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EFFECT OF CONCENTRATION OF OCIMUM SACTUM Linn (TULSI) LEAVES EXTRACT ON THE α- AMYLASE, α-GLUCOSIDASE ACTIVITY AND MICROORGANISM GROWTH

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Abstract

Medicines derived from medicinal plants prevent and cure many diseases. medicinal plants are used widely all over the world for natural medicines. Tulsi is known as “Queen of plants” and “The mother medicine of nature”. Ocimum sanctum has number of chemical compounds (beta carotene, calcium, vitamin C, estragol, linalool, eugenol, methyl chavicol and small quantities of methyl cinnamate, cineole, and other terpenes, tannins, camphor, flavonoids, triterpene: urolic acid) for curing and preventing diseases. This plant has valuable antimicrobial resources. These plants can produce a large number of secondary metabolites which can produce significant action against human pathogens. Ocimum extract inhibit the growth of Bacillus subtilis. From the diffusion method, the inhibition on microorganism with four zones (80, 70, 60 and 50 micro litres) sizes of 2.1cm, 1.8cm, 1.7cm and 1.4cm. From this, inhibition was increased with the concentration of Ocimum extract. Diabetes mellitus is one of the most common diseases characterized by hyperglycaemia. One approach is to reduce the production of glucose in the digestive tract and delay the absorption through the inhibition of alpha amylase enzyme and alpha glucosidase. The absorbance values of alpha amylase tubes with 0, 0.5, 1, 1.5, 2 and 2.5ml Ocimum extract were 0.34, 0.29, 0.21, 0.16, 0.12 and 0.06. The absorbance values of alpha glucosidase tubes with 0, 0.5, 1, 1.5, 2 and 2.5ml Ocimum extract were 0.63, 0.56, 0.48, 0.32, 0.17 and 0.13.Increasing the concentration of Ocimum extract decreases the activity of enzymes which play key role in carbohydrate hydrolysis in the digestive tract.

Introduction

The World Health Organization (WHO)) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world [16]. Plants are the primary source of medicines. Medicinal plants are considered to be very rich sources of secondary metabolites and oils which are of therapeutics importance. The important advantages of medicinal plants in various treatments are: their safety besides being less expensive, efficacy and availability throughout the world [7]. Plant extracts are potentially curative. Some of these extracts can boost the humoral [9] and cell mediated immunity [10] against viruses [11], bacteria [12], fungi [13], protozoa [14] and cancer [15]. Among the plants known for medicinal value, the plants of genus Ocimum belonging to family Labiatae are very important for their therapeutic potentials. *Ocimum sanctum L.* (Tulsi), known as ‘Tulsi’ in Hindi and ‘Holy Basil’ in English, is an erect softy hairy aromatic herb or under shrub found throughout India. Tulsi is commonly cultivated in gardens [2]. Leaves of tulsi contain number of active ingredients having very good medicinal value[3,4,5]. The leaves contain an essential oil, which contains eugenol, eugenal, carvacrol, methylchavicol, limatrol and caryophylline. The seeds contain oil composed of fatty acids and sitosterol. The roots contain sitosterol and three triterpenes A, B, and C. The leaves also contain ursolic acid and n-triacontanol. Eugenol, its methyl ether, nerol, caryophyllene, terpinen 4-decylaldehyde, selinene, pinenes, camphene and a-pinene have been identified in essential oil. Additionally, it also contains rosmarinic acid, thymol, linalool and methyl chavicol and citral etc.[8]. Eugenol is an allyl chain substituted guaiacol [3]. Eugenol is a member of the phenylpropanoids class of chemical compounds. It is a clear to pale yellow oily liquid extracted from certain essential oils especially from Holy basil (Tulsi) and bay leaf, clove oil, nutmeg, cinnamon etc. It is slightly soluble in water and soluble in organic solvents. It has a spicy, clove-like aroma [6]. Previous studies on volatile oils from leaves of *O. micranthum* Willd. reported the occurrence of three chemotypes. The Indian

chemotype which contain mainly eugenol, 1,8-cineole, β-caryophyllene and γ-elemene [1]. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post prandial blood sugar levels. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025. WHO has predicted that the major burden will occur in developing countries. Studies conducted in India in the last decade have highlighted that not only is the prevalence of diabetes high but also that it is increasing rapidly in the urban population [17]. Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction. Type I diabetes is caused due to insulin insufficiency because of lack of functional beta cells. Patients suffering from this are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes (insulin independent) are unable to respond to insulin and can be treated with dietary changes, exercise and medication. Type II diabetes is the more common form of diabetes constituting 90% of the diabetic population [18]. There are many approaches to treat diabetes, in those; one approach is to decreasing the postprandial hyperglycaemia. This can be achieved by delaying the absorption of glucose through the inhibition of carbohydrate hydrolysing enzymes alpha amylase and alpha glucosidase in the digestive tract [20]. This plant has valuable antimicrobial resources. These plants can produce a large number of secondary metabolites which can produce significant action against human pathogens [19]. The main objective of this paper is to find the effect of *Ocimum sanctum* leaves extract concentration on the alpha amylase, alpha glucosidase activity and microorganism growth.

Materials And Methods

Preparation of *Ocimum* Extracts

Leaves of *Ocimum Sanctum Linn* (Tulsi) collected in the campus of National Institute of Technology, Warangal, and Andhra Pradesh, India. Leaves of O. sanctum were dried in shade and powdered. The leaf powder (100 g) was refluxed with 750 ml of double distilled water for 1 h at 75–80_C. It was then cooled and filtered. This was repeated in three trials. The extracts were pooled and evaporated using lyophilizer.

Microbial growth

Anti microbial activity of Ocimum extract was determined using agar well diffusion method. For this, *Bacillus subtilis* culture was used. Nutrient agar medium was prepared and sterilized at 121^0C for 15min before inoculation. Sterile agar medium was poured into the sterile plates. The plates were incubated at 37^0C for one day. 1ml of inoculum was added to plates next day. The plates were allowed to set. Each plate was divided into four sectors and in each sector a bore of 5mm diameter was made using sterilized borer in the solidified medium. Four bores in different sector were loaded with 50, 60, 70 and 80 micro litres of Ocimum extract and allowed to diffuse at room temperature for 3hours. The plates were then incubated at 37^0C for 24hrs for Bacillus. The zone of inhibition of growth of Bacillus around the bore was measured with the scale.

Amylase and glucosidase activity

Starch solutions of 50g/l concentrations were prepared for both enzyme assays. 0.04g/l of both enzyme solutions were prepared. 6 test tubes were taken for each enzyme assay. For the amylase assay, First 1ml of starch solution is added to the test tube. To these, different concentrations (0, 0.2, 0.4, 0.6, 0.8 and 1ml) of plant extracts were added to tubes. Then 1ml of enzyme was added to those. The reaction mixture was allowed to incubate for exactly *10 minutes. 1ml of DNS (3, 5-dinitrosalicylic acid) reagent was added to each tube and covered the test tubes with aluminum foil. The contents were heated in the test tubes in a boiling water bath for 5 minutes and then tubes were cooled. 9ml distilled water was added to each test tube and mixed well. 1ml of this final solution was taken from test tube and transferred into different cuvettes and each cuvette was placed in a UV spectrophotometer and the absorption values of glucose by UV spectrophotometer at 540nm. The Glucose formed can be analyzed by Glucose standard curve by DNS method. Same procedure was repeated for glucosidase assay.*

Result And Discussion

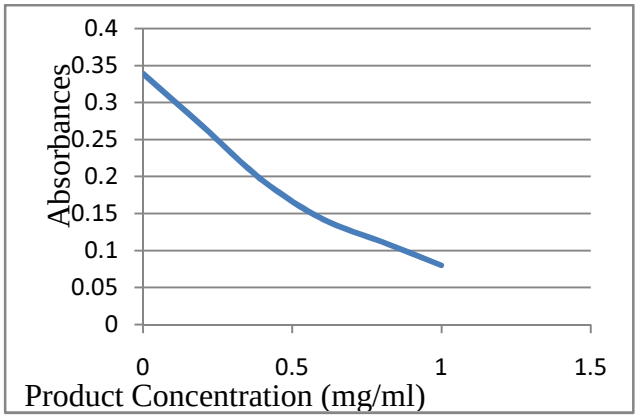

Figure 1: Effect of *Ocimum* on amylase activity

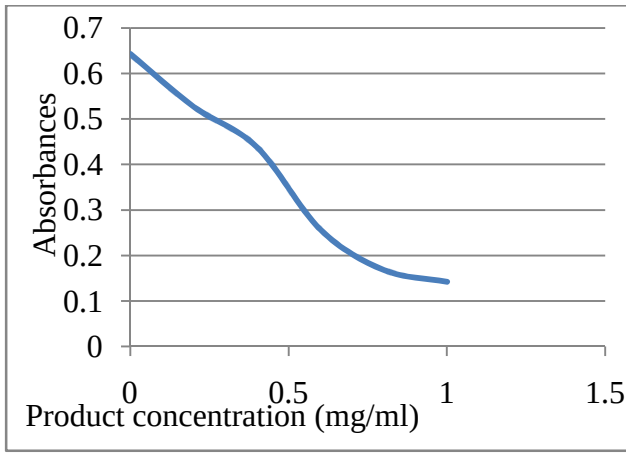

Figure 2: Effect of *Ocimum* on glucosidase activity

The effect of Ocimum *extract inhibited the growth of* Bacillus subtilis*. From the diffusion method, the inhibition on microorganism with four zones (80, 70, 60 and 50 micro liters) sizes of 2.1cm, 1.8cm, 1.7cm and 1.4cm. From this, inhibition was increased with the concentration of* Ocimum *extract. First zone was loaded with 50 micro liter had less inhibition where as 80 micro liter had maximum inhibition. The existence of two types of linkages, the alpha-1, 4 and the alpha-1, 6 different structures are possible for starch molecules. An unbranched, single chain polymer of 500 to 2000 glucose subunits with only the alpha-1, 4 glucosidic bonds is called* amylose*. On the other hand, the presence of alpha-1, 6 glucosidic linkages results in a branched glucose polymer called* amylopectin*. The degree of branching in amylopectin is approximately one per twenty-five glucose units in the unbranched segments. Alpha amylase and alpha glucosidase break down the starch solution at alpha-1, 4 bonds. Depending on the relative location of the bond under attack as counted from the end of the glucose chain (starch), the products of this digestive process are maltose, and glucose, etc. 3, 5-Dinitrosalicylic acid is an aromatic compound that reacts with reducing sugars and other reducing molecules which absorbs light strongly at 540 nm. From table 1, the product concentration is decreasing gradually with the increasing the concentration of* Ocimum *extract. The absorbance values of alpha amylase tubes with 0, 0.5, 1, 1.5, 2 and 2.5ml* Ocimum *extract were 0.34, 0.29, 0.21, 0.16, 0.12 and 0.06. The absorbance values of alpha glucosidase tubes with 0, 0.5, 1, 1.5, 2 and 2.5ml* Ocimum *extract were 0.63, 0.56, 0.48, 0.32, 0.17 and 0.13. At 0 concentration of* Ocimum *extract, enzymes had maximum activity because there was no inhibition on the enzyme activity. That means enzymes broke down starch completely, consequently the product formed concentration was high. The tubes of both alpha amylase and alpha glucosidase with 0.5ml concentration of* Ocimum *extract got 0.34 and 0.63. So at less concentrations of* Ocimum *extract the activity of the enzymes were high. The tubes of both alpha amylase and alpha glucosidase with 2.5ml concentration of* Ocimum *extract got 0.06 and 0.17. So at high concentrations of* Ocimum *extract the activity of the enzymes were low. At 2.5ml concentration of* Ocimum *extract, enzymes had least activity because there was maximum inhibition on the enzyme activity. That means enzymes could not break down starch, consequently the product formed concentration was low. Finally, increasing the concentration of* Ocimum *extract decreases the activity of enzymes which play key role in carbohydrate hydrolysis in the digestive tract.*

Conclusion

From the diffusion method, the inhibition on microorganism with four zones (80, 70, 60 and 50 micro liters) sizes of 2.1cm, 1.8cm, 1.7cm and 1.4cm. From this, inhibition was increased with the concentration of Ocimum *extract. The absorbance values of alpha amylase tubes with 0, 0.5, 1, 1.5, 2 and 2.5ml* Ocimum *extract were 0.34, 0.29, 0.21, 0.16, 0.12 and 0.06. The absorbance values of alpha glucosidase tubes with 0, 0.5, 1, 1.5, 2 and 2.5ml* Ocimum *extract were 0.63, 0.56, 0.48, 0.32, 0.17 and 0.13.Increasing the concentration of* Ocimum *extract decreases the activity of enzymes which play key role in carbohydrate hydrolysis in the digestive tract.*

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