

## FORMULATION, EVALUATION AND QUANTIFICATION OF DEACEYL GYMNEMIC ACID AND GALLIC ACID BY USING HPLC AND HPTLC OF ANTIDIABETIC POLYHERBAL GRANULES

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### ABSTRACT

The aim of present research work is to formulate and standardize *Gymnema sylvestre* containing polyherbal formulations. *Gymnema sylvestre* widely used as an anti-diabetic drug *Gymnema sylvestre* and other herbal drugs that having anti diabetic activity like *Momordica charantia*, *Embllica officinalis*, *Enicostemma littorale*, *Trigonella foenum-graecum*, *Azadirachta indica*, *Picrorhiza kurroa* were formulated into polyherbal granules by wet granulation technique and then evaluated for various parameters like angle of repose, bulk density, tapped density, car's index and hausner's ratio. The formulated granules exhibited excellent flow properties compared to

marketed formulation. Quantification of marker compounds present in various formulations done by HPTLC and HPLC. DAG-1 (Deacyl gymnemic acid) and Gallic acid content were found to be the highest in lab formulation and lowest in market formulation.

**KEYWORD:** Poly herbal, anti -diabetic activity, *Gymnema sylvestre*, wet granulation, DAG, Gallic acid.

### INTRODUCTION

Medicinal plants are of great value in the field of treatment and cure of disease. Over the years, scientific research has expanded our knowledge of the chemical effects and composition of active constituents, which determine the medicinal properties of the plants. About three quarters of the world's population rely on plant and plant extract for healthcare,<sup>[1,2]</sup>

Diabetes mellitus is the common endocrine disorder that affects more than 100 million People worldwide (6% of the population) and in the next 10 years it may affect about five times more people than it does now.<sup>[3,4]</sup> In India, the prevalence rate of diabetes is estimated to be 1-15%.<sup>[5-7]</sup> The disease was well known to the ancient Indian medical experts. All the renowned classic texts of Ayurveda like Charaka Samhita (1000 B.C.), Sushruta Samhita (600 B.C.) and subsequent works refer to this disease under the term *Madhumeha* or *Ikshumeha*.

The present scenario of global market is in urgent need of standardized and reproducible herbal preparations, which can be achieved by the formulation of modern herbal dosage forms and their evaluation by modern techniques. The main objective of the present study was to focus on the formulation and evaluation of poly herbal anti diabetic tablet by using *Gymnema sylvestre*, *Momordica charantia*, *Emblica officinalis*, *Enicostemma littorale*, *Trigonella foenum-graecum*, *Azadirachta indica*, *Picrorhiza kurroa* extracts based on the literatures these plants were selected for the formulation of polyherbal granules used for the treatment of Diabetes mellitus.

## MATERIALS AND METHODS

### Collection and authentication of plant material

Dried sample of *Gymnema sylvestre*, *Momordica charantia*, *Emblica officinalis*, *Enicostemma littorale*, *Trigonella foenum-graecum*, *Azadirachta indica*, *Picrorhiza kurroa* were collected from the local market. These were identified and authenticated on the basis of their various morphological as well as microscopical characters.

### Formulation of anti-diabetic herbal granules

Initially stated quantity of powder of each ingredient were taken and mixed by geometric mixing. Cross PVP was added as disintegrating agent. Then Povidon in alcohol (5%) was added till the dough is obtained, which was passed through 60 # sieve to get granules. They were dried in oven at 120° C for about half an hour. After that they were passed through 22# and 40# sieve and granules remained on 40# sieve were collected. Required quantity of lubricants like talc and Magnesium stearate were added and named as Lab Formulation abbreviate as LF.

**Table 1: The composition of formulation of herbal granules.**

Sr. no.	Ingredients	Quantity (GM)
1	Gymnema sylvestre	50
2	Momordica charantia	6
3	Emblica officinalis	20
4	Enicostemma littorale	6
5	Trigonella foenum-graecum	3
6	Azadirachta indica	6
7	Picrorhiza kurroa	6
8	Excipients	q.s.

### Quality analysis of all formulations

#### Determination of appearance, color, odor and taste

All the three formulations were evaluated for their appearance, color and odor. Results are shown in table 2.

#### Proximate analysis<sup>[8]</sup>

All the three formulations went through proximate analysis procedures for further comparison. Results are shown in table 3. These following parameters were checked.

##### (a) Determination of total ash

2 gm of accurately weighed powder was incinerated in a crucible at a temperature 500-600oc in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

##### (b) Determination of acid insoluble ash

The total ash of the powder obtained by the above procedure was subjected separately for the estimation of acid insoluble ash using the following procedure. The total ash obtained above was boiled for 5min with 25 ml of 2M hydrochloric acid and filtered using an ash less filter paper to collect insoluble matter. The ash obtained was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid-insoluble ash was calculated with reference to the air-dried powdered drug (60#).

##### (c) Determination of water soluble ash

The total ash of the powder obtained by the above procedure was subjected separately for the estimation of water soluble ash using the following procedure

The total ash was boiled for 5min with 25 ml of water and insoluble matter collected on an ash-less filter paper washed with hot water and ignited for 15 min at a temperature not exceeded 450<sup>0c</sup> in a muffle furnace.

Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powdered drug.

#### **(d) Determination of moisture content**

5 gm of gymnema powder was taken in a previously dried evaporating dish. Then drying was carried out in an oven at 60° C till constant weight was obtained. The difference in the weight before and after drying was calculated. Difference in weight was content of moisture in sample.

#### **Physical evaluation parameters<sup>[10]</sup>**

All the three formulations went through various evaluation parameters. Results are shown in table 4.

#### **(a) Determination of angle of repose**

The angle of repose is a relatively simple technique for estimation of the flow property of a powder. Powders with low angle of repose are free flowing and those with a high angle of repose are poorly flowing powders. 10 gm of granules were passed through funnel and the pile was formed. The angle of repose was calculated by using the formula

Angle of repose ( $\theta$ ) =  $\tan^{-1}$  height /radius.

#### **(b) Determination of tap density**

The measuring cylinder containing a known mass of blend was tapped for a fixed time. The minimum volume (Vt) occupied in the cylinder and the weight (M) of the blend was measured.

$$\rho_t = M / V_t$$

Where, M = Weight of powder

V t = Volume after tapping

$\rho_b$  = tap density

#### **(c) Determination of Hausner's ratio**

Hausner ratio is an indirect index of ease of powder flow

Hausner ratio =  $\rho_t / \rho_d$

Where,  $\rho_t$  = Tapped density

$\rho_d$  = Bulk density

#### **(d) Determination of Carr's index**

The simplest way of measurement of free flow of powder is compressibility. The indication of the ease with which a material can be induced to flow is given by compressibility index.

$$I = [(V_b - V_t) / V_b] \times 100$$

Where  $V_b$  = Bulk volume

$V_t$  = Tapped volume

I = Carr's index

### **Quantification of marker compounds in various formulations by HPTLC**

#### **Quantification of DAG-1**

Gymnemic acid is calculated as Deacyl Gymnemic acid.

#### **Preparation of standard solution**

Solution of Deacyl Gymnemic acid (200 $\mu$ g/ml) was prepared by dissolving 1 mg of reference compound in 5 ml of methanol.

#### **HPTLC analysis (Calibration curve)**

Semiautomatic spotter was used containing a syringe having capacity of 50 $\mu$ l. 10 $\mu$ l solution was filled in syringe and under nitrogen stream it was applied in form of bands of desired concentration (200ng/spot to 1000ng/spot) of standard solution on precoated plates. Plate was developed in stated mobile phase. Developed plate of Deacyl gymnemic acid were subjected to densitometric measurements in absorbance mode at wavelength 565 using Camag TLC scanner. Plots of peak area vs concentration of Deacyl Gymnemic acid was prepared.

#### **Estimation of DAG-1 in different formulations**

Test sample extract of different formulations were injected into chromatographic system. Peak areas of DAG-1 in the samples were noted and the concentration was determined from calibration curve using peak area. Results are shown in Fig 1.

### **Quantification of Gallic acid**

#### **Preparation of standard solution**

Solution of Gallic acid (100µg/ml) was prepared by dissolving 1 mg of reference compound in 10 ml of methanol.

#### **HPTLC analysis (Calibration curve)**

Semiautomatic spotter was used containing a syringe having capacity of 50µl. 10µl solution was filled in syringe and under nitrogen stream it was applied in form of bands of desired concentration (100ng/spot to 500ng/spot) of standard solution on precoated plates. Plate was developed in above mobile phase. Developed plate of Gallic acid was subjected to densitometric using Camag TLC scanner. Plot of peak area vs concentration of Gallic acid was prepared.

#### **Estimation of Gallic acid in different formulations**

Test sample extract of different formulations were injected into chromatographic system. Peak areas of marker compounds in the samples were noted and the concentration was determined from calibration curve using peak area. Measurement in absorbance mode at wavelength 285 nm Results are shown in fig 2.

### **Quantification of the DAG-1 in formulations by HPLC**

#### **Calibration curve for Standard DAG-1**

Appropriate volume of aliquots from standard (Deacyl gymnemic acid) was transferred to same volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol give a solution containing 10, 20, 30, 40, and 50 µg/ml standard. The standard solution was chromatographed for 10 minutes using mobile phase at a flow rate of 1.0 ml/min. Graph was plotted for peak area vs. concentration for the drug.

#### **Sample preparation**

10 gm of each powdered formulation were accurately weighted and extracted with petroleum ether twice. Then gymnemic acid was extracted with methanol each time. Methanol was evaporated. Treat this extract with 10 ml 2% NaOH and let it dissolve. It was heated on a waterbath for 1 hour. After cooling, con. HCl was added so that pH of solution is under 8. Methanol was added to make up the volume upto 25 ml. Then it was filtered and filtrate was evaporated to dryness. Dissolve 50 mg extract in 5 ml methanol. The resultant solution

was filtered through 0.45 micron membrane filter and the resulting solution was then injected and the peak areas of sample were measured at 273nm.

### Analysis of sample solution

20 micro litre of diluted sample solution were injected to HPLC system and peak responses were recorded for at least three replicate injections. Relative standard deviation was calculated, % relative std deviation was less than 2%. Chromatograph the sample preparation for at least 2 times and calculate. The % of marker compound in test solution were calculated by comparing the peak area of standard (API) with corresponding peak present in the Chromatogram of the test solution. Results are shown in fig 3 and table 7.

## RESULTS

**Table 2: Determination of color and odor**

Parameters	Color	Odor
Lab formulation	Greenish brown	Characteristic
MF-1	Green	Characteristic
MF-2	Brown	Characteristic

**Table 3: Proximate analysis of formulations.**

Sr. no.	Determination	Percentage w/w		
		LF	MF-1	MF-2
1	Total Ash	8%	9.33%	9%
2	Acid insoluble ash	2%	2.5%	3%
3	Water extractive value	30.4%	29.6%	30%
4	Alcohol extractive value	22.4%	22%	18.8%
5	Moisture content	6.2%	6%	5.5%

**Table 4: Evaluation Parameters of Formulations.**

Parameters	LF	MF-1	MF-2
Angle of repose	31.38°	39.50°	33.33°
Bulk density(g/cc)	0.277	0.408	0.645
Tap density(g/cc)	0.35	0.588	0.689
Compressibility index	41.66	30.612	6.452
Hausner's ratio	1.264	1.441	1.068

**Table 5: Estimation of deacyl Gymnemic acid in different Formulations.**

Formulation	Peak area	Concentration in µg/ml from calibration curve	% of deacyl Gymnemic acid
MF-1	1645.5	27.17	0.27
MF-2	1435.3	20.38	0.20
LF	4722.2	126.52	1.27



Table 6: Estimation of Gallic acid in different Formulations.

Formulation	Peak area	Concentration in $\mu\text{g/ml}$ from calibration curve	% of Gallic acid
MF-1	2040.7	57.61	0.12
MF-2	2021.2	57.12	0.11
LF	2000.9	169.84	0.34

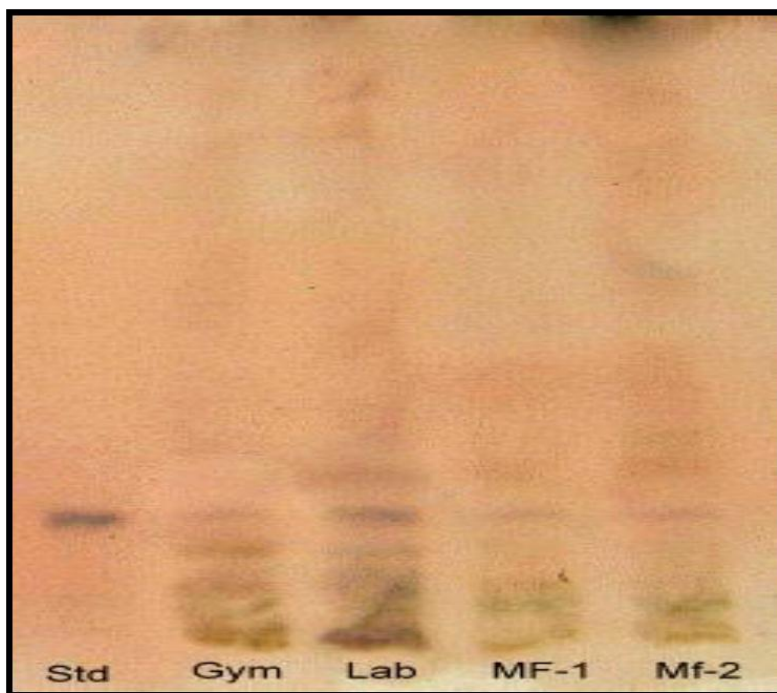


Figure 1: HPTLC chromatograph of quantification of DAG-1

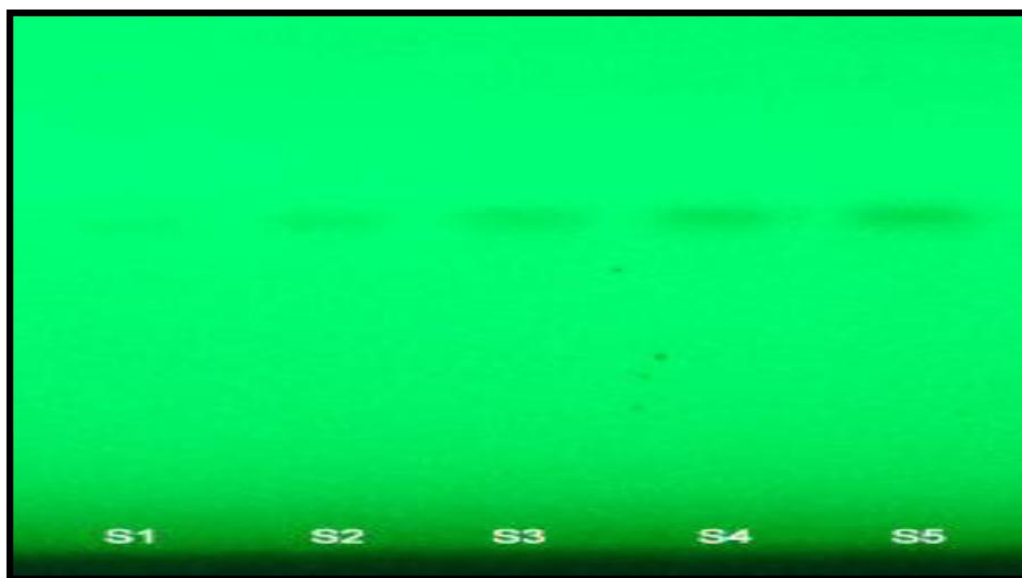
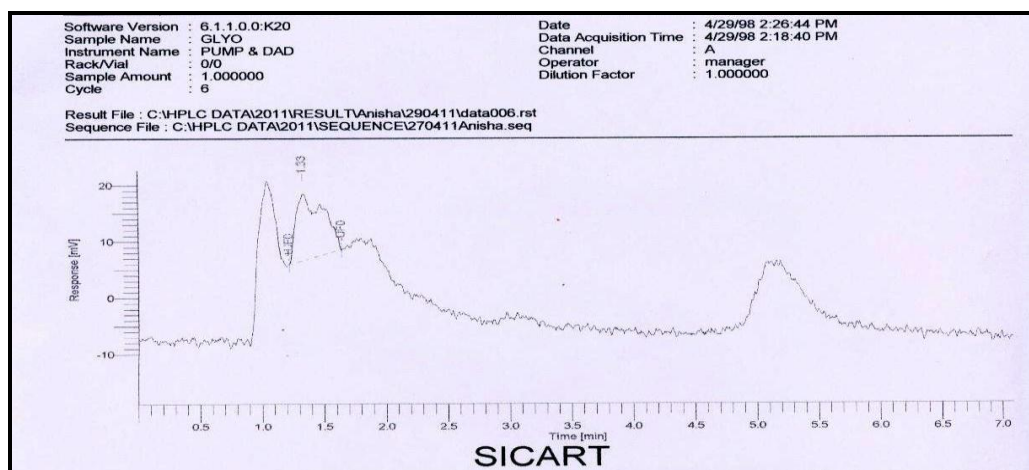


Figure 2: HPTLC chromatograph of calibration of standard Gallic acid.





**Figure 3: Quantifications of DAG-1 by HPLC**

**Table 7: Estimation of DAG-1 by HPLC in different Formulation**

Formulation	Peak area	Concentration in $\mu\text{g/ml}$ from calibration curve	% of DAG-1
MF-1	177366.2	131.0709626	1.31
MF-2	121652.2	83.34966355	0.83
LF	983940.96	245.3701584	2.45

## DISCUSSION

Polyherbal granules prepared by wet granulation method were tested for the preformulation studies. All the evaluated Preformulation parameters are shown in table 4. Quality analysis of all three batches are shown in table 2 and 3. Quality control parameters according to WHO guidelines were examined such as appearance, colour, ash value, extractive values, moisture content etc.

Total ash, acid insoluble ash, water soluble ash were found to be present in order MF-1>MF-2>LF. Lowest values were found in LF and highest values were in MF-1. Higher ash values indicate contamination due to extraneous materials which contain carbonates, phosphates, silicates and silica. High acid insoluble ash is due to presence of greater amount of silica especially sand and siliceous earth. The moisture content of all formulations was found to be highest in lab formulation and lowest in MF-2. Lower the moisture content, lower the microbial growth.

Water extractive values denote the amount of polar substances present in the formulation. The result shows that the lab formulation consisted of higher amount of water soluble substances.

Alcohol extractive values denote the amount of alcohol soluble constituents present in the formulation. The result shows that the lab formulation consisted of higher amount of alcohol soluble substances. It was found from the results that lab formulation showed good flow ability than market formulations. Thus a sensitive, precise and accurate HPTLC method was developed for estimation of DAG-1 and Gallic acid, HPLC method for estimation of Gymnemic acid. HPTLC chromatogram for Gallic acid and gymnemic acid shown in figure 1 and 2. Amount of Gallic acid and gymnemic acid quantified by HPTLC method in all three batches shown in table no. 5 and 6. Amount of DAG-1 present in all three batches shown in table no. 7 and HPLC chromatogram for DAG-1 shown in figure 3.

Percentage of DAG-1 was found to be 1.27% w/w, 0.27% w/w and 0.20% w/w and percentage of Gallic acid was found to be 0.34% w/w, 0.12% w/w and 0.11% w/w in lab formulation, market formulation-1, market formulation-2 respectively by HPTLC method. Percentage of DAG-1 was reported to be 2.45% w/w in lab formulation, 1.31% w/w in market formulation-1 and 0.83% w/w in market formulation-2 by HPLC method.

DAG-1 and Gallic acid content were found to be the highest in lab formulation and lowest in market formulation-2. Thus, quality control parameters for standardization of *Gymnema sylvestre* leaf and its formulations were developed. This would fulfill the need of standardization of formulation.

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