combination 2, the extract under study contained varying concentrations (10 – 1280 μg) of lemon peel extract and orange extract in quantity equal to its IC 50 values, i.e., 80.16 μg for amylase inhibitory study and 43.73 μg for glucosidase inhibitory study.

Combination 1 inhibited amylase between 22% to 92%, while the range of inhibition was between 15% to 90% for combination 2. The 50% inhibition of amylase activity by combinations 1 and 2 was found to be at the concentrations of 40 and 80 μg, respectively (Figure 3). Combination 2 inhibited glucosidase in ranges between 28% to 92% and the range of inhibition was between 20% to 92% by combination 2. The 50% inhibition of glucosidase activity by combinations 1 and 2 was found to be at the concentrations of 40 and 80 μg, respectively (Figure 4).

The results of this study establish that the hydroalcoholic extract of both the lemon and orange peel exhibits significant inhibitory potential against both the enzymes. Fruit peels are reservoirs of medical components and help to alleviate chronic diseases that have affected mankind since ages. Fruit peels play a vital role in herbal medicine (Nair *et al.*, 2013). Several flavonoids and related compounds were isolated from the unripe pulp and peel of banana (Lewis and Shaw, 2001). The phytochemical composition of persimmon peel has been studied and the result indicates the presence of high levels of dietary fibre and antioxidants with antidiabetic properties (Lee *et al.*, 2006). The peels of Punica granatum, commonly known as pomegranate, have long been used in traditional herbal medicine against diabetes. (James *et al.*, 2018) have reported the antidiabetic effect of fruit rind of *Terminalia bellirica*.

CONCLUSIONS

The study was designed to evaluate the individual and additive amylase and glucosidase inhibitory potentials of the extracts of the peels of *Citrus limon* and *Citrus sinensis.* The extracts exhibited remarkable potential comparable to the standard drug. The peels also proved to possess an additive effect in inhibiting the enzymes. The inhibitors of amylase and glucosidase are promising targets in the treatment of hyperglycemia in diabetics. Naturally available inhibitors are safe and do not exert the side effects presented by synthetic anti-hyperglycemic agents and hence the hydroalcoholic extract of the peels of citrus fruits can be evaluated for their antidiabetic value in animal models.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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