

**IN VITRO ANALYSIS OF ANTI- ADVANCED GLYCATION EFFECT  
OF POLYHERBAL COMPOUND *TRIPHALADI COMPOUND CAPSULE*  
AND *ELANEER KUZHAMBU ANJANA***

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**ABSTRACT**

**Background-** Non-enzymatic or Advanced glycation between reducing sugar and protein results in the formation of fluorescent and insoluble advanced glycation end products (AGEs), which is believed to play important roles in the pathogenesis of diabetic and aging complications. **Objectives-** To determine anti- advanced glycation activity of an *Anubhuta* (experienced) polyherbal formulation *Triphaladi* Compound capsule and *Elaneer Kuzhambu Anjana* a semi-solid drug of eye drop mentioned in *Ayurvedic* text *Sahastrayoga*, to

the reference Arbutin a known inhibitor of glycation reaction by using an *in vitro* glucose-bovine serum albumin (BSA) assay. **Material and Method-** Glucose and BSA were incubated at 60°C in the presence and absence of drug samples. Following a 24-hour incubation period, the Glycated BSA product was precipitated with Trichloroacetic acid (TCA) and redissolved in alkaline phosphate buffered saline (PBS). In spite of the sample, the phosphate buffer saline was used as the sample control and Arbutus was used as the reference standard. Negative control was carried out at the same time with BSA, phosphate buffer saline and drug sample incubated under the same conditions. The formation of Glycated BSA was relatively quantified based on fluoresce intensity by measuring fluorescence intensity. Excitation and emission wavelengths were at 370 nm and 440 nm, respectively. Each sample was analyzed in triplicate form. **Results-** *Triphaladi* Compound

capsule and *Elaneer Kuzhambu Anjana* showed a significant increase in anti advanced glycation activity with an increase in their concentration. **Conclusion-** The present study showed that *the Triphaladi* compound capsule and *Elaneer Kuzhambu Anjana* as an anti-glycation agent may prove beneficial in diabetic and aging complications.

**KEYWORDS:** *Elaneer Kuzhambu Anjana; Triphaladi compound capsule; Arbutin; advanced glycation end product; glucose-BSA assay.*

## 1. INTRODUCTION

Advanced glycation is a non-enzymatic condensation reaction between reducing sugars and amino groups of proteins that undergo rearrangements to stable ketoamines, leading to the formation of advanced glycation end products (AGEs).<sup>[1]</sup> It is a spontaneous reaction that is dependent on the degree and duration of hyperglycemia, the half-life of the protein and permeability of the tissue to free glucose.<sup>[2]</sup> This reaction was first studied by Maillard in the early 1900 & is known as Maillard reaction. This reaction was initially described in the context of food science where its products were found to impart changes in food texture, bioavailability, flavor & preservation. Increased glycation and build-up of tissue AGEs have been implicated in diabetic complications because they can alter enzymic activity, decrease ligand binding, modify protein half-life and alter immunogenicity.<sup>[3]</sup> There is accumulating evidence that AGEs could play an important pathogenic role in eye diseases like diabetic retinopathy, age-related macular degeneration, and cataract.<sup>[4]</sup> AGE formation is a key component of pathophysiological processes with links to a range of important human diseases.<sup>[5]</sup> However, it is not clear how AGEs may act as mediators, not only in diabetic complications but also in widespread age-related pathologies such as Alzheimer's disease<sup>[6]</sup>, decreased skin elasticity<sup>[7]</sup>, male erectile dysfunction, fibrosis<sup>[8]</sup>, and atherosclerosis.<sup>[9]</sup> The plants derived agents with the antiglycation and antioxidant activities are highly important in preventing diabetic complications and over the last few decades, the reputation of herbal remedies has increased globally due to its therapeutic efficacy, safety, minimal adverse effects, and low cost.

Many ingredients of both the test drugs have anti-glycation activity as listed in Table-1 & Table-2. So both the formulations were studied to know their anti-glycation activity in this study. *Elaneer Kuzhambu Anjana*<sup>[10]</sup> is extensively used clinically for centuries in Kerala by local physicians to treat ocular diseases. *Triphaladi* compound capsule contains eleven *Ayurvedic* drugs; the maximum among them was having anti-glycation activity.

## 2. MATERIALS AND METHODS

### A. Materials

The study was carried out as a PhD work at, Department of Shalakyatantra, Institute for Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat - 361008.

Bovine Serum Albumin for the assay this was procured from Hi-Media Company. Arbutin was procured from Sigma Aldrich and was used as a standard solution. Other agents like Glucose, Tri Chloroacetic Acid Phosphate Buffer like Sodium Dihydrogen Phosphate and Disodium Hydrogen Phosphate were of analytical standards. Test Drugs used were *Triphaladi* Compound Capsule and *Elaneer Kuzhambu Anjana* as shown in table 1 and table 2.

**Table 1: Ingredients of *Elaneer Kuzhambu Anjana* and their reported antiglycation activity.**

S. No	Name of Ingredients	Botanical name	Parts used	Reported Anti Glycation activity
1	<i>Daruharidra</i>	<i>Berberis aristata</i> DC.	Stem	Bhutkar, M et al. <sup>[11]</sup>
2	<i>Hareetaki</i>	<i>Terminalia chebula</i> Retz.	Pericarp	Lee, H. S. et al. <sup>[12]</sup>
3	<i>Amalaki</i>	<i>Emblica officinalis</i> Linn.	Pericarp	Nampoothiri SV et al. <sup>[13]</sup>
4	<i>Vibheetaki</i>	<i>Terminalia bellerica</i> Roxb.	Pericarp	Kasabri, V et al. <sup>[14]</sup>
5	<i>Madhuka</i>	<i>Glycyrrhiza glabra</i> Linn.	Root & stolons	Siddiqui, M. A. et al. <sup>[15]</sup>
6	<i>Nalikerajala</i>	<i>Cocos nucifera</i>	Water from fruit	
7	<i>Pitakarohini</i>	<i>Coptis teeta</i> Wal.	Rhizome	
8	<i>Sasi</i>	<i>Dryobalanops aromatic</i>	Sublimed extract	
9	<i>Saindhava Lavana</i>	Rock salt		
10	<i>Makshika</i>	Honey		

**Table 2: Ingredients of *Triphaladi compound capsule* and their reported antiglycation activity.**

S. No.	Botanical name	Common names	Parts used	Reported Anti Glycation activity
1	<i>Terminalia chebula</i> Retz.	<i>Hareetaki</i>	Pericarp	Lee, H. S. et al. <sup>[16]</sup>
2	<i>Terminalia bellerica</i> Roxb.	<i>Vibheetaki</i>	Pericarp	Kasabri, V et al. <sup>[17]</sup>
3	<i>Emblica officinalis</i> Linn.	<i>Amalaki</i>	Pericarp	Nampoothiri SV et al. <sup>[18]</sup>
4	<i>Glycyrrhiza glabra</i> Linn.	<i>Yashtimadhu</i>	Roots & stolons	Siddiqui, M. A. et al. <sup>[19]</sup>
5	<i>Tribulus terrestris</i> Linn.	<i>Gokshura</i>	Fruits	Siddiqui, M. A. et al. <sup>[20]</sup>
6	<i>Tinospora cordifolia</i> Miers.	<i>Guduchi</i>	Stem	
7	<i>Curcuma longa</i> Linn.	<i>Haridra</i>	Rhizomes	Sovia, E et al. <sup>[21]</sup>
8	<i>Berberis aristata</i> DC.	<i>Daruharidra</i>	Stem	Bhutkar, M et al. <sup>[22]</sup>
9	<i>Ocimum sanctum</i> Linn.	<i>Tulasi</i>	Whole plant	
10	<i>Boerhavia diffusa</i> Linn.	<i>Punarnava</i>	Whole plant	
11	<i>Zingiber officinale</i> Rosc.	<i>Shunthi</i>	Rhizomes	

## B. Methods

The study was conducted at L J Institute of Pharmacy, Sarkhej, Ahmedabad, Gujarat 382210. This study was a part of PhD work carried out at Institute of Post Graduate Teaching & Research in Ayurveda entitled as Further study on evaluation of anti cataract effect of *Triphaladi* Compound Capsule and *Elaneer Kuzhambu Anjana* in Senile Immature cataract w.s.r. *Timira* and In Vitro analysis of Anti- AGE (Advanced Glycation End product) activity of test drugs.” Clinical study was started after getting clearance from Institutional Ethics Committee (No. PGT/7/-A/Ethics/2015-16/2625) and study was also registered under CTRI (CTRI/2016/03/006708)

Antiglycation activity was determined using the Bovine Serum Albumin assay.

### a. Preparation of solutions

- I. **Standard solution:** The stock solution of Arbutin 1 mg/ml was prepared in absolute ethanol and the working solution of Arbutin (100 µg/ml) was made from the stock solution by dilution with the required amount of double distilled water.
- II. **Test solution:** Test Drugs (2gm) were macerated for 24 h with a 50% hydroalcoholic mixture and resultant solution (100 mg/ml) was used as a stock solution and working solutions were made from the stock solution by dilution with the required amount of 50% hydroalcoholic mixture.

### b. Assay Procedure

The final reaction volume was 10 ml and carried out in test tubes. Bovine Serum Albumin (BSA) 5 ml (1 mg/ml concentration), glucose 4 ml (500 mM) and 1 ml of sample were incubated at 60°C for 24 hours and terminated by adding 100 µl of 100% (W/V) Trichloroacetic acid (TCA). In spite of the sample, the phosphate buffer saline 1 ml was used as the sample control and 1 ml Arbutin used as the reference standard. Negative control was carried out at the same time with BSA 5 ml (1 mg/ml concentration), 4 ml phosphate buffer saline and 1 ml sample incubated under the same conditions. The TCA added mixture was kept at 4 °C for 10 minutes and centrifuged 4 minutes at 13000 rpm. The precipitate was redissolved with alkaline phosphate buffer saline (pH 10) and was quantified for the relative amount of Glycated BSA based on fluoresce intensity. The excitation and emission wavelength used were at 370 nm and 440 nm respectively. Each sample was analyzed in triplicate form. The percentage of inhibition was calculated using the formula given below.

$$\% \text{ inhibition} = \frac{\text{OD blank} - (\text{OD sample} - \text{OD sample negative})}{\text{OD blank}} \times 100$$

c. Test solutions were divided into the following groups;

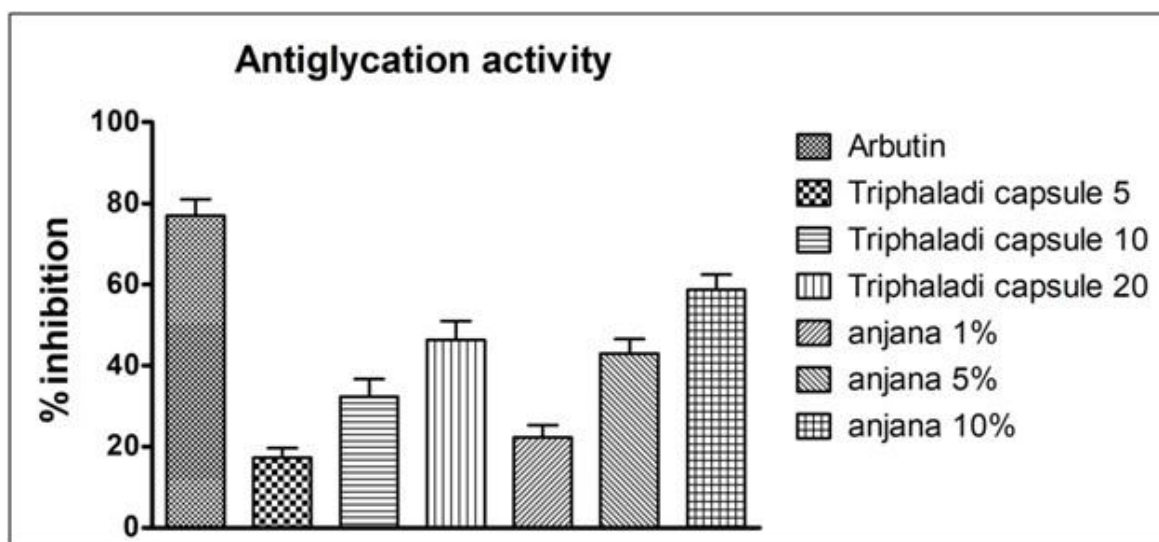
Sr. No.	Groups
1	Vehicle control (absence of drug)
2	Negative control (Saline Solution)
3	Standard control (Arbutin)
4	<i>Triphaladi</i> Compound Capsule (10 mg/ml)
5	<i>Triphaladi</i> Compound capsule (50 mg/ml)
6	<i>Triphaladi</i> Compound capsule (100 mg/ml)
7	<i>Elaneer Kuzhambu Anjana</i> (10 mg/ml)
8	<i>Elaneer Kuzhambu Anjana</i> (50 mg/ml)
9	<i>Elaneer Kuzhambu Anjana</i> (100 mg/ml)

### 3. Statistical analysis

Statistical analysis was performed by One Way Analysis of Variance (ANOVA) followed by Bonferroni's multiple comparison tests using Graph Pad Prism 5.0 software.

### 4. RESULTS

The results of the present study showed that *Triphaladi* Compound Capsule and *Elaneer Kuzhambu Anjana* possess the anti-advanced glycation end product activity. Both *Triphaladi* Compound Capsule and *Elaneer Kuzhambu Anjana* showed a significant increase in anti advanced glycation end product activity with increasing the concentration. (Fig.-1)



**Figure-1: Effect of *Triphaladi* Compound Capsule and *Elaneer Kuzhambu Anjana* on formation of Advanced Glycation End Products (AGEs).**

This anti advanced glycation end product activity of *Triphaladi* Compound Capsule and *Elaneer Kuzhambu Anjana* is comparable with the activity of Arbutin which was used as standard.

## 5. DISCUSSION

The study had highlighted the benefits of *Triphaladi* Compound Capsule and *Elaneer Kuzhambu Anjana* against the formation of Advanced Glycation End Products. Many components of the test drugs have been evaluated as inhibitors against the formation of Advanced Glycation End Products. *Triphala* (combination of *Terminalia chebula*, *Embolica officinalis* and *Terminalia bellerica*) extract effectively inhibits protein glycation which is contributed due to presence of tannins.<sup>[23]</sup> Total phenolic content, flavonoid and tannins contents are responsible for anti glycation activity of *Curcuma longa* likely by impeding further oxidation of glycated proteins and metal-catalyzed oxidation of glucose that leads to the formation of AGEs.<sup>[24]</sup> One of the study conducted on 26 medicinal plants revealed that, *Tribulus terrestris* and *Glycyrrhiza glabra* have properties against the in-vitro protein glycation. *Glycyrrhiza glabra* based herbal formulations are known to exhibit anti-AGEs activities. Additionally a pure substance (glycyrrhizic acid) from the roots of this plant showed anti-glycation potential in high fat diet treated rats. Major active component of *Glycyrrhiza glabra* include flavonoids, isoflavonoids, saponins, and tripentenenes. *Tribulus terrestris* contain phenols, saponins, alkaloids and sterols as active constituents.<sup>[25]</sup> Major constituents of *Boerhavia diffusa* are polyphenols and flavanoids. Ethanolic extract of *Boerhavia diffusa* has potent scavengers of these free radicals; it may also protect biological macromolecules to get damaged further, which in turn reverses or stops the glycation reaction.<sup>[26]</sup> The rhizome of *Zingiber officinale* contains over 20 phenolic compounds. The major active principles include zingiberene, bisabolene, gingerols, and shogaols. The aqueous extract of ginger shows both antiglycating activity and lens aldose reductase 2 (ALR 2) inhibitions.<sup>[27]</sup> Many components of the test drugs contain anti glycation activity and adverse effect on advanced glycation end products. Some important compounds such as phenols, tannins, flavonoids, saponins, tripentenenes, alkaloids, sterols etc. present in the constituents of the test drugs can explain the anti glycation activity of the test drug. So the test drugs may prove very useful in age related ocular disorders and diabetic ocular complications.

## 6. CONCLUSION

The results of the present study showed the beneficial effect of hydroalcoholic extract of *Triphaladi* Compound Capsule and *Elaneer Kuzhambu Anjana* as a traditional medicine may also provide protection against diabetic ocular complications and other senile ocular disorders.

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L.J. Institute of Pharmacy, L.J. Campus, Sanand Circle, Sarkhej - Gandhinagar Hwy, Sarkhej, Ahmedabad, Gujarat 382210.

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