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ORIGINAL ARTICLE

Development of a Herbal Tea with Potential Antiglycation Effects using *Phyllanthus emblica* (Indian Gooseberry), *Zingiber officinale* (Ginger), and *Coriander sativum* (Coriander)

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Abstract

The study was conducted to investigate the capability of developing a herbal tea using *Phyllanthus emblica* (PE) fruit, *Zingiber officinale* (ZO) rhizome, and *Coriander sativum* (CS) seeds and to assess its anti-glycation effects. *Phyllanthus emblica*, ZO, and CS are common and individually used materials in traditional medicine in Sri Lanka. No evidence is found in using combinations of these plant materials being used in commercial tea / herbal infusion production in Sri Lanka. The herbal tea was formulated with powdered form of PE fruits, ZO rhizome and CS seeds in three different formulations; PE-50% + ZO-25% + CS-25%; PE-25% + ZO-50% + CS-25%; and PE-25% + ZO-25% + CS-50%. The three formulated teas were subjected to a five-point hedonic scale sensory evaluation. The formula with PE fruits 50%, ZO rhizome 25%, and CS seeds 25% was selected for further analysis. The phytochemicals of the selected formulation were analyzed, and the results bared the presence of glycosides, alkaloids, terpenoids, saponins, and tannins, which ensures a high antioxidant quality of the formulated tea. The tested parameter for the shelf-life determination study concluded that the product is shelf-stable for 14 days under 0 ˚C temperature. An *in vitro* assay using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis was used to test the antiglycation effects. Results showed that the herbal tea was able to inhibit glycation-induced protein cross-linking over prolonged incubation periods with strong glycating conditions. Therefore, this product could also have a potential to be used as a home remedy to prevent diabetic complications.

***Keywords**: Antioxidants, Diabetes complications, Home remedy, Shelf-stable, Traditional medicine*

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1. Introduction

The Diabetes Association of Sri Lanka (DASL) shows that there are about four million patients with diabetes mellitus reported in Sri Lanka. The diabetes pervasiveness among the population older than 20 years of age was 16% in the urban populace and 8% among the rural populace, while it was 8.5% among the population younger than 20 years. According to DASL, the commonness of diabetes among urban populace had increased from 12.1% in 1996 to 16.4% by 2008. General commonness of some type of dysglycaemia had increased from 6.87% in 1987 to 30% by 2006 (Katulanda et al. 2008) Most glucose diminishing tablets have side-effects, which incorporate severe hypoglycemia, lactic acidosis and liver damage. Glycation is a non-enzymatic process initiated with a reaction between reducing sugars and free amino groups of various biomolecules. Therefore, this process proceeds at an accelerated speed with hyperglycaemia. Stable heterogeneous advanced glycation end products (AGEs) are formed during the later stages of glycation process. Advanced Glycation End-products (AGEs) are implicated as a major cause for the development of chronic diabetic complications such as nephropathy. One of the detrimental effects of AGEs is the formation of inter and intra molecular cross-linking causing structural and functional abnormalities of the affected molecule (Blough et al. 2015). The plants like *Phyllanthus emblica* (Gooseberry) (PE), *Zingiber officinale* (Ginger) (ZO), *Coriandrum sativum* (Coriander) (CS) have effects against diabetes and are hostile to the hyperglycemic movement. Past investigations demonstrated that parts of these plants can be utilized for the treatment of diabetes. There are suggestions on nutraceuticals present in tea to have an effect on controlling diabetes in human body (Al-Amin et al. 2006; Deepa and Anuradha 2011; Srinivasan et al. 2018).

Phyllanthus emblica, also known as Indian gooseberry or *Amla*, is widely distributed in the tropical and subtropical areas of Sri Lanka, India, China, and Thailand. It is widely cultivated for its fruits particularly in India, the Mascarene Islands, the West Indies, and Japan (Li et al. 2015). All parts of the plant are used in various Ayurveda medicinal herbal preparations, including fruit, seed, leaves, root, bark, and flowers (Krishanaveni and Mirunalini 2016). The fruit extract of *P. emblica* has been reported to possess anticancer, anti-inflammatory, anti-diabetic, cardioprotective, and hepatoprotective activities (Li et al. 2015).

Although, these fruits are reputed to contain high amounts of ascorbic acid (vitamin C), up to 445 mg per 100 g, the specific contents are disputed and the overall bitterness of *Amla* may derive instead of its high density of ellagitannins like emblicanin A, emblicanin B, punigluconin and pedunculagin. It also contains punicafolin and phyllanemblinin A, phyllemblin other polyphenols such as flavonoids, kaempferol, ellagic acid, and gallic acid (Zhang et al. 2003; Liu

et al. 2013) *Zingiber officinale* (Ginger) is a flowering plant whose rhizome is widely used as a spice or a folk medicine. Ginger is one of the most consumed dietary condiments in the world (Surh et al. 1998). The oleoresin from the rhizome of ginger contains many bioactive components such as [6]-gingerol, which is the primary pungent ingredient that is believed to exert a variety of remarkable pharmacological and physiological benefits. Ginger has been used for treatments of numerous ailments, such as colds, nausea, arthritis, migraines, and hypertension for thousands of years (Sharifi-Rad et al. 2017).

Ginger has been fractionated into at least 14 bioactive compounds, including [4]-gingerol, [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-paradol, [14]-shogaol, [6]-shogaol, 1-dehydro-[10]-gingerdione, [10]-gingerdione, hexahydrocurcumin, tetrahydrocurcumin, gingerenone A, 1,7-bis-(4′ hydroxyl-3′ methoxyphenyl)-5-methoxyhepthan-3-one, and methoxy-[10]-gingerol (Koh et al. 2009). The ratio of each individual component in ginger depends on the country of origin, commercial process, and whether the ginger is fresh, dried, or processed (Schwertner et al. 2006).

Coriandrum sativum (Coriander) is indigenous to the Mediterranean countries and today most of the commercial supply of this herb comes from Morocco, Romania, and Egypt, but commonly used in India, Latin America, China, Southeast Asia, and South Asia. Even though all parts of the plant are edible, the fresh leaves and the dried seeds of the plant are the parts most traditionally used in cooking (Nadeem et al. 2013). The coriander seeds include essential oil of triglycerides and petroselinic acid, a monounsaturated fatty acid (Kunicka 2003). The seed oil contains 53 compounds where the main compounds are linalool, geranyl acetate and gamma-terpinene (Ramezani et al. 2009).

Therefore, this research was conducted in order to develop a herbal tea with *Phyllanthus emblica* (PE) fruit, *Zingiber officinale* (ZO) rhizome and *Coriander sativum* (CS) seeds and to assess its anti-glycation effects. This would be a novel concept as there is no evidence of commercial production of a herbal tea formulated with *Phyllanthus emblica* fruits*, Zingiber officinale* rhizome and *Coriandrum sativum* seeds collectively.

2. Materials and Methods

Formulation of herbal tea powder

Mature fruits of PE, fresh rhizomes of ZO, dry seeds of CS were taken as main ingredients for the development of the herbal tea. These materials were obtained from herbal gardens. The above plant parts were washed properly to remove any extraneous matter present. The outer skin of ZO rhizome was removed by scraping. The PE fruits and ZO rhizomes were sliced manually using a sterilized knife into thin slices of about 1 mm thickness. All three cleaned and sliced plant

parts were sun dried. The dried samples were then powdered employing a household electric dry grinder. The powdered samples were packed into polyethylene sip lock bags and stored under refrigerated conditions. Three herbal tea formulations were developed by mixing the powdered PE fruits, ZO rhizome and CS seeds, in different compositions; formulation no 405: PE-50%, ZO-25%, CS-25%; formulation no 225: PE-25%, ZO-50%, CS-25%; formulation no 807: PE-25%, ZO-25%, CS-50%.

Preparation of herbal tea extraction of prepared samples

An amount of 2 g from the prepared herbal tea powder formulations was placed in a 500 ml Erlenmeyer flask, and a volume of 200 ml of boiled water was added and refluxed gently for 2 hrs. After cooling, the mixture was filled up to 500 ml and was filtered through dry filter paper (Whatman filter paper, Garde 1, circles, diam. 25mm). A volume of 50 ml of the filtrate was taken into a weighing bottle of known weight and evaporated to dryness on a steam bath. The mixture was dried at 98–100 ℃ to a constant weight (Anon 2000). The same procedure was carried out for the other formulations of herbal tea powder, separately.

Sensory Evaluation

Five-point hedonic scale sensory evaluation was used to evaluate the three different herbal tea formulations namely 225, 405, and 807. The samples were analysed for colour, aroma, mouthfeel, and overall likeness using the Kruskal-Wallis test in Minitab statistical software. A sample ballot for a ranking test was adopted. The ballot listed the ranks from 5 = most preferred to 1 = least preferred (Lawless & Heymann 1999). The evaluators were instructed to taste all three herbal tea formulations and rank them according to the first sensory impression and re-testing was done to be certain of the correct ranking. The formulation ranked as best was used for further analysis.

Phytochemical Analysis

Under phytochemical analysis, the formulation chosen from the sensory evaluation was tested for glycosides, phenols, flavonoids, alkaloids, sterols, terpenoids, saponins, tannins, and anthraquinones according to AOAC (2000) instructions. The presence of the above-mentioned types of phytochemicals was detected qualitatively.

Proximate Analysis

Moisture content was analysed using (ISO 712:2009) method, total ash using (ISO 2171:2007) method, water soluble ash content was analysed using (AOAC 2000) method, ash soluble ash content was analysed according to (AOAC 2000) method, and crude fibre content was determined using (AOAC 2000) method as well.

Microbiological Parameters

Total plate count, and yeast and mould count of the prepared herbal tea samples were analysed by using (ISO 516:1991) method.

Assessment of glycation-induced protein cross-linking inhibitory effects

An *in vitro* electrophoresis-based method (Perera & Ranasinghe 2015) was used to investigate the protein glycation inhibitory potential of the developed herbal tea. The formation of products of glycation-induced cross-linking were detected using SDS-PAGE.

Working solution of chicken egg lysozyme, fructose, and brewed herbal tea samples were prepared using 200 mM phosphate buffer (pH 7.4) containing 0.02% sodium aside. Lysozyme was incubated with 500 mM fructose in the presence of brewed herbal tea samples for 3 months at 37 ℃. The final concentration of the brewed herbal tea samples used was 0.005 to 0.16% (w/v) in the reaction mixture. Standard inhibitor AG (1 mg mL^{-1}) was included as the positive control. Negative control was prepared with buffer, lysozyme, and fructose. Parallel blanks for tests and controls were prepared without fructose. Aliquots were collected and stored at -40 ℃ until further analysis. These aliquots were analysed for the presence of high molecular weight products using sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE). Aliquots from the incubation mixtures were heated with the SDS sample buffer at 95 ℃ for 3 mins, before loading to the gel. Broad range molecular weight markers were included to assess the approximate size of the high molecular weight products. After separation, protein bands were visualized by staining with Coomassie brilliant blue. Appearance of high molecular weight products of lysozyme was assessed by visual observation (Perera & Ranasinghe 2015).

3. Results and Discussion

Sensory evaluation

According to the results (Table 1 and Fig. 1) obtained for the three different formulae from Kruskal-Wallis test for five-point hedonic scale sensory evaluation, H_{stat} values for overall taste, colour, aroma, mouth feel, and overall likeness were larger (p<0.05) than H_{table} values.

According to the Fig. 1, preference of most panellists was higher for the 405 (PE-50%, ZO-25%, CS-25%) formulation. Therefore, formulation 405 was selected as the most preferable herbal tea product out of the three formulations. Therefore, the rest of the experiments were conducted by using the herbal tea formulation no 405.

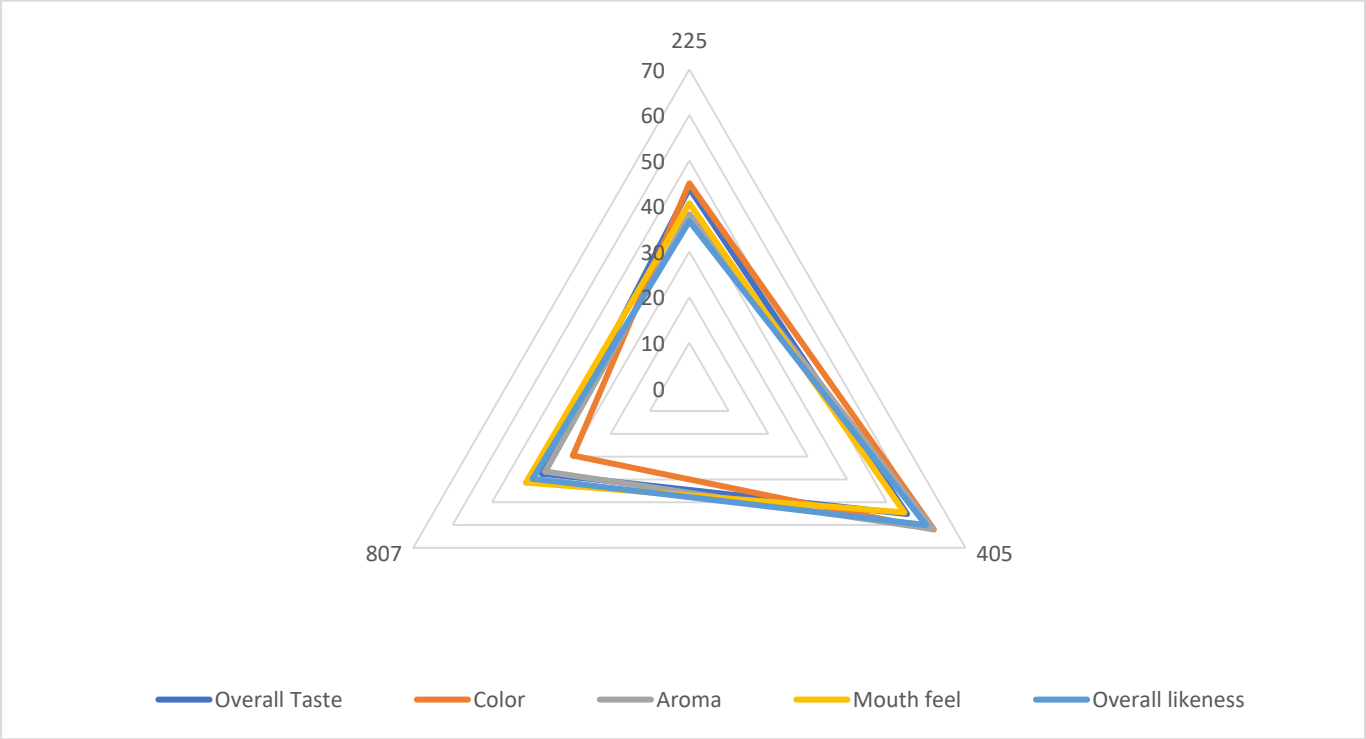

Figure1: Panelist score for sensory attributes

Phytochemical Analysis

According to results of phytochemical test (Table 1), the selected herbal tea mixture (formulation no 405) showed positive responses for glycosides, flavonoids, alkaloids, terpenoids, saponin and tannin tests. The herbal tea also showed negative responses for phenol, sterols and anthraquinones tests.

Table 1: Presence or absence of phytochemicals in the formulated herbal tea

Metabolites/Test	Presence (+) or Absence (-)
Glycosides	+
Phenols	-
Flavonoids	+
Alkaloids	+
Sterols	_
Terpenoids	+
Saponins	+
Tannins	+
Anthraquinones	-

\+ = Presence of relevant phytochemical compound
\- = Absence of relevant phytochemical compound

Table 2: Proximate analysis test results

Parameter	% (W/W)
Moisture	8.06 ± 0.060
Total Ash	4.1
Water soluble Ash	45.9
Acid insoluble Ash	0.1
Crude Fibre	5.96 ± 0.308

According to results of proximate analysis, the selected herbal tea mixture (formulation no 405) resulted values given in the Table 2. These values were in within the standard range of methods used for proximate analysis.

Microbiological Parameters

According to the SLS standards of the specification for "Sri Lankan origin teas" (ISO 516: part 2: 1991/ISO 21527-2:2008 and ISO 516: Part 1: 1991/ISO 4833:2003), respectively indicate that when considering the hygienic quality, it should be less than 1,000 cfu g^{-1} yeast and mould count and less than 10,000 cfu g^{-1} total plate count. The calculated result of the total plate count of the herbal tea was 2.32×10^2 cfu g^{-1} and the yeast and mould count were 2.16×10^2 cfu g^{-1}, which was lower than the maximum limit of SLS standards. Therefore, the herbal tea is acceptable for consumption.

Shelf-Life Evaluation

During storage time of 14 days in 0 °C temperature, both the total plate count and yeast and mould count had increased over time, and it had recorded a rapid acceleration of total plate count in 1st week and yeast and mould count between 1st and 2nd week (Fig. 2 and Fig. 3). In the evaluation of the values obtained for the tested parameters of the shelf-life determination study, the product was shelf-stable for 14 days without the use of any preservation method.

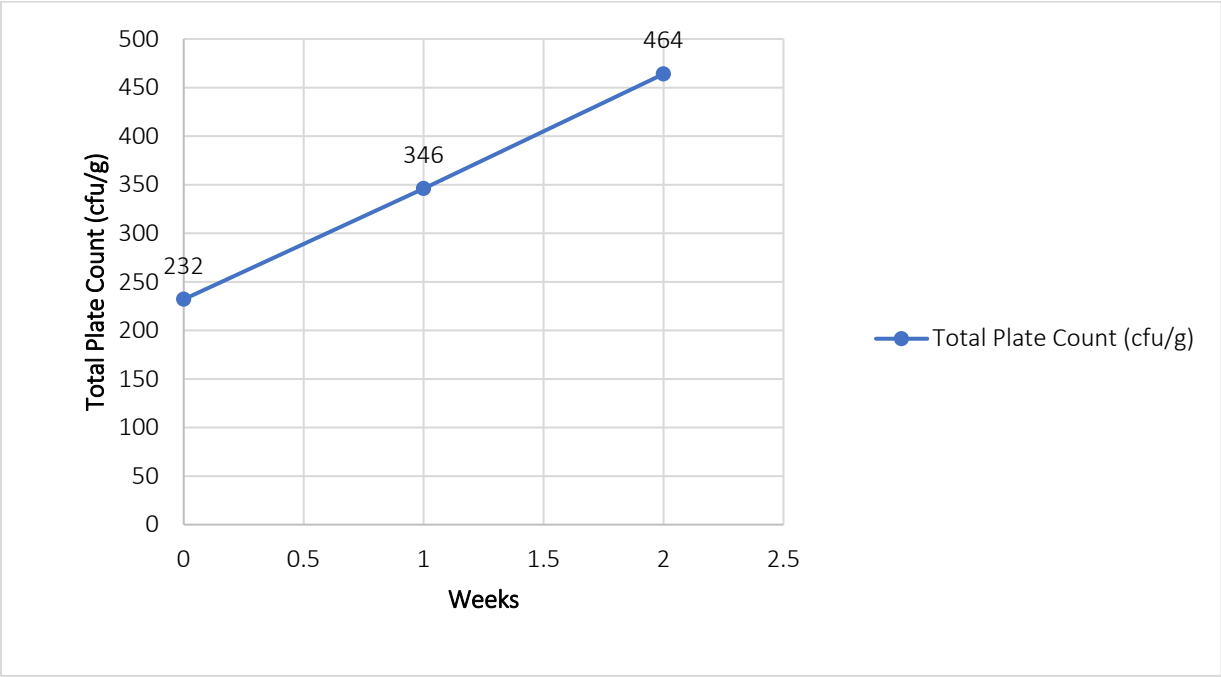

Figure 2: Total plate count variation during storage

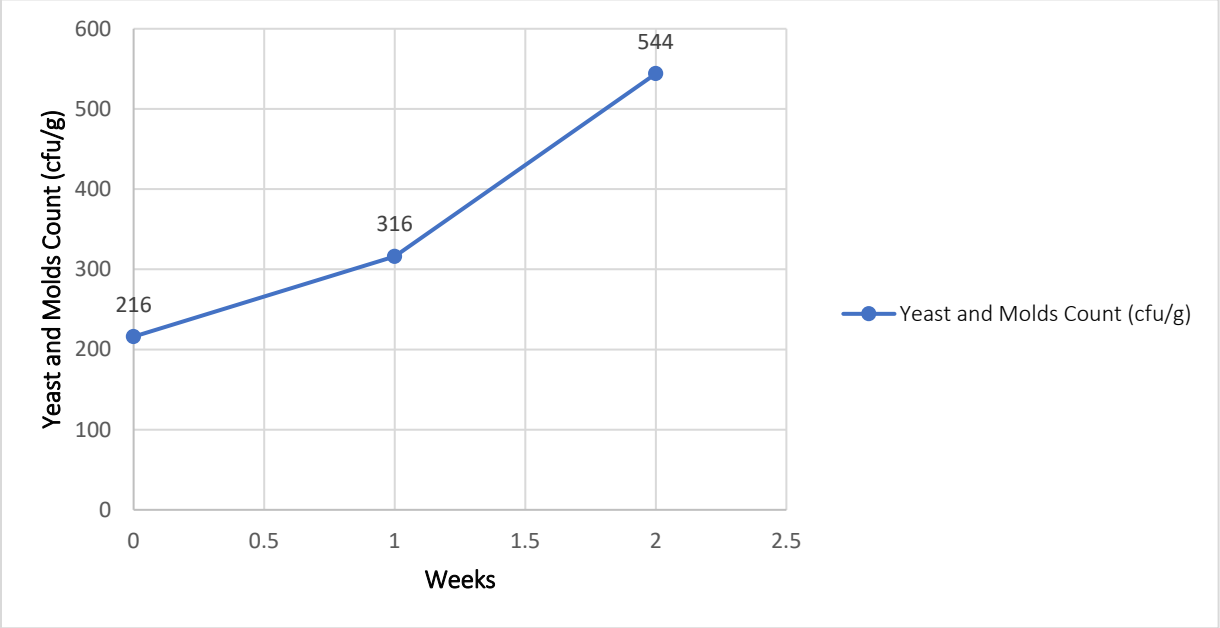

Figure 3: Yeast and Mould count variation during storage

Assessment of glycation-induced protein cross-linking inhibitory effects

SDS-PAGE showed the presence of high molecular weight products, which form as a result of inter molecular cross-linking of lysozyme, when the protein was incubated with fructose. Varying degree of high molecular weight bands corresponding to dimer (24 KDa), trimer (36 KDa), tetramer (48 KDa) and other products of the lysozyme (12 KDa) were observed when compared with the molecular weight markers. Previous studies have shown that the appearance of high molecular weight bands is proportional to the degree of glycation induced cross-linking, and that the formation of these products increase with time (Perera & Ranasinghe 2015; Perera & Handuwalage 2015).

The effects of the presence of the herbal tea in incubated samples at seven days and three months of incubation are shown in Figure 4. Appropriate controls were used for the comparison of the appearance of high molecular protein products to assess the cross-linking inhibitory effect of the herbal tea samples. As shown in Figure 4, controls included mixtures of only lysozyme (CB), herbal tea sample + lysozyme (PB), lysozyme + fructose (CT) and lysozyme + fructose + Aminoguanidine standard inhibitor (AG). The fructose added CT showed the appearance of high molecular weight products as multiple bands on the stained gels. Controls without fructose added (CB and PB) showed no such products. The standard inhibitor AG showed only one band, which was of less intensity as the bands of CT. This confirmed the proper establishment of controls and that the experiment produced results as expected in tea theory. All concentrations of the herbal tea samples showed no appearance of such high molecular weight products after seven days of incubation. Patterns observed were similar to those of samples incubated with the standard inhibitor AG. The AG, however, was of a much higher concentration than the herbal tea samples (1 mg mL^{-1}). A clear inhibition of glycation-induced cross-linking has occurred in all tested samples (Fig. 4). Inhibitory effects matched with that of AG remained even after 90 days of incubation at concentrations ≥0.01% (W/V) herbal tea CB: Lysozyme, PB: Lysozyme + herbal tea, CT: Lysozyme + fructose, AG: Lysozyme + fructose + aminoguanidine, MW: Molecular weight markers. Herbal tea was used at 0.16% (1), 0.08% (2), 0.04% (3), 0.02% (4), 0.01% (5) and 0.005% (6) (W/V) final concentrations. Samples were incubated for seven and 90 days.

Accelerated speeds of glycation as seen in hyperglycemia lead to formation of inter and intramolecular cross-linking due to the production of AGEs. Thus, in the absence of sugar, cross-linking does not occur, and no high molecular weight products are formed (Perera & Ranasinghe 2015). Hence, only a single band representing the monomer appeared in the absence of fructose.

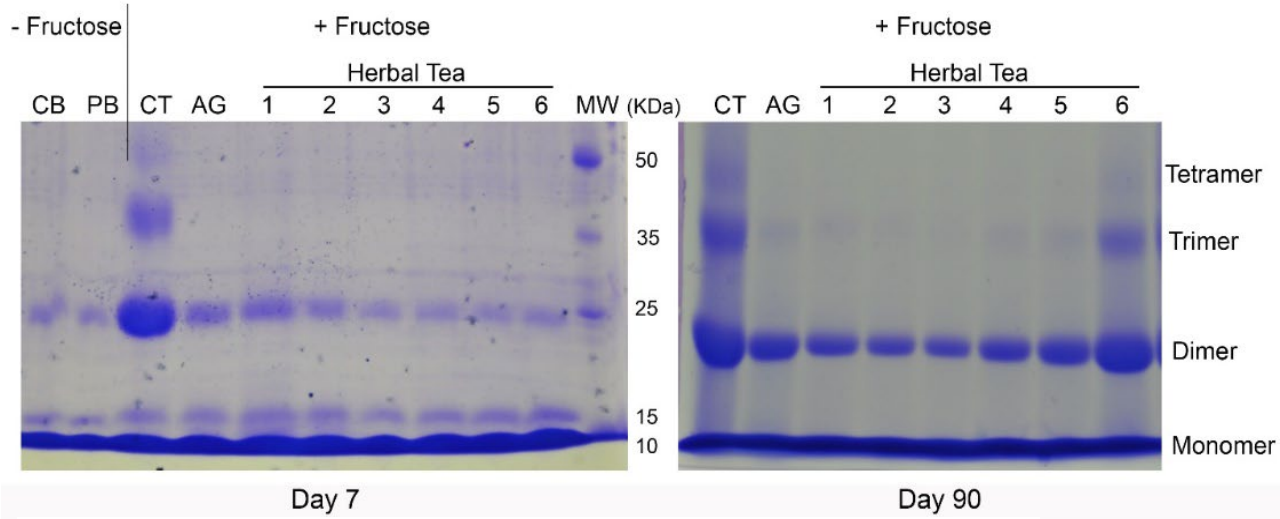

Figure 4: Inhibitory effects of developed herbal tea on the appearance of high

In the presence of a standard inhibitor or a substance with glycation inhibitory effects, the process of glycation-induced cross-linking was inhibited, and the degree of the appearance of high molecular products was reduced (Perera & Ranasinghe 2015) as observed in the sample incubated with aminoguanidine standard inhibitor. The herbal tea samples tested, had prevented the appearance of high molecular protein bands during a prolonged incubation period of three months in a strong glycating medium with 500 mM fructose, indicating that these were able to inhibit glycation induced protein cross-linking.

4. Conclusions

Herbal tea product can be successfully prepared by using gooseberry, coriander, and ginger.

The herbal tea formula with higher gooseberry concentration was selected as the most preferable herbal tea product out of the three formulations tested. The best herbal tea sample composition was formulated using 1 g of gooseberry, 0.5 g of ginger, and 0.5 g of coriander. Phytochemical analysis resulted positive responses for glycosides, flavonoids, alkaloids, terpenoids, saponins, and tannin tests and has shown negative responses for phenol, sterols, and anthraquinones tests. According to the proximate and microbial analysis, the developed herbal tea was in the range of standard reference values. Until three weeks of time, the herbal tea product was chemically and microbiologically safe and stable during storage. Thus, this tea can be recommended for day-to-day consumption. According to the SDS-PAGE assay, the tea was able to inhibit protein cross-linking for prolonged

periods under strong glycating conditions which establishes its strong anti-glycation effects. Therefore, this product has the potential to be used as an herbal drink and a home remedy to prevent diabetic complications.

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Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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