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**IN-VITRO PHARMACOLOGICAL SCREENING OF ANTI-DIABETIC
AND ANTI-HYPERLIPIDEMIC ACTIVITY OF POLYHERBAL
EXTRACT**

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ABSTRACT

Diabetes Milletus is a metabolic condition by the common trait of chronic hyperglycemia. Hyperlipidemia is an abnormally elevated levels of any or all lipids or lipoproteins in the blood. An in-vitro study was carried for polyherbal extract to determine the combined effect of Anti-diabetic and Anti-Hyperlipidemic effect. The activity was confirmed using Alpha- glucosidase inhibition method and HMG-CoA reductase inhibition assay method. In the α - Glucosidase Inhibition assay was performed on various concentration like 10, 20, 40, 80, 160, 320 ($\mu\text{g/ml}$). The effect of the extract at various concentrations were compared with standard Voglibose. The maximum inhibitory concentration IC 50 value of the sample (TEE) and the standard drug (Voglibose) was found to be 22.8 $\mu\text{g/ml}$ and 11.39 $\mu\text{g/ml}$. The Hyperlipidemic aactivity was determined by HMG-CoA inhibition assay at various concentrations. The percentage inhibition of Polyherbal extract is compared with the standard drug Atorvastatin. The IC₅₀ value of the sample was found to be 89.54 $\mu\text{g/ml}$ and the standard (Atorvastatin) was 20.46 $\mu\text{g/ml}$, respectively. Overall, the study provides more information about the combination effects for the treatment of Diabetes milletus and Hyperlipidemia conditions, but more researches on animal studies will be more helpful to understand its therapeutic potential and safety profile.

Keywords: Poly herbals, *in-vitro* anti-diabetic, anti-hyperlipidemic activity

INTRODUCTION

According to the WHO, Diabetes mellitus is the most important disease which causes death and high mortality in people of any age group, or sex [1]. Especially in case of diabetes is a raised blood glucose level, which is a common cause of the disease, which may also lead to various secondary complications like increase in obesity, Cardiovascular diseases [2]. The major factors of the diabetes are the Type 1 and Type 2 Diabetes, the reason for type I is associated with interaction between genes and various environmental factors but the exact reason was not known [3]. The type II diabetes is caused by various reasons like stress, Lack of Physical activity and unusual eating habits, Smoking and drinking [4]. The people with type II diabetes have higher risk of getting cardiovascular diseases, especially due to hyperlipidemia conditions. Diabetes patients have a CVD death rate of 1-3 in males and 2-5 in females compared to non-diabetic individuals [5]. Higher waist circumference and body mass index (BMI) are linked to a higher risk of type 2 diabetes, albeit this association may alter amongst groups populations in south-east Asia [6]. The complex relationship between diabetes and hyperlipidemia poses a challenge for treating both conditions. Diabetes, dyslipidemia,

hypertension, and obesity are common risk factors which may cause serious complications among people. Diabetes patients often have numerous causes of CVD, including neuropathy, endothelial dysfunction, coagulation enhancement, and increased oxidative stress which are due to the presence of high lipid profile and excessive Body mass index [7]. In 2021, Type 2 diabetes was responsible for 96.0% (95.1-95.8) of diabetes cases and 95.0% (94.9-95.9) of diabetes DALYs globally. In 2021, high BMI accounted for 52.2% (25.5-71.8%) of type 2 diabetes-related DALYs worldwide. The impact of high BMI on type 2 diabetes DALYs increased by 24.3% from 1990 to 2021 (18.5-30.4%) globally [8] [9]. Several plants have been acknowledged in traditional medicine for their effectiveness in treating various systemic disorders. Traditional herbal remedies are naturally occurring plant-derived substances that have undergone minimum or no industrial processing and have been utilized to treat illness in local or regional healing traditions. While many traditional or indigenous medicinal techniques are regarded as more beneficial than modern medicine, they face difficulties due to a lack of comprehensive standardization. Polyherbal compositions may increase pharmacological

activity while lowering the amounts of individual herbs, hence decreasing unwanted effects. It is still difficult to discover a successful medicine, whether alone or in combinations. The selected plant materials are *Nigella sativa* [10], *Eugenia jambolana* [11], *Cajanus cajan* [12], *Cassia auriculata* [13], *Linum usitatissimum* [14], *Garcinia cambogia* [15], *Vitis vinifera* [16], and all these seven plants have traditional claim in treating Diabetes and Hyperlipidemia. Hence the study was aimed on Polyherbal extract for treating Diabetes and Hyperlipidemia conditions through In-vitro methods.

MATERIALS AND METHODS

Collection of plant material

The plant materials *Nigella sativa* (Seeds), *Eugenia jambolana* (Seeds), *Cajanus cajan* (Seeds), *Cassia auriculata* (Flowers), *Garcinia cambogia* (Fruits), *Linum usitatissimum* (Seeds), *Vitis vinifera* (Seeds) was collected from the local market, Chennai, Tamilnadu on October 2023.

Identification and Authentication

The Plant materials used in this study was botanically identified authenticated (Certificate No.702.28122301-07) by Dr. K.N.Sunil Kumar, Research officer and Head of Pharmacognosy, Centre Council for Research in Siddha, Chennai, Ministry of

AYUSH, Government of India, Arumbakkam, Chennai-600106.

Processing of the plant materials:

The obtained leaves were thoroughly cleaned with distilled water, cleaned, and cut into pieces before being air dried. The pieces were then pulverized in a grinder and passed through a sieve (mesh no.4) to produce powder of the same size. The powder was employed throughout the extraction process.

Preparation of plant Extract

The plant materials *Nigella sativa* (Seeds), *Eugenia jambolana* (Seeds), *Cajanus cajan* (Seeds), *Cassia auriculata* (Flowers), *Garcinia cambogia* (Fruits), *Linum usitatissimum* (Seeds), *Vitis vinifera* (Seeds) were powdered. These powdered plant materials were extracted using Ethanol which are proven to be a universal solvent. Ethanol was opted for the extraction of the active constituents. The method of extraction is **COLD MACERATION** process. The process was continued for Seven days. After Seven days the extract was filtered using Whatman NO.1 filter paper, which was then submerged in the rotary vacuum evaporator at 40° C, under reduced pressure. The semisolid mass was kept at 4° C in storage containers. The dried extract of seven plants was mixed in equal ratios and labeled as polyherbal extract

(PHE). The percentage yield of 50% ethanol is calculated.

IN- VITRO STUDIES OF ANTI HYPERLIPIDEMIC ACTIVITY HMG-CoA REDUCTASE INHIBITORY ASSAY [17, 18]

The in vitro HMG-CoA reductase activity was measured by using the Sigma-Aldrich HMG-CoA reductase assay Kit under the conditions recommended by the manufacturer's specified conditions. This kit includes of HMG CoA reductase assay buffer, HMG COA reductase, NADPH, and an inhibitor (Atorvastatin).

HMG CoA reductase is a key enzyme that regulates the manufacture of glaucy CoA (HMG COA) 10 mevalonate and cholesterol biosynthesis from acetyl CoA. This enzyme reduces -hydroxy, 3-methyl

Cholesterol cannot be produced because this enzyme is inhibited. This *in vitro* model evaluates the hypolipidemic activity of a chemical moiety. The current tarin

derivatives display hypolipidemic action via this enzyme inhibition mechanism.

Inhibiting HMG CoA reductase increases the production of LDL receptors in the liver, lowering cholesterol levels in the blood. This is a reaction that depends on NADPH. Commercial kits for HMG CoA assays are available.

The inhibitory action of plant extracts against HMG-CoA reductase was assessed using spectrophotometric techniques.

Various concentrations of sample (10, 20, 40, 80, 160, 320 µg/ml) was mixed with the reaction mixture containing 60µg/ml of NADPH (400 µM), HMG-CoA substrate (400µµM) and Pottasium phosphate buffer (100mM. pH 7.4) containing KCI (120mM) EDTA (1mM) and DTT (5mM) followed by addition of HMG-CoA reductase (180 l). Atorvastatin was used as a standard. The reactants were incubated at 340nm after 10 mins.

Use the calculation below to get the percentage of inhibition

$$\% \text{ HMG-CoA inhibition} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

**IN VITRO STUDIES OF ANTI DIABETIC
ACTIVITY
ALPHA GLUCOSIDASE INHIBITORY
ASSAY [19, 20]**

Sample extraction

2.5 grams of powdered material were extracted with ethanol using a soxhlet equipment. The extract was collected and condensed under reduced pressure in a rotary vacuum evaporator before being stored at 4 degree celsius

The inhibition action of alpha-glucosidase was determined.

Materials required

Phosphate buffer : 50mM, pH 6.8

Sodium carbonate : (0.1M).

PNPG : 1Mm

Sample: Concentrations of extracts range from 20 to 100 µg/m.

Alpha-glucosidase : lu/ml-SRL

Alpha-glucosidase Inhibitory assay:

The effect of plant extracts on alpha-glucosidase activity was assessed using the alpha-glucosidase enzyme, as described by Apostolidis *et al* [1]. The substrate solution, p-nitrophenyl glucopyranoside (PNPG), was produced in 100 Mm phosphate buffer and pH 6.8. 200 µL of a alpha-glucosidase was pre-incubated with different concentrations of the extracts (10,20,40,80,160, and 320) for 10 minutes. To initiate the reaction, add 400 µL of 5.0 mM (pNPG) substrate diluted in 100mM phosphate buffer (pH 6.8). After 20 minutes of incubation at 37 degree Celsius, the reaction was halted by adding 1 mL of Na₂CO (0.1M). The yellow coloured reaction mixture, 4-nitrophenol, produced from PNPG was detected at 405 nm using a UV-VIS spectrophotometer. The inhibitory activity of alpha glucosidase was determined using the following formula, with voglibose as a positive control.

$$\text{Percentage Inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

Calculation:

$$\text{Percentage Inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

Voglibose was utilized as a positive control. The inhibitory activity of a alpha-glucosidase.

% Inhibition-[(Abs Control - Abs Sample)/ Abs Control) x 100

CALCULATION:

Inhibition (%) = $\frac{\text{Absorbance. Control} - \text{Absorbance. Sample}}{\text{Abs. control}} \times 10$.

RESULTS:

The percentage of successful Anti-Hyperlipidemic activity for Poly herbal extract was determined based on spectrophotometric measurements on different concentrations of sample (10, 20, 40, 80, 160 and 320 µg/ml), Atorvastatin was used as a standard. Absorbance was recorded and shown in **Table 1**. When Hyperlipidemia was treated with various concentrations 10, 20, 40, 80, 160 and 320 µg/ml, of the test sample, i.e., 89.54 percent, was observed. When compared with the control group (Atorvastatin), the percentage inhibition was observed to be 20.46. **Figure 1** depicts the

percentage inhibition of the 50% ethanolic extract with the standard (Atorvastatin).

The Alpha- glucosidase inhibition assay was carried to determine the *in-vitro* Anti-diabetic activity of Poly herbal extract and Voglibose was used as the reference drug in this study. The *in- vitro* anti-diabetic activity of a 50% ethanolic extract of Poly herbal extract was subjected to various concentration such as (10, 20, 40, 80, 160 and 320 µg/ml), which was compared with the standard drug voglibose. The percentage inhibition of test drug was 22.28 %, which is compared with the standard drug (Voglibose) was recorded at various concentrations the percentage inhibition of standard was 11.39%. which is shown in **Table 2**. Percentage of inhibition after treatment with different concentrations of the 50% ethanolic extract and standard drug is shown in **Figure 2**.

IN- VITRO STUDIES OF ANTI HYPERLIPIDEMIC ACTIVITY HMG-CoA Reductase Inhibition Assay

Table 1: HMG-COA reductase inhibitory assay

SAMPLE	CONCENTRATION	S.D	IC50
ATORVASTATIN	10	25.245002	
	20	48.8827911	
	40	75.9310074	20.46
	80	85.2606821	
	160	93.6887495	
	320	95.6879655	
EXTRACT	10	11.368091	
	20	19.325755	89.54
	40	31.28185	
	80	47.785182	
	160	60.603685	
	320	76.636613	

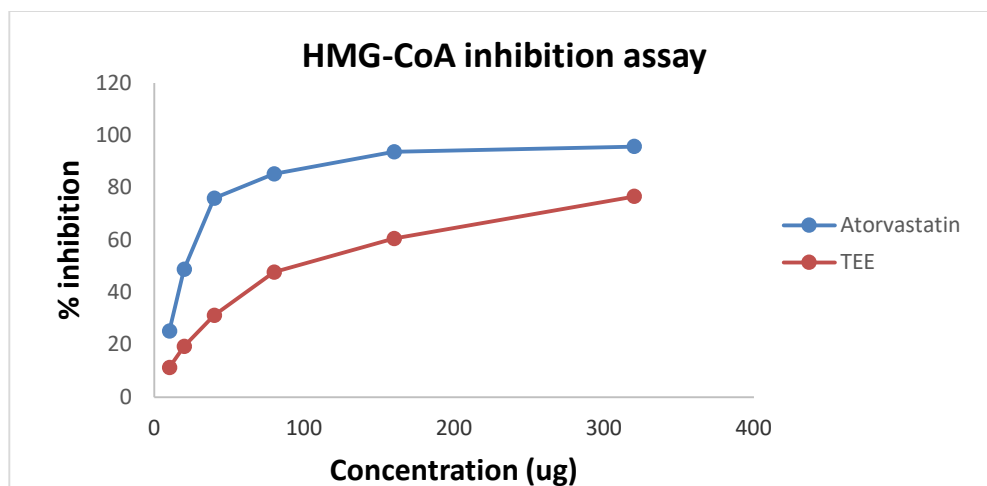


Figure 1: Graphical representation of HMG –CoA reductase inhibitory assay

IN VITRO STUDIES OF ANTI DIABETIC ACTIVITY

ALPHA GLUCOSIDASE INHIBITION ASSAY:

Table 2: Alpha-glucosidase Inhibition assay:

SAMPLE	CONCENTRATION	S.D	IC50
VOGLIBOSE	10	0.989632	
	20	0.588595	
	40	0.571365	11.39
	80	0.431911	
	160	0.355789	
	320	0.424128	
EXTRACT	10	0.294298	
	20	0.374046	22.28
	40	0.294298	
	80	0.45446	
	160	0.431911	
	320	0.282752	

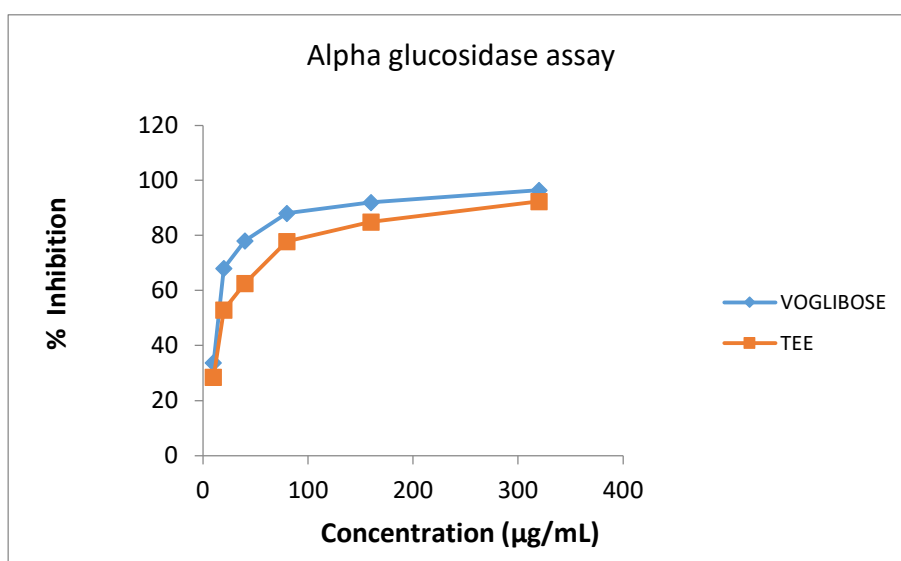


Figure 2: Graphical representation of Alpha glucosidase Inhibition assay

DISCUSSION

Even though significant advances and technologies has been developed for the treatment of Diabetes and Hyperlipidemia. Due to lack of standardization procedures and increased side effects and adverse effects has made people to switch on traditional medicine. When compared to the single drug, poly herbal extract has greater pharmacological actions and synergistic, additive effects. *in vitro* assays are used to determine the effectiveness of extract towards the diseases. They can provide insight into the effectiveness of the Poly herbal extract and whether it has potentially therapeutic effects. Poly herbal extract contains *Nigella sativa*, *Eugenia jambolana*, *Cajanus cajan*, *Cassia auriculata*, *Linum usitatissimum*, *Garcinia cambogia*, *Vitis vinifera* were significantly reduced blood glucose levels and lipid profile at various concentrations. The polyherbal extract has greater potency when compared to the standard drugs. The current investigation suggested that poly herbal extract may have Anti- diabetic and Anti-Hyperlipidemic effect.

CONCLUSION

Our study demonstrates that the diabetes has a greatest impact in people in all over the country which leads to secondary complications like Hyperlipidemia condition.

Due to the increasing population with Diabetes will also have unwanted conditions like hyperlipidemia are main cause of the mortality and death. So, the experiment focusses on the both diseases and their prevention and control. Polyherbal extract highlight the therapeutic potential for the treatment of Anti-diabetic and Anti-Hyperlipidemic effect.

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DECLARATION

Conflict of interest

According to the authors, there are no conflicts of interest in this work.

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