Original research paper

Evaluation of the Cookies Formulated with *Costus igneus* Plant Material for Antidiabetic Activity

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Abstract

Diabetes Mellitus is a fast-growing disorder in the current scenario commonly affecting all age groups especially elder groups. The high blood glucose can be kept in control with nutraceutical foods. The present study is aimed to formulate a nutritionally rich cookie with *Costus igneus* leaf extract and to determine the effect of cookie consumption on decreasing blood glucose levels in type 2 diabetic patients. Cookies made with *Costus igneus* leaf powder etc. were analyzed for proximate analysis and phytochemical analysis with standard procedures. A total of 30 Type-II diabetic patients were selected for the study whose demographic variables and blood glucose levels were measured on the first day and after 15 days, 30 days of cookies consumption. The proximate and physic-chemical analysis showed cookies contain high amount of secondary metabolites including antioxidant compounds. Among the 30 study subjects, the mean levels of FBS and PBS were decreased significantly in all the four groups from day 0 to day 30 which indicates that *C.igneus* cookies consumption had good effect in reducing the blood glucose levels. However the HbA1C has been decreased by one unit in all the four study groups. The cookies made with *Costus igneus* plant material have good antimicrobial, antioxidant and antidiabetic activities and can be used as a therapeutic and functional food source for the treatment of overweight, obesity and diabetes.

Key words: *Costus igneus, cookie, proximate analysis, phytochemicals, antioxidant, post prandial, glycated hemoglobin.*

Introduction

The incidences of deficiency diseases are on decline phase due to the incorporation of fortified foods into our diet. However, the prevalence of obesity, diabetes mellitus (DM) and cancer are escalating due to food and lifestyle changes. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia (high level of sugar in the blood for a prolonged period), resulting from defects in insulin secretion, or insulin action or both. Nutritional management plays a key role in managing diabetes. Foods with a lower glycemic index (GI) scores are ideal for helping to keep blood sugar levels stable. Several studies have reported that the consumption of fiber-rich and low-GI foods like oats, ragi, soya, bajra, almonds, flax seeds, etc. will help to keep blood sugar level stable and also significantly decrease in total cholesterol, LDL-cholesterol, and triglyceride levels. Antioxidants like polyphenols and ascorbic acid are vital substances which possess the ability to protect the body from damage caused by free radical-induced oxidative stress. [1-5] The use of antioxidants from food sources is an active area of study in diabetes research.

Costus igneus is an herbaceous plant, its alternative names include insulin plant, spiral flag or fiery *Costus*. It belongs to the *Costaceae* family. It is widely grown and

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used in South India, to treat diabetes due to its antidiabetic property. The various ethanolic and methanolic extracts of *C. igneus* have been reported for many pharmacological activities such as antidiabetic in controlling the blood glucose levels, antibacterial, antifungal, antioxidant, hypolipidemic, hepatoprotective, anti-inflammatory, antiproliferative and many more and these properties are due to presence of secondary metabolites such as alkaloids, quercetin, diosgenin, steroids, beta-carotene, flavonoids, terpenoids and phenolic compounds in the leaf extracts. [6-8] Most of the individuals living in both urban and rural areas of Visakhapatnam who were suffering with type 2 diabetes practicing of consuming 1-2 leaves of *C.igneus* per day daily in their diet. Hence the present study is intended to develop a nutritional rich cookie formulated with *C. igneus* leaf powder and to determine the effect of consuming *Costus igneus* cookies on decreasing blood glucose levels in type 2 diabetic patients.

Materials and Method

Collection of plant extract: *Costus igneus* plant leaves were collected from Ambasamudram, Tirunelveli, Tamil Nadu, India. Fresh leaves of *Costus igneus* were collected, cleaned and shade dried. The dried leaf was made into powder using a mechanical grinder. The powdered samples were extracted with methanol using a Soxhlet apparatus.

Preparation of cookie: In the present study the following raw materials were used to prepare cookies which include *C. igneus* leaf powder, multigrain flour (oatmeal, ragi, bajra, soya, wheat flour), almonds, flax seeds, maida, butter, vegetable oil, milk, sweetener. Insulin plant leaves were shade dried, grinded and sieved into a fine powder. Oatmeal, ragi, bajra, soya were roasted and grinded into fine flour. A known amount of dry mix was prepared by mixing multigrain flour and insulin plant leaf powder in a vessel. Wet mix was prepared in a separate vessel by mixing a known amount of vegetable oil and melted butter. Salt, sweetener, baking powder, and a drop of vanilla essence for the flavor were added to the wet mix. Prepared dry mix was added slowly to the wet mix and mixed well to make the dough. The dough was kneaded well and made into shapes. The shaped dough was baked at 180°C. Cookies were packed into airtight cover and stored in the cool and dry place.

Figure 1: Cookie prepared with *Costus igneus* leaf powder

Proximate analysis: In proximate analysis total moisture, total solids, total ash, water-soluble ash, acid-insoluble ash, sulphated ash and fat content of *Costus igneus* cookie were analysed with standard procedures [9-11]. Moisture content and solid content of the sample were analyzed in moisture analyzer MB45. Estimation of protein was carried out by standard Lowry's method (1951).[12] Total Carbohydrate content was estimated by modified anthrone method (Hedge and Hofreiter, 1962). The total reducing sugar content of *Costus igneus* cookie was evaluated with 3,5-dinitrosalicylic acid (DNS) method. [13] α-Amylase inhibitory activity of the extract and fractions was carried out according to the standard method with few modifications.[14]

Phytochemical analysis: In the present study, several phytochemical constituents were evaluated qualitatively using standard protocols [15-17].

Antioxidant activity:

Estimation of Ascorbic Acid: Ascorbic acid estimation was carried by the volumetric method described by Pisoschi et al. (2008) [18]. Amount of ascorbic acid present is calculated by below formula

$$\text{Ascorbic acid} = \frac{0.5}{V1} \times \frac{V2}{5} \times \frac{100}{wt.of\ the\ sample} \times 100$$

Determination of β-carotene: Estimation of beta-carotene in cookie sample was performed according to Mustapha and Babura (2009). The following formula was used to calculate

$$\beta\text{-carotene } (\mu g/100gm) = \frac{OD\ at\ 452nm \times 13.9 \times 104 \times 100}{Wt.of\ sample \times 560 \times 1000}$$

Total Phenolic Content (TPC): The total phenolic content (TPC) of *Costus igneus* cookie sample

was determined by Folin-ciocalteu method.[19] TPC quantification and results were expressed as mg/g (Gallic Acid Equivalent (GAE)/ dry weight).

DPPH radical scavenging assay: The radical scavenging activity of cookie sample was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) using the method described by Shimada et al. (1992). The percentage inhibition was calculated by the following equation.

$$\% \text{ radical scavenging activity} = \frac{(absorbance\ of\ blank - absorbance\ of\ sample)}{(absorbance\ of\ blank) \times 100}$$

Evaluation of cookies for controlling the blood glucose levels in Type-II diabetic patients: The study was a randomized clinical trial done to evaluate the effect of consuming *C.igneus* cookies on decreasing the blood glucose levels in 30 Type-II diabetic patients. From the 30 subjects, blood samples were collected and sent for analysis of FBS, PBS and HbA1C using C111 Cobas analyzer. The inclusion criteria of the study were Age > 35 years, non-pregnancy, non-allergic to herbal products, not taking any herbal products during the study, no acute infection, HbA1C > 7, FBS > 140 mg/dl, PBS > 200 mg/dl and non-insulin dependent. *C.igneus* cookies were prepared in a way that each cookie weighs 20 g and each contains 500mg of *C.igneus* plant extract. After explaining the main objective of the study, study protocol and importance of consuming *C.igneus* cookies in controlling the diabetes, a written consent form was taken from each individual. Each subject was instructed to consume a single cookie after half-an-hour of breakfast, lunch and dinner along with their routine regular diabetic drugs for 15 days initially. After 15 days, FBS, PBS and HbA1C tests were performed. Again they were instructed to take the same quantity of cookies at three different time intervals in a day for another 15 days and their blood glucose levels were again monitored. Data was analyzed using SPSS Version 24.0. Results were expressed as Mean ±SD of three replicates determinations.

Results and Discussion

Proximate analysis is an important index to classify the nutritional value of a food material. The proximate compositions determined in the cookie samples were summarized in Table 1. It shows that the cookie sample has a moisture content (7.94 ± 0.2), total ash (70.7 ± 0.1), high protein content (4.24 ± 0.4), high fiber (8.03 ± 0.007) content; and a relatively lower fat content (3.75 ± 0.3). This indicates that *C. igneus* cookies are good source of mineral elements as they contained a high percentage of ash. The presence of high protein content in cookie promotes body development, growth control, maintenance of fluid balance, strong immune function etc. As the fibre content of the cookie was high which may helpful for the better digestion process. It was found that, the total carbohydrate content of the cookie sample was high (80.2 ± 1.1) which are major contributors of energy. From the present study, it has been shown that cookies made with *Costus igneus* plant leaf have less reducing sugar (0.07 µg/ml) when compared with standard (1.05µg/ml). The results of the present study on in-vitro anti-diabetic activity of *Costus igneus* cookie are shown in Figure 2. Cookies made with *Costus igneus* have good α-amylase inhibitory activity at 500 µg/ml. The present study had proved that cookies made with *Costus igneus* leaf had shown good anti-diabetic property which can better control the blood glucose levels in diabetic patients because α- amylase inhibitors also called as starch blockers tend to prevent or slow down the absorption of starch into the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose and other simple sugars.

Costus igneus cookie was subjected to preliminary phytochemical analysis and the results showed the presence of phytochemicals which include flavonoids, alkaloids, terpenoids, tannins, steroids, quinones, polyphenols, phenols, saponins, glycosides and coumarins and this in turn indicates it have good antibacterial, anti-inflammatory, anticancer, and antidiabetic activities and cardiac failures. The results obtained with *Costus igneus* cookie revealed the ascorbic acid content as 4.2µg/g hence act as a powerful antioxidant which fights against free-radical induced diseases. β-carotene was estimated, and the results showed that *Costus igneus* cookies have high β-carotene content 1.49µg/g and vitamin content 2.48µg/g. The results showed that the total phenolic content of cookie was 5.44 mg of GAE/g of dry sample. As the cookie showed higher phenolic content, it is likely to be a potential antioxidant. DPPH radical scavenging activity of cookie was showed in Table 2. Cookie at 10 µg/ml showed the lowest inhibition with 9.92%. The IC_{50} value for DPPH, the *Costus igneus* cookie shows

maximum antioxidant activity with 62.3% (Table 3).

All the 30 subjects fasting serum blood glucose levels, post-prandial serum blood glucose levels, HbA1C levels before and after intervention were showed in the Table 4. The mean levels of FBS and PBS were decreased significantly in all the four groups from day 0 to day 30 which indicates *C.igneus* cookies consumption had good effect in reducing the blood glucose levels. However the HbA1C has been decreased by one unit in all the four study groups. Akhila et al. (2010) found that leaves of *Costus igneus* reduced the fasting and postprandial blood sugar levels, bringing them towards normal, in dexamethasone-induced hyperglycemia in rats [20]. The study was mainly designed and investigated based on the consumption of *C.igneus* leaves by the local inhabitants of Visakhapatnam to control their blood glucose levels by both type I and type II diabetic patients. The *Costus igneus* plant was cultivated in their own houses and daily they were consuming 1-2 leaves per day. However further research has to be carried out to know the effect of *C.igneus* cookies in Type-I diabetic patients.

Table 1: Proximate analysis of *C. igneus* cookies

Parameter	**Percentage dry weight basis**	**Mean**	**SD**	**SEM**
Moisture (%)	7.94± 0.2	8.27	0.56	0.37
Solids (%)	92.06±0.5	92.05	0.05	0.03
Regain (%)	-8.63±-1.0	-8.61	0.9	0.56
Total ash (%)	70.7±0.1	70.7	0.01	0.05
Water-soluble ash (%)	43.65±0.8	43.6	0.08	0.04
Acid-insoluble ash (%)	82.35±0.6	82.3	0.06	0.03
Sulphated ash (%)	80.47±1.1	80.3	1.24	0.72
Fat (%)	3.75±0.3	3.75	0.05	0.028
Fiber (%)	8.03 ± 0.007	8.01	0.01	0.001
Protein (%)	3.24 ± 0.4	3.24	0.05	0.029
Total carbohydrate content (%)	80.2 ± 1.1	80.06	1.006	0.58
'p' ≤ 0.05 Significant				

Values are expressed as mean ± SD of the three replicates. SD: Standard deviation, SEM: Standard error of means

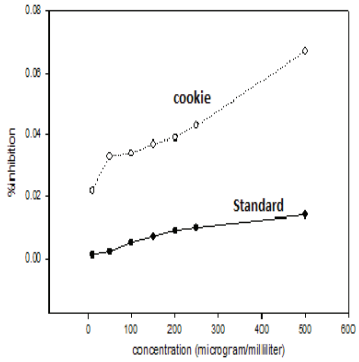

Figure 2: α–Amylase inhibitory assay of *Costus igneus* leaf and Cookie

Table 2: DPPH radical scavenging assay of *Costus igneus* cookie

Concentration (µg/ml)	IC50 Values (%)
10	9.92
20	11.25
30	12.37
40	13.19
50	14.52
100	18.81
500	62.3

Table 3: Antioxidant activity of *C.igneus* cookie

Ascorbic acid content (µg/g)	β-carotene content (µg/g)	Total phenolic content (mg/g)	DPPH activity (%)	'p' Value
4.2±1.3	1.49±0.1	5.44±0.7	62.3±1.9	0.9371

'p' value: Probability value

Table 4: Effect of cookies in controlling blood glucose levels in Type-II diabetic patients

S.No	Age (years)	Fasting serum glucose levels (Mean ± SD) (mg/dl)			Post prandial serum glucose levels (Mean ± SD (mg/dl)			HbA1C levels (Mean ± SD) (%)		
		Day 0	Day 15	Day 30	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
1.	35-40	185±3	141±4	123±9	199±1	168±7	151±4	8.9±0.2	8.2±0.4	7.9±0.3
2.	41-45	178±2	154±5	131±5	215±5	180±6	148±5	8.7±0.6	8.5±	7.8±0.1
3.	46-50	163±8	138±2	110±4	238±6	189±3	159±3	8.3±0.3	7.8±0.1	7.6±0.2
4	51-55	184±6	159±2	125±3	256±4	197±8	163±4	9.1±0.2	8.9±0.1	8.4±0.3

Conclusion

Due to high antioxidant potential and phenolic content of the *Costus igneus* cookie, it can be used as a therapeutic or functional food source for the treatment of overweight, obesity and diabetes. The study also concluded that, regular consumption of *Costus igneus* cookies reduced serum glucose levels and glycated hemoglobin levels in people with type 2 diabetes.

Conflict of Interest: Conflict of interest declared none.

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Ethical Clearance: This study was reviewed and approved by The Ethics Committee of GITAM Institute of Medical sciences and Research, Tertiary care hospital, GITAM (Deemed to be University)

Conflict of Interest: The study declared "No conflict of interest"

References

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes care, 2019;32(1):62-67
2. QingtaoHou, Yun Li, Ling Li, Gaiping Cheng, Xin Sun, Sheyu Li, and Haoming Tian. The Metabolic Effects of Oats Intake in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis. Nutrients, 2015; 7(12): 10369–10387.
3. Jason Kam, Swati Puranik, Rama Yadav, Hanna R Manwaring, Sandra Pierre, Rakesh K Srivastava, and Rattan Yadav S. Dietary Interventions for Type 2 Diabetes: How Millet Comes to Help. Frontiers in Plant Science, 2016; 7: 1454.
4. Golbidi S, Ebadi SA, Laher I. Antioxidants in the treatment of diabetes. Current Diabetes Rev, 2011; 7(2):106-25.
5. Rajeshwar Y, Senthil Kumar GP, Gupta M, Mazumder UK. Studies on in vitro Antioxidant activities of methanol extract of Mucunapruriens (Fabaceae) seeds. Eur Bull Drug Res, 2005;13:31–9.
6. Meena S Deogade, Anita W, Seema C Lohakare. Pharmacognostical and Phytochemical study of *Costus igneus* NEBr leaf. Journal ISM, 2014; 2 (4): 174-178.
7. Prakash K Hegde, Harini A Rao, and Prasanna N Rao. A review on Insulin plant

(*Costus igneus* Nak). Pharmacognosy Review, 2014; 8(15): 67-72.

8. Kalailingam P, Kaliaperumal R and Shanmugam K. Efficacy of methanolic extract of *Costus igneus* rhizome on hypoglycemic, hypolipidimic activity in Streptozotocin (STZ) diabetic rats and HPTLC analysis of its active constituents. International Journal of Bioscience, Biochemistry and Bioinformatics, 2011; 5(1):318-321.
9. Saraswathi R, Upadhyay L, Venkatakrishnan R, Meera R and Devi P. Isolation and biological evaluation of steroid from stem of *Costus igneus*. Journal of Chemical and Pharmaceutical Research, 2010; 2: 444-448.
10. Adamul AU, Paul ED, Gimba CE, and Ndukwe IG. Phytochemical And Proximate Analysis Of Aspil L Ia Kotschyi (Sch.Bipex, Hochst) Oliv, Nigerian. Journal of Technology, 2017;36(4):1135 – 1137.
11. Audu Idi Wakawa, Audu Bala Sambo, Suleiman Yusuf. Phytochemistry and proximate composition of root, stem bark, leaf and fruit of desert date, Balanites aegyptiaca. The Journal of Phytopharmacology, 2018; 7(6): 464-470.
12. Lowry OH, Rosenbrough NJ, Ferur AL, and Randall RJ. Protein measurement with Folin phenol reagent. Journal of Biological chemistry, 1951; 193: 65-275.
13. Miller GL. Use of Dinitrosaiicyiic acid reagent for determination of reducing sugar. Journal of Analytical Chemistry, 1959; 31(3): 426-428.
14. Talent Chipiti, Mohammed A Ibrahim, Moganavelli Singh, and Shahidul Islam. In vitro α-amylase and α-glucosidase Inhibitory and Cytotoxic activities of extracts from Cissuscornifolia Planch Parts. Pharmacogn Mag, 2017;13(Suppl 2): 329-333.
15. John Reddy P, Sneha Sri M, SrinivasaVarma K, Anitha P, RavindraBabu P. Chromatographic analysis of phytochemicals in *Costus igneus* and computational studies of flavonoids. Formatics in Medicine Unlocked, 2018; 13: 34-40.
16. Vasantharaj S, Sathiyavimal S and Hemashenpagam N. Antimicrobial potential and screening of antimicrobial compounds of Costus Igneus using GC-MS. IJPSR, 2013; 4(5): 1815-1819.
17. Bakari S, Amal D, Samir F, Slim S, Neji Gharsallah, Adel K. Proximate analysis, mineral composition, phytochemical contents, antioxidant and antimicrobial activities and GC-MS investigation of various solvent extracts of cactus cladode, Food Science and Technology, 2017; 37(2): 286-293.
18. Pisoschi AM, Danet AF, Kalinowski S. Ascorbic Acid Determination in Commercial Fruit Juice Samples by Cyclic Voltammetry. 2008; 2008: 1-8
19. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture, 1965; 16, 144-158.
20. Akhila J Shetty, Divya Choudhury, Rejeesh, Vinod Nair, Maria Kuruvilla, and Shashidhar Kotian. Effect of the insulin plant (*Costus igneus*) leaves on dexamethasone-induced hyperglycemia. Int J Ayurveda Res., 2010; 1(2): 100–102.