ORIGINAL ARTICLE

Development and Evaluation of Herbal Tea for the Management of Diabetes Mellitus Using Novel Combination of Herbal Ingredients

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Abstract

Introduction: The present study was aimed at the development of an antidiabetic dietary supplement in the form of herbal tea comprising green tea, gudmar, and asparagus followed by its evaluation by physical, chemical, and *in vitro* biological studies. **Materials and Methods:** Individual raw materials of all the three selected crude drugs were standardized for extractable matter, foreign organic matter, heavy metals, and microbial contamination. Flavonoid content, tannin content, *in vitro* non-enzymatic hemoglobin glycosylation, and α-amylase inhibition assays were also carried out as per standard methods. Standardized powdered materials of *Camellia sinensis* (tea), *Gymnema sylvestre* (gudmar), and *Asparagus racemosus* were blended in different ratios and evaluated for antioxidant activity by DPPH, O-Phenanthroline, superoxide radical scavenging, and nitric oxide radical scavenging assays. **Results and Discussion:** Of all the ratios, the batch 1 having 600, 250, and 150 of each of tea, gudmar, and asparagus, respectively, showed better antioxidant activity with an IC_{50} value of 130.36, 564, and 296 in the superoxide, O-Phenanthroline, and DPPH radical scavenging assays, respectively. The ratio of the combination was based on the results of α-amylase inhibitory assay (gymnema: 109.17 ± 2.0 and tea: 282.32 ± 2.1) and hemoglycosylation activity (gymnema: 595.09 ± 1.8; tea: 400.64 ± 1.6; asparagus: 391.1 ± 2.3) of individual drugs. **Conclusion:** From our study, it was observed that the herbal tea made by green tea, gudmar, and asparagus had a hemoglycosylation and α-amylase inhibition activities and showed balanced good antioxidant activity and therefore concluded as perfect supplement for diabetic patients.

Keywords: *Asparagus racemosus*, *Camellia sinensis*, diabetes mellitus, *Gymnema sylvestre*

INTRODUCTION

Diabetes mellitus is a metabolic disorder of the endocrine system, classified into Type-1 and Type-2 based on impairment in insulin secretion or its utilization. The cause of diabetes is a mystery, although both genetic and environmental factors such as obesity and lack of exercise appear to play a role.[1] The prevalence and incidence of diabetes are increasing worldwide, more so in the developing countries.[2] The type-2 diabetes remains a leading cause of cardiovascular disease, blindness, end-stage renal failure, amputations, and hospitalization.[3]

Any rise in glycemia is the net result of glucose influx exceeding glucose outflow from the plasma compartment. In fasting, state hyperglycemia is directly related to increased hepatic glucose production. In post-prandial state, glucose excursion results from combination of insufficient suppression of the glucose output and defective insulin stimulation of glucose disposal in target tissues, mainly skeletal muscle. Interventions designed to impact an individual's physical activity levels and food intakes are critical parts of Type-2 diabetes management. Weight reduction, achieved through dietary means alone or with adjunctive medical or surgical intervention, improves

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glycemic control and other cardiovascular risk factors. Prescription drugs include biguanides such as metformin; sulfonylureas such as gliclazide; meglitinides such as repaglinide; thiazolidinediones such as pioglitazone; alpha-glucosidase inhibitors such as acarbose.

The resurgence in the herbal remedies in the recent past has opened avenues to fight diseases at grassroot levels.[4] Herbs have been proved to be beneficial safe and healthy option.[5] The WHO has authenticated the use of herbal remedies for the treatment of diabetes.[6] Some of the potential antidiabetic plants (Afolayan and Sunmonu 2010) are the following *Artemisia afra, Catharanthus roseus, Chilianthus olearaceus, Helichrysum odoratissimum, Hypoxis colchicifolia,* and *Momordica balsamina.* The polyherbal formulations are logical mix of herbs and therefore have advantages over monoherbal products the logic being synergistic effects and potentiation due to bioenhancment etc. Polyherbal formulations contain blend of herbs which may work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects.[7] According to Ayurveda, a combination of substances is used to get the enhanced described action and eliminated unwanted side effects.

Therefore, the current study was planned and executed to develop an antidiabetic dietary supplement in the form of herbal tea and to evaluate its physical and chemical characteristics along with *in vitro* biological activities.

MATERIAL AND METHODS

Collection of plants

Camellia sinensis (green tea) was purchased from Korakundah Jayachamararajendra Tea Estate, Ooty. *Gymnema sylvestre* and *Asparagus racemosus* were purchased from a local vendor Jogappa Shanbhag.

Authentication

The drugs were authenticated by Dr. Gopalakrishna Bhat, Professor, Department of Botany, Poornaprajana College, Udupi.

A voucher specimen for each of the crude drug (specimen no PP-581A for *A. racemosus*, specimen no PP-564 for *G. sylvestre*, and specimen no PP-563.for *C. sinensis)* was deposited in the herbarium in the Department of Pharmacognosy, MCOPS, Manipal.

Standardization

Extractive values

Extractive values were carried out by following two cold maceration methods.

Cold maceration

Ethanol soluble extractive value[8]

5 g of previously weighed air-dried drug was taken in a stoppered conical flask and 100 ml of 95% ethanol was added to it. It was shaken continuously for 24 h on an electric shaker. It was then filtered rapidly taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed Petri dish, dried at 105°C and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

Water soluble extractive[8]

5 g of previously weighed air dried drug was taken in a stoppered flask and 100 ml chloroform water (1:99) was added to it. It was taken continuously for 24 h on an electric shaker. It was then filtered rapidly taking precaution against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed Petri dish, dried at 105°C and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

Foreign organic matter[8]

100–500 mg of the crude drug was accurately weighed and spread out as a thin layer. The sample was inspected with the naked eye first, followed by a ×10 lens and all the foreign matter was removed manually. The percentage of foreign organic matter was determined with reference to the weight of the drug taken.

Microbial contamination[9]

Escherichia coli

Quantity of the homogenized material was transferred to lactose broth, prepared and incubated and 1 g or 1 ml of the material being examined, to 100 ml of MacConkey broth and incubated at 43–45°C for 18–24 h.

Prepared a subculture on a plate with MacConkey agar and incubated at 43–45°C for 18–24 h. Growth of red, generally non-mucoid colonies of Gram-negative rods, sometimes surrounded by a reddish zone of precipitation, indicates the possible presence of *E. coli.*

Salmonella spp.

The solution, suspension, or emulsion of the material was incubated at 35–37°C for 5–24 h for enrichment.

10 ml of the enrichment culture to 100 ml of Tetrathionate Bille Brilliant Green Broth and incubated at 42–43°C for 18–24 h. It was then subcultured on Deoxycholate citrate agar and brilliant green agar media; and incubate at 35–37°C for 24–48 h.

Small, transparent, opaque, pink or white colonies confirm the presence of *Salmonella.*

Heavy metal analysis[9]

Heavy metal analysis for lead and cadmium

Sample preparation

The plant material was powdered and placed in a silica crucible, the digesting mixture consisting of 2 part of nitric oxide and 1 part of perchloric acid was added and heated for 100°C for 3 h, followed by 120°C for 2 h and heating was continued for 240°C for 4 h. The leftover inorganic residue was dissolved in nitric acid and the solution was subjected to atomic absorption spectroscopy.

Arsenic limit test[10]

The test solution of the crude drug was introduced into the wide mouthed bottle, 1 g of KI and 10 g of ZincAsT was then added. Immediately assemble the apparatus and immerse the flask in a water bath at a temperature such that uniform evolution of gas is maintained. After 40 min, stain produced on the mercuric chloride paper (if any) by the extracts was compared with that of a standards prepared by treating 1.0 ml of arsenic standard solution (10 ppm) diluted to 50 ml with water.

Estimation of total phenolic content[11]

Total phenolic content was estimated by Folin–Ciocalteu colorimetric method using gallic acid as a standard phenolic compound.

Reagents

1. Folin–Ciocalteu reagent (0.2 N).
2. Saturated sodium carbonate (75 g/L).

Procedure

To 0.2 ml of different concentrations of the extract (25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, and 400 µg/ml), 1.0 ml of Folin–Ciocalteu reagent and 0.8 ml of 0.7 M sodium carbonate was added. The absorbance of the resulting solution was then measured at 765 nm. Blank was carried out with 0.2 ml extract with 1.0 ml water instead Folin reagent and 0.8 ml of 0.7 M sodium carbonate. The absorbance of the resulting solution was then measured at 765 nm.

Blank was carried out with 0.2 ml extract with 1.0 ml water instead Folin reagent and 0.8 ml of 0.7 M sodium carbonate.

The total phenolic content was calculated as mg of gallic acid equivalent (GAE)/g of dry weight of extract or fractions calculated by the following formula.

$$T = C.V/M$$

Where, T= total content of phenolic compounds, mg/g plant extract, expressed as GAE
C= The concentration of gallic acid established from the calibration curve, mg/ml
V= The volume of extract or fraction in ml
M= The weight of fraction or extract in gram.

Estimation of total flavonoid content[12]

Quercetin is used as standard. To 0.5 ml of different concentrations of standard, 1.5 ml of methanol was added followed by addition of 0.1 ml of potassium acetate (1 M), 0.1 ml aluminum chloride (10% w/v), and 2.8 ml distilled water. This was incubated at room temperature for 30 min and read the absorbance at 415 nm.

Standard blank was carried out with 0.5 ml methanol without standard.

Preparation of sample

Sample preparation was carried out with extract concentration being 1000 µg/ml and adding 1.5 ml of methanol, 0.1 ml of potassium acetate (1 M), 0.1 ml aluminum chloride (10% w/v), and then added 2.8 ml distilled water incubated this mixture at room temperature for 30 min and read the absorbance at 415 nm.

The percentage of flavonoids was calculated from the standard plot.

Estimation of tannin content[13,14]

Reagents

1. Folin–Denis reagent.
2. Saturated sodium carbonate solution. (35 g in 100 ml water).

Extract solution 10 mg of extract was dissolved in 10 ml of methanol.

Procedure

Standard preparation

1 ml of different concentrations of tannic acid was pipette into the 10 ml of standard volumetric flask containing 7.5 ml of water, 0.5 ml of Folin–Denis reagent, and 1 ml of sodium

carbonate. The solution was mixed and absorbance was determined at 760 nm after 30 min. The blank consists of all the reagents without sample.

Sample preparation

Sample preparation was carried out with extract concentration being 1000 µg/ml, to which added 7.5 ml of water, 0.5 ml of Folin-Denis reagent, and 1 ml of sodium carbonate. Absorbance of the resulting mixture was determined at 760 nm after 30 min.

The percentage of tannin content was calculated by the standard plot.

Drug content (high-performance thin-layer chromatography [HPTLC] estimation of catechin and gymnemic acid)

HPTLC was performed on 10 × 10 aluminum plates coated with silica gel 60 F_{254}. Sample and standard solutions were applied on the same chromatographic plates as bands of 2–4 µl volume using CAMAG Linomat V sample applicator equipped with a Hamilton syringe. Ascending chromatographic development was performed in Camag glass twin trough chamber previously saturated with mobile phase vapor. After development, the dried plates were scanned with CAMAG TLC scanner-3 and the phytoconstituents were calculated using the formula:

$$\% \text{ content} = \frac{\text{AUC of sample} \times \text{Conc of standard} \times 100}{\text{AUC of standard} \times \text{Conc of standard}}$$

Preparation of standard solutions

The stock solution of catechin was prepared by dissolving 1.0 mg accurately weighed standard in 10 ml methanol and was sonicated for 10 min and 10 µl of the sample was applied.

Preparation of sample solutions

20 mg of the sample was dissolved in methanol and sonicated for 10 min and 10 µl of the sample was applied.

General HPTLC chromatographic conditions

Stationary phase	: Pre-coated silica gel aluminum plate 60 F_{254}
Chamber saturation time	: 20 min
Slit width	: 6mm × 0.45 mm
Temperature	: 25 ± 2°C
Humidity	: 60% ± 5
Thickness	: 200 µm
Syringe	: 100 µL Hamilton syringe
Development chamber	: CAMAG twin trough chamber
Detection	: UV-VISIBLE spectrophotometer

Specific HPTLC chromatographic for catechin

Marker compound	: Catechin
Mobile phase	:Toluene:ethyl acetate:acetic acid :water (12:6:6:0.5)
Detection wavelength	: 560 nm

Specific HPTLC chromatographic conditions for gymnemic acid

Marker compound	: Deacyl gymnemic acid
Mobile phase	:Ethyl acetate:acetic acid: methanol:water(6.1:1:2:0.5)
Detection wavelength	: 560 nm

In vitro antidiabetic studies of individual crude drugs

In vitro non-enzymatic hemoglobin glycosylation method[15]

The antidiabetic activities of extracts and fractions were investigated by estimating the degree of non-enzymatic hemoglobin glycosylation, measured calorimetrically at 520 nm.

Materials

Gentamycin and hemoglobin from bovine blood and D-glucose.

Procedure

The assay was performed by the addition of 1 ml of glucose solution (0.2%), 1 ml of hemoglobin (0.06%), and gentamycin (0.02%) in 0.01 M phosphate-buffered saline (pH 7).

This mixture was incubated in the dark at room temperature for 72 h. The degree of glycosylation in the presence of different concentrations of standard and extracts was measured calorimetrically at 520 nm. Gallic acid was used as a standard.

% inhibition was calculated as follows

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}}$$

In-vitro α-amylase inhibition assay method[16]

1 ml substrate solution, that is, potato starch (1% w/v) was added to 1 ml of various concentrations of 250, 500, 750, and 1000 ml of extracts. To this, 1 ml of α-amylase solution (1% w/v) and 2 ml of acetate buffer (0.1 M, PH

7.2) were added and incubated at room temperature for 1 h. Starch solution, α-amylase solution, and drug solutions were prepared in acetate buffer (820.3 mg sodium acetate and 18.7 mg sodium chloride in 100 ml distilled water). After incubation, 0.1 ml iodine–potassium iodine indicator (635 mg iodine and 1 g potassium iodide in 250 ml distilled water) was added to the mixture. Absorbance of starch-iodide complex formed was measured at 565 nm.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}}$$

Formulation

Based on the result of *in vitro* antidiabetic assay, different concentrations of the selected raw material were blended into six different batches [Table 1].

The powdered crude drugs in the given quantities were blended uniformly using amortar and pestle.

1 g of the blend was filled into a muslin cloth bag to make a tea bag.

Tea bags were dipped separately in 100 ml hot water for 10 min. The hot infusion was evaporated to dryness to estimate water-soluble extractive of the blend.

The above-dried extract was used for *in vitro* antioxidant assay.

In vitro antioxidant activity of formulations

DPPH radical scavenging assay[17]

1. The assay was carried out in 96-well microtiter plate.
2. To the sample wells, 10 µl of each of the test drug or standard drug dilutions were added to 200 µl of DPPH.
3. Sample blank was performed by adding 10 µl of each of the test or standard dilutions to 200 µl of standard.
4. To the control wells, 10 µl of DMSO was added to 200 µl of DPPH solution, whereas in control blank wells, 10 µl of DMSO was added to 200 µl of methanol.
5. The plate was covered with aluminum foil and incubated at 37°C for 20 min and absorbance of each well was measured at 540 nm using ELISA reader. IC_{50} (inhibitory concentration), that is, the concentration of an extract required to scavenge 50% of DPPH free radicals was calculated using following formula:

$$\% \text{ scavenging} \quad \frac{\text{O.D. of control} - \text{O.D of test}}{\text{O.D. of control}}$$

Superoxide scavenging assay[18]

To 0.3 ml of various concentrations of extracts in DMSO, 1 ml alkaline DMSO and 0.1 ml NBT were added. The absorbance was measured at 560 nm. The experiment was performed in triplicate.

The percentage of scavenging was calculated by the formula

$$\% \text{ scavenging} = \frac{\text{O.D. of control} - \text{O.D of test}}{\text{O.D. of control}}$$

Nitric oxide radical scavenging assay[19]

Procedure

Griess reagent preparation

Solution A: 1% sulfanilamide in 5% orthophosphoric acid

Solution B: 0.01% Naphthyl ethylenediamine in distilled water

Solution A and solution B were mixed equal volumes within 12 h of use.

Sodium nitroprusside 5 mM (0.0373 g in 25 ml) was prepared in phosphate buffer PH 7.4. To 1 ml of various concentrations of extract, 0.3 ml of sodium nitroprusside was added in the test tubes. The test tubes were incubated at 25°C for 5 h. After 5 h, 0.5 ml of Griess reagent was added. The absorbance was measured at 546 nm. The experiment was performed in triplicate.

O-Phenanthroline assay[20]

The reaction mixture 1 ml O-Phenanthroline (0.005 g in 10 ml methanol), 2 ml ferric chloride 200 µM (3.24 mg in 100 ml distilled water), and 2 ml of various concentrations of the extract. The mixture was incubated at ambient temperature for 10 min and then the absorbance of the same

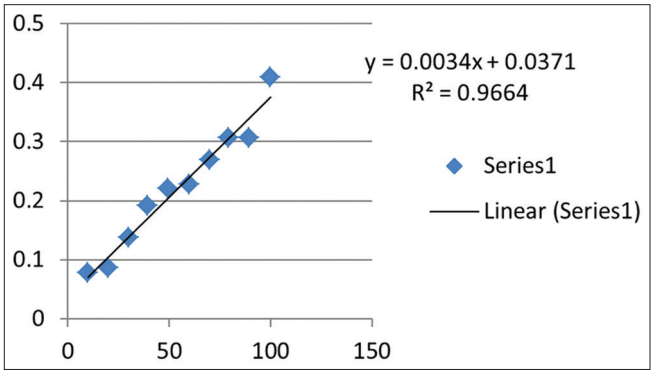

Figure 1: Standard plot of gallic acid

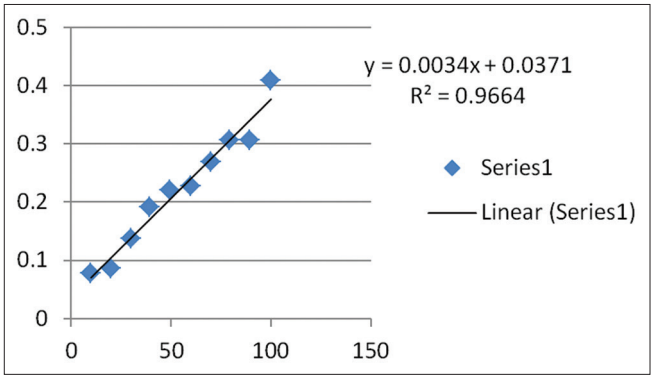

Figure 2: Standard plot of quercetin

was measured at 510 nm. The experiment was performed in triplicate.

$$\% \text{ of scavenging} = \frac{\text{O.D. of control} - \text{O.D of test}}{\text{O.D. of control}}$$

RESULT

Extractive value; foreign organic matter; heavy metal analysis

Extractive values (both alcohol and water soluble), foreign organic matter, and heavy metals of all the crude drugs were determined and tabulated in Table 2.

Table 1: The ratios of selected drugs

Batches	*C.sinensis* (mg/g)	*G.sylvestre* (mg/g)	*A.racemosus* (mg/g)
F1	600	250	150
F2	600	100	300
F3	600	200	200
F4	600	300	100
F5	600	150	250
F6	600	220	180

C. sinensis: Camellia sinensis, G. sylvestre: Gymnema sylvestre, A. racemosus: Asparagus racemosus

Microbial contamination

In the microbial load testing for *E. coli* and *Salmonella,* the crude drugs and the formulation showed the absence in all the dilutions tested.

Phenol content; flavonoid content; and tannin content [Table 3 and Figures 1-3]

In vitro antidiabetic study of crude drugs [Table 4]

Phenol, Flavonoid and Tannin content of C. sinensis, G. sylvestre and A. racemosus determined and tabulated in Table 3.

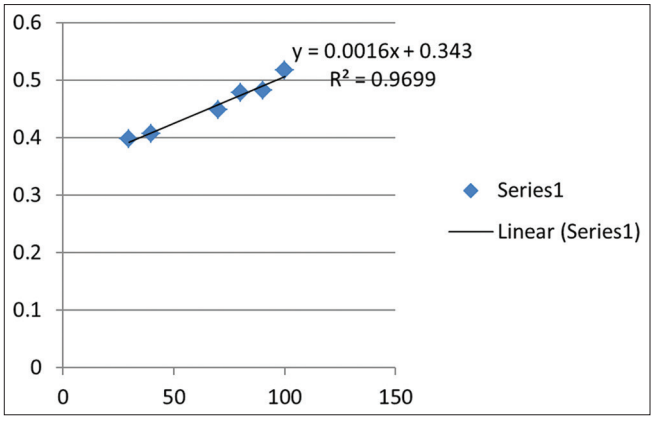

Figure 3: Standard plot of tannic acid

Table 2: Values for extractable matter (water and alcohol), foreign matter, and heavy metals)

Crude drug	Wat solub extractive (%w/w)	Alc solub extractive (%w/w)	Foreign matter	Heavy Metals
C. sinensis	23.0±0.0429	14.0±0.78	0%	Absent
G. sylvestre	33.0±0.039	24.0±0.62	0.8%	24 mg/kg
A. racemosus	38±0.5	15.0±0.8	0.5%	1.44 mg/kg

C. sinensis: Camellia sinensis, G. sylvestre: Gymnema sylvestre, A. racemosus: Asparagus racemosus

Table 3: Values of phenol content, flavonoid content, and tannin content

Crude drugs	Phenol cont. (mg/G GA equival.)	Flavonoid cont. (mg/G quercet equival.)	Tannin cont. (mg/G equival. of tannic acid)
C. sinensis	285.0	39.6	640.0
G. sylvestre	66.0	12.4	56.0
A. racemosus	35.5	--	--

C. sinensis: Camellia sinensis, G. sylvestre: Gymnema sylvestre, A. racemosus: Asparagus racemosus

Table 4: Values of both the methods

Extract	α-amylase inhibitory activity IC_{50}	Hb glycosylation IC_{50}
Gallic acid	113.41±1.0	95.12±1.3
G. sylvestre	109.17±2.0	595.09±1.8
C. sinensis	282.32±2.1	400.64±1.6
A. racemosus		391.1±2.3

C. sinensis: Camellia sinensis, G. sylvestre: Gymnema sylvestre, A. racemosus: Asparagus racemosus

1. *In vitro* non-enzymatic hemoglobin glycosylation method [Figure 4].
2. *In vitro* α-amylase inhibition assay method [Figure 5].

Drug content of the individual formulation (dipped for 10 min) [Table 5]

The real time extractable matter to the actual content was determined by dipping the blend for 10 minutes in hot water. The results are tabulated in Table 5

IC_{50} value of formulations for antioxidant assays [Table 6]

The IC_{50} Value of different batches of the formulation were tested. F1 showed optimum activity in three antioxidant assays [Figures 6-9]. Results tabulated in Table 6.

Drug content (Estimation of gymnemic acid and catechin) [Tables 7 and 8]

The Formulation 1 (F1) was standardized to gymnemic acid and catachins by HPTLC estimation. Catachins [Figures 10-14] and Gymnemic acid [Figures 15-18] were found to be 2.41% and 0.59% respectively.

DISCUSSION

Today, a large number of diabetes have been reported of which Type-2 is highest. The disease has become a national burden owing to its management cost which is a lifelong menace. A wide array of medicines are available for the management of diabetes but unfortunately have their own side effect which adds up to the morbidity.

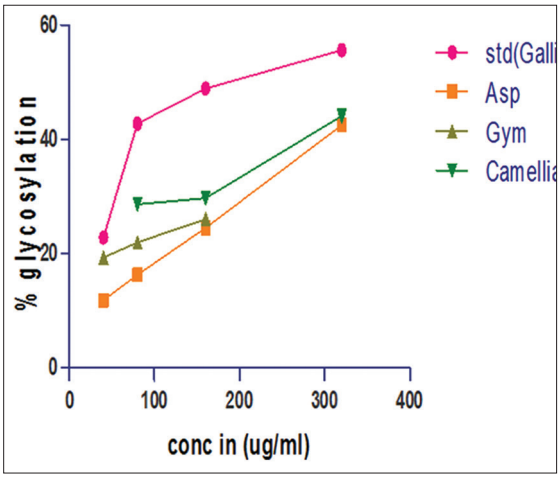

Figure 4: Hb glycosylation assay

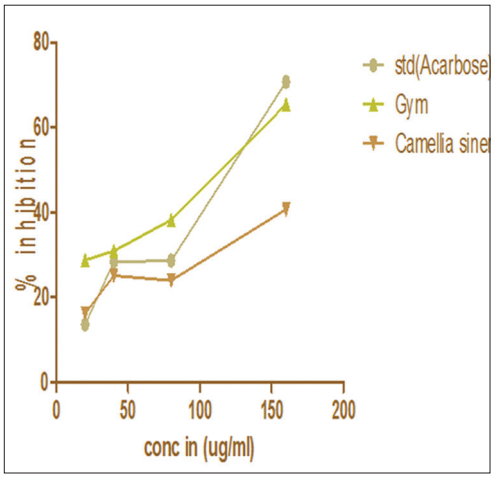

Figure 5: α-amylase inhibition assay method

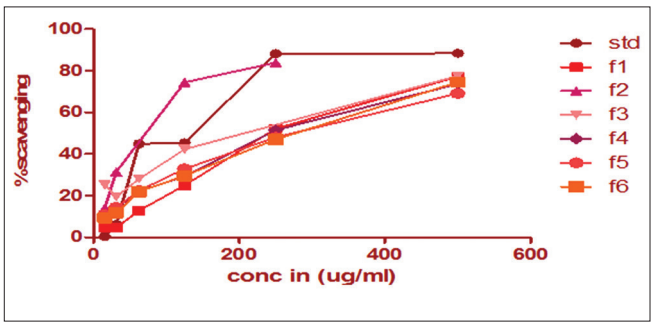

Figure 6: DPPH radical scavenging assay

Table 5: The drug content of the formulations and values of their extractable matter

Formulation (G>T + Gym+Asp)	Extractable matter (10 min)
F1 (600+250+150)	0.118±0.05
F2 (600+100+300)	0.114±0.03
F3 (600+200+200)	0.114±0.02
F4 (600+300+100)	0.116±0.05
F5 (600+150+250)	0.114±0.025
F6 (600+220+180)	0.113±0.05

Table 6: IC_{50} value of formulations

Antioxidant assay	Std (Ascorbic acid)	F1	F2	F3	F4	F5	F6
DPPH radical scavenging assay	134	296.58	102.19	249.82	292.63	308.38	298.33
O-Phenanthroline assay	401.76	564.07	656.15		902.22	985.29	818.09
Superoxide radical scavenging assay	86.48	130.36	220.3	213.36	271.48	332.64	353.65
Nitric oxide radical scavenging assay	77.98			116.875			

Therefore, the current study was planned and executed to develop an antidiabetic dietary supplement in the form of a herbal tea and to evaluate its physical and chemical characteristics along with *in vitro* biological activities.

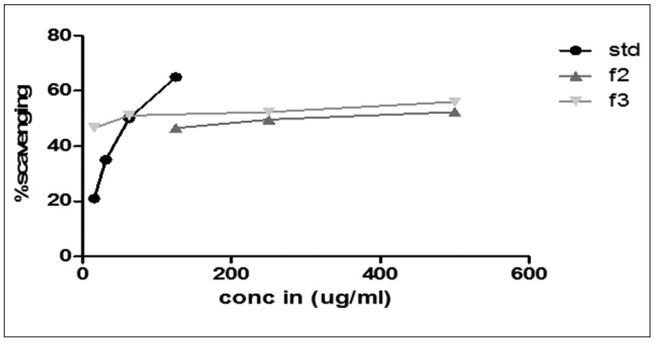

Figure 7: No radical scavenging assay

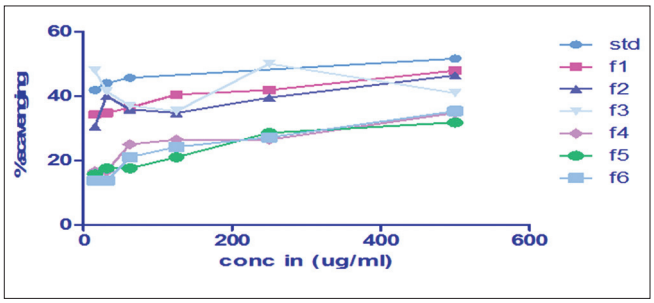

Figure 8: O-Phenanthroline assay

In our study, we collected the herbal raw material from authenticated sources and had them identified by a botanist. The crude drugs were evaluated for their identity and purity by standardization methods such as extractive values,

Table 7: Values of AUC and % content of catechin in extract and formulation estimation of gymnemic acid content

Sl no	Fraction	AUC	% content
1	Catechin standard	3954.4	98
2	*C. sinenis* extract	2636.95	3.33
3	Formulation 1 (1a)	1913.9	2.41

C. sinensis: Clonorchis sinensis, AUC: Area under the curve

Table 8: Values of AUC and % content of gymnemic acid in extract and formulation

Sl no.	Fraction	AUC	% content
1	Gymnemic acid std.	5328.6	98
2	Gymnema aq. extract	616.1	1.15
3	F1 aq. extract	312.5	0.59

AUC: Area under the curve

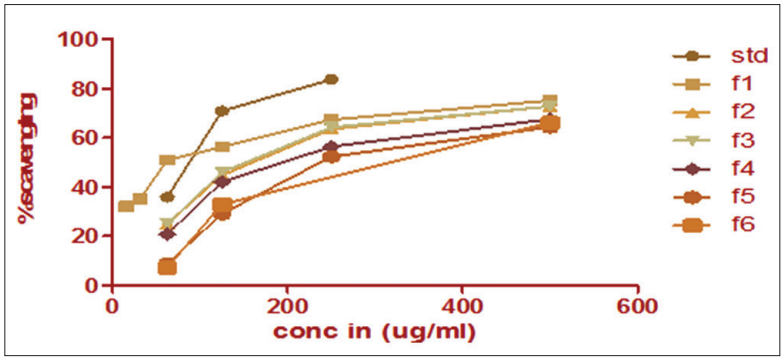

Figure 9: Superoxide radical scavenging assay

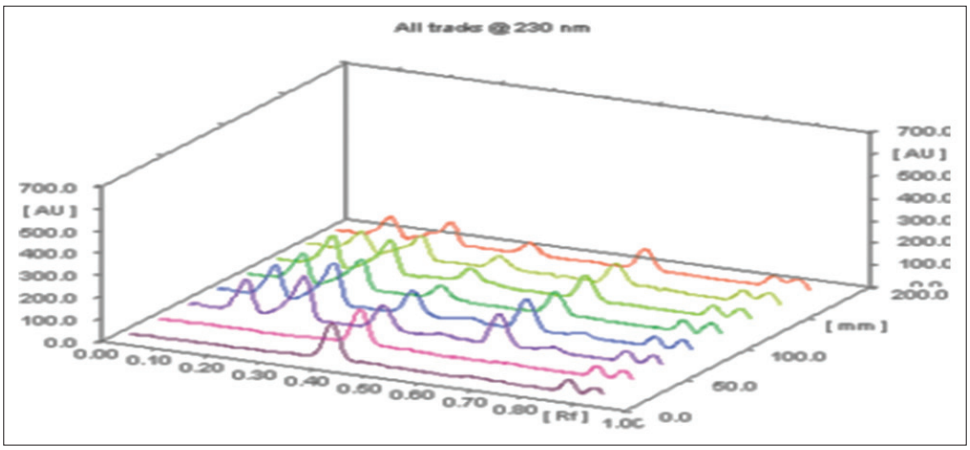

Figure 10: 3D chromatographic profile of standard and fractions

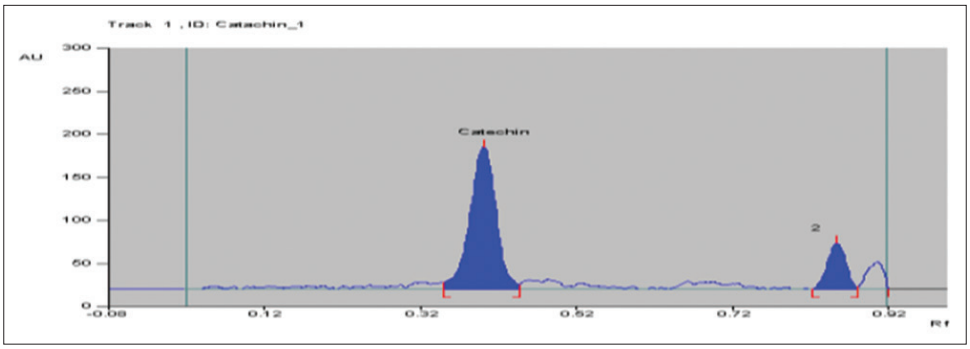

Figure 11: Standard catechin

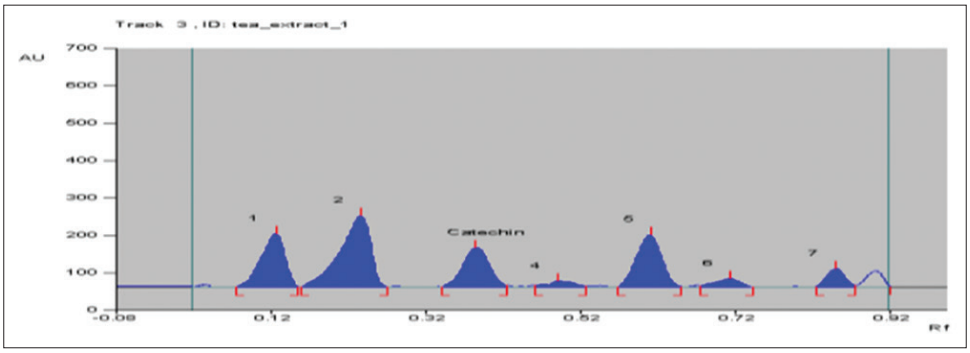

Figure 12: *Camellia* extract

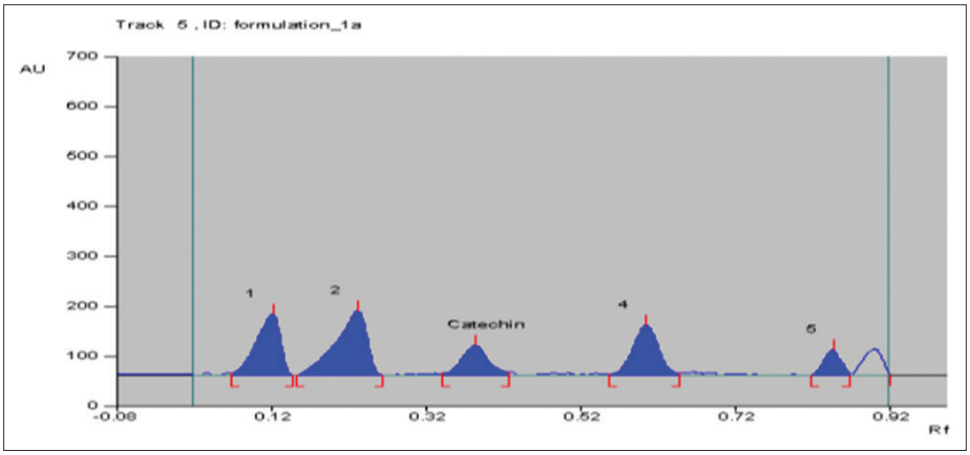

Figure 13: Formulation 1

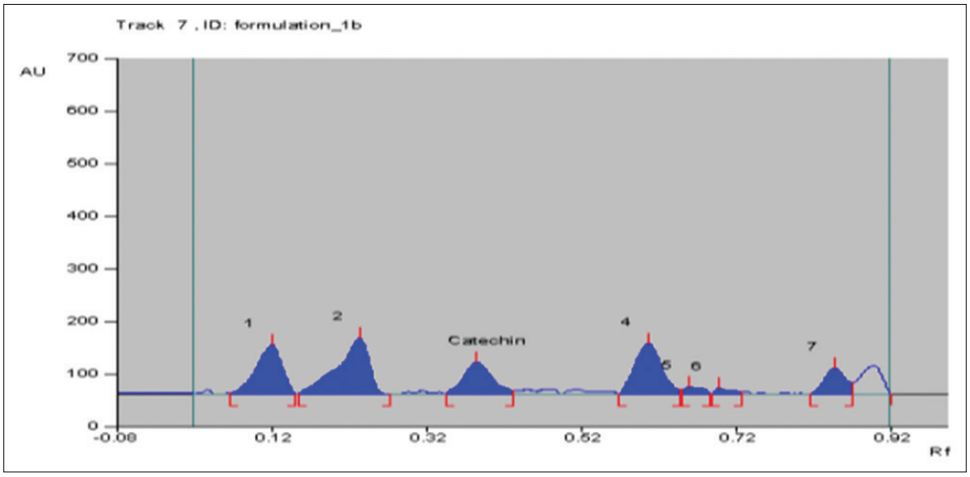

Figure 14: Formulation 2

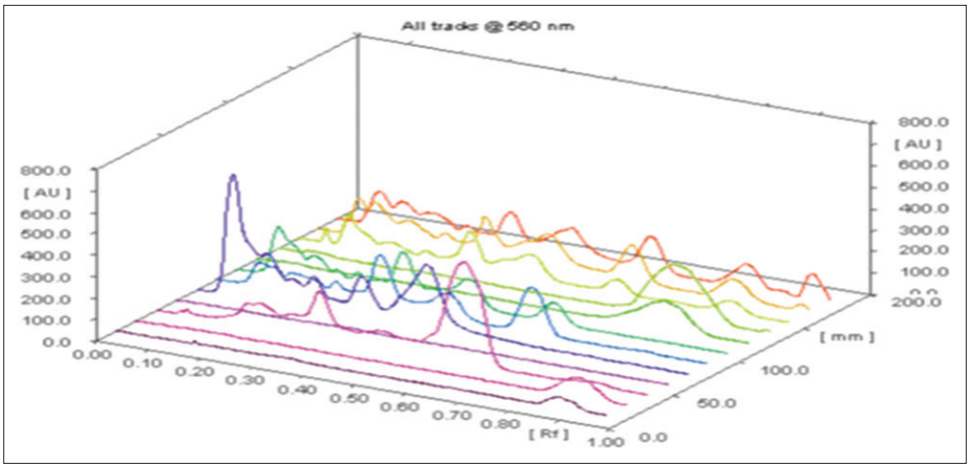

Figure 15: Chromatographic profile of standard and fractions

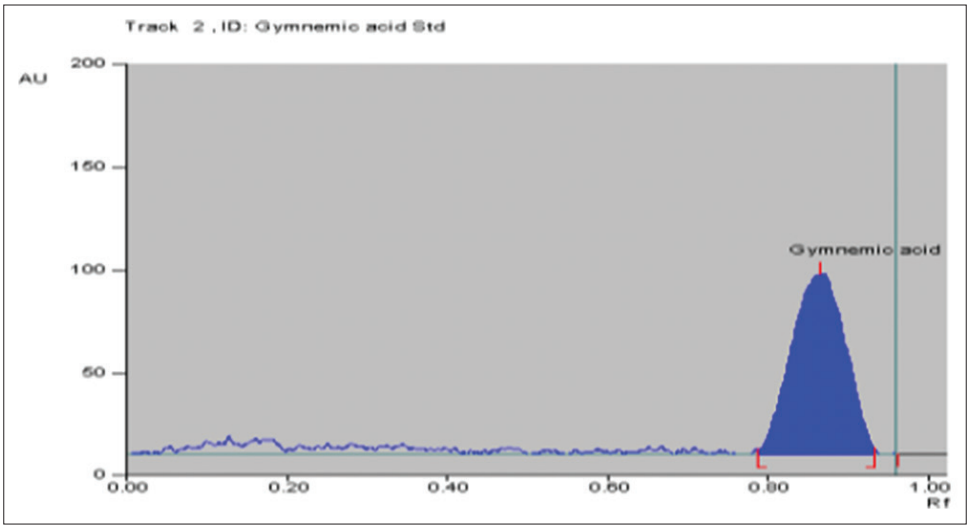

Figure 16: Standard gymnemic acid

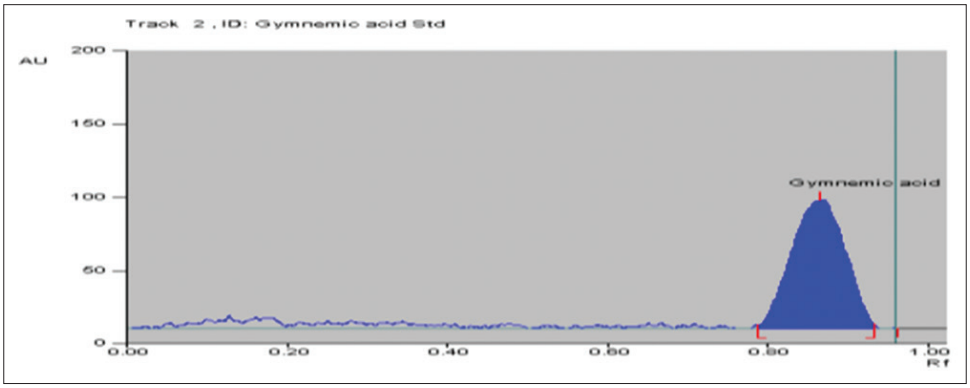

Figure 17: Gymnema aqueous extract

foreign organic matter, and heavy metal analysis. Phenolic, flavonoid, and tannin content were also established as these phytoconstituents were shown to be responsible for antioxidant potential of the drugs.

In vitro antidiabetic assays were carried out for the assessment of antidiabetic potency of drugs.

In this study, we see that the IC_{50} value of green tea was higher in both assays (*In vitro* non-enzymatic hemoglobin glycosylation method and *In vitro* α-amylase inhibition assay method) and hence concentration was decided to be more than the other two drugs. Further green tea also serves as the base or medium for incorporating other two drugs *G. sylvestre* and *A. racemosus*. A composition was developed accordingly

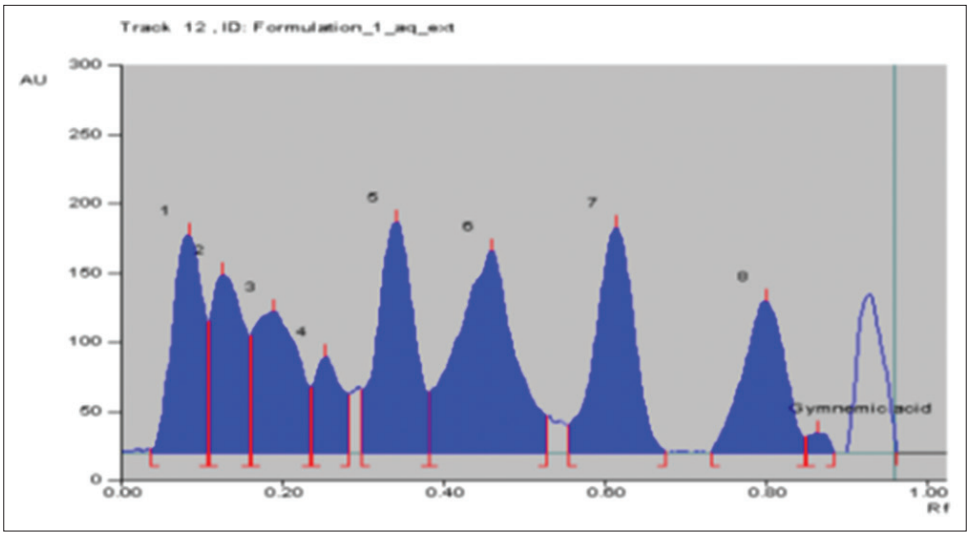

Figure 18: Formulation 1 aqueous extract

with different combinations in a total of 6 batches. This was evaluated for antioxidant activity.

The antioxidant assay was done by four different methods such as DPPH, O-Phenanthroline, superoxide scavenging, and nitric oxide scavenging. Batch 1 and batch 2 of the formulations to be effective batches when compared to other batches. The batch 2 showed a maximum scavenging of free radicals in DPPH with an IC_{50} value of 102.19. Batch 1 showed potential superoxide radical scavenging with an IC_{50} value of 130.36. In nitric oxide radical scavenging assay, formulation 3 showed potential activity with IC_{50} value of 116 while others showed none.

From the studies, it was clear that the composition of batch 1 was an effective composition and therefore subjected to HPTLC standardization using markers catechin and gymnemic acid, the product was standardized to 2.41% catechin and 0.59% gymnemic acid.

CONCLUSION

To conclude, we have developed and evaluated a herbal tea for the management of diabetes which can address not only diabetes but also the complications such as cholesterol and triglyceride. The *in vitro* studies adapted in our work indicated that the developed herbal tea is effective in controlling diabetes considerably. The tea was found to prevent hemoglycosylation in *in vitro* assays.

We planned to carry out *in vivo* and clinical studies on the developed product in the future.

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