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In Vitro Antiglycation Activity of "*Cympobogon Citratus*" and "*Cynodon Dactylon*"

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Abstract: Plant is an important source of medicine and plays a key role in human health. Medicinal plants having both antiglycation and antioxidant activity can be of good therapeutic value in reducing complications of diabetes and slowing down aging process. Advanced Glycation End product (AGE'S) formation is due to non-enzymatic glycation and oxidative stress, which has been demonstrated in the pathogenesis of diabetic complication and aging processes. In this study we have investigated the antiglycation effect of *Cynodon dactylon* and *Cympobogon citratus* plants which are being used in the treatment of diabetes mellitus. Results indicated that the concentration of the *Cympobogon citratus* extract increases and the absorbance decreases from 0.027 ± 0.011 **and reaches minimum 0.012** ± 0.0011 **at 390** μg/ml**. The same trend is observed with *Cynodon dactylon* extract too which reaches its minimum of 0.006** ± 0.0028 **at concentration of 390** μg/ml **from maximum absorbance of 0.024** ± 0.006**. Antiglycation activity of *Cympobogan citratus* is more as compared to *Cynodan dactylon*. The result also show that precent inhibition of glycation reaction by *Cympobogon citratus* is lower than *Cynodon dactylon*.**

Keywords: Advanced Glycation End products (AGEs), Antiglycation, *Cympobogon citratus*, *Cynodon dactylon*, Diabetes mellitus.

I. Introduction

Non enzymatic glycation takes place when elevated levels of reduced sugars react with amino groups of proteins and is called as Advanced Glycation End products (AGEs) are responsible for Diabetes Mellitus [1, 2, 3]. Accumulation of AGE's in different parts of the body like heart, muscle and large blood vessels, result in the promotion and progression of diabetic complication like nephropathy, neuropathy, cardiovascular disease and atherosclerosis [4, 5, 6]. During diabetes mellitus AGE's are formed at an accelerated rate because of the hyperglycaemic condition in the body. Therefore any drug or agent that could stop AGE's formation can be of therapeutic value [7, 8]. Diabetes mellitus is associated with increase production of free radical leading to oxidative stress. Thus disturb the balance between radical formation and radical neutralization leads to oxidative damage of cell component such as protein, lipid and nucleic acid [9]. Glycation of protein and its further oxidation alter its conformation, stability and induce protein aggregation and immobilization through cross linkage [7, 8, 10]. On the other hand Collagen elasticity is responsible for the proper shape of the body. Glycation result in loss of collagen elasticity and thus cause in arterial stiffness decreased myocardial compliance and hence aging [11, 12]. One of the ways to reduce diabetic complication and slowing down aging is to stop glycation and oxidation process. This is only possible by using compounds or agent that have antiglycation and properties. So far various synthetic and natural compounds have been tested for their antiglycation activity. However there are many herbs, plants are being used for testing various ailments. Since ancient time and well mentioned in scipts. To support and validate use of herbal compound for treating glycation. The present investigation is an attempt to explore, evaluate and comparing antiglycation activity of *Cynodon dactylon* and *Cympobogon citratus.*

Cympobogon citratus better known as lemon is a genus of Asian, African, Australian and Tropical Island plants in the grass family [13, 14] Common names include lemon grass, lemongrass, barbed wire grass, citronella grass, *cha dartigalongue*, fever grass, *hierba Luisa*, or *gavtichaha*, amongst many others [15, 16]. Lemongrass is widely used as a culinary herb in Asian cuisine and also as medicinal herb in India. It has a subtle citrates flavour and can be dried and powdered, or used fresh. It is commonly used in tea, soups and curries. It is also suitable for use with poultry, fish, beef, and seafood. It is often used as a tea in African countries. Lemongrass oil is used as a pesticide and a preservative. Research shows that lemongrass oil has antifungal properties. Its ability to repel some insects, such as mosquitoes, its oil is commonly used as a "lure" to attract "honey bees"

[17]. The leaves of *Cymbopogon citratus* have been used in traditional medicine and are often found in herbal supplements and teas. In traditional medicine of India the leaves of the plant are used as stimulant, sudorific, and periodic, and anticatarrhal, while the essential oil is used as carminative, depressant, analgesic, antipyretic, antibacterial, and antifungal agent [18].

According to *Ayurveda*, India's traditional pharmacopoeia, *Cynodon plant* is pungent, bitter, fragrant, heating, appetizer, vulnerary, anthelmintic, antipyretic, alexiteric. It destroys foulness of breath, useful in leucoderma, bronchitis, piles, asthma, tumors, and enlargement of the spleen [19]. *Cynodon dactylon* also known as *vilfastellata*, durva grass, *dhubbarmuda grass*, dogs tooth grass, Bahama grass, Indian *doab*, wiregrass [20]. *Cynodon dactylon* Pers, a hardy perennial grass, is one of the most commonly occurring weeds in India. Although a problem for farmers, *doobghas* is a valuable herbal medicinal plant and used as first aid for minor injuries. Farmers traditionally apply crushed leaves to minor wounds as a styptic to stop bleeding similar to *Tridax Procumbens* [21, 22] *Achyranthesaspera, and Blumeaiacera*. *Cynodon* has a renown position in Indian systems of medicine and many parts of the plants are assumed to have medicinal properties. A traditional use of *Cynodon*is for eye disorders and weak vision; the afflicted are advised to walk bare foot on dew drops spread over *Cynodon dactylon* plant each morning.

II. Material and Method

A. Plant Materials

The leaves of *Cympobogon citratus* and *Cynodon dactylon* were selected for study. The leaves of *Cympobogon citratus* and *Cynodon dactylon* were collected in the month of December nearby Nagpur region Maharashtra, India. Plants were identified and authenticated from department of botany Kamla Nehru College, Nagpur.

B. Preparation of Plant Extract

The leaf of *Cympobogon citratus* and *Cynodon dactylon* were thoroughly washed and kept in shade for drying. Shade dried material was then chopped and grounded to powder and kept in air tight container for further use and extraction was done by using cold maceration method, where 3 mg dried powder was suspended overnight in 1 ml 0.1 M PBS. The extract was then filtered through muslin cloth and centrifuge for 15 min at 5000 rpm the supernatant was used in next experiments.

C. Phytochemical Screening

Qualitative phytochemical studies of plant extracts of *Cympobogon citratus* and *Cynodon dactylon* leaves were done using standard procedure to identify the phytoconstitutents [23, 24, 25].

D. Evaluation of In Vitro Antiglycation Activity

Antiglycation activity of *Cympobogon citratus* and *Cynodon dactylon* plant extracts was determine by using the method of Matsuura *et al.*, 2002 [26] was followed with little modifications. The plant extracts were prepared by dissolving 3 mg in 1 ml of alkaline PBS. Through this solution 30 µg/ml, 150 µg/ml, 270 µg/ml and 390 µg/mL solutions were mixed with a 1ml solution containing 0.001 g BSA and 200 mM glucose followed by Incubation at 65 °C for next 48 hours. BSA and glucose without any inhibitor was used as control. After incubation 10 µL of 100% w/v TCA was added to the reaction mixture and centrifuged at 14500 rpm at 4 °C for 4 minutes and the pellet was re-suspended in 1 ml alkaline PBS. Here we used aminoguanidine as a standard inhibitor.

Using fully automated UV double beam spectrophotometer (schimatzu UV 1800 ENG 240 V, SOFT), the degree of absorbance for both the control and the test reaction mixtures were taken at 350 nm. Percent inhibition was calculated using the following formula:

$$\text{Percent inhibition} = [1 - (As - Ao)/(Ab - Ao)] \times 100$$

where As is absorbance of test samples, Ab is absorbance of reaction mixture without plant extract and Ao is absorbance of blank control [2].

E. Qualitative Phytochemical Screeing of the Sample Extract

Phytochemical screeing of the sample extract for Sterol test [27], Alkaloids test [28], Tanins test [29], Phenol test [30], Sugar test [30], Saponins test [31], Flavonoid test [31], Protein test [31], was performed.

III. Results

A. Antiglycation Activity Plant Extracts With Standard Aminoguanidine

Table I: Antiglycation Activity of Aminoguanidine, Cympobogon Citratus and Cynodon Dactylon at Various Extract Concentration Mean ± SD (N = 3)

	30 µg/ml	*150 µg/ml*	*270 µg/ml*	*390 µg/ml*
Aminoguanidine	0.02 ± 0.001	0.014 ± 0.004	0.011 ± 0.001	0.004 ± 0.0015
Cynodon Dactylon	0.024 ± 0.006	0.017 ± 0.0064	0.016 ± 0.0057	0.006 ± 0.0028
Cympobogon Citratus	0.027 ± 0.011	0.023 ± 0.0026	0.019 ± 0.0081	0.012 ± 0.0011

B. Antiglycation Activity

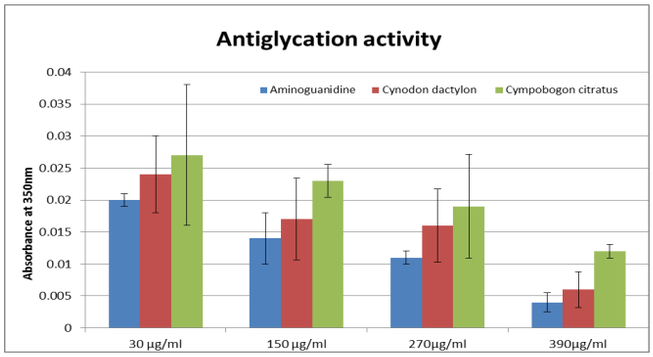

Fig. 1: Antiglycation Properties at Different Concentration of *Cympobogon Citratus*, *Cynodon Dactylon* and Aminoguanidine Mean ± SD (n = 3)

C. Percent Inhibition

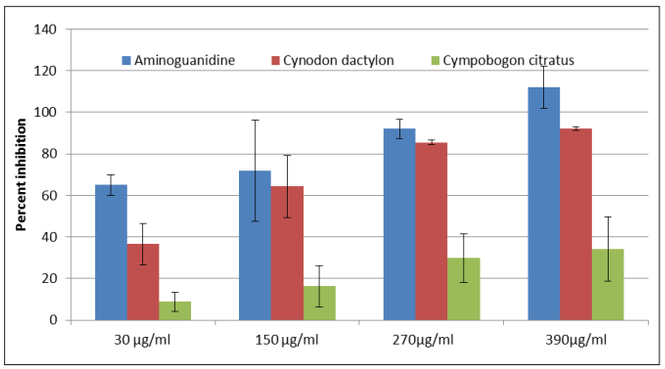

Fig. 2: Percent Inhibition at Different Concentration of *Cynodon Dactylon*, *Cympobogon Citratus* and Aminoguanidine

IV. In Vitro Antiglycation Assay

Plants have been employed for therapeutic purposes since ancient time. Traditional healers from across the globe practise the use of different plants and plant parts to aid in the alleviation of afflictions that result from various types of ailments. The phytochemical analysis carried out of plant extract of *Cympobogon citratus* and *Cynodon dactylon*. The extract showed the presence of Sterol, Saponins, Tanins, Phenol, Protein.

The antiglycation activity of plant extracts of leaves of *Cympobogon citratus* and *Cynodon dactylon* were measured by the method described by Matsuura *et al*., 2002 Using fully automated UV double beam Spectrophotometer (schimatzu UV 1800 ENG 240 V, SOFT). The results obtained under experimental circumstances from antiglycation activity of *Cynodon dactylon and Cympobogon citratus*. The UV double beam spectrophotometric analyses of the reaction and test mixtures of *Cynodon Dactylon* and *Cympobogon citratus* are shown in the Fig. 1. From Fig. 1 one can easily figure out that the experimental tubes treated with extract of *Cynodon Dactylon* showed significant decrease in absorbance from 0.024 ± 0.006 to 0.006 ± 0.0028 in a dose dependent manner i.e. from concentration of 30 µg/ml to 390 µg/ml. The same trend was observed in experiments treated with *Cympobogon citratus* extract too, where absorbance 0.027 ± 0.011 of 30 µg/ml subsequently decrease to 0.012 ± 0.0011 of 390 µg/ml.

From the observations it can be seen that different concentration of plant extract of *Cympobogon citratus* and *Cynodon Dactylon* in two reaction mixture decrease absorbance this gives an indication of increase in antiglycation activity and decrease glycation activity. Antiglycation activity of *Cympobogon citratus* is much higher than *Cynodon Dactylon* as shown in Table I.

The percent inhibition of glycation activity was shown in Fig. 2. On comparing percent inhibition of glycation of both plant extract *Cympobogon citratus* and *Cynodon dactylon* with aminoguanidine the extract showed that Percent inhibition of glycation reaction by *Cympobogon citratus* is decrease as compared to standard aminoguanidine by 50.58% and by the 39.47% than *Cynodon dactylon*. However percent inhibition of *Cynodon dactylon* was found to be 18.35% decrease than the standard aminoguanidine. Decrease in the percent inhibition as compare to aminoguanidine a potent known standard inhibitor confirmed that both the plants viz. *Cympobogon citratus and Cynodon dactylon* possess antiglycation activity.

V. Discussion

The current study was initiated because of the ever-increasing possibilities of Diabetes in most of the people. Plant extracts and compounds are of new interest as antiglycation agents. Plants have been employed for therapeutic purposes since ancient time. Traditional healers from across the globe practice the use of different plants and plant parts to aid in the alleviation of afflictions that result from various types of ailments [32, 33]. Different plants and plant parts are being use to treat various ailments since time immemorial, despite being advancement in modern medicines herbal remedies or herbal treatments are regularly in practice by herbalists and many doctors even a popular reason behind use of plant and plant products might be because of less side effects and its synergistic activities.

It is also assumed to be formed by sequential glycation and oxidation reactions called glycoxidation. The role of advanced glycated end-product proved to be significantly responsible in diabetic micro vascular complications of retinopathy, neuropathy and nephropathy as well as their role in the accelerated hyperlipidemia. There is an evidence that glycosylation leads to biochemical modification of Immunoglobulin, and other macromolecules and is cause for pathogenesis of diabetic complications [34].

Risks of diabetes have been subsequently increased in many folds since last three decades. Overexertions, sedentary lifestyle, food habits are now become major risk factors for the diabetes. Early detection of diabetes can be treated by available synthetic drugs. However consumption of these antidiabetic drugs becomes a regular practice and one has to consume them to their entire life span as a result there will be several adverse effects. Therefore, the present investigation was carried out to explore the antiglycation activity of *Cympobogon citratus* and *Cynodon dctylon* two known plants of having diversified therapeutic applications.

It is said that curative properties of medicinal plants are due to presence of various metabolites such as Sterol, Saponin, Tanin, Phenol, Protein [35]. Our preliminary qualitative analysis for phytochemicals reveals that both plant extracts possess Sterol, Saponin, Tanin, Phenol, Protein phytocompounds, suggestive of presence of medicinal properties.

During starvation or hypoglycaemic conditions body requires extra glucose for its normal physiological process. Elevated blood glucose level further controlled by secretion of insulin. Insulin, a protein hormone secreted by β-pancreatic cells. Insulin regulates increased sugar level by activating a protein cascade that further transported out glucose from the blood into cytoplasmic region of a cell with the help of transporter molecule known as GLUT4 [35, 36]. A normal antiglycation activity is regulated by sufficient concentration of insulin level in the blood. However, in diabetic condition where insulin level is significantly depleted that affect glucose uptake in the cell. On the other hand a dose dependent study showed that increasing concentration of plant extracts *Cympobogon citratus* and *Cynodon dactylon* if a concentration 30 µg/ml, 150 µg/ml, 270 µg/ml, 390 µg/ml resulted prominant increase in antiglycation activity. The antiglycation activity of plant extract *Cympobogon citratus* and *Cynodon dactylon* was found to be significantly higher ($P < 0.05$) when compared with standard aminoguanidine , the possible reason for increase in antiglycation activity of plant extract is due to activation of more number of GLUT4 molecules [37] that allows glucose to translocate inside the cell. It is further confirmed by calculating percent inhibition elevated glucose level is etiologic agent for the pathogenesis of diabetic complications by increasing protein glycation and formation of advanced glycation end products (AGEs). Insoluble advanced glycation end product that accumulate on long lived protein thus compromising the physiological function. Oxidation plays an important role in the formation of advanced glycation end product and plant derived agent with the antiglycation activities are highly important in preventing diabetic complication [34].

In this study, the active phyto components of *Cympobogon citratus* and *Cynodon dactylon* was studied and further antiglycation activity of different concentration of plant extract of *Cympobogon citratus* and *Cynodon dactylon* was also tested. The percent inhibition of glycation reaction by aminoguanidine and the two plant extract verses the amount of test sample used. It was further confirmed that they both possess antiglycation capacity, where *Cympobogon citratus* extract had shown higher antiglycation activity (0.012 ± 0.0011) at dose of 390 µg/ml as compared to *Cynodon dactylon* (0.006 ± 0.0028).

VI. Conclusion

The present investigation was an attempt made towards exploring antiglycation activity of *Cympobogon citratus* and *Cynodon dactylon* the study reveals that both plant extract possess antiglycation activity. The increase amount of glucose uptake by the cells was observed in a set treated by *Cympobogon citratus* was higher than *Cynodon dactylon*. This may be due to presence of certain phytochemicals which might have property to activate GLUT4 transport system, which can further be studied in details by isolating different compounds and elicit the desire function. Upcoming results would be a great support in the development of potent antidiabetic molecule.

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