

# **STUDIES ON MEDICINAL PROPERTIES OF DIFFERENT MULBERRY VARIETIES**

## **SUMMARY of THESIS**

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## Summary

The present thesis entitled “Studies on medicinal properties of different mulberry varieties” contains research work and its outcome in respect of anti-diabetic property of a few selected mulberry varieties as the mulberry plant is said to hold good amount of phytochemicals and antioxidants. These plant chemicals are supposed to work as efficient anti-diabetic medicines. Medicinal plants are gaining considerable importance due to their special attributes as a large source of therapeutic phytochemicals. These naturally occurring chemicals may lead to formulation of side effect free novel drugs. Most of the phytochemicals from plant sources such as phenolics and flavonoids are found to have incredible capacity to prevent diabetes disorder and maintain good health.

Plant sourced drugs are gaining more popularity among the urban population now a days. Mulberry leaves which are known as the only food source of silkworm till now, are finding greater recognition and utility in the medicinal field too. The mulberry leaves are found to contain antioxidant, antimicrobial and anti-inflammatory, anti-diabetic and cancer healing properties. Mulberry leaves are being found useful to treat hyperglycemia, inflammation, cough, hypertension, cancer and fever as they have diaphoretic and emollient effects. As the medicinal properties of mulberry plants are getting identified for treatment of many ailments their commercial/medicinal utilities are attracting the attention of the pharmaceutical industry. Availability of Mulberry (*Morus*, Moraceae) plants are in plenty distributed worldwide and thus the raw material is quite abundant.

The current study is thus oriented towards evaluating certain medicinal properties of selected mulberry varieties. Therefore, for the present study ten mulberry varieties namely BC2-59, C-776, C-763, DD, K-2, MR-1, MR-2, S-799, S-1708, and TR-10 were selected based on the recommendation made by the scientists working in the field of mulberry.

This study was carried out in three phases namely, 1. The Phytochemical study, 2. The Anti-oxidant study and 3. The Anti-diabetic study.

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The first phase was to determine the presence of Phytochemical compounds in selected mulberry varieties and to carry out quantitative analysis. The second phase was to evaluate the anti-oxidant properties in the same selected mulberry varieties through *in-vitro* studies. The third phase was to investigate the anti-diabetic property of the said selected mulberry varieties through *in-vivo* studies.

The first phase of the study was carried out through HPLC method where HPLC profiles were created for the ten varieties of mulberry along with the standard 7-hydroxycoumarin and quercetin dehydrate solvents. The solvents taken were for comparison purpose. The HPLC profiles of each of the mulberry varieties were measured and compared for drawing conclusions. The detailed procedure is enumerated in chapter II.

The second phase of the study was carried out through *in vitro* studies using DPPH and FRAP assay methods with methanol and ethanol solvents under five different concentrations (50, 100, 200, 300, 400 µl/mL) of the selected ten varieties of mulberry leaf extracts along with Ascorbic acid. The Ascorbic acid served as the control for comparisons. Measurements were made on the DPPH radical scavenging activity and FRAP activity under both the solvents. The detailed procedure is explained in chapter IV.

The third phase of study was carried out through *in-vivo* process by administering selected mulberry leaf extracts to the male park strains mice which were induced with diabetes through STZ well in advance. The diabetes induced mice were kept as the control specially to observe the effect of selected varieties of mulberry leaf on the high level blood glucose induced. The Normal mice (non-diabetic) and Diabetic mice with insulin treatment also served as reference points. Measurements were made on the body weight, fasting blood glucose levels and enzymatic activities such as Catalase, G6PDH, SGPT and SGOT. Detailed methodology is explained in Chapter VI.

#### **Screening of selected mulberry varieties for phytochemical content.**

The profiles of mulberry leaf extracts obtained through HPLC evaluation and comparison of this with the standard profiles of 7- hydroxycoumarin and quercetin dehydrate has indicated that mulberry varieties namely, **C-763**, **MR-1**, **TR-10** have expressed a character of possessing Phytochemical of the kind 7- hydroxycoumarin and varieties like

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**BC-2-59, MR-2 and S-1708** have expressed character of possessing Phytochemical of the kind quercetin dihydrate. Thus out of the ten varieties screened, profiles of these six varieties have agreement with the standard profiles. This is a positive inference. The other four varieties though have not matched the profiles with the standard ones may have an acceptance with standard chemicals away from the present ones which need to be explored.

#### **Evaluation of antioxidant properties of different mulberry varieties (*in-vitro* study).**

Based on the evaluation, it has been found that most of the mulberry varieties have commendable antioxidant properties. Under the two solvents namely, methanol and ethanol, five concentrations (50, 100, 200, 300, 400 µl/mL) of the homogenates and two evaluation methods (DPPH, FRAP), it is found that varieties like, **BC-2-59, C-763, K-2, MR-1, S-1708** have fared well under DPPH- methanol combination. Varieties like **C-763, MR-1, S-1708** fared well under DPPH- ethanol combination. Varieties such as **BC2-59, C-763, DD, S-1708, TR-10** did well under FRAP- methanol set and varieties namely, **BC2-59, C-763, DD, K-2, MR-1, S-1708, TR-10** have done well under FRAP- ethanol set.

Secondly, though both the solvents namely, methanol and ethanol exhibited positive response for identification of the antioxidant properties, methanol was found to be the most efficient solvent for detection of antioxidants from mulberry leaves in all the assays used. It was also observed that higher the concentration, higher was the percent inhibition or ferric ion reduction power. This implies that the effect will be more at higher concentrations of the extracts.

#### **Evaluation of anti-diabetic property of different mulberry varieties (*in-vivo* study).**

Mulberry varieties selected in the study have also been subjected to anti-diabetic property evaluation. The experiment was conducted on male park strains mice where in they were induced with diabetes in the beginning and later administered with selected mulberry leaf extracts. Data were recorded in respect of body weight, fasting blood glucose levels,

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enzyme activities (Catalase, G6PDH, SGPT and SGOT). Compiled data have given interesting output as follows.

**Body weight:**

With regard to body weight, it is required that the weight must increase with the intake of mulberry leaf extract. It did happen in case of insulin treatment. From the data, it is observed that the mulberry varieties namely, **C-763, MR-1, S-799, S-1708 and TR-10** were also found to be very much effective in providing a substantial body weight gain on par with that of normal group and the insulin treated group. The other five varieties have not provided such an encouraging result with the weight gain.

**Fasting Blood Glucose level:**

Observation of the fasting blood glucose controlling activity data has revealed that mulberry varieties such as **C-763, MR-1, MR-2, S-799, S-1708 and TR-10** have indicated better capacity to act on the pancreas and liver cells for regulating the glucose levels in blood. They have the capacity to bring down the glucose levels to normal levels. However, the other varieties such as BC-2-59, C-776, DD and K-2 have though expressed some anti-diabetic property they seem to possess insufficient phytochemical/antioxidant load to contain the glucose levels in blood.

**Catalase activity:**

Data with regard to catalase enzyme activity indicated that the mulberry varieties like **C-763, MR-1, S-799, S-1708** have been extraordinarily useful in reducing the enzyme activity which help normal functions of the body by cutting down the glucose levels. The other six varieties have not been found to be as encouraging as these four.

**G6PDH:**

Regarding the G6PDH, it is essential that this activity need to be augmented many fold than that of the diabetic mice. This function has been efficiently performed by the

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varieties **C-763, MR-1, S-799 and S-1708** and thus they could be considered as the most promising ones. The other varieties such as BC-259, C-776, DD, K-2, MR-2 and TR-10 have lagged behind with lesser scores. Though TR-10 has indicated positive improvement, it has not reached the required level. Similarly MR-2 variety though has indicated potential; the score has not come up to the necessary level. However, as it appears these two varieties may catch up in the longer run.

#### **SGPT:**

Regarding the SGPT activity, the treatment has to bring down the enzymatic value that has been largely pushed up due to influence of higher level of blood glucose (diabetes). On observation of the experimental data, it is found that **C-763, MR-1, MR-2, S-799, S-1708 and TR-10** mulberry varieties have efficiently contributed for lowering the SGPT values to the near normal level and even less than the levels under insulin treatment.

#### **SGOT:**

Similarly in case of SGOT measures also, the above said varieties have contributed substantially to reduce the enzymatic values to the normal level and even less than the normal level. In addition to this the effect of them is proved to be better than the insulin treatment. Hence under this measure the varieties **C-763, MR-1, MR-2, S-799, S-1708 and TR-10** are regarded as highly promising.

The histopathological study also has suggested that mulberry leaf has the capacity to augment regeneration of liver tissue. The experimental results emphasize that mulberry varieties namely **C-763, S-1708, S-799, MR-1** possess excellent anti-diabetic properties. These investigations will be helpful for pharmacological area for formulation of suitable drugs for treatment of diabetic disorder.

On perusal of the above results and out of the many performing varieties, it will be essential to pick some that have shown a very consistent performance over all the three

phases detailed above. Performances of all the varieties over the parameters are thus tabulated here under to assist making a final conclusion.

From the table below it will be easy to identify those varieties which have performed consistently. Those varieties which got a high consistency score could be regarded as the best that with stood all the three tests here. On the basis of this clause, the varieties are thus classified into three categories as poor, moderate and best performers.

### Performance of varieties over different variables

Mulberry varieties	Phyto-chemical study		Anti-oxidant study ( <i>in-vitro</i> )				Anti-diabetic ( <i>in-vivo</i> study)						Total consistency score
	HPLC		DPPH		FRAP		Body wt.	FBG	CAT	G6PDH	SPGT	SGOT	
	7-HC	Q. D.	M	E	M	E							
BC2-59	-	Y	Y	-	Y	Y	-	-	-	-	-	-	4
C-776	-	-	-	-	-	-	-	-	-	-	-	-	0
C-763	Y	-	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11
DD	-	-	-	-	Y	Y	-	-	-	-	-	-	2
K-2	-	-	Y	-	-	Y	-	-	-	-	-	-	2
MR-1	Y	-	Y	Y	-	Y	Y	Y	Y	Y	Y	Y	10
MR-2	-	Y	-	-	-	-	-	Y	-	Y	Y	Y	5
S-799	-	-	-	-	-	-	Y	Y	Y	Y	Y	Y	6
S-1708	-	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11
TR-10	Y	-	-	-	Y	Y	Y	Y	-	-	Y	Y	7

Note: 7- Hydroxycoumarin (7 HC), Quercetin Dihydrate (Q. D.), M-Methanol, E- Ethanol, CAT- Catalase and Y - Indicates satisfactory performance.

Hence the varieties falling under the three categories are:

1. Poor performers : BC2-59, C776, DD and K-2
2. Moderate performers : MR-2, S-799 and TR-10
3. Best performers: C-763, MR-1, S-1708.

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So based on the above research study it could be convincingly reported that the mulberry leaf is emerging as an important herbal medicinal source for treatment of many chronic human ailments. Some of the mulberry varieties are even more efficient than insulin in containing the excess blood glucose due to the combined impact of natural antioxidant compounds present in them. Finally, the mulberry varieties namely, **C-763**, **MR-1** and **S-1708** have proved to be extraordinary with Phytochemical, Anti-oxidant and Anti-diabetic ingredients and thus would stay longer to benefit the human health through the pharmaceutical industry. Out of the three the two varieties C-763 and S-1708 have performed exceedingly well with higher rank of performance as evidenced by the data. Thus, the anti-diabetic effect of these mulberry varieties could be well exploited to provide relief without any side effects to millions of patients suffering from Diabetes Mellitus all over the globe.

Therefore, it is essential that the pharmaceutical industries consider the importance of the medicinal values of these mulberry plants. The bioavailability of antioxidants could be utilized in food and natural medicine systems as well. In addition it is vital that the potential, exploitable, beneficial effects and life safety tests for human consumption need to be proved through proper clinical trials.

Further, these studies have opened up new scope for future investigations such as exploring some more mulberry varieties for added medicinal properties, subjecting the poor performers listed above for a different kind of investigation so as to explore their intrinsic potentialities and thirdly taking the best performers further up in the ladder of investigation for making them available to benefit the human society.

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