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Explore the therapeutic potential and bioevaluation of giloy leaves *(Tinospora cordifolia)* powder for the management of type 2 diabetes

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Abstract: Giloy (Tinospora cordifolia) is an important Ayurvedic medication. Numerous illnesses, including general senility, fever, diabetes, dyspepsia, urinary infections, jaundice, and skin conditions are treated with it. The biological description and chemical components of *cordifolia* are critically reviewed in this essay, with a focus on its ayurvedic properties and pharmaceutical applications. The goal of the current study was to investigate the chemical, phytochemical and mineral profile and anti-diabetic potential of giloy leaves powder. The results showed that the moisture content was 6.2%, ash content was 13.12%, crude protein was 17.27% and fiber was 5.5%. While in mineral analysis, sodium was 22.12±1.78, magnesium was 15.78±1.70, calcium was 9.78±1.27, potassium was 32.24±1.40, iron was 8.37±1.078 and zinc was 4.87±0.89. Furthermore, total phenolic content was 156.78±1.18 and total flavonoid content was 45.78±0.57. Afterwards, the anti-diabetic potential was analyzed by givingthe giloy leaves powder to human experimental group G1 and G2 at adose of 400 mg/kg and 800 mg/kg, respectively. The effect of giloy leaves powder on diabetes patients' blood sugar levels was monitored every seventh day for 2 months, and HbA1c tests were done initially and after 2 months. Random blood sugar and HbA1c were significant in ANOVA.

Keywords: Flavonoids, anti-diabetic, HbA1C, Giloy leaves.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM), also known as "premature ageing disease," is a collection of abnormal metabolic paradigms characterized by hyperglycemia as a core characteristic. Despite extensive and coordinated efforts by the World Health Organization (WHO), the International Diabetes Federation, and several social science agencies, the incidence of T2DM is increasing globally at a concerning rate [Kumar *et al*., 2015]. Research hasbeen approved that numerous plants have therapeutic properties especially in the control of certain chronic illnesses i.e., diabetes on a very reasonable cost. Diabetes and its different kinds have been a deep-rooted infection for doctors for quite a long time. Naturally available plants are cost effective and have decreased side effects and have proven more efficient in diabetes mellitus [Singletary 2010]. To find a treatment and control this disease globally, more thorough study is urgently required. The advantages of Ayurveda, a lengthy system of conventional treatment, are now widely acknowledged. It's interesting to note that many medications used now in medicine have plant origins. *Tinosporacordifolia* (*T. cordifolia*) "Giloy" stands out as a unique source with a wide range of therapeutic advantages among the numerous helpful herbal plants [Kumar and Ashaq, 2021]. By increasing insulin secretion, cordifolia reduced oxidative stress and elevated blood sugar levels in rats [Kumar, 2015]. *In vivo* and *in vitro* studies show that the presence of active phytochemicals in giloy that is isoquinoline alkaloid fragment which includes jatrorrhizine, monofluoride and palmatine, has insulin lowering effects [Patle *et al*., 2021]. One more isoquinoline alkaloid called berberinehasbeen tested on diabetic humans, which proved that it reduced the glucose level just like metformin tablet [Stackhouse *et al*., 2020]. The effects of *T. cordifolia* stem administration was recently studied by Vishnu Kumar *et al*. in alloxan-treated diabetic rats and they found that it helped to reverse hyperglycemia, dyslipoproteinemia and oxidative stress [Kumar, 2021. The purpose of the current study was to evaluate *T. cordifolia*'s impact on diabetic patients considering the considerations [Kumar, 2015].

MATERIALS AND METHODS

Collection and preparation of giloy (Tinospora cordifolia) leaves powder

Healthy (free from diseases as well as insecticidal and pesticidal effects) stems of *T. cordifolia* were gathered from the vicinity of the nursery, examined, and taxonomically recognized with the assistance of a

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taxonomist. Under shade, leaves were dried and ground in a lab mill to a fine powder.

Characterization of giloy leaves powder
Giloy leaves powder was subjected to various assays defined as follows:

Proximate analysis
Giloy powder was assessed for moisture, ash, crude, fat, protein, crude fiber and nitrogen extract as done by according to their standard procedures described by the Association of official analytical chemists (Mishra *et al*., 2015).

Minerals analysis
Suitable minerals analysis of corn silk such Na, Mg, K, Ca, iron and zinc was analyzed by specific protocols (Soler *et al*., 2016).

Antioxidant features
Total phenolic content was analyzed by the Folin Ciocalteu method and total flavonoid content was determined by spectrophotometer through the colorimeter activity of aluminum chloride (Pandey *et al*., 2016).

Bio-evaluation of giloy leaves powder to check its therapeutic potential against diabetes
The Ethical approval has been taken from diabetic patients

Selection of diabetic subjects
30 type 2 diabetic subjects (Female- 30-60) were selected from the Diabetes Outpatient Department of General Hospital, Lahore.

Exclusion criteria
Patients taking insulin or other drugs that might have an impact on glucose metabolism were not allowed. Women who were lactating or pregnant were also excluded from the study.

Inclusion criteria
All diabetic patients underwent a thorough medical evaluation by a doctor, which included a thorough medical history and physical examination. The study included females between the ages of 30 and 60 who had fasting blood glucose levels between 135 and 150mg/dl.
Subjects were divided into three groups of 30 subject each, with the following conditions:

Group 1: Diabetic Control: not giving any treatment
Group 2: Diabetic treated: 400kg/kg of giloy leaves powder body weight of patients
Group 3: Diabetic treated: 800mg/kg body leaves powder weight of patients

Collection of blood samples
Blood was drawn from the subjects' antemedial cubital veins before and after the study period, after an overnight fast. Disposable plastic syringes were used, and all aseptic precautions were taken. To prevent hemolysis, blood was gently pushed into a dry, clean plastic test tube right away. Blood was drawn from each of the three groups and tested for HBa1c (Vermeulen *et al*., 2011).

Ethical approval
The authors declared that there are no ethical issues in this paper.

STATISTICAL ANALYSIS

Using SAS version 6.12, the ANOVA along with Duncan Multiple Range Test were used to determine the significance of the data (SAS 1989).

RESULTS

Proximate analysis
The quality of the raw materials being used is significantly influenced by the determination of proximate composition. The quality characteristics of giloy leaves powder, including moisture, ash, fibre, proteins, and lipids along with nitrogen free extract, were examined. The six qualities that make up proximal analysis are of different components. The moisture content of 5-gram sample of *Tinospora cordifolia* is about 6.2$\pm$0.45 and it contains a good amount of protein in it which is 17.27$\pm$0.07, which is good for insulin metabolism and prevents from the loss of the muscle mass in body. It also plays an important role in many body functions. It contains a minimal amount of lipid, which is about 6.7$\pm$0.3 and this fat is considered as healthy fat. It contains a fiber in an amount of 5.5$\pm$0.5 and this dietary fiber is good for health and reduces many health issues. The amount of ash in the sample is 13.12$\pm$0.17 and nitrogen free extract is present in the amount of 51.8$\pm$0.72 in the given sample. The results of the current study, which focused on the close characterization of giloy leaf powder, were largely consistent with the ranges identified in the literature with a few minor exceptions. Environmental factors like climate and location may be the cause of the variance in values. Additionally, differences in genetic make-up may also contribute to differences in values (table 1).

Table 1: Proximate analysis of Giloy leaves *(Tinospora cordifolia)* powder

Parameter	Mean ± Std. E
Moisture (%)	6.2 ± 0.45
Fiber (%)	5.5±0.5
Protein (%)	17.27 ± 0.07
Ash (%)	13.12 ± 0.17
NFF (%)	51.8 ± 0.72
Lipid (%)	6.7 ± 0.3

Table 2: Mineral composition of Giloy leaves *(Tinospora cordifolia)* powder

Minerals	Mean (ug/g) ± Std
Sodium	22.12 ± 1.78
Magnesium	15.78 ± 1.70
Calcium	9.78 ± 1.27
potassium	32.24 ± 1.40
Iron	8.37 ± 1.078
zinc	4.87 ± 0.89

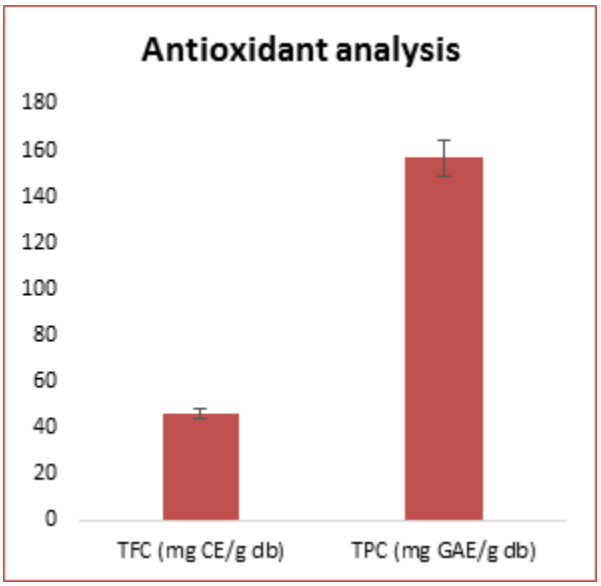

Fig. 1: Antioxidant features (TPD, TFC) of giloy leaves powder

Minerals analysis

The calcium content of 5-gram sample of *Tinospora cordifolia* is about 9.78±1.27, which is important in preventing the risk of osteoporosis, with which diabetes is correlated. It contains huge amount i.e. 8.37±1.078 of iron, which plays an important role in the prevention of kidney damage, with which diabetes is associated. However, many scientists conducted amineral analysis of giloy leaves powder. However, our results are in accordance with Pandey *et al.* who measured the mineral analysis of giloy leaves powder (Pandey *et al*., 2016). There is an important role of trace elements and minerals in human health and immune function. Iron, which is present in abundant amount in the giloy leaves powder shows that this plant can also be used as supplement. (table 2)

Antioxidant analysis (TFC, TPC)

TPC and TFC content of giloy leaves powder was determined by Pandey *et al.* (2016) and our results are similar to theirs. 2-gram sample of *Tinospora cordifolia* leaves powder contains total phenolic content (TPC) of 156.78±1.18 and total flavonoid content (TFC) is 45.78±0.57. By regulating glucose metabolism, activities of hepatic enzymes and profile of lipid, flavonoids increase the diabetic complications and pathogenesis. (fig. 1).

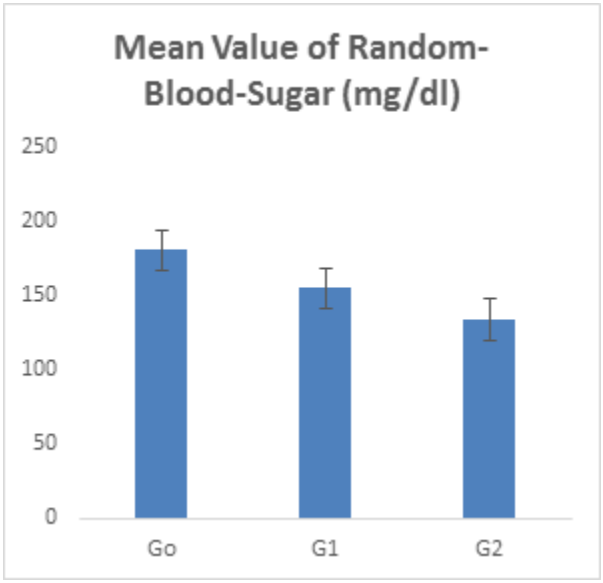

Fig. 2: Mean Value of Random Blood Sugar (mg/dl) from 0-60 days

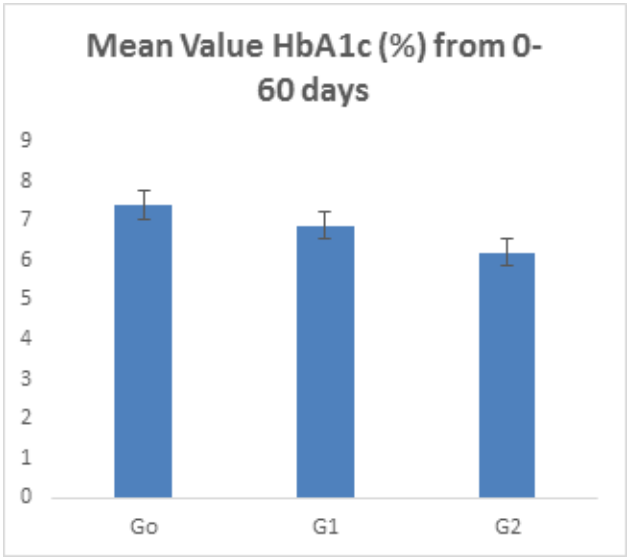

Fig. 3: Mean Value of HbA1c (%) from 0-60 days

Bioevolution of giloy leaves powder for the management of blood sugar level in human female subjects

The purpose of this bio evaluation conducted was to determine the nutraceutical worth of giloy leaves powder against diabetes in human female subjects. Bioevaluation was conducted for a period of 60 days. Patients of control group Go were not given giloy leaves powder and were only recommended to use their regular medications. G_1 (diabetes treatment group) was given giloy leaves powder of 400mg per day along with their regular medicines for a time span of 60 days. G_2 (diabetes treatment group) was given adose of 800mg/day of giloy leaves powder of

along with their regular medicines for 60 days. Random blood sugar levels of diabetic patients were checked every seventh day for 2 months.

Random blood sugar levels

Results showed that at primary stage of treatment, there was not any significant result but according to control, the results were significant. However, after 60 days, the random blood sugar level was checked forall patients and the results were highly significant. The control patients showed 181mg/dL but group 1, which contained 400mg giloy leaves powder showed 155mg/dL and group 3 that contained 800mg giloy leaves powder showed 134mg/dL random blood sugar levels. So, these results showed that 800mg giloy leaves powder was more significant as compared to all other treatments.

HbA1c

In the primary stage of treatment there was not any significant result but according to control, the results were significant. However, after 60 days, HbA1c was checked forall patients and the results were highly significant. The control patients showed 7.4 HbA1c but group 1 which contained 400mg giloy leaves powder showed 6.89 HbA1c and group 3 that contained 800mg giloy leaves powder showed 6.2 HbA1c. So, these results showed that 800mg giloy leaves powder was more significant as compared to all other treatments (Liang *et al*., 2019).

T. cordifolia study was carried out to scrutinize the levels of blood glucose in patients who suffered from type 2 diabetes. In this study, 30 subjects with type 2 diabetes were engaged and divided into 2 groups A and B. Group A was considered as control group and they were asked to continue with their medication of diabetes while group B was given 400 milligrams of *T. cordifolia* dose thrice a day with meal. After six months, their HbA1c and glucose levels before and after meal were documented. Statistical analysis clearly highlighted diminution in glycosylated hemoglobin, postprandial and fasting glucose in B group without showing any side effects (p less than or equal to 0.05).

DISCUSSION

The incidence of diabetes has risen due to changes in lifestyle that encourage aging, sedentary lifestyles, and also due to the lack of exercise. Herbal medicine has gained much progression in the past few centuries due to their natural source of origin and least side effects. The investigation of compositional and mineral content of giloy leaves reflects that it has good amount of protein which is important in muscle building and good amount of iron can be added in diet to prevent anemia and specially the women who has reached menopause having low calcium deposits. The effects of *T. cordifolia* stem administration was recently studied by Vishnu Kumar *et al*. in alloxan-treated diabetic rats and they found that it helped to reverse hyperglycemia, dyslipoproteinemia and oxidative stress [Kumar, 2021. The purpose of the current study was to evaluate *T. cordifolia*'s impact on diabetic patients considering the considerations. TPC and TFC were estimated for investigating the antioxidant potential and it was found that this herb is rich in these compounds which are good for removing oxidative stress in the body due to any disease, and also remove free radicals in the body. Our study was conducted to investigate the effect of giloy leaves on type 2 diabetes. 1000mg/day dose of giloy leaves powder for 60 days showed highly significant results against random blood sugar levels and HbA1c without showing any adverse effects.

CONCLUSION

One of the most significant medicinal plants, giloy (*Tinospora cordifolia*), is used to make a key medicine in the Ayurvedic medical system. Numerous illnesses, including general senility, fever, diabetes, dyspepsia, urinary infections, jaundice, and skin conditions are treated with it. The biological description and chemical components of *Cordifolia* are critically reviewed in this essay, with a focus on its ayurvedic properties and pharmaceutical applications. The goal of the current study was to investigate the chemical, phytochemical and mineral profile and anti-diabetic potential of giloy leaves powder. The giloy leaves *(Tinosporacordifolia)* powder was found significant effective in dealing with type 2 diabetes.

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