COMPARISON OF SWERTIAMARIN CONTENT IN DIFFERENT ANTIDABETIC HERBAL MARKETED PREPARATION CONTAINING ENICOSTEMMA LITTORALE

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ABSTRACT

Swertiamarin is one of the phytoconstituents present in Enicostemma littorale Linn. which is known for its hypoglycemic activity from ancient times. In our previous studies we have reported new HPLC method of analysis of swertiamarin in herbal preparations containing E. littorale. In the present study we have utilized this method for standardization of marketed formulations of E. littorale using swertiamarin and compared with the one formulated in our laboratory. This HPLC method was found to be reproducible, accurate and precise and could detect swertiamarin concentration at microgram level. The developed HPLC method would be an important tool in the way of acceptability of quality control method of polyherbal formulations. Swertiamarin was found to be in the range of 0.5mg per pills with the highest amount of found in cold extract prepared by lyophilisation(1.2mg) and in Saptarangiadi Pills (3.3mg/pills).

KEYWORDS: Swertiamarin in herbal preparations containing E. littorale.

INTRODUCTION

Traditional medicine system of India comprises of varieties of plants which play a significant role in curing disease from ancient times. India has a rich heritage of usage of medicinal Plants in the Ayurvedic, Siddha and Unani system with a mention of about 45,000 plants.[1- 2] Diabetes mellitus and obesity are one of the most common chronic endocrine disorders associated with increased incidence of morbidity and mortality due to cardiac and renal
complications and are recognized as serious global health problems. Diabetes is a heterogeneous chronic metabolic disorder characterized by hyperglycemia resulting from defect in insulin secretion and/or deficiency of insulin secretion. Large number of polyherbal formulations (PHFs) is available and being prescribed nationwide even by registered doctors for diabetes mellitus. The rational for the use and compliance are either not available or known for these herbal formulations. Majority of herbal drugs are polyherbal combinations, whose rationality is yet to be proved in all the cases. Standardization is an essential measurement for ensuring the quality control of the herbal drugs.

Recently, E littorale Blume is mentioned to be useful in diabetes mellitus. Previously studies from our laboratory have established anti-diabetic and anti-obesity activities of E. littorale with swertiamarin as a lead compound.\(^1\) Also, it has been reported that the patients of type 2 diabetes taking E.littorale tablets for more than five years not only had sustained normoglycemia, normal lipid profile and cardiac functions but there was an evidence of genoprotective effect as compared to controls.\(^2\) It was further reported by us that swertiamarin is potent HMG Co-A reductase inhibitor and increase adiponectin expressions as evident from 3T3L1 cell line studies.\(^3\) After successful characterization, semi synthetic analogues of swertiamarin were synthesized to obtain a compound more potent than swertiamarin.\(^3\) Docking experiments were also undertaken with the help of other docking software.\(^4\) These compounds also produced favourable effects in diabetic animal model in vitro cell lines.\(^5\) Further in vivo as well as in vitro analysis has shown that gentianin, a metabolite of swertiamarin, is also active for anti-diabetic and anti-obesity effects all these observations and results prompted us to go further and develop a novel drug candidate taking swertiamarin as the lead.\(^6\) In our previous studies we have reported new HPLC menthod of analysis of swertiamarin in herbal preparations containing E. littorale. In the present study we have utilized this method for standardization of marketed formulations of E.littorale using swertiamarin and compared with the one formulated in our laboratory.

![Figure 1: Swertiamarin and E littorale Plant](image-url)
Chemical Name: (5R,6S)-4a-hydroxy-6-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)-5-vinyl-4,4a,5,6-tetrahydropyranol[3.4-c]pyran-1(3H)-one.

EXPERIMENTAL
Chemicals and solvents
Swertiamarin was obtained from Dr. Hitesh Vaidya, St John’s University, Canada. HPLC grade or spectrophotometric grade solvents like acetonitrile and methanol were obtained from Fisher Scientific India Pvt. Ltd. Distilled water was used was prepared in-house using Milli-Q-UV Plus purification system (Millipore Corp., Billerica, MA, USA). Ammonium acetate (98%) was purchased from Mallinckrodt (Phillipsburg, NJ, USA). All the solutions for analysis were prepared and analyzed freshly. The selected marketed formulations A, B, & C were purchased from market and they were prepared by Shree Narayan Ayurvedic Pharmacy, Changodar, Ahmedabad, Tosol, Ahmedabad and Shree Yash Remedies, Ahmedabad and Amines Biotech Pvt. Ltd. Dry powder used was prepared in our laboratory.

Instrumentation and analytical conditions
Chromatography was performed using an Agilent HPLC 1200 Infinity (Agilent Technologies) equipped with 1260 Infinity Binary, an autosampler and Agilent 1260 Dual wavelength absorbance detector. Data Chromatography was performed using EZ Chrome automation system software. The methods were conducted using an isocratic reverse phase technique. The analytical conditions (mobile phase composition, flow rate and analytical wavelengths) for the three drugs have been summarized in Table 1. The mobile phases were prepared freshly, filtered through 0.45µm membrane and e filter (Millipore, USA) and sonicated (Ultrasonic Cleaner) for 30 min before use in order to deaerate.

The estimation was performed on Agilent HPLC 1200 Infinity for measuring the area of drug. A Mettler Toledo electronic balance AG245 a C18 Hiqsil C18 column (5 m, 4.6 mm × 250 mm) was used for analysis of standard.

Preparations of Standards and Sample solutions
Preparation of Calibration Solution for validation of HPLC method
1ml of each working standards stock solution of 1µg/ml, 5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml and 160 µg/ml was taken and transferred into 10ml of volumetric flask and milli-Q water was added to make it 10 ml. The solution was filtered with 0.45 µm Whatman
filter paper. 1 ml of solution was taken in 5 ml tubes and 2 ml of Acetonitrile solution was added. These tubes were vortexed for 2 min after which they were centrifuged at 8000rpm, 24°C for 10min. The resultant mixture was freezed at -80°C fir 10min. Finally the acetonitrile was transferred to 3 ml tarson tube for nitrogen evaporation. The remaining part was then reconstituted in to 200 µl mobile phase solution.

Primary stock solutions swertiamarin (1000 µg/ml) was prepared in ultra pure water and further diluted with water to obtain working standards in the concentration range of 1–160 µg/ml. Quality control (QC) samples were run with each batch of working standards in order to calculate the validation parameters. QC samples were prepared in ultra pure water spiked with analytes at different concentrations (3, 70 and 140 µg/ml) following the same procedure as for calibration standards, using a different primary stock. The samples were analyzed with reagent blanks. All the solutions were prepared in triplicates to reproducibility, accuracy and validating the proposed method. The correlation coefficient, coefficient of variance and the linearity of results were calculated.

**Preparation of sample solution of Marketed Formulation**

One Pill each of three marketed preparation were taken (A,B & C) and weighed crushed separately and transferred each in to separate 10ml volumetric flask and milli-Q water was added to make it 10 ml. The solution was filtered with 0.45 µm Whatman filter paper. 1 ml of solution was taken in 5 ml tubes and 2 ml of acetonitrile solution was added. These tubes were vortexed for 2 min after which they were centrifuged at 8000rpm, 24°C for 10 min. The resultant mixture was freezed at -80°C for 10min. Finally the acetonitrile was transferred to 3 ml tarson tube for nitrogen evaporation. The remaining part was then reconstituted in to 200 µl mobile phase solution.

**Preparation of dry powder extract at our laboratory**

100 gm of dried plant of *E.littorale*, crushed the leaf was taken in a steel vessel; to this 500 ml distilled water was added and rested for at room temperature. Further 1000 ml distilled water was added then this was boiled on light flame at a temperature of 70-80°C for 2-3 hr. It was then allowed to cool at room temperature for 15- 16 hr and then filtrated. The filtrate (380ml) which the aqueous extract of *E.littorale*. The extract was then freeze dried for 73-80 hr, as to obtain dried extract powder this will be used for formulation development.
Analysis of samples for swertiamarin
Swertiamarin isolated from three marketed formulations preparations using and then followed by acetonitrile solution, was reconstituted in ammonium acetate buffer and water (85:15). This was run in C18 column using solvent system consisting of mixture of ammonium acetate and acetonitrile (85:15) as a mobile phase in isocratic flow induce elution mode followed by UV detection at 238nm. The developed method was validated using International Conference on Harmonisation (ICH) guidelines.

RESULTS AND DISCUSSION
A sharp peak was obtained with the retention time 7.2min at a flow rate of 1ml/min, in 5 µl injection volume. Limit of Detection (LOD) and limit of quantification (LOQ) were calculated to be 0.3 µg/mL and 1 µg/mL. Swertiamarin was found to be in the range of 0.5mg per pills (Table 1) with the highest amount of found in cold extract prepared by lyophilisation 1.2mg and 3.3mg/pill.

Calibration curves offered good linear regression (r² 0.998) within the test ranges. Interday assay showed 1.2% maximum relative standard deviation (RSD) whereas the intraday assay showed maximum 1.17% RSD. The method was also found to be accurate with intraday values 2.44%, 1.62% and 0.6416% RSD for lower ((3 µg/mL), medium (70 µg/ mL) and higher (140 µg/mL) concentrations respectively of swertiamarin. In the interday assay, the maximum RSD values were found to be 0.4276%, 2.73% and 0.04322% for low, medium and high concentration respectively.

We also observed that dry powder extract marketed preparations greatly vary in the swertiamarin content. From the marketed preparation analysis we find that lowest concentration of swertiamarin observed in Formulation B (0.2402mg) than Formulation B 0.3192mg and maximum content observed in Formulation C (3.302mg). The lyophilied dry powder extract prepared at PERD centre had 11.6% yield of swertiamarin so suggesting that this formulation may be better efficacious. However, clinical studies are required to substantiate the statement.
Figure 2: Chromatogram of swertiamarin in different formulations (A, B and C) and Layophilized Dry Powder and Mamejava Pills *E.litorale* containg marketed formulation with retention of at 238 nm.
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(Note: Kindly observe the drug peak with its height and scale rather than visual. Saptrangi samples contain impurity at end time of run so that run time was increased specifically for it from 12 min to 20 min showed in figure d).

Table 1: Marketed Sample Analysis Result

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Number sample</th>
<th>Average Amount Found (μg/ml)</th>
<th>Swertiamarin in formulation(mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation A</td>
<td>6</td>
<td>10.26</td>
<td>0.1026 ± 0.00825 (mg/10mg)</td>
</tr>
<tr>
<td>Lyophilized Dry extract at PERD Centre</td>
<td>5</td>
<td>116.6</td>
<td>1.166 ± 0.00222 (mg/10mg)</td>
</tr>
<tr>
<td>Formulation B</td>
<td>1</td>
<td>24.02</td>
<td>0.2402 (mg/vati)</td>
</tr>
<tr>
<td>Formulation C</td>
<td>1</td>
<td>330.2</td>
<td>3.302 (mg/pills)</td>
</tr>
<tr>
<td>Mamejava Pills of PERD</td>
<td>5</td>
<td>31.92</td>
<td>0.3192 ± 0.0018 (mg/pills)</td>
</tr>
</tbody>
</table>

Figure 3: Comparision of Swertiamarin Content in (a) Dry Powder Extract (b) Marketed Polyherbal formulation

CONCLUSIONS

Our data suggest that the developed method can be utolized for the sample analysis of the marketed formulations. Swertiamarin content may vary in different anti diabetic herbal formulation containing *E.littorale*. The reults of nalysis may be correlated with the clinical studies.

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