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IN-VITRO ANTIDIABETIC ACTIVITY OF *CHAMAECOSTUS CUSPIDATUS*

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Insulin plant, Chamaecostus, anti-diabetic, Glycation, *Costus igneus*

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ABSTRACT: Background: The most common disease of endocrine disorder is diabetes due to underlying causes and pathophysiologies. It is spreading swiftly worldwide and is expected to reach up to 4.4% in the world by 2030. There are numerous synthetic glucose-lowering drugs on the market, but they all have detrimental effects. As a result, this necessitates the search for an alternative anti-diabetic agent from a natural source, *i.e.*, herbal remedies that have been used traditionally and have a safe profile. **Aim of the study:** The purpose of this study was to look into the anti-diabetic activity of *C. cuspidatus* methanolic leaves extract on the glycation of Bovine Serum Albumin by measuring fluorescence intensity at various concentrations (1 mg/ml, 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, 10 mg/ml) using positive control 1mM of Aminoguanidine (1 mg/ml, 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, 10 mg/ml). **Methodology:** Phytochemical screening of *Chamaecostus cuspidatus* for determination of Phytoconstituents. BSA- Glucose Assay for *in-vitro* Glycation- methodology (Brownlee *et al.*, 2013). BSA (10 mg/mL) was incubated with glucose (500mM) in phosphate-buffered saline (PBS) (5mL total volume, pH 7.4) and extract containing 0.02% sodium azide at 37 °C with a final concentrations of BSA (2mg/mL), glucose (40 mM), sample (0.1 to 0.5mg/mL). All the reagents and samples were sterilized by filtration through 0.2 µm membrane filters. **Results:** The phytochemical screening shows the presence of flavonoids, saponins, terpenoids, tannins, and chlorophyll. The BSA- Glucose Assay for *in-vitro* Glycation shows that after 15 days of incubation with methanolic extract of *Chamaecostus cuspidatus* and aminoguanidine the fluorescence intensity was significantly decreased in a concentration-dependent manner. **Conclusion:** The reductions in fluorescence intensity reveal that methanolic extract of *Chamaecostus cuspidatus* can inhibit the formation of AGEs and provide protection from diabetic complications.

INTRODUCTION: Diabetes is a group of metabolic diseases characterized by hyperglycemia caused by insulin secretion, insulin action, or a combination of the two. Diabetes chronic hyperglycemia is linked to long-term damage, dysfunction, and failure of various organs, particularly the eyes, kidneys, nerves, heart and

blood vessels (Kharroubi and Darwish 2015) ⁴. Diabetes and its complications are rapidly gaining prominence as the leading cause of morbidity and mortality worldwide. The negative effects of persistently elevated plasma glucose levels on various body parts differ depending on the cell type.

Glucose forms covalent adducts with plasma proteins during a long-term hyperglycemic state in diabetes mellitus *via* a non-enzymatic process known as glycation (Gkogkolou and Böhm 2012) ⁵. Protein glycation and the formation of advanced glycation end products (AGEs) are important in the pathogenesis of diabetic complications such as

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retinopathy, nephropathy, neuropathy, cardiomyopathy, and other diseases rheumatoid arthritis, osteoporosis, and ageing (Singh *et al.*, 2014)⁶. Protein glycation disrupts normal functions by altering molecular conformation, altering enzymatic activity, and interfering with receptor function. AGEs form intra and extracellular cross-links with proteins and other endogenous key molecules such as lipids and nucleic acids, which contribute to the development of diabetic complications (Chen *et al.*, 2018)⁷. There are numerous synthetic glucose-lowering drugs on the market, but they all have drawbacks. As a result, this necessitates the search for an alternative anti-diabetic agent from a natural source, *i.e.*, herbal remedies that have been used traditionally and have a safe profile.

Chamaecostus cuspidatus Nak (syn. *Costus pictus* Don, *Costus mexicanus* Liebm or *Costus congenitus* Rowle), commonly known as Fiery costus, Step ladder, Spiral flag, or Insulin plant, is native to South and Central America (Jyothi *et al.*, 2015)¹. It is widely grown in gardens as an ornamental plant in South India and also grows wild in many places. The plant belongs to the family Costaceae (Hedge *et al.*, 2014)². *Costusigneus* is a synonym of *Chamaecostus cuspidatus*, which is an accepted name. The Costaceae was first raised to the rank of the family by Nakai based on spirally arranged leaves and rhizomes being free from aromatic essential oils (Hedge *et al.*, 2014)². In Siddha medicine, the plant is used for diabetes; leaves are chewed twice daily or dried powder of leaves taken 1/2 to 1 gram twice daily. Used by tribal people of Kolli hills of Tamil Nadu for diabetes. Leaves are consumed as fresh, dried, and powder leaf forms. In Mexico, infusion of aerial parts is used to treat renal disorders (Hedge *et al.*, 2014)².

Scientific Classification

Domain: Eukaryota

Kingdom: Plantae

Subkingdom: Viridaeplantae

Phylum: Trahceophyta

Subphylum: Euphyllophytina

Class: Liliopsida

Subclass: Commelinidae

Order: Zingiberaceae

Family: Costaceae

Subfamily: Asteroideae

Tribe: Coreopsidae

Genus: *Chamaecostus*

Specific Epithet: *igneus*.

Botanical Snippet: The family Costaceae consists of four genera and 200 species. The genus *Costus* is the largest family, with about 150 species of mainly tropical distribution (Jyothi *et al.*, 2015)¹. *Chamaecostus cuspidatus* is a perennial, upright, spreading plant growing to a height of 2 feet, with the tallest stems falling and lying on the ground (Hedge *et al.*, 2014)². Leaves are simple, alternate, entire, oblong, 4-8 inches long with parallel venation, spirally arranged around the stems (Hedge *et al.*, 2014)², dark green leaves have a light purple underside. Flowers are orange, 1.5 inches in diameter, on cone-like heads at the tips of branches. Fruits are green colored, less than 0.5 inches.



FIG. 1: AERIAL PARTS OF *CHAMAECOSTUS CUSPIDATUS*

Phytochemistry: Phytochemical investigations revealed the presence of carbohydrates, terpenoids, proteins, alkaloids, tannins along with flavonoids. Quercetin is the major flavonoid (Hedge *et al.*, 2011)² isolated from methanol extract of *Chamaecostus cuspidatus*. Phytoconstituents with anti-oxidant potential such as ascorbic acid, α -tocopherol, β -carotene, terpenoids, steroids, and flavonoids have been reported (Khan *et al.*, 2014)³. Reports also showed that leaves of *Chamaecostus cuspidatus* contain Tigogenin, gracillin, sitosterol, Dglucoside. A number of constituents like roseoside, epigallocatechingallate, beta-pyrazol-1-ylalanine, cinchonainIb, leucocyandin-3-O-beta-d-galactosyl, cellobioside, leucopelargonidin - 3 - Oalpha - L rhamnoside, glycyrrhetic acid,

dehydro-trametenolic acid, strictinin, isostrictinin, pedunculagin, epicatechin, christinin-A have been isolated (Rao *et al.*, 2016)¹³ and reported to possess significant insulin-mimetic activity along with the significant antidiabetic activity.

MATERIAL AND METHODS:

Preparation of Extract: To prepare the methanolic extract of *Chamaecostus cuspidatus* Soxhlet apparatus has been used. First, defatting with petroleum ether has been done to remove all the oil substances from the extract. Then, after drying the marc or completely removing the petroleum ether, methanolic extract was prepared.

Preliminary Phytochemical Screening of *Chamaecostus cuspidatus*:

Petroleum Ether Extract of *Chamaecostus cuspidatus*: For phytochemical screening, the sample is mixed with more petroleum ether and then at 60 °C gets heated for confirmation of chlorophyll in the sample (Jangid *et al.*, 2021)¹².

Methanolic Extract of *Chamaecostus cuspidatus*:

For determination of flavonoids (Alkaline reagent test, Lead acetate test, Ferric chloride test) (Rao *et al.*, 2016)¹³, alkaloids (Dragendroff's reagent test) (Gul *et al.*, 2017)¹⁴, tannins (Gelatin test, Matchstick test) (Ukoha *et al.*, 2011)¹⁵, terpenoids (Salkowski test) (Das *et al.*, 2014)¹⁶ and saponins (Foam test, Lead acetate test) (Angone *et al.*, 2013)¹⁷ tests has been performed.

Bovine Serum Albumin (BSA) – Glucose Assay for *In-vitro* Glycation:

The methodology was based on that of (Brownlee *et al.*, 2013). BSA (10 mg/mL) was incubated with glucose (500mM) in phosphate buffered saline (PBS) (5mL total volume, pH 7.4) and extract containing 0.02% sodium azide at 37°C with a final concentrations of BSA (2mg/mL), glucose (40 mM), sample (0.1 to 0.5mg/mL). All the reagents and samples were sterilized by filtration through 0.2 µm membrane filters. The protein, the sugar, and the prospective inhibitor were included in the mixture simultaneously.

Amino guanidine was used as a positive inhibitor control. Reactions without any inhibitor were also setup. Each solution was kept in the dark in a capped tube. After 15 days of incubation, fluorescence intensity (excitation wavelength of 370nm and emission wavelength of 440 nm) was measured for the test solutions.

$$\% \text{ Inhibition} = (1 - \text{Fluorescence of test sample} / \text{Fluorescence of the control}) \times 100$$

OR

$$\% \text{ Inhibition} = 100 - \{(\text{Fluorescence of test sample} / \text{Fluorescence of the blank}) \times 100\}$$

D. M. Rutherford, M. P. Nielsen, S. K. Hansen, *et al.*, 1992.

RESULTS: Percentage yield of methanolic extract of *Chamaecostus cuspidatus*.

$$\% \text{ Age yield} = \text{weight of extract} / \text{weigh to frawmaterial} \times 100$$

$$= 12/35 \times 100$$

$$= 34.2\%$$

The percentage yield of petroleum ether extract of *Chamaecostus cuspidatus*:

$$\% \text{ Age yield} = \text{weigh to extract} / \text{weigh to raw material} \times 100$$

$$= 10.26/35 \times 100$$

$$= 29.3\%$$

Preliminary Phytochemical Screening of Petroleum Ether Extract of *Chamaecostus cuspidatus*:

The preliminary phytoconstituents screening of petroleum ether is done. It confirms the presence of chlorophyll only in **Table 1 Fig. 6**.

Preliminary Phytochemical Screening of Methanolic Extract of *Chamaecostus cuspidatus*:

Flavonoids **Fig. 2**, Terpenoids **Fig. 3**, Saponins **Fig. 4**, Tannins and Alkaloids **Fig. 5** have been screened as preliminary phytoconstituents **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING OF CHAMAECOSTUS CUSPIDATUS

S. no.	Name of constituents	Phytochemical test	Observation	Results
1	Flavonoid	Alkaline reagent test	Solution becomes colorless	Present
		Lead acetate test	Yellow precipitates	Present
		Ferric chloride test	Intense green color	Present
2	Terpenoids	Salkowski test	Reddish brown coloration of the interface	Present

3	Tannins	Gelatin test Matchstick test	White buff color precipitates The matchstick wood turns pink or red	Absent Absent
4	Saponin	Foam test Lead acetate test	Development of stable foam White precipitates	Present Present
5	Alkaloid	Dragendorff's reagent test	Red- orange precipitates	Present
6	Chlorophyll	Sample+ pet ether & heat	Green residue	Present

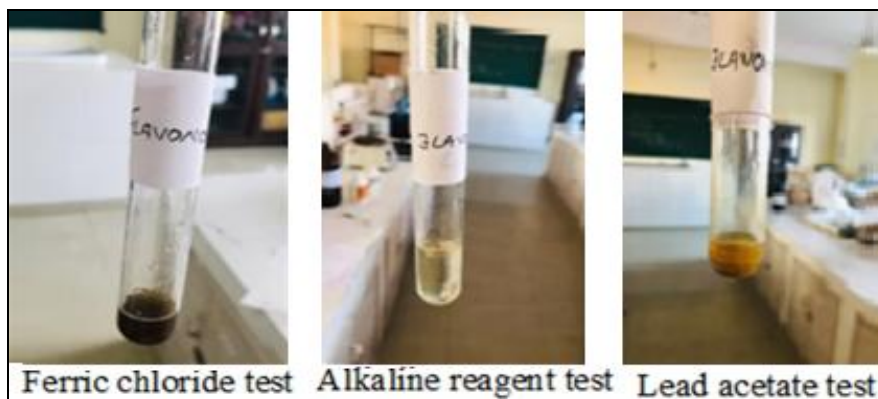


FIG. 2: IMAGES OF PRELIMINARY PHYTOCHEMICAL TEST FOR CONFIRMING THE PRESENCE OF FLAVONOIDS



FIG. 3: IMAGE OF PRELIMINARY PHYTOCHEMICAL TEST FOR CONFIRMING THE PRESENCE OF TERPENOIDS

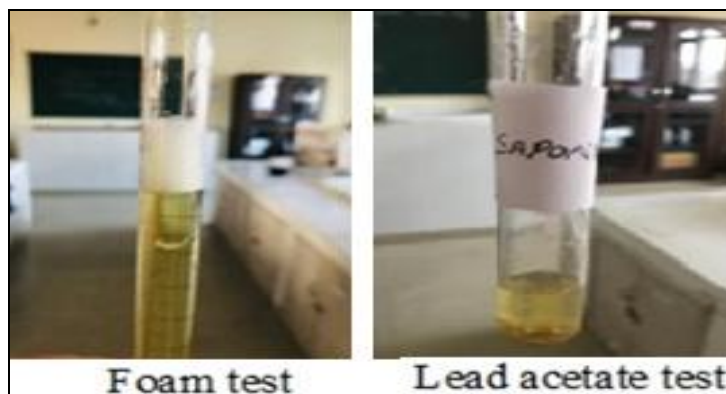


FIG. 4: IMAGES OF PRELIMINARY PHYTOCHEMICAL TEST FOR CONFIRMING THE PRESENCE OF SAPONINS



FIG. 5: IMAGES OF PRELIMINARY PHYTOCHEMICAL TEST FOR CONFIRMING THE PRESENCE OF ALKALOIDS

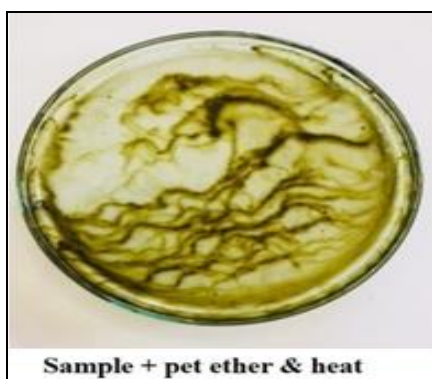


FIG. 6: IMAGES OF PRELIMINARY PHYTOCHEMICAL TEST FOR CONFIRMING THE PRESENCE OF CHLOROPHYLL

Effects of Methanolic extract of *Chamaecostus cuspidatus* on Glycation of BSA: The formation of AGEs was monitored for 15 days by measuring the fluorescence intensity of the BSA-glucose solutions. When BSA was incubated with glucose, a significant increase in fluorescence intensity was observed after 15 days of the experiment. After the methanolic extract was added to reaction media containing BSA/glucose system, the fluorescence intensity was significantly decreased in a concentration-dependent manner **Fig. 7**. Amino guanidine was used as a positive control **Fig. 8**.

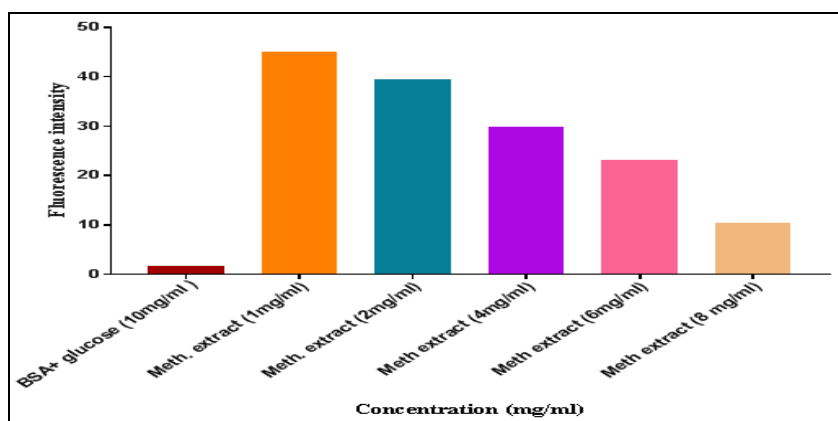


FIG. 7: EFFECTS OF METHANOLIC EXTRACT OF CHAMAECOSTUS CUSPIDATUS ON GLYCATION OF BOVINE SERUM ALBUMIN REPRESENTED IN A BAR GRAPH

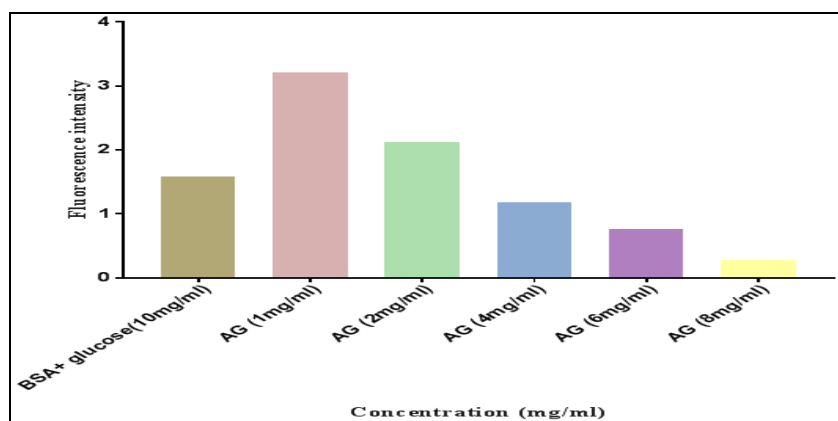


FIG. 8: EFFECT OF AMINOGUANIDINE (POSITIVE CONTROL/STANDARD DRUG) ON GLYCATION OF BOVINE SERUM ALBUMIN

DISCUSSION: Hyperglycemia is thought to be the primary cause of micro and macrovascular complications in diabetes mellitus; persistent hyperglycemia increases the concentration of advanced glycation end products (AGEs) (Chawla *et al.*, 2016)⁸. This increase in AGEs has been linked to the ageing process as well as the pathogenesis of age-related disorders such as Alzheimer's disease and diabetic complications. AGEs are formed by a series of complex reactions

between the carbonyl group of a reducing sugar or aldehyde and the free amino group of a protein, which results in the reversible formation of Schiff bases. Covalently bound Amadori products are then formed, inducing further oxidation to form dicarbonyl compounds, including fluorescent cross-links and nonfluorescent cross-links collectively referred to as AGEs (Kim *et al.*, 2017)⁹. Although aminoguanidine, a synthetic glycation inhibitor, reduces the development of diabetic complications,

adverse effects have been reported in clinical trials involving this drug (Narayanan, 1993)¹⁰. Thus, the discovery of novel inhibitors of the protein glycation reaction from natural sources represents a promising therapeutic strategy for the prevention of diabetes complications. The effect of methanolic extract of *Chamaecostus cuspidatus* on the formation of total AGEs was evaluated using the fluorescence property. The results indicate that methanolic extract of *C. cuspidatus* inhibits the glycation reaction effectively because oxidative reactions favor the formation of advanced glycation end products, methanolic extract of *C. cuspidatus* may inhibit their formation by reducing reactive oxygen species (ROS) or by trapping ROS formed *in-vitro* during the autoxidation of sugars and/or the oxidative degradation of Amadori products such as Fructosamine.

Our results indicate the presence of various phytochemicals such as flavonoids, terpenoids, saponins, alkaloids, *etc.* From previous studies, all these phytochemical constituents inhibit the formation of AGEs (Shad *et al.*, 2014)¹¹. Therefore, from BSA-glucose assay, methanolic extract *C. cuspidatus* plant reduces the fluorescence intensity in a concentration-dependent manner, revealing the inhibition of AGEs formation.

CONCLUSION: Based on the findings presented above, we can infer that methanolic extract of the *C. cuspidatus* plant is a viable therapeutic intervention in the treatment of diabetes and the prevention of AGEs in diabetic complications.

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CONFLICT OF INTEREST: Nil

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