



ORIGINAL RESEARCH PAPER

Pharmacology

IN VITRO ANTIDIABETIC ACTIVITY OF METHANOL EXTRACTS OF OCIMUM GRATISSMUM LINN

KEY WORDS: Ocimum gratissimum, alpha amylase, alpha glucosidase, Antidiabetic

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ABSTRACT

In the present study the methanol extracts of *Ocimum gratissimum* was studied for alpha (α) - amylase and alpha (α)-glucosidase inhibition using an in vitro experimental model. Methanol extracts of the leaves were prepared by using maceration techniques. They were then tested for inhibition of (α) - amylase activity by DNSA colour reagent and for their ability to hinder diffusion of glucose across a dialysis membrane. The methanol extract of *Ocimum gratissimum* showed maximum inhibition of (α)- amylase activity and a strong hindrance to diffusion of glucose across a dialysis membrane. *Ocimum gratissimum* was found to possess maximum anti-diabetic properties. The findings indicate that the plant leaves possess antidiabetic properties too varying degrees. They can be used to develop natural drugs which may be used in lieu of commonly used strong allopathic drugs which possess a number of harmful side effects.

INTRODUCTION:

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, resulting in hyperglycaemia leading to acute and chronic complications. The incidence of diabetes mellitus is on rise all over the world. Control of plasma glucose concentrations is vital to decrease the incidence and severity of long term diabetes complications. Synthetic drugs are likely to give serious effects in addition they are not suitable for intake during conditions like pregnancy. Apart from conventional diabetes therapy, several studies have shown that some plants used in traditional medicine have beneficial effects in diabetic patients⁵. More than 400 plants worldwide have been documented as beneficial in the treatment of diabetes. The majority of traditional antidiabetic plants await proper scientific and medical evaluation for their ability to improve blood glucose control.



Fig.1 Ocimum Gratissimum

Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit, and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals, which produce definite physiological actions on the human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds. *Ocimum gratissimum* Linn is grown for the essential oils in its leaves and stems. Eugenol, thymol, citral, geraniol and linalool have been extracted from the oil. Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties. The anti nociceptive property of the essential oil of the plant has been reported. The whole plant and the essential oil are used in traditional medicine especially in Africa and India. The essential oil is also an important insect repellent. *O. gratissimum* is germicidal and has found wide use in

toothpastes and mouth washes as well as some topical ointments. It is used as an excellent gargle for sore throats and tonsillitis. It is also used as an expectorant and a cough suppressant. The plant extract is used against gastrointestinal helminths of animals and man. In addition, *O. gratissimum* carminative properties make it a good choice for upset stomach. It is used as an emetic and for hemorrhoids. The plant is also used for the treatment of rheumatism, paralysis, epilepsy, high fever, diarrhea, sunstroke, influenza, gonorrhoea and mental illness¹. The present work has been designed to evaluate the antidiabetic potential of extracts from the leaves of *O. gratissimum* and to explore the basis for its traditional use.

MATERIALS AND METHODS:

Plant Materials:

The leaves of *Ocimum gratissimum* was collected from Thiruvallur district Tamilnadu, India and authenticated from plant anatomy Institute, Tambaram. The voucher specimens were preserved in Madha Medical college department Museum (Voucher No.46/2018). The plant parts were shed-dried, pulverized and stored in an airtight container for further extraction.

Extraction Of Plant Material:

The air-dried and coarse powdered plant sample of *Ocimum gratissimum* (10 g) was extracted with 100 ml methanol by maceration on an orbital shaker with agitation for five days at room temperature. The extract was filtered and residue was again extracted with same solvent for another five days and filtered. The filtered extracts were combined and concentrated using a rotary evaporator, under reduced pressure at approximately 40°C and lyophilized to obtain the powdered extract. The powdered extract were analysed for their α -glucosidase and α -amylase inhibition assays⁵.

Alpha- Amylase Inhibition Assay:

Alpha-amylase activity can be measured in-vitro by hydrolysis of starch in presence of α -amylase enzyme. This process was quantified by using iodine, which gives blue colour with starch. The reduced intensity of blue colour indicates the enzyme-induced hydrolysis of starch in to monosaccharides. α -Amylase was premixed with the *O. gratissimum* leaves extracts at various concentrations (20-100 μ g/ml) and 0.5% starch solution was added at 37°C for 5 min to start the reaction and terminated by addition of 2 ml of 3,5-dinitrosalicylic acid (Holecheck et al. 1982). The reaction mixture was heated for 15 min at 100°C and diluted with 10 ml of distilled water in an ice bath α -Amylase activity was determined by measuring spectrum at 540 nm and IC50 value was measured⁵.

Alpha-Glucosidase Inhibition Assay:

The inhibition of -glucosidase activity was determined using the modified published method. One mg of - glucosidase was dissolved in 100ml of phosphate buffer (pH 6.8). To 100 µl of (2, 4, 8, 10, 15 µg/ml) plant extracts, 200 µl - glucosidase were added and the mixture was incubated at 37°C for 20 min. To the reaction mixture 100 µl 3mM -nitrophenyl -Dglucopyranoside (p-NPG) was added and incubated at 37 °C for 10 min. The reaction was terminated by the addition of 2ml Na2CO3 0.1M and the alpha-glucosidase activity was determined spectrophotometrically at 405nm on spectrophotometer UV-VIS (Shimadzu UV-1800) by measuring the quantity of - nitrophenol released from p-NPG. Acarbose was used as positive control of amylase and glucosidase inhibitor. The concentration of the extract required to inhibit 50% of alpha amylase and alpha glucosidase activity under the assay conditions was defined as the IC50 value².

RESULTS AND DISCUSSION:

There was a dosage-dependent increase in percentage inhibitory activity against alpha amylase enzyme. Alpha-amylase catalyses the hydrolysis of alpha-1, 4-glycosidic linkages of starch, glycogen and various oligosaccharides. Alpha-glucosidase is a glucosidase located in the brush border of the small intestine that acts upon 1, 4-alpha bonds. This is in contrast to beta-glucosidase. Alpha-glucosidase breaks down starch and disaccharides to glucose. Alpha-glucosidase inhibitors work by preventing the digestion of carbohydrates (such as starch and table sugar) by inhibition of enzyme alpha glucosidase. Carbohydrates are normally converted into simple sugars (monosaccharides), which can be absorbed through the intestine. Hence, alpha-glucosidase inhibitors reduce the impact of carbohydrates on blood sugar alpha-glucosidase further breaks down the disaccharides to simple sugars, readily available for intestinal absorption. The inhibition of their activity in the digestive tract of humans is considered to be effective tool to control diabetes.

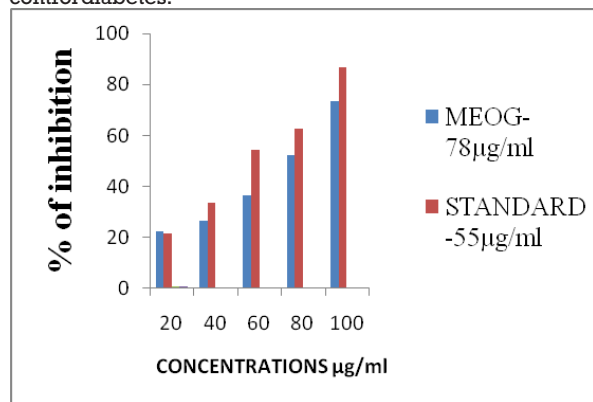


Fig 2: Alpha Amylase Activity

The methanol extracts of *O.gratissimum* leaves have exhibited potent inhibition of alpha-amylase and alpha-glucosidase enzyme activity. In addition, MEOG was able to inhibit both the enzymes at lower concentration. Further studies are required to elucidate whether *O.gratissimum* have antidiabetic potential by in vivo for validating the traditional claim of the plant.

CONCLUSION:

In this present study we evaluated in vitro alpha amylase and alpha glucosidase activity of methanol extract of *Ocimum gratissimum* leaves. The plant showed significant antidiabetic activity, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of antidiabetic agent.

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