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#### ANTIDIABETIC AND ANTIHYPERLIPIDAEMIC ACTIVITY OF ETHANOL EXTRACT OF

#### **MELASTOMA MALABATHRICUM L. LEAF IN ALLOXAN INDUCED DIABETIC RATS**

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Abstract: The ethanol extract of *Melastoma malabathricum* leaf (Family: Melastomataceae) was investigated for its antihyperglycemic and antihyperlipidaemic effect in Wistar Albino rats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg, i.p). The ethanol extract of *Melastoma malabathricum* at a dose of 150 and 300mg/kg of body weight were administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of Melastoma malabathricum leaf on blood glucose, serum insulin, urea, creatinine, glycosylated haemoglobin, serum lipid profile [total cholesterol (TC), triglycerides (TG), low density lipoprotein – cholesterol (LDL-C), very low density lipoprotein – cholesterol (VLDL-C), high density lipoprotein – cholesterol (HDL-C) and phospholipid (PL)] serum protein, albumin, globulin, serum enzymes [Serum Glutamate Pyruvate Transaminases (SGPT), Serum Glutamate Oxaloacetate Transaminases (SGOT), and Alkaline Phosphatase (ALP)] were measured in the diabetic rats. The ethanol extract of *Melastoma malabathricum* leaf elicited significant reductions of blood glucose (p<0.05), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C. The extracts also caused significant increase in serum insulin (p<0.05) in the diabetic rats. From the above results, it is concluded that ethanol extract of Melastoma malabathricum possesses significant antihyperglycemic and antihyperlipidaemic effects in alloxan induced diabetic rats.

**Keywords:** *Melastoma malabathricum*, Antidiabetic, Antihyperlipidaemic, Alloxan, Glibenclamide, SGOT, SGPT and HbA<sub>1</sub>C



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#### INTRODUCTION

Diabetes mellitus is an epidemic occurring in adults throughout the world and is the leading cause of kidney failure, heart attack, blindness and lower limb amputation. It is the fourth main cause of death in most developed countries. The prevalence of diabetes is estimated to reach 330 million by the year 2025, according to international Diabetes Federation, with the greatest potential increase being in Africa and Asia. This numerical increase will occur in developing countries. This by the year 2025 over 75% of people with diabetes will reside in developing countries, as compared to 62% in 1995<sup>1</sup>.

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides. Many of them have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigations<sup>2</sup>. Many herbs and plants have been described as possessing hypoglycemic activity when taken orally <sup>3</sup>. According to this World Health Organization (WHO) there is more than 1200 plant species worldwide used in the treatment of diabetes mellitus and substantial number of plant showed effective hypoglycemic activity after laboratory testing <sup>4</sup>.

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies. Antihyperglycemic activity of the Plats is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids etc that are frequently implicated as having antidiabetic effect<sup>5</sup>. However, the study of plant for hypoglycemic, antioxidant and hypolipidemic activities may give new pharmacological approaches in the treatment of diabetes mellitus<sup>6</sup>.

Melastoma malabathricum belongs to the Melastomataceae family. It is also called the Singapore Rhododendron or Sendudok. It is a erect shrub or small tree 1.5 to 5m tall. It was traditionally used to treat diarrhoea, dysentery, leucorrhoea, hemorrhoids, wounds, infection during confinement, toothache, flatulence, sore legs, and thrush and also it is used by the Jah hut people in Malaysia to cure diarrhea<sup>7</sup>. There is no on the antidiabetic report and antihyperlipidaemic potential of this plant etract so far. The main objective of this study was to assess the antidiabetic and



antihyperlipidaemic effect of ethanol extracts of leaf of *Melastoma malabathricum* in alloxan induced diabetic rats.

#### **Materials and Methods**

#### Plant Material

The leaves of *Melastoma malabathricum* L. were freshly collected from Daudeli, Joide Taluk, Hubli District, North Karnataka. With the help of local flora, a voucher specimen were identified and retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

# Preparation of plant extract for phytochemical screening and antidiabetic studies

The *Melastoma malabathricum* leaf was shade dried at room temperature and the dried leaf was powdered in a Wiley mill. Hundred grams of powdered leaf was packed in a Soxhlet apparatus and extracted with ethanol. The extracts were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures <sup>8,9</sup>. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

#### Animals

Normal healthy male Wistar Albino rats (180- 240g) were housed under standard environmental conditions at temperature (25±2°C) and light and dark (12: 12 h). Rats

were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*. Study was carried out as per IAFC approval No: 82/PHARMA/SCRI, 2010.

#### Acute Toxicity Study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study <sup>10</sup>. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals. then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100, and 2000mg/kg body weight.

# Induction of Diabetes in Experimental animal

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg)<sup>11</sup>. Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access



to water and pellet diet and maintained at room temperature in plastic cages.

#### **Experimental Design**

In the present investigation, a total of 30 rats (24 diabetic surviving rats and 6 normal rats) were taken and divided into five groups of 6 rats each.

Group I: Normal untreated rats

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *Melastoma malabathricum* leaf (150mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of *Melastoma malabathricum* leaf (300mg/kg body weight)

Group V: Diabetic rats given standard drug glibenclamide (600µg/kg body weight).

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes.

# Estimation of insulin, glucose, urea, creatinine and glycosylated haemoglobin

Serum glucose was measured by the Otoluidine method <sup>12</sup>. Insulin level was assayed by Enzyme Linked Immuno Sorbant Assay (ELISA) kit <sup>13</sup>. Urea estimation was carried out by the method of Varley <sup>14</sup>; serum creatinine was estimated by the method of Owen *et al* <sup>15</sup>. Glycosylated haemoglobin (HbA<sub>1</sub>C) estimation was

carried out by a modified colorimetric method of Karunanayake and Chandrasekharan <sup>16</sup>.

# Estimation of protein, albumin, globulin, SGPT, SGOT, ALP

Serum protein <sup>17</sup> and serum albumins was determined by quantitative colorimetrically method by using bromocresol green. The total protein minus the albumin gives the glutamate globulin, serum pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel<sup>18</sup>. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong<sup>19</sup>.

#### Estimation of lipids and lipoprotein

Serum total cholesterol (TC)<sup>20</sup>, total triglycerides (TG)<sup>21</sup>, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL- C) <sup>22</sup>, high density lipoprotein cholesterol (HDL-C)<sup>23</sup> and phospholipids<sup>24</sup> were analyzed.

#### **Statistical Analysis**

The data were analyzed using student's ttest statistical methods. For the statistical tests a p values of less than 0.01 and 0.05 was taken as significant.

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#### RESULT

#### **Phytochemical constituents**

The phytochemical screening of ethanol extract of *M.malabathricum* leaf revealed the presence of alkaloid, catachin, coumarin, flavonoid, phenol, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein.

#### Acute toxicity test

The extract was safe up to a dose of 2000mg/kg body weight. Behavior of the animals was closely observed for the first 8h then at an interval of every 4h during the next 48h, the extract did not cause mortality on rats during 48h observation or any behavioral change.

#### Body weight and fasting blood glucose

In the present study, alloxan induced diabetic rats showed significant (p < 0.01) reduction in body weight. Administration of ethanol extract of *M.malabathricum* leaf (150 and 300mg/kg) and glibenclamide (600mg/kg) significantly (p<0.05) increased the body weight within 14 days (Table 1). Fasting blood glucose levels of the diabetic control rats were higher than those of normal rats. A significant (p<0.05) dose dependent decrease in blood glucose levels was observed in the diabetic treated group from an initial level of 232.14mg/dl to the level of 144.36mg/dl and from 213.12 mg/dl to 124.33mg/dl after the treatment at a dose of 150mg/kg 300mg/kg and respectively for 14 days (Table 1).

 Table 1: Effect of Melastoma malabathricum leaf extract on the body weight and fasting blood glucose in normal, diabetic and diabetic treated rats.

Treatment groups	Mean initial body weight (g)	Mean final body weight (g)	Mean weight Gain (G↑ ) / Ioss(L↓) (g)	Fasting Bloodglucose (mg/dl)	
				Initial	Final(after 2wks)
Group I	189.54±5.11	197.65±6.36	8.12↑	69.88±1.92	73.95±1.64
Group II	192.63±8.24	173.94±5.24	18.69↓**	248.56±2.84	263.16±5.36
Group III	188.31±5.36	196.47±4.14	8.16 <b>↑</b> a	232.14±2.54	144.36±4.86b
Group IV	184.51±5.33	194.93±4.38	10.42↑a	213.12±5.28	124.33±4.37b
Group V	196.58±9.34	216.42±7.39	19.84↑a	229.66±6.16	104.53±4.35b

Each Value is SEM of 5 animals \* p < 0.05 comparison with Normal control vs diabetic and drug treated : a p < 0.05 Diabetic control vs drug treated : b p < 0.05 comparison with initial vs final



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#### Blood glucose level and other parameters

Table 2 shows the levels of blood glucose, creatinine serum insulin. urea. and glycosylated hemoglobin of normal, diabetic control and drug treated rats. There was a significant (p < 0.01) increase blood glucose level in alloxan induced diabetic rats (Group II) when compared with normal rats (Group I). Administration of leaf extract of M.malabathricum (Group III& IV) and glibenclamide (Group V) tends to bring the parameters significantly (p<0.05, p<0.01) towards the normal. Serum insulin level of diabetic control group was significantly (p < 0.01) decreased when

compared to normal control group (Group I). The extract and glibenclamide group of diabetic rats significantly (p<0.01) increased the serum insulin. A significant (p < 0.05) elevation in urea and creatinine was observed in alloxan induced diabetic rats (Group II), when compared to control rats. The *M. malabathricum* extracts were administrated orally to diabetic rats for 14 days reversed the urea and creatinine level to near normal. Administration of ethanol extract of М. malabathricum leaf (300mg/kg) and glibenclamide significantly (p<0.05) reduced HbA<sub>1</sub>C level compared to diabetic control rats.

Table 2: Effect of *Melastoma malabathricum* leaf extracts on the serum insulin, glucose, urea, creatinine and HbA1C level of normal, diabetic induced and drug treated rats

Parameter	Insulin (mlu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	GlycolytedHb (%)
Group I	21.83±1.13	73.81±3.54	12.05±1.04	0.73±0.12	4.61±0.52
Group II	9.19±0.948**	198.39±2.61**	29.63±1.85*	2.45±0.35*	9.89±0.25**
Group III	14.30±1.67 <sup>a</sup>	134.66±1.56 <sup>a</sup>	21.54±1.26	1.86±0.48	7.26±0.76ns
Group IV	18.51±1.25 <sup>aa</sup>	114.51 ±1.84 <sup>aa</sup>	14 .45±1.38	1.29±0.15	5.16±0.81 <sup>a</sup>
Group V	19.73±1.09 <sup>aa</sup>	109.38±1.84 <sup>aa</sup>	13.05±1.56 <sup>a</sup>	1.05±0.11 <sup>a</sup>	5.21±0.62 <sup>a</sup>

Each Value is SEM of 5 animals: \*Comparison made between normal control to diabetic control and drug treated groups; \* p < 0.05, \*\*p < 0.01 a, Comparison made between diabetic control to drug treated groups .a: p < 0.05; aa p < 0.01: ns: Not significant.

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#### **Biochemical parameters**

The level of total protein, albumin, globulin and liver marker enzymes such as SGPT, SGOT and ALP in the serum of diabetic rats are presented in the Table 3. A significant reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic rats (Group II), when compared to control rats (Group I). On administration of ethanol extract of *M.malabathricum* leaf to the diabetic rats, protein albumin and globulin levels were found to be restored in normal. Also, the SGPT, SGOT and ALP levels were elevated significantly in alloxan induced diabetic rats compared to control rats. Both the doses of *M.malabathricum* leaf extracts and glibenclamide treatment significantly reduced above parameters compared to diabetic control rats.

Table 3: Effect of *Melastoma malabathricum* leaf extracts on the serum protein, albumin, globulin, SGPT, SGOT and ALP level of normal, diabetic induced, and drug treated rats.

Groups	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/I)
Group I	7.93±0.16	4.63±0.14	3.30±0.11	18.53±1.62	20.16±1.33	184.93±3.65
Group II	6.02±0.19*	3.84±0.21*	2.18±0.24	97.18±3.54*	89.25±1.91*	226.16±4.84*
Group III	6.96±0.15 <sup>a</sup>	4.12±0.11	2.84±0.14	38.56±1.26	49.66±1.76	204.08±4.56 <sup>a</sup>
Group IV	8.26±0.65 <sup>a</sup>	4.86±0.53	3.40±0.52	27.59±1.84	30.51±1.94	191.56±2.89 <sup>a</sup>
Group V	7.96±0.38ns	4.03±0.45	3.93±0.24	21.94±1.12	25.88±1.65	173.94±2.16 <sup>a</sup>

Each Value is SEM of 5 animals: \*Comparison made between normal control to diabetic control and drug treated groups; \* p < 0.05, \*\*p < 0.01 a, Comparison made between diabetic control to drug treated groups. a: p < 0.05; aa P < 0.01: ns: Not significant

#### Lipid profiles

Table 4 shows the levels of TC, TG, LDL-C, VLDL-C, HDL-C and PL in the serum of diabetic rats showed significantly (p<0.01) increased serum lipid profiles except HDL-C when compared with normal rats. The ethanol extract of *M.malabathricum* leaf treated rats showed a significant (p<0.01,

p < 0.05) decrease in the content of lipid profiles when compared with diabetic induced rats. Similarly HDL-C level decreased in alloxan induced diabetic rats compared with normal when rats. Administration of ethanol extract of *M.malabathricum* leaf and glibenclamide to the diabetic rats. HDL-C level was found to be restored to normal.



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Table 4: Effect of *Melastoma malabathricum* leaf extracts on the serum Lipid profile of normal, diabetic induced, and drug treated rats

Groups	TC (mg/dl)	TG(mg/dl)	HDL(mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)	PL (mg/dl)
Group I	103.34±2.18	93.61±1.64	42.98±2.16	41.64±1.32	18.72±1.03	159.97±3.51
Group II	214.26±4.76**	198.16±4.27**	26.36±1.65**	148.27±3.68**	39.63±1.26*	258.69±5.33**
Group III	164.13±2.48 <sup>aa</sup>	156.31±1.74 <sup>aa</sup>	31.14±2.14 <sup>a</sup>	96.64±1.34 <sup>a</sup>	34.44±1.34 <sup>a</sup>	224.36±2.36 <sup>a</sup>
Group IV	141.65±2.27 <sup>aa</sup>	136.18±1.13 <sup>aa</sup>	36.39±2.31 <sup>a</sup>	78.02±1.16 <sup>aa</sup>	27.24±1.48 <sup>a</sup>	194.06±2.17 <sup>a</sup>
Group V	119.57±1.21 <sup>aa</sup>	124.74±2.68 <sup>aa</sup>	23.54±1.88	71.08±1.21 <sup>aa</sup>	24.95±1.23 <sup>a</sup>	174.41±1.92 <sup>aa</sup>

Each Value is SEM of 5animals: \*Comparison made between normal control to diabetic control and drug treated groups; \* p < 0.05, \*\*p<0.01 a, Comparison made between diabetic control to drug treated groups. a: p<0.05; aa p<0.01: ns: Not significant

#### DISCUSSION

Diabetes mellitus is one of the most familiar chronic diseases associated with carbohydrate metabolism. It is also an indication of co-morbidities such as obesity, hypertension and hyperlipidemia which are metabolic complications of both clinical and experimental diabetes. Despite the fact, that diabetes mellitus has high prevalence, morbidity and mortality globally; it is regarded as a non curable but controllable disease. Different synthetic drugs, plant remedies and dietary modification play an efficient role in the reduction of the suffering that it causes. The potential role of medicinal plants as hypoglycemic agents has been reviewed by several authors<sup>25, 26</sup>.

Pancreas is the primary organ involved in sensing the organism's dietary and

energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas.

Alloxan causes a massive reduction in insulin release by the destruction of  $\beta$ -cells of the islets of langherhans, thereby inducing hyperglycemia. Insulin deficiency leads to various metabolic alterations in the animals viz increased cholesterol, increased levels of alkaline phosphate and transaminases<sup>27, 28</sup>.

In diabetic condition, elevated blood glucose, reduction in body weight, polyuria, polydipsia and polyphagia are commonly observed. In the present study, induction of



diabetes by alloxan produced increase in blood glucose level, decrease in body weight and polyuria. In diabetic rats, observed reduction in body weight was possible due to catabolism of fats and protein <sup>29</sup>. The administration of ethanol extract of *M.malabathricum* leaf improves body weight compared to diabetic control rats which indicates preventive effect of *M.malabathricum* on degradation of structural proteins. The increase in blood glucose level after alloxan administration may be due to insulin deficiency or resistance state in diabetic rats. Administration of ethanol extract of *M.malabathricum* leaf significantly reduced blood glucose level in diabetic rats which represents reversal of insulin resistance or increasing insulin secretion possibly by regeneration of damaged pancreatic β-cells in alloxan-induced diabetic rats <sup>30</sup>. Earlier many plants have been studied for their hypoglycemic and insulin release stimulatory effects <sup>31-36</sup>.

In diabetes, elevated levels of serum urea and creatinine are observed which may be due to renal damage caused by abnormal glucose regulation or elevated glucose and glycosylated protein tissue levels <sup>37</sup>. In present study, significant increase in serum urea and creatinine levels were observed in diabetic rats compared to normal control rats which indicate impaired renal function in diabetic rats. The treatment with ethanol extract of *M.malabathricum* lowered the above parameters significantly compared to diabetic control rats and it showed protective effect of ethanol extract of *M.malabathricum* on the kidneys.

In diabetes, HbA<sub>1</sub>c is considered as a diagnostic marker and helps to know about degree of protein glycation, long-term blood sugar level and correlation of diabetes associated complications <sup>38, 39</sup>. Glycosylated haemoglobin has been found to be increased over a long period of time in diabetes. During diabetes, the excess of glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin <sup>40</sup>. The rate of glycation is proportional to the concentration of blood glucose. In present study, alloxan induced diabetic rats showed significant increase (p<0.01) glycosylated haemoglobin (HBA1C) level compared with normal rats. The ethanol extract of *M.malabathricum* whole plant treated rats showed a significant decrease (p<0.05) in the content of glycosylated haemoglobin that could be due to an improvement in glycemic status.

diabetic condition, In occurrence of reduction in protein and albumin may be due to proteinuria, albuminuria or increased protein catabolism, which are clinical markers in diabetic nephropathy<sup>41</sup>. The protein and albumin level was reduced after the induction of diabetes and treatment of ethanol extract of M.malabathricum increased both levels considerably in diabetic rats towards normal level. This action possibly is through increase in the insulin mediated amino acid uptake, enhancement of protein synthesis

and/or inhibition of protein degradation<sup>42</sup>. Also, increased serum SGOT, SGPT and ALP levels were reported in diabetes and it may be due to liver dysfunction<sup>43</sup>. In this study, increased level of SGOT, SGPT and ALP was observed in alloxan-induced diabetic rats which may have occurred by leakage of enzymes from the liver cytosol into the blood stream; it represents the toxicity of alloxan on liver. Diabetic rats treated with ethanol extract of *M.malabathricum* leaf significantly reduced both enzyme levels which represents the protective action of ethanol extract of *M.malabathricum* leaf liver in diabetic condition.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia<sup>44</sup>. The abnormal high concentrations of serum lipids in diabetic animals are mainly due to an increased mobilization of free fatty acids from peripheral fat depots<sup>45</sup>. In the present study, significantly increased levels of serum TC, TG, VLDL and LDL as well as marked reduction in serum HDL level in diabetic rats. Administration of both the doses of ethanol extract of M.malabathricum leaf decreased levels of TC, LDL, VLDL and TG levels as well as increased the level of HDL in diabetic rats. The above action could be beneficial in preventing diabetic complications like coronary heart diseases and atherosclerosis diabetic in condition. Increased phospholipids levels in serum were reported by Anitha et al.,46 in alloxan induced diabetic rats. Administration of ethanol extract of *M.malabathricum* leaf glibenclamide decreased the levels of phospholipids.

In the present study, the administration of M.malabathricum leaf extracts to alloxan induced hyperglycemic rats demonstrated prominent reduction in blood sugar level, normalization of serum biochemical profile including lipid content, as compared to alloxan control rats. The phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, terpenoids, glycosides, steroids, saponin and phenols. Several authors reported that flavonoids, steroids/terpenoids, phenolic acids are known to be bioactive antidiabetic principles<sup>47,46</sup>. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues <sup>48</sup>. In the present study, the phytochemical analysis of ethanol extract of *M.malabathricum* leaf clearly prints out the presence of above said principles. preliminary active The investigation on the antihyperglycemic, antihyperlipidaemic and antioxidant efficacy of ethanol extract of *M.malabathricum* leaf will be significant to proceed further in this path for the isolation of active principles responsible for the antidiabetic activity.

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