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. The prevalence and incidence of diabetes are increasing worldwide, more so in the developing countries. The type-2 diabetes remains a leading cause of cardiovascular disease, blindness, end-stage renal failure, amputations, and hospitalization.

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...
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 grassroots levels. Some of the potential antidiabetic plants (Afolayan and Sumonu 2010) are the following *Artemisia afra*, *Catharanthus roseus*, *Chillianthus 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 bioenhancement etc. Polyherbal formulations contain blend of herbs which may work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects. 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 Jayachamarajendra 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**

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 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.

**Water soluble extractive**

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**

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**

**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.

\[
T = \frac{C \cdot V}{M}
\]

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.
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 : 6 mm × 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

\[
\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100
\]

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 iodide 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 an mortar 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:

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

**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

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...
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.

**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](image-url)

**Table 1: The ratios of selected drugs**

<table>
<thead>
<tr>
<th>Batches</th>
<th><em>C. sinensis</em> (mg/g)</th>
<th><em>G. sylvestre</em> (mg/g)</th>
<th><em>A. racemosus</em> (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>600</td>
<td>250</td>
<td>150</td>
</tr>
<tr>
<td>F2</td>
<td>600</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>F3</td>
<td>600</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>F4</td>
<td>600</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>F5</td>
<td>600</td>
<td>150</td>
<td>250</td>
</tr>
<tr>
<td>F6</td>
<td>600</td>
<td>220</td>
<td>180</td>
</tr>
</tbody>
</table>


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

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


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

<table>
<thead>
<tr>
<th>Crude drugs</th>
<th>Phenol cont. (mg/G GA equival.)</th>
<th>Flavonoid cont. (mg/G quercet equival.)</th>
<th>Tannin cont. (mg/G equival. of tannic acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. sinensis</em></td>
<td>285.0</td>
<td>39.6</td>
<td>640.0</td>
</tr>
<tr>
<td><em>G. sylvestre</em></td>
<td>66.0</td>
<td>12.4</td>
<td>56.0</td>
</tr>
<tr>
<td><em>A. racemosus</em></td>
<td>35.5</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>


**Table 4: Values of both the methods**

<table>
<thead>
<tr>
<th>Extract</th>
<th>α-amylase inhibitory activity IC(_{50})</th>
<th>Hb glycosylation IC(_{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>113.41±1.0</td>
<td>95.12±1.3</td>
</tr>
<tr>
<td><em>G. sylvestre</em></td>
<td>109.17±2.0</td>
<td>595.09±1.8</td>
</tr>
<tr>
<td><em>C. sinensis</em></td>
<td>282.32±2.1</td>
<td>400.64±1.6</td>
</tr>
<tr>
<td><em>A. racemosus</em></td>
<td>391.1±2.3</td>
<td></td>
</tr>
</tbody>
</table>

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.

**IC₅₀ value of formulations for antioxidant assays [Table 6]**

The IC₅₀ 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 catechins 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.

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

<table>
<thead>
<tr>
<th>Formulation (G&gt;T + Gym+Asp)</th>
<th>Extractable matter (10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 (600+250+150)</td>
<td>0.118±0.05</td>
</tr>
<tr>
<td>F2 (600+100+300)</td>
<td>0.114±0.03</td>
</tr>
<tr>
<td>F3 (600+200+200)</td>
<td>0.114±0.02</td>
</tr>
<tr>
<td>F4 (600+300+100)</td>
<td>0.116±0.05</td>
</tr>
<tr>
<td>F5 (600+150+250)</td>
<td>0.114±0.025</td>
</tr>
<tr>
<td>F6 (600+220+180)</td>
<td>0.113±0.05</td>
</tr>
</tbody>
</table>

**Table 6: IC₅₀ value of formulations**

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

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**

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Fraction</th>
<th>AUC</th>
<th>% content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Catechin standard</td>
<td>3954.4</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>C. sinenis extract</td>
<td>2636.95</td>
<td>3.33</td>
</tr>
<tr>
<td>3</td>
<td>Formulation 1 (1a)</td>
<td>1913.9</td>
<td>2.41</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Fraction</th>
<th>AUC</th>
<th>% content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gymnemic acid std.</td>
<td>5328.6</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>Gymnema aq. extract</td>
<td>616.1</td>
<td>1.15</td>
</tr>
<tr>
<td>3</td>
<td>F1 aq. extract</td>
<td>312.5</td>
<td>0.59</td>
</tr>
</tbody>
</table>

AUC: Area under the curve
Figure 11: Standard catechin

Figure 12: *Camellia* extract

Figure 13: Formulation 1

Figure 14: Formulation 2
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.
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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<sub>50</sub> value of 102.19. Batch 1 showed potential superoxide radical scavenging with an IC<sub>50</sub> value of 130.36. In nitric oxide radical scavenging assay, formulation 3 showed potential activity with IC<sub>50</sub> 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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