Research Article

Comparative Evaluation of Anti-Diabetic Activity of Lemon Grass Oil and Tulasi Oil

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ABSTRACT

The aim of the study is to evaluate the Anti-diabetic activity of Lemon grass oil and Tulasi oil. Diabetes mellitus is a serious health problem that has adverse complication for individuals. Essential oils plays important role in hypoglycaemic effect. Lemon grass oil and Tulasi oil is of great interest due to commercial and economical value in traditional medicine. The *in vitro* enzymatic inhibition assay of essential oil is carried out. The reason of the study is to validate the efficacy of essential oils in Anti-diabetic treatment, since diabetes is much concern in the society. Comparative evaluation of lemon grass oil and Tulasi oil has anti-diabetic properties.

Keywords: Cymbopogon citraus, Ocimum sanctum, Antidiabetic activity, amylase inhibition activity and alpha glucosidase activity.

INTRODUCTION

Diabetes mellitus is a global health crisis which has been persistently affecting the humanity irrespective of the socioeconomic profile and geographic location of the population[1]. Diabetes mellitus is one of the common metabolic disorder acquiring around 2.8% of the world's population and is anticipated to cross 5.4% by the year 2025. Since long back herbal medicines have been the highly esteemed source of medicine therefore, they have become a growing part of modern and high tech medicine. Diabetes mellitus is one of the most common endocrine metabolic has caused significant morbidity and mortality due to microvascular (retinopathy,neuropathy,and nephropathy) and macrovascular (heart attack, storke, and peripheral vascular disease) complication[2]. Now a days, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies[3]. Plants have been always a very good source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information suggests that about 800 plants may possess anti-diabetic potential among all of them Momordica charanta, Pterocarpus marsapium and Trigoella foenum have been reported to be beneficial for treatment of type 2 diabetes[4,5]. Lemongrass (Cymbopogon citrarus) a perennial plant with long, thin leaves,is one of the largely cultivated medicinal plants for its essential oils in parts of tropical and subtropical areas of Asia, America and Africa[6]. It contains 1-2% of-essential oil on dry basis[7]. The chemical composition of Lemongrass essential oil is varying widely upon genetic diversity, habitat and agronomic treatment of the culture. The leaves of Lemongrass contains lemony characteristic flavour due presence of citral. Citral is a combination of neural and geranial isomers and is used as a raw material for the production of ionone, vitaminA and beta-carotene[6]. There were a number of studies carried out to prove the anti-oxidant, anti-microbial and anti- fungal activities of Lemon grass[9-11]. Ocimum sanctum (Tulasi) is a annual herb belonging to the mint family with 150 varieties worldwide. Tulasi emits a spicy scent. The roots, leaves and seeds of Tulasi possess several medicinal properties. Tulasi is pungent and bitter in taste, pungent in the post digestive effect and has hot potency. Tulasi is also called by names like Manjari/Krishna Tulasi (Sanskrit), Trittavu (Malayalam), Tulshi (Marathi) and Tulasi (Tamil and Telugu). Tulasi is used in Ayurvedic properties for treating various aliments. Tulasi leaves contains a bright yellow volatile oil which is useful against insects and bacteria.

MATERIALS AND METHODS

Materials

Lemon grass oil and Tulasi oil are purchased from cyprus enterprise.

Methods

In vitro Anti-diabetic Activity

Essential oil samples of *Lemon grass* and *Tulsi* leaves were assessed for *in vitro* anti-diabetic activity by the α-amylase, glucose uptake by yeast cells and α-glucosidase inhibition.

α-amylase Inhibition Activity

The α-amylase inhibition assay was performed using the 3, 5-dinitrosalicylic acid (DNSA) method (Miller, 1959)[12]. Starch solution (0.25% w/v) was prepared by stirring 0.125 g of tapioca powder in 50 mL of 20mM sodium phosphate buffer containing 6.7mM sodium chloride at pH 6.9.

One unit of α-amylase enzyme solution was prepared by mixing 0.0253 g of α-amylase in 100 mL of cold distillation water. Tulsi and lemongrass oils were dissolved in DMSO to give concentrations (50, 100, 150µg/ml). The color

reagent was prepared by mixing sodium potassium tartrate solution (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH) and 96mM of 3,5-dinitrosalicylic acid solution (0.4381 g of 3,5-dinitrosalicylic acid in 20 mL of deionized water).

One unit of α-amylase solution and Tulsi and lemongrass oils were mixed thoroughly in a tube and incubated for 15 min. Then 500µL of the starch solution was added into each tube and incubated for 15 min.

The reaction was terminated by addition of 500µL DNSA reagent, placed in boiling water bath for 5 min. The mixture was cooled to ambient temperature, diluted with 5 mL distilled water, and the absorbance was measured at 540 nm using a visible spectrophotometer.

The blank control of reaction showing 100% enzyme activity was conducted by replacing the essential oil with DMSO (1.0 mL). To eliminate the absorbance effect of essential oil, a blank solution was also used and the reaction was terminated by DNSA before adding the starch solution.

Acarbose solution (diluted in DMSO to 80 – 400 µL/mL) was used as a positive control. The production of maltose will decrease with α-amylase inhibitory activity which will result in reduced absorbance intensity.

The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the following equation:

$$\%\ Relative\ enzyme\ activity = \frac{Enzyme\ (Maltose) activity\ of\ test}{Enzyme\ activity\ of\ control} \times 100$$

$$\%\ of\ \alpha - amylase\ inhibition\ activity = 100 - \%\ Relative\ enzyme\ activity$$

[Table 1, Figure 1]

α-glucosidase Inhibition Activity

Alpha-glucosidase inhibitory activity of essential oils was carried out according to method of Bachhawat with slight modification[14].

In a 96-well plate, reaction mixture containing 50µl phosphate buffer (50mM, pH= 6.8), 10µl alpha-glucosidase (1U/ml) [SRL] and 20µl of varying concentrations of oils was pre-incubated at 37°C for 15 min.

Then 20µl p-nitrophenyl-α-DGlucopyranoside (PNPG) (1mM) [SRL] was added as a substrate and incubated further at 37°C for 30 min. The reaction was stopped by adding 50µl sodium carbonate (0.1M). The yellow color produced was read at 405nm using visible spectrophotometer. Acarbose at various concentrations (50-150 µg/ml) was included as a standard.

The control samples were prepared without any essential oil. The result is expressed as percentage inhibition, which was calculated as,

$$\%\ Inhibition = \frac{Absorbance\ of\ Control - Absorbance\ of\ test\ sample}{Absorbance\ of\ control} \times 100$$

Acarbose (Sigma, U.S.A) a well-known α-glucosidase and α-amylase inhibitor wasused as reference drug for the inhibitory activity. [Table 2, Figure 2]

Glucose Uptake by Yeast Cells

Yeast cells were prepared according to the method of Cirillo, 1962[13].

Briefly, commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water.

Various concentrations of oils (50, 100, 150µg/ml) were added to 1 mL of glucose solution (5, 10 and 25mM) and incubated together for 10 min at 37 °C.

Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37 °C for 60 min.

After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant by DNSA method.

Metronidazole was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$Inhibition\ \% = \frac{Abs\ sample - Abs\ control}{Abs\ sample} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample.

The amount of glucose lingering in the medium after a specific time serves as a marker of the glucose uptake by the yeast cells. [Table 3,4,5, Figure 3]

RESULTS AND DISCUSSION

Alpha Amylase Inhibition Activity

Table 1.1: % of α-amylase Inhibition Activity

Concentration (µg)	Control	Lemon grass	Tulsi	Acarbose	% Relative enzyme activity Lemon	% Relative enzyme activity Tulsi	% Relative enzyme activity Acarbose
50	0.871	0.482	0.514	0.528	55.33869116	59.01262916	60.61997704
100	0.871	0.397	0.427	0.408	45.57979334	49.02411022	46.84270953
150	0.871	0.302	0.343	0.305	34.6727899	39.38002296	35.01722158

Table 1.2: % of α-amylase Inhibition Activity

Lemon Grass	Tulsi	Acarbose
44.66130884	40.98737084	39.38002296
54.42020666	50.97588978	53.15729047
65.3272101	60.61997704	64.98277842

Table 2: % of α-glucosidase Inhibition Activity

Concentration (µg)	Control	Lemon grass	Tulsi	Acarbose	% inhibition activity Lemon grass	% inhibition activity Tulsi	% inhibition activity Acarbose
50	0.796	0.407	0.434	0.508	48.86934673	45.47738693	36.18090452
100	0.796	0.319	0.349	0.388	59.92462312	56.15577889	51.25628141
150	0.796	0.228	0.274	0.251	71.35678392	65.57788945	68.46733668

Table 3: % Uptake of Glucose

Concentration (µg)	Control	Lemon grass	Tulsi	% uptake of glucose Lemon grass 5mM	% uptake of glucose Tulsi 5mM
50	0.458	0.727	0.698	37.00137552	34.38395415
100	0.458	0.994	0.891	53.92354125	48.59708193
150	0.458	1.354	1.098	66.17429838	58.28779599

Table 4%: Uptake of Glucose

Concentration (µg)	Control	Lemon grass	Tulsi	% uptake of glucose Lemon grass 10mM	% uptake of glucose Tulsi 10mM
50	0.458	0.665	0.642	31.12781955	28.66043614
100	0.458	0.882	0.779	48.07256236	41.20667522
150	0.458	1.187	0.948	61.41533277	51.68776371

Table 5: % Uptake of Glucose

Concentration (µg)	Control	Lemon grass	Tulsi	% uptake of glucose Lemon grass 25mM	% uptake of glucose Tulsi 25mM
50	0.458	0.599	0.552	23.53923205	17.02898551
100	0.458	0.752	0.681	39.09574468	32.74596182
150	0.458	0.955	0.784	52.04188482	41.58163265

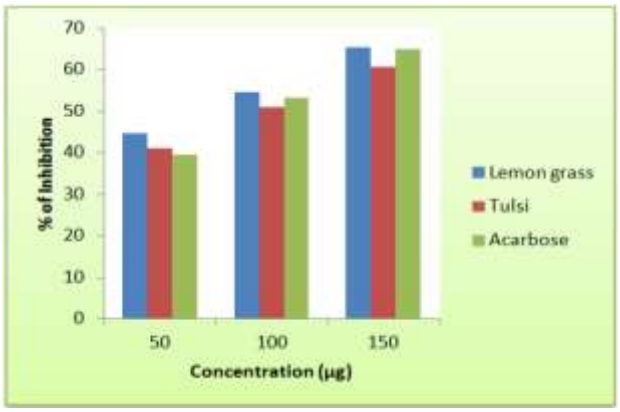

Figure 1

The percentage of Alpha amylase inhibition activity of Lemon grass oil is 44.66%, Tulasi oil is about 40.98 %. The alpha amylase activity of Lemon grass oil is slightly grater than the alpha amylase activity of Tulasi oil. The gradual increase in the percentage of alpha amylase inhibition activity when the concentration is increased from 50microgram to 150microgram and when the concentration at 150 microgram, the percentage of alpha glucosidase activity of Lemon grass oil, Tulasi oil is 65.32, 60.61.

Alpha Glucosidase Inhibition Activity

The percentage of alpha glucosidase inhibition activity of Lemon grass oil is 48.86, Tulasi oil is 45.47. The percentage of alpha glucosidase activity of Lemon grass oil is sightly great than that of Tulasi oil.

The gradual increase in the percentage of alpha glucosidase activity,when concentration is increased from 50microgram to 150microgram. When the concentration at 150 microgram, the percentage of alpha glucosidase activity of Lemon grass oil, Tulasi oil is 71.35, 65.57.

Alpha Glucosidase Inhibition Activity

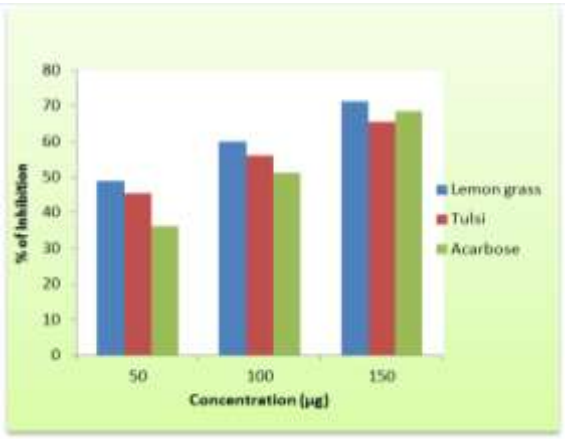

Figure 2

Glucose Uptake by Yeast Cell

The rate of uptake of glucose into yeast cells was linear in all the 3 glucose concentrations. The percentage of Glucose uptake by Lemon grass oil and Tulasi oil in 5m**M**is 37 and 34.38. The gradual increase in the percentage of glucose uptake by Lemon grass oil and Tulasi oil in 5m**M** when the concentration is increased from 50microgram to 100microgram. The percentage of Glucose uptake by Lemon grass oil and Tulasi oil in 10m**M** is 31.12 and 28.66. The gradual increase in the percentage of glucose uptake by Lemon grass oil and Tulasi oil in10M when the concentration is increased from 50microgram to 100microgram.

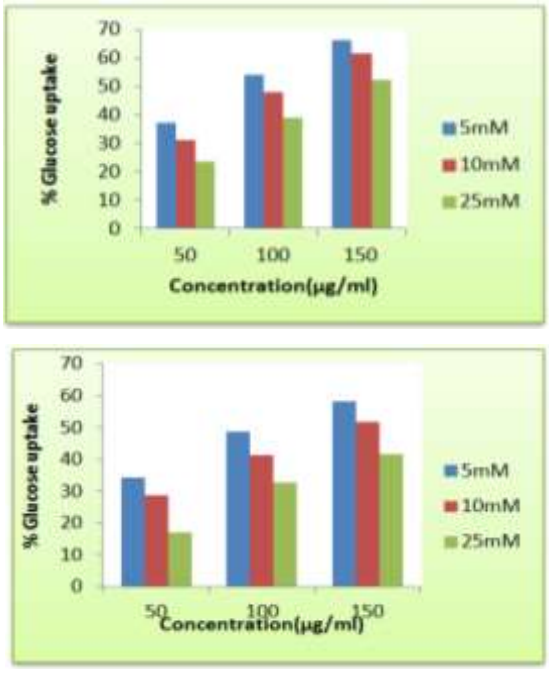

Figure 3

Inhibition of Glucose uptake by yeast cells: Lemon Grass and Tulasi oil

The percentage of glucose uptake by Lemon grass oil and Tulasi oil in 25m**M** is 23.23 and 17.02. The gradual increase in the percentage of glucose uptake by Lemon grass oil and Tulasi oil in 25m**M** when the concentration is increased from 50microgram to 100microgram. When the concentration at 150microgram. The percentage of glucose uptake by Lemon grass oil and Tulasi oil in 25m**M** is 52.04 and 41.58.

CONCLUSION

The present study concludes that the lemon grass oil and Tulasi oil have anti hypoglycaemic properties. It showed that these plants have hypoglycaemic effects. Use of lemon grass oil and Tulasi oil on a regular basis by diabetic patients can be beneficial in lowering the blood glucose and potentiates other therapies used in diabetes treatment. Since the number of patients with diabetes is increasing, to reduce the amount spent on the treatments such simple plants available can be used. In future, we can done the experiment by *in vivo* anti-diabetic activity.

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