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*Invitro*antidiabetic Activity of Nerolidol: An Active Compound Isolated From *Alpinia Calcarata*

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Abstract: ***Diabetes mellitus is a well known clinical ailment leading to various late complications like retinopathy, neuropathy, nephropathy etc. The results of the work indicate that the nerolidol characterized from Alpinia calcarata possessed considerable invitro antidiabetic activity. Alpinia calcarata Roscoe is known for its therapeutical use in Indian traditional medicine. It is therefore essential to investigate the inherent profile of phytocompounds which confer therapeutic value. This analysis focuses on the antidiabetic potential of the selected species. Synthetic inhibitor often causes side effect such as abdominal pain, diarrhoea etc. Nerolidol seems promising for the development of a phytomedicine for Diabetes mellitus. It has been found that the IC 50 of Acarbose (standard) is 220µg/ml and while in the case of nerolidol it exhibits much more activity ie, IC 50 is 130µg/ml.***

Keywords: Diabetes mellitus, nerolidol,Alpha amylase inhibition, Acarbose, hyperglycemia, IC_{50}.

1. Introduction

Diabetes mellitus is one of the most common and serious chronic disease and is characterized by hyperglycemia[1-2] and a disorder of carbohydrate, fat and protein metabolism attributed to diminished production of insulin or mounting resistance to its action[3]. The presence of Diabetes mellitus confers increased risk of many devastating complications such as cardiovascular diseases, peripheral vascular disease[4-5]complications such as coronary artery disease, stroke, neuropathy, renal failure, retinopathy amputations and blindness[6].Diabetes is an endocrine dysfunction resulting from insulin deficiency or incapability of peripheral tissues to respond to insulin[7-8].Diabetes results in abnormal levels of glucose in the blood stream. A person suffering from diabetes is expected to rise to 366 million by 2030[9-10]. Scientific validation of several Indian plant species has proved the efficacy of the botanicals in reducing the sugar level and many remain to be scientifically investigated[11-13].The main disadvantages of the currently available drugs are that they have to be given throughout the life and produce side effects[14]. More than 1200 plant species have been used to treat diabetes in folk medicine[15-16]and there are 136 plant species clearly showed the anti-diabetic effects[17-18].The bioactive compounds of medicinal plants are used as antidiabetic, chemotherapeutic, anti-inflammatory, anti-arthritic agents where no satisfactory cure is present in modern medicines[19].Herbal medicines are getting more importance in the treatment of diabetes as they are free from side effects, less expensive cost affordable when compared to synthetic hypoglycemic agents[20-21].

Alpinia calcarata Roscoe (Family: Zingiberaceae), is a rhizomatous perennial herb, which is commonly used in the traditional medicinal systems. *A. calcarata* is cultivated in tropical countries, including Sri Lanka, India, and Malaysia[22]*A. calcarata* rhizomes are known to possess a broad spectrum of medicinal properties. Experimentally, rhizomes of *A. calcarata* are shown to possess antibacterial[23], antifungal[24] anthelmintic, antinociceptive[25], antioxidant[26], aphrodisiac[27], gastroprotective[28]**,** antidiabetic activities[29], rheumatism, fever, stomachache[30] and anticancer activity. It is also widely used to relieve colds and reducing swellings[31-32] etc. In India, the dried rhizomes form a major ingredient of several Ayurvedic drug formulations such as Rasnadhi Kazhayam, Rasnadhi Choornam, Rashnadhi Thailam and Ashawagandharishtam[33]. Drugs from the rhizomes of *Alpinia calcarata* are used in treatment of rheumatism, bronchial catarrh and asthma. The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds[34-36].Because of the medicinal importance of the *Alpinia calcarata*, the present study is an attempt to isolate and identify the active components responsible for antidiabetic activity. The objective of this work was to evaluate the *invitro* antidiabetic activity of Nerolidol.

2. Materials and Methods

Collection of the Plant Sample

Fresh *Alpinia calcarata* rhizomes were cut into small pieces, air dried for 12-15 days in the shade and coarsely powdered. A voucher specimen was deposited in the Rapinat herbarium and centre for molecular systematics, Tiruchirappalli, Tamilnadu.

Preparation of Plant Extract

The coarsely powdered rhizome 80g was subjected to successive soxhlet extraction with solvents of increasing polarity Petroleum ether, Acetone, Methanol and Water respectively. Each extract were evaporated to dryness in a rotary evaporator. The extracts were preserved in an airtight container. Thin layer chromatography was used to determine the suitable solvent systems for column chromatography. The methanol extract showed a high antimicrobial activity, so this extract was subjected to the column chromatography for the separation of the compounds. For purification and isolation, the active methanolic plant extracts were fractionated and each fraction or pure compound was subjected to alpha amylase Inhibition assay for evaluating the activity. The structure of isolated, purified active compound was determined by spectroscopic methods like UV, IR, NMR, Mass Spectrum etc.

Invitro method involved in antidiabetic study

Experimental procedure for alpha amylase Inhibition assay

A total of 500μl of test sample and standard drug (100-1000μg/ml) were added to 500μl of 0.20mM phosphate buffer(pH 6.9) containing α-amylase(0.5mg/ml) solution and were incubated at 25^0 C for 10min.After these,500μl of a 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25^0C for 10 min. The reaction was stopped with 1.0ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling waterbath for 5min, cooled to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540nm.Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

Calculation of 50% inhibitory concentration (IC50)

The concentration of the nerolidol required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at 6 different concentrations of the compound. Percentage of inhibition was calculated by

$$\text{Inhibition \%} = \frac{\text{Ac 540 (control)} - \text{As 540 (sample)}}{\text{Ac 540 (control)}} \times 100$$

Where,

Ac = Absorbance of the control at 540nm
As = Absorbance of the sample at 540nm

The IC 50 values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitor. All tests were performed in triplicate.

Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± S.D.

3. Results and Discussion

The IR, ^{1}H-NMR and ^{13}CNMR spectra of the extracted fraction was used for the identification and structure of the isolated compound *i.e.*, nerolidol. The structure of the nerolidol is given in fig.1.

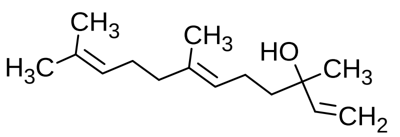

Figure 1: Chemical structure of Nerolidol

Column chromatic single fractions of methanolic extracts of *Alpinia calcarata* (AC1,AC2,AC3) were subjected to antidiabetic acivity, but nerolidol (AC1) only showed invitro alpha amylase inhibition activity.Compared to the standard acarbose the nerolidol showed much significant activity against diabetes (Table1). Alpha amylases play an important role in starch breakdown in human beings and animals, the presence of such inhibitors in food stuffs may be responsible for impaired starch digestion.The α-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in breakdown of starch. α- amylase inhibitors offer an effective strategy to lower the levels of post- prandial hyperglycemia via control of starch breakdown. Hence retardation of starch digestion by inhibition of enzymes such as α-amylase plays a key role in the control of diabetes. Inhibitors of pancreatic α-amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post-prandial serum glucose levels. Alpha amylase inhibitors are used to achieve greater control over hyperglycemia in type II Diabetes mellitus. Hence our finding reveals that nerolidol efficiently inhibits α-amylase enzyme *invitro* and it has potential to emerge as new remedy for treatment of diabetes.

Table 1: *Invitro*screening of α-amylase inhibition

Sample	*Con. (μg/ml)*	*% Inhibition*	*IC_{50} μg/ml*
Nerolidol	0 100 200 300 400 500	0 32.78±0.35 65.57±1.94 79.32±0.11 89.51±1.16 94.11±0.08	150±5.7
Acarbose (Standard)	0 100 200 300 400 500	0 40.73±1.39 49.34±1.04 55.62±0.81 63.48±1.22 65.97 ±1.39	230.71±7.89

All determinations were carried out in triplicate and values are expressed as the mean ± SEM. The IC50 value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions.

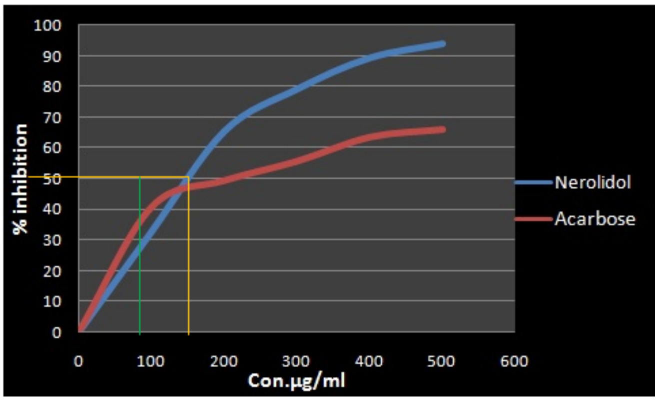

Figure 2: IC50 values of Acarbose (standard) and nerolidol

4. Conclusion

Diabetes mellitus is a global health. The Nerolidol is proved to possess significant antidiabetic activity so that it can be used as an adjuvant along with allopathic treatment of medicine to treat diabetes as well as to delay the late complications of diabetes. Our studies have confirmed that the nerolidol possessed high *invitro* antidiabetic properties. The present investigation has also opened avenues for further research especially with reference to different compounds present in *Alpinia calcarata* and their therapeutic value.

Current knowledge on altered body metabolism during diabetes mellitus can be utilized for development of new trends in herbal antidiabetic research. The future scope of the study is the bioactivity of the nerolidol have a promising role in controlling blood glucose level. Also the study reveals that *Alpinia calcarata* has significant antidiabetic activity and the nerolidol seems promising for the development of a phytomedicine for diabetes mellitus. Nerolidol play a significant role in management of diabetes, which needs further exploration for nesscessary development of drugs and neutraceuticals from this resource.

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